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Social Determinants of Health – A Comparative Study of Bosnian Adolescents in Different Cultural Contexts

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ABSTRACT

This study investigated the effects of sociocultural contexts on health and the psychological well-being of immigrant adolescents, aged 15 to 18 years, originally from Bosnia and Herzegovina and now living as displaced persons either in Bosnia, or immigrants in Croatia and Austria. The study addresses the social determinants of health with a specific focus on five factors in the social environment that might have an influence on health status: gender, socio-economic status (SES), perceived discrimination and exposure to violence, social support and religious commitment. Dependent variables included self-rated health, a count of self-reported objective health problems and a range of indices of psychological well-being (somatic stress, anxiety, depression and self-esteem). The purpose of the study was to examine whether social risk factors have an effect on health, which factors mediate these effects on self-rated health and to assess whether these effects differ by gender. Results indicate that perceived discrimination and violence are related to poor health through psychological stress as a major mechanism with stronger effects for girls in the study. Differences across the three socio-cultural contexts reveal the complexity and specificity of the relationships between analyzed factors as the association between discrimination and health was attenuated for some groups due to the protective resources of immigrants.

Key words: adolescents, acculturation, discrimination, health, psychosocial well-being

Introduction

Previous studies confirm that immigration is a strong stressor for psychological and physical health¹. Immigrants must immerse themselves in a new culture and often must undergo a great deal of social and personal change that could affect their general and psychological health. Research has consistently found that displaced or immigrant persons are usually faced with disadvantaged conditions, including lower overall socioeconomic conditions, discrimination and violence^{3,4,5}. Their poor socioeconomic conditions in many health studies have proved to be a meaningful and important factor of health and health behavior. In that sense, adequate income represents the ability to access the necessities of good health, such as adequate housing, a nutritious diet and educational opportunities⁶. On personal level, immigrant or refugee status of a longer duration and developmental stage (e.g. adolescence) interfere with developmental pro-

cesses of individuation-separation, and also with other tasks of adolescence, in the same time with integration in new society⁷.

Based on the existing literature, the relationship between migration and overall health especially among adolescents is a complex one and involves a multiplicity of factors while the relationships and the relative importance of these factors are rarely studied simultaneously. Besides, many studies associated with migration and health have clearly shown that in spite of hardships and difficulties the majority of migrants make positive adaptation outcomes due to the interplay of protective and risk factors at various levels of adolescent's environment⁸.

An international comparative study related to the quality of life and health outcomes of adolescent youth,

with particular focus on youth with immigrant experience was carried out in different socio-cultural contexts across European countries⁸. Project goals were to determine risk and protective factors of socio-cultural integration and health of adolescent immigrants, and assess the role of contextual effects through cross-cultural comparison in six countries. The general hypothesis of the research was that the sociocultural context and socio-economic conditions in different countries representing the macrosystem of the transactional model of health, with varying degrees of cultural and linguistic familiarity and contact history for immigrants and different ethnic attitudes will influence differently the process of adjustment of adolescent refugees and potential effects on their health and psychosocial well being. The possible effects of migration and exile on adolescent health and well being were assessed within the ecological model of health based on the dynamic interplay of the different factors and mechanisms (social and intraindividual) and situational specificity of the symptoms,⁹ which constitute a holistic view of the child and her environment. The environment of an individual is seen as being composed of several coexisting layers: macrosystem (cultural beliefs and values), exosystem (community, school, peers, neighborhood) and microsystem (family and close friends).¹⁰

In this article, we address the social determinants of health with a specific focus on five factors in the social environment that might have an influence on health status: gender, socio-economic status (SES), perceived discrimination and exposure to violence, social support and religious commitment. The purpose of the study was to examine whether social risk factors have an effect on subjective health status, which factors mediate these effects on self-rated health and to assess whether these effects differ by gender and specific socio-cultural contexts.

Within this culturally anchored ecological framework the study design contrasts four groups of Bosnian-origin adolescents living in three different cultural contexts, in their home country (Bosnia and Herzegovina) and as refugees/immigrants in receiving countries (Croatia and Austria). These groups indicate different degrees of cultural similarity with receiving societies, while each migrant group and each context has its own characteristics that have to be considered in the analysis. In addition to considerable socioeconomic differences related to various transition stages toward democracy and market economy, each of these countries is situated on a cultural continuum ranging from high traditionalism and collectivistic values to individualism. These orientations are directly linked to changes in family systems, socialization values, parenting styles and childrearing orientations (dependence and obedience, autonomy and self-reliance) which may affect psychosocial health.

Methods

Participants

Data for these analyses were drawn from a larger international study related to the quality of life and health outcomes of adolescent youth, with particular focus on youth with immigrant experience⁸. The sample of the current study comprised a total of 1282 respondents born in Bosnia & Herzegovina who were interviewed in three different contexts during 2003. The sample consisted of 553 boys and 729 girls. The mean age of the respondents was 16.9 years ($SD = 1.5$ years). The demographic characteristics of the sample divided by adolescent groups are shown in Table 1.

TABLE 1
DEMOGRAPHIC CHARACTERISTICS OF THE SAMPLE BY GROUP

	Displaced Bosnians in B&H	Bosnians in Croatia	Bosnians in Austria	Host population in B&H
Total N by group	N=359	N=301	N=198	N=424
Mean age (years)	17.3	17.0	16.84	16.84
Gender				
Females	217 (60.45%)	164 (54.49%)	102 (51.52%)	246 (58.0%)
Males	142 (39.55%)	137 (45.51%)	96 (48.48%)	178 (42.0%)
Religion				
Muslim	100%	18.6%	42.9%	92.9%
Catholic	0.00%	80.1%	21.7%	3.5%
Other	0.00%	1.3%	35.4%	3.6%
Parental employment				
Unemployed	9.19%	8.97%	0.00%	5.90%
Homemaker	65.46%	10.63%	0.00%	10.61%
Retired	10.86%	23.26%	2.02%	29.72%
Part-time employed	12.26%	26.91%	18.18%	30.66%
Fully employed	2.23%	30.23%	79.80%	23.11%

Adolescents still living in Bosnia and Herzegovina were divided in two groups, the first including Bosnian displaced youth, all Muslims of Bosniac nationality, and the second including the host adolescents living in the Tuzla – Canton area, consisting of 92.9% Muslims, 3.5% Catholics and 3.6% of other denominations. The third group consists of Bosnian immigrants living in the towns of Zagreb and Knin in Croatia. They include 18.6% Muslims of Bosniac nationality, 81% Catholics of Croatian nationality and 1.3% of other denominations, while the fourth group includes Bosnian immigrants living in Austria, mainly in the area of Linz, with 42.9% Muslims, 21.7% Catholics and 35.4% of other denominations.

The cluster sampling method was used as the most appropriate for this type of cross-sectional study. Trained assistants administered the self-report questionnaires to students of secondary schools during regularly scheduled class time.

Between-group analyses indicated that the displaced Bosnians were older than host adolescents and their counterparts in Austria. With regard to parental employment, there are also significant differences between groups reflecting different socioeconomic conditions in each country. Parents of displaced adolescents in Bosnia have significantly the lowest rates of both part-time (12.26%) and full-time employment (only 2.23%), while those in Austria are either fully-employed (79.8%) or have a part-time job (18.18%) ($p < .001$).

Measures

Dependent health variables

Perceived health problems. This variable is constructed from the question, »How would you rate your overall physical health? Is it excellent, good, fair or poor?« This measure is treated as a continuous variable and a value of 1 represents a self-report of excellent, whereas a value of 4 represents a self-report of poor health⁶.

Objective health problems. The second dependent variable in our analysis is a composite measure of three variables concerning medication use, utilization of health services and absence from school because of illness (adapted from WHO, HBSC⁶). Medication use during the last month was assessed for the following symptoms: headache, stomach ache, nervousness, indigestion, or other problems. The utilization of medical services was assessed by the frequency of visits to a doctor and hospitalization during the past twelve months. Absence from school is used as an additional indicator of general health status. Respondents were asked to report absence from school in days on account of illness during the preceding 6 months, on a scale ranging from 1 (0 days) to 4 (5 or more days). All items were summed up into a one-item indicator of objective health problems that ranges from 1 (low objective problems) to 3 (high objective problems).

The *index of psychological distress* was measured using the indices of the most common anxiety symptoms, depressive behavior and somatic complaints, based on

Hopkins Symptom Checklist 25,^{11,12} and RADS – Reynolds Adolescent Depression Scale.¹³ In this study count variables of anxiety, depression and somatic symptoms were used as separate variables. *Anxiety* (5 items) is assessed by difficulty relaxing, nervous arousal, tension, irritability and feeling of threat. *Depression* (20 items) items refer to dysphoric mood, sadness, loneliness, sleep disturbance, anhedonia, pessimism, self-injurious or suicidal tendency, self-depreciation, reduced speech, worry, social withdrawal, loss of interest, appetite disturbance, helplessness, confusion. *Somatic symptoms* (10 items) include those most frequently related to stress. The items are rated on a four point a Likert scale ranging from 1 (almost never) to 4 (most of the time) depending on the extent to which specific states are experienced. Higher score on every scale indicates higher level of distress.

Self-esteem was measured using the Self-Esteem Scale by Rosenberg.¹⁴ It has 10 items answered on a four point a Likert scale ranging from strongly disagree to strongly agree. Studies have demonstrated both a one-dimensional and a two-factor (self-confidence and self-deprecation) structure to the scale.

Independent environmental predictors

Several indicators of *socioeconomic status* were used in the survey, to obtain more reliable data from the adolescents. Economic status was measured by two variables: family affluence scale (FAS) consisting of home ownership, adolescent's own bedroom occupancy, family car ownership and family holidays (adapted from WHO Cross National Study⁶), and employment status of father and mother. The FAS scale ranged from 5–20, while higher score on scale indicates higher level of family affluence. The employment status scale included the following options: fully employed (5), part-time employed (4), retired (3), homemaker (2), unemployed- looking for job (1). The third variable was parental education, ranging from low (1) to high (3) education levels. The level of mother's and father's *education* (elementary, secondary, university) was used as a proxy for social status which, particularly in transitional countries and for immigrant populations does not necessarily imply adequate economic status, but can be an important factor for general adolescent well-being.

Demographic variables included items related to gender, age and status according to origin and place of residence.

Risk environmental factors on health and psychological stress were measured by three scales including perceived discrimination, peer violence and adult violence. Perceived personal discrimination due to ethnic, religious, linguistic and gender differences was measured by frequency of experience during the past six months (adapted from CHKS¹⁵). The exposure to peer violence was measured by a four-point scale asking respondents about the frequency of their exposure to physical and verbal forms of violence during the past six months (adapted from CHKS¹⁵). Higher scores on last two scales indicate higher levels of peer violence and personal discrimina-

tion. A separate item measured any lifetime experience of physical violence from an adult. A variable is constructed from the question, »During your life, has any adult ever intentionally hit or physically hurt you?« The answer ranged from 1 (negative) to 2 (positive).

Protective environmental factors on health and psychological stress related to social support were measured by three scales, family and school connectedness and neighborhood attachment. The scale of family connectedness (adapted from CHKS¹⁵) consists of 9 survey items with a four point Likert scale response option. They measure emotional support, instrumental support, high expectations, clear rules and boundaries and meaningful participation in the family. The measure of school connectedness¹⁴ includes 9 items rated on a four-point scale, which refer to emotional and instrumental support, high expectations and meaningful participation. Higher scores on above scales indicate higher levels of family and school connectedness. Neighborhood Scale (adapted from Corrigan¹⁶) explores emotional attachment, sociability in the neighborhood, neighborhood safety, and respondent’s involvement with neighbors. The scale includes 5 items rated on a four-point scale.

Religious commitment was measured with a composite measure of two scales. One scale refers to service at-

tendance or involvement in religious community activities measured on a four-point scale from 1 (never) to 4 (once a week or more), while the other refers to subjective feeling of one’s own religious devotion measured on a five-point scale from 1 (don’t know) to 5 (very religious).

Results and Discussion

Mean differences between the groups

The differences between the four groups of Bosnian adolescents in socio-economic status, risk factors (perceived discrimination, peer violence and adult violence), protective factors (family and school connectedness, neighborhood attachment and religious commitment), perceived health problems, objective health problems, somatic stress, depression, anxiety and self-esteem were assessed by two-way, 4 (group) × 2 (gender) ANCOVAs, with age as a covariate. The results of these analyses are shown in Table 2.

The four immigrant groups differed significantly from each other in all analyzed variables except one, somatic stress. Post-hoc comparisons indicated that displaced Bosnians in Bosnia and Herzegovina had the signifi-

TABLE 2
RESULTS OF ANCOVA ANALYSIS

Variables	Group Means				ANCOVA, Main Effects	
	Displaced Bosnians in B&H	Bosnians in Croatia	Bosnians in Austria	Host population in B&H	Group F (35.68)	Gender F (26.14)
SOCIO-DEMOGRAPHIC FACTORS						
Parental education	1.44 ^{a,b,c}	1.97 ^{a,d}	2.01 ^{b,e}	1.82 ^{c,d,e}	107.55***	6.07*
Family affluence scale	1.66 ^{a,b,c}	2.24 ^{a,d}	2.37 ^{b,d,e}	2.21 ^{c,e}	320.77***	
Parental employment	2.30 ^{a,b,c}	3.43 ^{a,d}	4.64 ^{b,d,e}	3.36 ^{c,e}	226.12***	
RISK FACTORS						
Peer violence	1.16 ^a	1.15 ^b	1.09 ^{a,b,c}	1.15 ^c	5.37**	4.68*
Adult violence	1.41 ^a	1.39 ^b	1.23 ^{a,b,c}	1.39 ^c	6.75***	6.02*
Perceived discrimination	1.18 ^a	1.24 ^b	1.16	1.08 ^{a,b}	10.13***	4.48*
PROTECTIVE FACTORS						
Family connectedness	3.37 ^{a,c}	3.53 ^{a,b}	3.34 ^{b,d}	3.55 ^{c,d}	13.30***	24.04***
School connectedness	2.95 ^{a,b}	2.64 ^{a,c}	2.68 ^{b,d}	2.90 ^{c,d}	31.97***	
Neighborhood attachment	3.14 ^{a,b,c}	2.89 ^{a,d,e}	2.56 ^{b,d,f}	3.32 ^{c,e,f}	60.27***	4.02*
Religious commitment	2.79 ^{a,b,c}	3.13 ^{a,d,e}	2.40 ^{b,d,f}	2.59 ^{c,e,f}	39.37***	
PSYCHOSOCIAL HEALTH						
Perceived health problems	1.91 ^a	1.75 ^{a,b}	1.86	1.91 ^b	4.27**	50.54***
Objective health problems	2.76 ^{a,b}	3.07 ^{a,c,d}	3.30 ^{b,c,e}	2.77 ^{d,e}	22.06***	6.47*
Somatic symptoms	1.79	1.83	1.78	1.82		168.74***
Depression	2.00 ^{a,b,c}	1.86 ^a	1.90 ^b	1.90 ^c	4.50**	127.66***
Anxiety	2.12 ^a	2.10 ^b	1.88 ^{a,b,c}	2.13 ^c	5.43**	102.99***
Self-esteem	2.89 ^a	2.98 ^b	3.29 ^{a,b,c}	2.92 ^c	45.72***	55.16***

^{a,b,c} t tests with Bonferroni’s adjustment; the means that share the same superscript differ significantly ($p < .05$).

* $p < .05$, ** $p < .01$, *** $p < .001$

cantly lowest socioeconomic status of all groups concerning parental education, parental employment and family affluence, while Bosnians in Austria had significantly higher scores than all other groups in parental employment and family affluence. As to risk factors, Bosnians in Austria experienced less peer and adult violence than the other groups, whereas displaced Bosnians and those in Croatia reported more perceived discrimination.

With respect to group differences in protective factors, Bosnians in Croatia and host adolescents in B&H experienced significantly more support from their families, than other two groups, while both displaced and host adolescents in B&H were more connected to school and attached to their neighborhood than their counterparts in Austria and Croatia. Bosnians in Croatia expressed the highest religious commitment, followed by displaced adolescents in Bosnia, while those in Austria reported the lowest values. With regard to self-rated health, both groups in Bosnia perceived more health problems than the other two groups. On the other hand, Bosnians in Croatia and Austria reported significantly more objective health problems than both groups in Bosnia and Herzegovina. In addition, displaced Bosnians in B&H reported a higher level of depression than all other groups, while the level of anxiety was higher for both groups in B&H and Croatia than for those in Austria. Adolescents in Austria, in turn, expressed significantly higher self-esteem than all other groups.

The gender effect was found for all health variables, all risk factors as well as for family connectedness and neighborhood attachment. The girls perceived more health problems and reported more somatic, depressive and anxiety symptoms than the boys, while boys experienced more violence and discrimination and had higher self-esteem than the girls.

The significant Group X Gender interactions included family connectedness, neighborhood attachment and all health variables. Displaced boys in B&H and those in Austria were significantly less connected to their families than boys and girls in other groups, while girls in Croatia and both boys and girls in Austria were less attached to their neighborhoods than other groups. The greatest gender difference was observed in perceived health between girls and boys in Austria. These girls had the highest rate of reported poor health among all groups, while boys reported the most favorable levels of health, significantly higher than boys in Bosnia. Both girls and boys in Austria and Croatia also reported significantly more objective problems than their counterparts living in Bosnia. Adolescents of both gender in Austria experienced significantly lower anxiety levels than all other groups, while displaced girls in B&H reported significantly higher depression than girls in other groups. Boys and girls in Austria and boys in Croatia reported significantly higher self-esteem than all other boys and girls.

Perceived health problems

We modeled perceived health problems through the specification of five linear regression models (Table 3).

Results from Model 1 indicate significant positive effects of all three risk factors on perceived health problems, with a stronger effect of perceived discrimination than those of peer and adult violence. Model 2 adds controls for the three analyzed groups of Bosnian adolescents living in different cultural contexts. The only significant effect is found for Bosnians in Croatia who tend to report more favorable levels of health. The observed insignificant effects show that the group in Austria has less perceived health problems than the one in Bosnia and Herzegovina. The initial effects of risk factors on perceived health are only slightly changed with a small increase in the effect of discrimination on health. Model 3 includes controls for demographic factors of gender and age. Whereas age does not affect perceived health, it is in expected direction, while gender expectedly has a highly significant effect. Simultaneously, the effect of perceived discrimination is slightly decreased, and those of peer and adult violence are slightly increased, net of demographic factors. The fourth model controls for the important factors of socio-economic status. They are all in the expected direction, with family affluence scale having a significantly stronger effect than parental education and parental employment. The group effects in this model are significant and negative for Bosnians in Croatia, and significant but positive in the Austrian group. The effects of risk factors are slightly decreased except for adult violence, net of socio-economic status. Model 5 controls for important protective factors that may affect perceived health problems and moderate the impact of risk factors through social support. However, only school connectedness and religious commitment are significantly associated with self-rated health, with adolescents who are less attached to school and less religious reporting worse health. The inclusion of social support in the model reduces somewhat the effects of both peer and adult violence, with a slight increase in discrimination effect. On the other hand, the effect of group turns insignificant, that of gender is slightly decreased, while in addition to family affluence parental education becomes significant.

Finally, model 6 tests the hypothesis whether the effects of environmental social variables on subjective health are mediated through objective health problems and psychosocial health problems manifested as symptoms of somatic stress, anxiety, depression and self-esteem. As it can be seen in Table 3, all indices of objective and psychological health except for anxiety are significantly related to poorer reports of perceived health. In addition to a highly significant effect of objective health, the strongest effect is found for somatic stress, while adolescents exhibiting higher levels of depression and lower levels of self-esteem also report more perceived health problems. Simultaneously, the effects of all risk factors, gender, socio-economic status and protective factors are considerably reduced and most of them cease to be statistically significant. The exceptions are perceived discrimination, parental education and the effect of religious commitment which are also reduced but remain statistically significant. This model is significant for Bosnians in Croatia

TABLE 3
LINEAR REGRESSION COEFFICIENTS OF PERCEIVED HEALTH PROBLEMS ON PREDICTOR VARIABLES

Variables	Model 1	Model 2	Model 3	Model 4	Model 5	Model 6
<i>1. Risk factors</i>						
Perceived discrimination	0.115***	0.119***	0.116***	0.113***	0.116***	0.063*
Peer violence	0.080*	0.079*	0.091**	0.085**	0.066*	-0.018
Adult violence	0.063*	0.063*	0.075*	0.080**	0.062*	0.006
<i>2. Country – group</i>						
Displaced Bosnians in B&H		0.054	0.040	-0.089	-0.065	-0.025
Bosnians in Croatia		-0.136***	-0.133**	-0.095*	-0.070	-0.100**
Bosnians in Austria		-0.024	0.041	0.096*	0.050	-0.041
<i>3. Socio-demographic factors</i>						
Sex (female)			0.190***	0.187***	0.182***	0.038
Age			0.030	0.024	0.018	0.021
<i>4. Socio-economic status</i>						
Parental education				-0.047	-0.068*	-0.059*
Parental employment				0.007	-0.004	-0.045
Family affluence scale				-0.120*	-0.118*	-0.048
<i>5. Protective factors</i>						
Family connectedness					-0.022	0.008
School connectedness					-0.106**	-0.047
Neighborhood attachment					0.088	0.021
Religious commitment					-0.120***	-0.097***
<i>6. Psychosocial health</i>						
Objective health problems						0.234***
Somatic stress						0.238***
Anxiety						0.026
Depression						0.094*
Self-esteem						-0.108***
R ²	0.038	0.050	0.085	0.097	0.123	0.285

*** p<0.001, ** p<0.01, * p<0.05

as opposed to displaced adolescents in Bosnia and Herzegovina and Austria. Additionally, it adds to perceived health problems in Austrian adolescents, while it works in a negative direction for other groups. As compared to previous five models, model 6 accounts for 29% of variance and clearly indicates that social and environmental factors such as discrimination and violence, lower socio-economic status and a lack of social support operate indirectly through objective health problems, somatic stress, depression and low self-esteem to affect subjective self-rated health.

Predictors of psychological well-being

To test the above mediating role of objective health and psychosocial problems in the relationship between social determinants and subjective health, we further performed stepwise multiple regression analyses for each of the five health measures to determine the extent to

which (a) social risk factors, measured as perceived discrimination, peer and adult violence, (b) cultural contexts measured through country of residence, (c) socio-demographic factors, in terms of gender and socio-economic status, and (d) social protective factors, measured as family and school connectedness, neighborhood attachment and religious commitment, accounted for the variance in psychological well-being. The results of the regression analyses are presented in Table 4. It can be seen that the two risk factors, including perceived discrimination and peer violence, as well as gender affect significantly all five health variables. Additionally, adult violence and low connectedness to school are significantly related to somatic stress, anxiety, depression and self-esteem. The more the adolescents perceived discrimination and experienced violence, the less they were attached to school, the higher their somatic stress, anxiety and depression, and the lower their self-esteem. Religious com-

TABLE 4
STEPWISE MULTIPLE REGRESSION COEFFICIENTS OF MENTAL HEALTH INDICATORS ON PREDICTOR VARIABLES

Variables	Objective health	Somatic stress	Anxiety	Depression	Self-esteem
R ²	0.76	0.24	0.24	0.32	0.27
F	11.67***	34.22***	34.11***	50.51***	38.35***
<i>1. Risk factors</i>					
Perceived discrimination	0.083**	0.141***	0.115***	0.160***	-0.130***
Peer violence	0.063*	0.171***	0.192***	0.174***	-0.084**
Adult violence		0.110***	0.121***	0.088***	
<i>2. Country – group</i>					
Displaced Bosnians in B&H	-0.188***				-0.101*
Bosnians in Croatia	0.067				-0.104**
Bosnians in Austria	0.261***		-0.136***		0.372***
<i>3. Socio-demographic factors</i>					
Sex (female)	0.081**	0.359***	0.307***	0.333***	-0.220***
<i>4. Socio-economic status</i>					
Family affluence scale				-0.095**	0.140***
<i>5. Protective factors</i>					
Family connectedness				-0.139***	0.165***
School connectedness		-0.092***	-0.112***	-0.117***	0.148***
Neighborhood attachment				-0.077**	
Religious commitment			-0.095***	-0.104***	0.051*

*** p<0.001, ** p<0.01, * p<0.05

mitment had a significant negative impact on anxiety and depression, while it positively affected self-esteem. In addition, the lower socio-economic status of adolescents measured by family affluence scale, and the lack of social support provided by family, school and neighborhood, the higher their depression levels and the lower their self-esteem. The only index of psychological problems affected by neighborhood attachment was depression.

As to group specific results, a significant negative effect was found for displaced Bosnians in B&H and a significant positive effect for adolescents in Austria with respect to objective health problems. In other words, higher perceived discrimination and experience of violence are associated with more objective health problems in Austrian Bosnians, but not in displaced youth in B&H. Also, more perceived discrimination, and experience of both peer and adult violence are also negatively associated with anxiety levels in Bosnians in Austria as opposed to other groups.

Significant group effects were found for all three groups with respect to self-esteem. Less perceived discrimination and experience of peer violence, higher economic status and stronger family and school connectedness as well as higher religious commitment are positively associated with self-esteem of Bosnians in Austria, while for youth in Bosnia and Croatia, and especially girls the effects are in negative direction.

Conclusions

In this study we explored the social determinants of health in young immigrants with a specific focus on five factors in the social environment that might have an influence on health status: gender, socio-economic status (SES), perceived discrimination and exposure to violence, social support and religious commitment. We wanted to examine not only the possible influences on health, but also which factors mediate these effects on self-rated health and to assess whether these effects differ by gender and specific socio-cultural contexts. Our analyses reveal significant effects of risk factors including discrimination and violence on self-ratings of health. Importantly, these risk factors are primarily mediated by objective health, somatic stress, depression and self-esteem in the interaction with socioeconomic status and religious commitment. The regression models performed indicate clearly that psychological health works as a key mechanism through which social risk factors in the interplay with protective factors affect subjective health status. While school connectedness represents an important factor for all indices of psychological health, family connectedness is additional protective factor in depression and self-esteem, while religious commitment plays important role in anxiety, depression and self-esteem.

In line with other similar studies, our analyses also revealed consistent differences in gender in all health

variables, all risk factors as well as for family connectedness and neighborhoods attachment. The girls perceived more health problems and reported more somatic, depressive and anxiety symptoms than the boys, while boys experienced more violence and discrimination but had higher self-esteem than the girls.

We also anticipated that specific contexts in particular countries would moderate the associations between risk and protective factors, and health. Our results with respect to differences in perceived health status and objective health between adolescents in Bosnia and those in Austria and Croatia seem to reflect particular contextual factors, such as different socioeconomic conditions, availability of medical services and general cultural attitudes towards medication use and usage of medical services.

These differences in the adolescents' adjustment are probably due to differential response from the broader society as well as to its differential effects on family and other type of support to these groups.

The macro-system contexts in this study refer to three countries that differ politically, economically and culturally. Austria is a highly developed country, with relatively stable democratic polity, and market economy in place. Two other countries are post-conflict countries in different transition stages, faced with economic shifts, destabilisation of institutions and value change in the society at large. All these national specifics through the initial institutional configurations, have an influence on individual values, beliefs and attitudes, which is mediated in different ways by the support of the family and other processes at the micro-level. In Bosnia and Herzegovina the situation is further compounded by high unemployment rates affecting more than a third of the population and high levels of poverty manifesting itself not only in low income, but also importantly in limited access to and poor quality of health services. Youth in that country are more likely, compared to those in other two countries, to lack health insurance coverage, mainly because of its high cost and lack of employer coverage, as evidenced by Bosnian youth in Bosnia, both displaced and native, reporting higher levels of fair or poor health, but lower rates of objective health problems when compared to Bosnians in Croatia and Austria.

Dramatic socioeconomic changes bring attitudinal and norm shifts, value confusion and conflict, and individual disorientation for both adult and adolescent citizens, with resulting feelings of uncertainty, insecurity and lack of confidence. All this results in a more problematic adolescence for youth in all post-socialist countries as evidenced in our samples by high levels of anxiety and low self-esteem experienced by youth regardless of origin in

Bosnia and Herzegovina and Croatia. Additional factor that contributes to the feelings of uncertainty and insecurity is the lack of public security, weak judicial system and increase in crime, reflected in our samples by higher rates of both adult and peer violence reported by youth in Bosnia and Herzegovina and Croatia.

As to moderating effects of protective factors, cultures develop different beliefs and parenting patterns in response to immediate environmental risks and demands, with resulting variations in different types of desirable behaviour. Connectedness to school is especially critical, since it is the major socializing institution in all countries and the main source of secondary cultural experiences outside the family. Lower connectedness to school is associated with both perceived discrimination and violence, as well as with higher rates of poor health and psychological problems, particularly for Bosnians in Croatia. Aspects of the neighbourhood context include youth's familiarity and association with neighbours, and the cohesiveness and safety of the neighbourhood. Our analyses showed its negative association with depression and significantly higher values of neighbourhood attachment and its protective quality for both immigrants and hosts in a more traditional society in Bosnia and Herzegovina, in which the immediate neighbourhood is likely to be of the same ethnic origin, while in contrast, for Bosnians in Austria, the neighbourhood attachment is considerably lower. On the other hand, family connectedness, which is also negatively associated with depression, though rather high in all groups, is significantly lower for immigrants in Austria and displaced Bosnians in Bosnia. These results reflect considerable variations in developmental orientations related to traditional collective values and individual autonomy, that are directly linked to changes in family systems, socialization patterns and parenting styles. As shown by significantly higher self-esteem of Bosnians in Austria, in the new individualistic cultural context, with changing life styles, autonomous orientations become more functional in coping with more specialized tasks requiring individual responsibility and decision making rather than pursuing traditional values.

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DRUŠTVENE ODREDNICE ZDRAVLJA: USPOREDNO ISTRAŽIVANJE BOSANSKIH ADOLESCENATA U RAZLICITIM KULTURNIM KONTEKSTIMA

S A Ž E T A K

U radu se analiziraju utjecaji sociokulturnih faktora na zdravlje adolescenata u dobi od 15–18 godina, porijeklom iz Bosne i Hercegovine, koji žive kao izbjeglice u Bosni i Hercegovini, te kao doseljenici u Hrvatskoj i Austriji. Istraživanje društvenih odrednica zdravlja usmjereno je na pet društvenih faktora koji utječu na zdravstveni status: rod, socio-ekonomski status, percepciju diskriminiranosti i izloženost nasilju, društvenu podršku i religioznost. Zavisne varijable uključuju, subjektivnu procjenu zdravstvenog statusa, objektivne pokazatelje zdravstvenih problema te četiri mjere psihološkog zdravlja (somatski stres, anksioznost, depresivnost i samopoštovanje). Istraživanjem se nastoji utvrditi u kojoj mjeri navedeni društveni faktori utječu na zdravlje, mehanizme interakcije faktora te moguće razlike obzirom na rod i zemlju boravka. Dobiveni rezultati pokazuju da je psihološko zdravlje ključni mehanizam kroz koji rizični društveni faktori (percepcija diskriminiranosti i izloženost nasilju) utječu na nižu subjektivnu procjenu zdravstvenog statusa, a ta povezanost je statistički značajnija u djevojaka. Razlike između bosanskih adolescenata u tri različite zemlje ukazuju na složenost utjecaja na zdravlje kroz specifičnosti pojedinog sociokulturnog konteksta koji utječe na interakciju društvenih rizičnih i zaštitnih faktora.

A Veil (*hijab*) as a Public Symbol of a Muslim Woman Modern Identity

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ABSTRACT

In this article the author explains the social role of Muslim woman in a postmodern society through a public symbol of her identity – the veil. The article's thesis is that the Muslim women's manifestation of their Islamic denomination through veiling and wearing appropriate clothes (in the case of men through growing beards and wearing clothes considered appropriate for them) signifies an expression of a new, Islamic shaped identity. This is a postmodern identity based on modernity rather than a fundamental reaction to modernity. The veil, a public symbol of Muslim identity, is often given a different meaning by its observers than the person actually wearing it. Therefore, the intention of this article is to analyze the elements of a particular, postmodern identity that a Muslim woman's veil, as a public symbol, represents.

Key words: Islam, woman, feminism, tradition, identity

Introduction

El-Din al Hilali, the chief mufti of Australia, became the object of public criticism when he said that *women that go uncovered are much like exposed meat that attracts sexual predators*. The statement brought down on him women's fury throughout Australia and it resulted in calls for resignation of the leading Australian Muslim cleric. Has the mufti, as the official representative of the Australian Muslim community, slipped his tongue or has he expressed the opinion of the Muslim majority? Is this a misunderstanding or deeply rooted difference that makes Muslims different from the rest of the citizens of today's global world? Do these differences make Muslims incapable of participating in this modern world or do we just not understand what message the Muslims want to send to the world, even through the Australian mufti?

It is about covering women. This case has reminded us of the discussion in European, American and Muslim public about the right or prohibition of wearing the veil as a religious symbol. Where are the borders between freedom, morality and institutional coercion?

The veil, as a demarcation line between the opposing sides, is the core of disagreement between socially acceptable behavior and socially unacceptable behavior, between our perception of what is advanced and what is backward.

Veil (*hijab*) is a cover for head. It is women's equivalent to men's hat, by which women cover themselves, their hair, face and neck. In a modern society, covering the head is usually a custom related to civil servants (soldiers, police officers, nurses, nuns) traditional society, rural background, lower education and older age. In a modern society, the veil, as a symbol of distinction, worn with big sunglasses, was promoted by grand movie stars like Grace Kelly and Sophia Loren. Yet, in the same modern society, the veil is still perceived as a symbol that implies something old, traditional, historical, religious, rural, and backward.

Therefore, it is undoubtedly surprising to see how young women voluntarily accept the veil as a symbol of particular identity in modern societies like those of USA and EU. It is rather confusing when young, urban and highly educated Muslim women wear such a thing. It becomes even more confusing when we realize that these women were in fact born and educated in those countries, speak the language, participate in local culture and have the citizenship. In the eyes of a contemporary western observer such a status is considered to be higher than these Muslim women would have in their own Muslim societies. From his perspective, wearing a veil symbolizes a position of women, which is associated with terms like backward, closed, oppressed, hindered, etc.

However, from the point of view of women who wear it, the veil has transformed itself from a symbol of social oppression into a public symbol of distinguished social identity and a symbol of freedom to choose this very identity. In non-Muslim societies of Western Europe and the USA, the veil is perceived as a publicly insulting just like Gay pride or Mohawk hairstyle. Muslim women by simple act of wearing the veil have transformed it from a sign of backwardness into a symbol of pluralism of identity, which demands to be recognized as equal in the modern pluralistic society.

The Veil as a symbol of Muslim women's social role is often used as an argument in attempts to prove the backwardness of Muslim societies and Islam as a whole, and as a proof of failure of their modernization. This thesis is not appropriate. Namely, today all societies are modern. The modern frame of societal community is materialized through nation-state and in the relation toward her. Therefore, the explanation of the women's social role, and of Muslim women's too, should be looked for within the framework of nation-state and not within a dogma of a universal religion, such as Islam.

Prior to that, it is necessary to distinguish between societies in which a form of Islamism is the basis for a governing ideology (Iran, Afghanistan, Pakistan, Saudi Arabia, Malaysia, Sudan) and societies in which the Muslims are just a religious minority of an ethnic origin different from the domicile one (EU, USA, Australia) or groups entirely excluded from the political sphere. There is also the third type of societies in which Muslims form a majority of the population but a ruling establishment is against religion.

This article is focused on West European and American societies in which the Muslims are immigrants and just a religious minority of various ethnic origins. In these societies the veil worn by Muslim women is perceived in this article as a form of public expression of different kind of modern identity, protest and message. In these immigrant communities the veil, as a head cover, was worn by the first generation and only during religious ceremonies of the community that were taking part in the private sphere. In the public sphere the Muslims followed a dress code proscribed by the domicile society. It was not until political Islam emerged as social agency of modernization that the immigrant Muslim population became more self aware and more ready to cross from the private sphere into the public one. This article explains the consequences of this »coming of the closet«.

The Veil in the Islamic Tradition

When did the veil enter the Islamic tradition? The veil had first been worn by women from the Prophet Muhammad's household, in order to prevent potential rumors

about their relationships outside the household. It is the strong egalitarian Islamic culture that inspired other Muslim women outside the household to start wearing the veil. When Christian women who lived near Muslim population saw how much respect Muslim women enjoyed in their community, they also started to follow the trend.

Islam is unique in Abraham's religions because it pays a lot of attention in its basic religious texts to women and man-woman affairs. How does Qur'an define the relationship between men and women?

Men are the maintainers of women because Allah has made some of them to excel others and because they spend out of their property; the good women are therefore obedient, guarding the unseen as Allah has guarded; and (as to) those on whose part you fear desertion, admonish them, and leave them alone in the sleeping-places and beat them; then if they obey you, do not seek a way against them; surely Allah is High, Great.¹

Aside from the above-mentioned *sura* (chapter) *An-Nisa* (woman), Qur'an speaks about the woman and the relationship between a man and a woman in many places and also about the ways it is regulated. These issues also appear in *Hadith* (sayings) of Muhammad, which is the second fundamental text of the *sharia* law*.

In the Islamic interpretation of the world the sexes are not equal. They are rather complementary. Qur'an emphasizes the economic base of a man's social role. In return, a woman is obliged to sustain the morality, family and property which Qur'an emphasizes the reproductive and moral base of woman's social role. The Qur'anic revelation changed the perception of the woman's social role. In the 7th century Qur'an granted women the right to own property and to divorce. Given the time of appearance, these Qur'anic provisions were revolutionary. Egalitarianism, typical of Islam, has also found its expression in the issues of women's rights and in the time of its creation it has provided women a high degree of equality with men.

Modern interpretations of men-women relationships in Islam have a tendency to explain the Qur'an's precedence of man over woman as a confirmation of men's aspiration for dominancy and exploitation of women. At the same time, these interpretations fail to analyze the man's social role at the time and the reasons why he has been granted such a position in society. The man's social role contains some rights but also some responsibilities. What distinguishes a child from an adult are the duties and the responsibilities that each of them has in their society and a broader social surrounding.

The traditional pattern of man-woman relationship, created in the traditional patriarchal societies of the Mid-

* The foundation of *sharia* law, as the holy Muslim law, is made of Qur'an as the revelation of God's word and of *Hadith* and *Sunna* of the Prophet Muhammad. *Hadith* is collection of Muhammad's sayings and *Sunna* contains the description of Muhammad's deeds, conduct and behavior.

dle East including the subordination of women and the reduction of their existence to the private sphere only, became islamised with time.

In their fight for women's rights, Islamic feminists emphasize that this pattern of behavior is not originally Islamic, so they call for return to the authentic Islam of the Prophet Muhammad and his *rashiduna* (rightful caliphs). The feminists say that the Prophet's wife Aisha had participated in battles with men as well as in *diwan* (council) that used to discuss the most important issues concerning the survival of *umma* (Muslim community). Therefore, from the Islamic perspective, the women are not demanding some new right but rather the renewal of the rights that they have already had. In that way the golden age of Islam becomes their golden age too. By accepting the modern Islamic identity, the Muslim women use it as a toll for advancement of their demands for equality.

Traditional society, including the Muslim one, looks at a woman's social role within the traditional framework of a man-woman relationship. Throughout a woman's life, her social role can be divided in a couple of stages: child, girl, woman (mother), old woman (grandmother). The first stage is pre-reproductive and the last stage is post-reproductive. By entering the reproductive age a woman gains a higher social value, which is confirmed by her motherhood. The ability of giving birth guarantees the reproduction of the community. The age of reproduction is also the age of sexuality of both woman and man. Islam is aware of human sexuality and imperfection, so the ways of conducting social as well as sexual relationships are proscribed. In the Islamic dogma, life and deeds of Muhammad serve as a model according to which the Muslims should behave and organize their lives. Along side that, there are many roles that determine the social behavior of Muslims. The Muslims are aware of their inability to reach their Prophet's level of faith so they try to imitate him in their behavior and conduct. The life of Muslims is regulated by a whole set of rules and advices. The set encompasses prayer, diet, housing, personal and public hygiene, dress code, and system of social relations within and outside the community. Therefore, it is understandable why many people call Islam a way of life.

Sexuality is also regulated in the web of social relationships. Its regulation is often confusing for a non-Muslim observer since it allows polygamy**. From the observer's point of view the polygamy is heaven for men and it is a source of fantasies and numerous prejudices that the Westerners have had about Muslims throughout history. In reality, around 99% of Muslim families are monogamous. Polygamy occurs as rare as in Western societies and it exclusively depends on the man's economic

ability to sustain many wives. The Qur'an permits polygamy but it also regulates it extensively.

Islam, as community religion, propagates marriage. Aware of human limitations, Islam propagates marriage and family as institutions and advocates marriage of young people. In the background of that there are two kinds of concerns: biological and social stability. Orthodox Islamic texts believe that it is necessary for young people to marry so they could consume sex within the framework of marriage. Islam is permissive of human sexuality but it proscribes the harshest punishment for adultery and sex consumed outside the marriage. The reason for drastic punishment of adultery is instability that such an act causes to the whole community; therefore such acts should be suppressed by the harshest measures.

According to Western scale of measurement, Islam is highly sexual but this is primarily an impression of a Western observer. It should be more appropriate to analyze the cause of the modern Western sexual frustration then to shift the responsibility to Islam. Emphasizing the sexuality of a certain society or a system of beliefs has an additional function. It is for the observer to emphasize the backwardness and inferiority of the observed. This is evident in prejudices about Afro-Americans in which the movements for the white purity use euphemisms like: *they mate like animals*. A distance from corporality is considered to be a sign of racial superiority. If people are controlled by animalistic instincts, and that is usually ascribed to blacks and Muslims, then their position on the cultural scale is lower.

This is different from the traditional Islamic understanding of man. Islam is aware of a man's imperfection and sinfulness but also of his position towards God in respect to other God's creatures. Man is God's emissary on Earth. However, what God empowered him with has not been given to him to destroy but rather to preserve it for God, the only master of the world.

Balance and moderateness represent the foundation of Islamic understanding of life, which is also reflected in a dress code. A man's temperament and violent nature are familiar to Islam. Within the basic intention of Islam, which is the creation of a just society, the human nature is taken into consideration and is put in the frames of socially acceptable behavior. Therefore, the attitude of the Islamic orthodoxy is permissive towards human sexuality as long as this sexuality is practiced within the framework of socially acceptable behavior. The framework is heterosexuality in marriage. Dichotomy between private and public is very emphasized in Islam. The private sphere is represented in family and home and it is an invulnerable space. According to the traditional Islamic in-

** And if you fear that you cannot act equitably towards orphans, then marry such women as seem good to you, two and three and four; but if you fear that you will not do justice (between them), then (marry) only one or what your right hands possess; this is more proper, that you may not deviate from the right course.

The Holy Qur'an. Translated by M.H. Shakir. Tahrike Tarsile Qur'an, Inc. 1983). 4.3.

terpretation, a woman is the biggest treasure of a man; she is the ruler of the private space. Therefore, her rarely goes to the public sphere and when she does she is protected. The main purpose of the veil and other clothes is to protect her innocence and to prevent lust of observers in the public space. In the various local forms, such a pattern was dominant until the 20th century.

Modern Society and a Muslim Woman

Coole² traces the roots of feminism in a big transformation of social, economic and family relations that started in Great Britain in the 17th century and in the culture of individuality and self-reflexion that are related to it. It was the foundation on which the women started to identify themselves as the unjustly deprived category. Feminism entered the 20th century as women's movement equipped with feminist ideology. Despite numerous changes that the 20th century was going to bring to the women's policy, identity and aspirations the primary goal of feminism was and still is to abolish the discrimination or exclusion based on sex.

Therefore, the feminist perspective approaches to the analysis of a Muslim woman's social role by looking for the cause and manifestations of her submission to a man. Muslim women are subject to double discrimination. As women they are discriminated against in a Muslim society and as Muslim women they are discriminated against in an immigrant society.

However, along side feminism, Islamism as social movement is considered to be the most important movement in the 20th century. Is it possible that such an important social movement really insists on sexual discrimination? It is quite the opposite. The women issue is among the issues where Islamism brakes away from Muslim traditionalism.

Islamism is seen as the social agency of modernization in the Muslim societies. Its role is to transmit the idea of modernism to the Muslim masses and to modernize them in a way understandable to them. For this purpose ideologists of Islamism have articulated the idea of modernism using the language understandable to the Muslim masses. Women have the crucial role in the materialization of the ideologists' ideas.

There are over a billion Muslims in the world. They are all over the globe and almost in all societies. Half of that number are women. Over half a billion women. It is impossible to forcefully unify such a big amount of people and make them act only according to one pattern unanimously accepting one social identity with no differences. Islam is one but there are billion Muslims and just as much ways of thinking and practicing of Islamic religion. They all stick to the same pattern but their interpretation and practice are individual.

Islamism offers a modern, unified interpretation of the pattern. The interpretation is adjusted to the modern social conditions. The activism of the Islamic women is also visible. Their public appearances occur worldwide:

from demonstrations of armed and veiled women in Iran to demonstrations of Muslim women in European and American cities that happen for various reasons. Islamists have created an elite group of women intellectuals who write and whose texts are published.

Islamic woman enters politics too although she is not allowed to perform certain functions like being a judge or president of state. The core of Islamic activism is not returning women home but rather separation of sexes in the public. This is why Islamists design special rooms for women in mosques and other public places.

As a result, Roy³ explains how this new dress code of Muslim women (veil, coat, gloves) enables women to fulfill two mutually opposing goals. Women go out of isolation while at the same time keep their feeling of modesty by wearing the veil. Roy therefore concludes that *hijab* is not a modern adaptation of a traditional veil. It rather signifies a new place for a woman in the social order where the Western model renounces all of the traditional values. This is how a Muslim woman accepts Islamic identity by wearing the veil and at the same time keeps her essence by participating in the modern world.

What kind of an identity is that? Castells⁴ classifies identity types in the following way:

- Identity of legitimacy – it was introduced by dominant social institutions in order to expand and rationalize their domination towards a society's protagonists:
- Identity of resistance – it is created by subjects that in positions/conditions in which they feel degraded or stigmatized by the logic of domination:
- Projective identity – appears when social agencies, on a basis of whatever cultural material available, build a new identity, which redefines their position in a society and by doing so look for a transformation of overall social structure.

Islamism is considered to be the identity of resistance. Its public manifestations since the 1970s are oriented towards resistance to domination. The first identity manifestation of the Muslim women in the EU, *the Veil Affair*, happened in the fall 1989 in France. Many manifestations of this kind soon followed. Kepel⁵ believes that *Union des organisations islamiques de France* (UOIF) had leading role in the protest. Through its public action the organization wanted to assure its position of the mediator between the Islamic community and the authority.

In return for concessions, this organization offered the authorities a control over potentially unstable youth and a fight against drugs, crime and violence. Such a reasoning of the community made Islam the part of internal and no longer of foreign policy.

The controversy about the veil has been presented to the democratic public in France as the issue of freedom of belief and expression. The media had special emphasis on the young Muslim women who insisted on their desire to obtain modern education while wearing the veil as a protection of values of their religion from the external contamination. For them the republican model was a fail-

ure of promise of integration of an individual. Kepel emphasizes that the children of the immigrants, although entirely accustomed through education and assimilated into the French society but pressured by crime and unemployment, have expressed solidarity on the Islamic bases with the community as an alternative to the crime and unemployment. In that respect, wearing the veil represents the symbolic message of a different public identity – the religious one.

It is common to believe that identity is developed in relation to nation, religion, sex, language, socioeconomic position and life style. During the identity construction it is possible that one constitutive element (nation in the case of secular nationalism) excludes public religious identity. According to this view, Ismail⁶ claims that secular national identity cannot be in accord with public religious identity. Namely, the public space does not tolerate symbols of religious identity. In this case the veil in public does not carry a religious message but represents a public appeal for recognition of the right to equality of diversity.

This appeal is the base of the modern Islamic identity. Islamism is a modern political movement whose aim is to win the power and shape the society according to the Islamic ideal – creation of unified Muslim modern identity. Islamism can be seen as an answer to modernism. As such, it is often called Islamic fundamentalism. It is a modern shaped identity and this is what makes it different although to a non-Muslim it often seems like a public expression of traditional identity.

It is a group identity. According to the definition of Deschamps and Devos⁷ a group identity is observed through the fact that an individual sees himself as equal to others that are of the same origin (we), but it also relates to the difference, uniqueness that we experience in our relations with members of other groups or categories (they).

The stereotype in interpretation of the woman's social status implies an oppressed person with no freedom of choice and ability to change her status. However, there is a difference between the stereotype and the real situation on the ground. The author's experience with Muslim families in Croatia and Bosnia testifies how women, not men, are in many cases real core of the family: not only that they care for children and household but they also run business affairs and set the lead for the whole family.

Women's detention in the private sphere in Muslim societies is relative. Namely, this stereotype exists in the West. It is the result of woman's absence from public life. It is visible in societies like Afghanistan in time of Soviet occupation. It is possible to see young five-years-old girls but after that age they disappear. Journalist Robert Kaplan⁸ notices how in Afghanistan there are no women.

In the company of men in the Afghan society it is not even polite to mention women. However, Afghanistan is definitely not the pattern of Islamic society according to which all the societies where Muslims live should be compared, especially the European societies.

The trend of wearing the veil (*hijab*) is used by many analysts as an example of the growing Islamic enthusiasm. Is it really so? Judith Miller⁹ explains how many women that she came across while traveling in Muslim countries liked that style and not necessarily for political reasons. Some women told her that they cannot afford themselves a weekly visit to hairdresser or fancy make-up as some Arab middle-class women take for granted.

In Kasba, Algerian town, families sleep in shifts and women share the bathroom not only with male members of their family but with male neighbors and even strangers. In these situations the veil serves as psychological and physical protection. In overcrowded and uncomfortable Middle Eastern cities, full of young, frustrated men that came in search for job, the veil creates psychological and physical border, which no sexually active young man dares to desecrate. The veil (*hijab*) is sending a message: *I am a religious woman. Leave me alone.*

This example confirms how the veil represents a public statement. Woman that wears it manifests her identity. Giving the fact that she does that in public, she expresses her political attitude as well. There must be a distinction between wearing the veil in overwhelmingly Muslim society and in society where Muslims are minority although in both cases the veil signifies public expression of belonging to Islam and as such it carries a symbolic message.

It is believed here, according to the data and situation's development that Muslim women, by insisting on their distinctiveness, want to be accepted as such and as equal citizens of the society in which they live. They do not want to revive the traditional identify as opposed to the secular society in which they live but rather to set their modernly articulated Muslim identity as equal to other modern identities that they meet every day in the societies they live in.

Rise of consciousness about Muslim women's identity that they gained throughout the process of cultural adjustment, shows how they acquired enough self-consciousness that enables them to expose their private identity in public, asking for its equality. Sometimes the wearing of veil is wrongly interpreted. Namely, non-Muslim authors, looking at the issue from their perspective, try to impose their attitude as the correct one. Therefore, the modern identity of Muslim women, which includes the wearing of the veil, is primarily the identity of resistance to the values than individuals find foreign to them and as such imposed on them.

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MARAMA (HIDŽAB) KAO JAVNI SIMBOL MODERNOG IDENTITETA MUSLIMANKE

SAŽETAK

U članku autor interpretira društvenu ulogu muslimanke u postmodernom društvu kroz javni simbol identiteta – maramu. Teza rada je da manifestiranje islamske pripadnosti kod muslimanki pokrivanjem maramom i nošenjem odgovarajuće odjeće (u slučaju muškaraca nošenjem brade i odgovarajuće odjeće) predstavlja iskaz novog, islamistički oblikovanog identiteta. Riječ je o postmodernom identitetu utemeljenom u suvremenosti, a ne o fundamentalističkoj reakciji na modernost. Marami, kao javnom simbolu muslimanskog identiteta, promatrači često pridaju značenje drukčije od značenja koje joj pridaje osoba koja je nosi. Stoga namjeravamo razmotriti elemente zasebnog, postmodernog identiteta koji marama muslimanke, kao javni simbol, predstavlja.

Evaluation of the Development of Psychosomatic Medicine in a Large University Hospital in Turkey

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ABSTRACT

The purpose of this study was to evaluate the development of psychosomatic medicine at our university hospital in Istanbul, which has an inpatient capacity of 3.000. Changing patterns of utilization of psychiatric service were analyzed in two 1-year surveys five-year intervals (1998, n=888) – (2003, n=1609). Psychiatric referrals were analyzed with regard to rate of consultation, demographic characteristics, departments making referrals, reasons for referral, psychiatric diagnoses and patterns of psychiatric intervention. Psychiatric consultation request, consultation reply and medical psychiatric examination forms were used. In evaluating the data, consultation rate was seen to have doubled over the five intervening years. Significant changes were also noted in the demographic characteristics of patients (e.g., more men, older mean age). The most prevalent disorders in both groups were depressive disorder and adjustment disorders. Alcohol and substance abuse remained as a small group. The gradual increase in the utilization of psychiatric services can be attributed to service and education-related variables.

Key words: psychosomatic medicine, consultation liaison psychiatry, psychiatric diagnosis, general hospital, consultation rate, Turkey

Introduction

Historical and cultural roots of consultation liaison psychiatry in Turkish medicine

Turkish psychiatry and medicine are centuries old and have developed through the ebb and flow of empires. As such, they are culturally and geographically a synthesis of both east and west. They come from an area that cultivated the likes of Hippocrates, Galen and Aesclepiades.

The history of mental health in Turkey begins with the Temple of Aesklepion – the first mental hospital in the world. It continues with treatment centers for the mentally ill in the form of the *Darülsifas* and *Bimarhanes* established during the Seljuk and Ottoman periods. Contemporary mental health care is now provided in Turkey¹. Such indigenous pioneers as Hippocrates and Galen have been influential in the development of contemporary medicine and psychiatry in Turkey. Historical Turkish thinkers like Mevlana and Ibni Sina (Avicenna) emphasized the integrity of, and holistic interaction between, the mind, body and brain. Avicenna (980–1037), whom many scholars believe to be the founder of Turkish psychiatry, wrote about mind-body connections and

asked that the insane be treated humanely. In his treatment of the mentally ill, he employed free association and a combined method of persuasion, psychotherapy and pharmacotherapy.

Hospitals integrating the cultural heritage of the geography and aiming to provide psychosocial treatment to medical patients were opened in Anatolia in the 1200s. What characterized these places was the integration of mental and physical care. They served both mentally and physically ill people, taking special consideration not to separate mental from physical health². Early forms of therapy used in the treatment of mental patients included music therapy and water therapy. Psychiatric care began to be provided in general hospitals in the 1300s in Kayseri (central Anatolia). One example of this is the Gevher Nesibe Hospital, the first institution to integrate a medical school, and the practice of both psychiatry and general medicine. At the time, the mentally ill were protected and treated with kindness – and not discriminated, stigmatized or ostracized – by society.

The history of contemporary Turkish psychiatry dates back to the mid-1800s, a time marked by reforms

designed to westernize the country. Turkish psychiatry has a special place in the 200 years of westernization of what is now Turkey. The discipline represents both westernization and a mode of development that incorporates the best of east and west. It has also been able to take advantage of the long tradition of looking at medicine and psychiatry holistically. The awareness that combines medicine and psychiatry exists in the cultural background of the region and peoples. It is within this structure of thought that preserves a holistic approach and that is in line with 150 years of contemporary development, that Consultation Liaison Psychiatry (CLP) has been able to make progress within a westernizing Turkish psychiatry.

Subsequent to the founding of the modern Turkish republic in 1923, and the university reforms in 1933, Turkish psychiatry began to make enormous progress. Adopting western values and standards, it has come to make major contributions in biological, dynamic and descriptive psychiatry³. Currently, there are five mental health hospitals and 50 medical faculties in Turkey. The total number of psychiatric beds in the country is around 6,000, with more than 5,000 of them in mental hospitals⁴. The majority of the medical faculties have psychiatry beds. Approximately 1/4–1/3 of all psychiatrists work in general hospitals.

The establishment of medical schools has paved the way for the integration of psychiatric departments into general hospitals. It has also enabled psychiatry to be taken as a functional discipline by non-psychiatric departments.

The development and institutionalization of our CLP department

The first modern psychiatric department in a general hospital in Turkey was established in 1954 at the Istanbul University Faculty of Medicine. Up until the 1980s, psychiatric consultations in university hospitals were haphazard and mostly limited to emergencies (suicide attempts, psychotic excitations) as well as to cases where »no organic pathology« was detected. CLP was pioneered at the Istanbul University Faculty of Medicine, where the first CLP unit was formally established in 1989. It was officially recognized and approved as a specific academic discipline in 1997. This department has been integral in pioneering CLP in Turkey, where it is a rapidly developing area of practice and research. With the emphasis of CLP on the mind-body connection, the incorporation of consultation psychiatry into Turkish psychiatry brings psychiatry back to its historical and cultural roots³.

Since 1998, the department of CLP at the Istanbul University Faculty of Medicine has undergone major transformations – particularly with respect to the characteristics and organization of CLP service. Two full-time senior faculty psychiatrists have joined the team. Professionals from different disciplines (liaison nurses, psychologists) have begun to participate in the provision of services and resident rotation has become regular.

This has resulted in a dramatic improvement in the composition of the team from a small multidisciplinary medical consultation model to a much larger multidisciplinary one. There has also been an extension of liaison services throughout the hospital. Thus, routine joint case discussions and the making of ward rounds in the medical, surgical, oncology units have been made possible. Systematic training and educational programs have been added to major medical specialty clinics. Monthly seminars have been conducted at various medical departments and weekly multidisciplinary seminars have been held at the department of CLP. The hospital CLP bulletin has been published and distributed and educational programs for hospital nurses have been organized. Postgraduate courses have been conducted. The National Congress of CLP and Psychosomatics and most recently, The 8th Annual Scientific Meeting of The European Association of CLP and Psychosomatics were organized by the department and held in Istanbul.

By evaluating the changing patterns of psychiatric referrals over a five-year period, we intended to get an idea of the effect of patterns of service delivery, characteristics and composition of CLP service and educational programs on the rate, nature and context of psychiatric in-patient referrals.

Materials and Methods

All the consultations requested in 1998 and in 2003 were evaluated with regard to demographic characteristics, the source of referral, reason for referral (adopted from Mayou⁴), psychiatric diagnoses (according to DSM-IV⁵), and suggested treatment modalities. During the time of the study, hospital characteristics (the number of beds, physicians, nurses of the hospital, the number of hospital departments) were basically the same. For all consultations, we made use of a standard psychiatric consultation request form filled in by the referring physician, a psychiatric consultation reply form, and a medical psychiatric examination form that included physical-psychiatric interaction axes. The psychiatric consultation request form contained information concerning socio-demographic features, medical diagnosis, and reasons for psychiatric referral, laboratory data, and observation as to the behavioral characteristics of the patient. Psychiatric diagnosis was formalized according to the DSM-IV⁵. The reasons for referral are evaluated according to a system developed by Mayou⁴, with the addition of two axes.

Data were analyzed using SPSS version 9.0. In this comparison study, significant differences between two groups were calculated using χ^2 .

Results

Consultation rate

The number of patients referred for psychiatric consultation was 888 in 1998 and 1609 in 2003. The total number of patients admitted to the hospital during the

respective periods was 34,715 (1998) and 34,175 (2003). This means that the consultation rate in 1998 was 2.55, whereas in 2003, it was 4.70. The consultation rate nearly doubled from 1998 to 2003.

Demographic characteristics

The percentage of women referrals decreased in the five-year period. Over time, more men were referred than women ($p < 0.01$). The mean age increased from 47.33 ± 18.36 to 53.08 ± 18.18 . The percentage of older patients referred increased from 26.5% to 37.0% ($p < 0.001$). The age distribution differed in both groups in that the percentage of consultations from old age group (>60) meaningfully increased over the five-year period (Table 1).

Referring clinics

There was a significant difference in the distribution of psychiatric requests in relation to the clinics of the hospital between 1998 and 2003 (Table 2). Psychiatric referrals increased with regard to the departments of internal medicine ($p < 0.001$), cardiology ($p < 0.05$), algology ($p < 0.05$), obstetrics & gynaecology ($p < 0.05$). Psychiatric referrals decreased from the departments of oncology ($p < 0.001$), respiratory disorders clinic ($p < 0.05$), and emergency surgery ($p < 0.001$).

Patterns of reasons for referral

Table 3 shows the comparison of the patterns of referrals in years 1998 and 2003. A significant increase in groups 1, 2 and 6 were recorded. A meaningful increase in referrals for psychiatric consequences of physical disorder ($p < 0.05$), and co-morbid psychiatric disorder ($p < 0.05$) is noted. There is a decrease in referrals for psychiatric disorders presenting with physical symptoms ($p < 0.01$).

Psychiatric diagnostic characteristics

In the distribution of psychiatric diagnoses of referred patients, depressive disorder was the most prevalent psychiatric disorder in 1998. This was replaced by adjust-

ment disorder in 2003. The percentage of dementia increased ($p < 0.05$) from 1998 to 2003. The ranking of the category »No psychiatric diagnosis« remained roughly the same, as did the distribution of such psychiatric diagnoses as substance abuse, personality disorder, and psychosis (Table 4).

Past psychiatric history

In majority of referred cases, no psychiatric history was defined in either group.

80% of patients that developed psychiatric problems in medical settings did not have a past psychiatric history.

Psychiatric treatment patterns

In both groups, psychotropic medication was the most prevalent intervention. This has increased to an even greater extent over the five-year period (72.7%–75.8%) ($p < 0.01$). The application of supportive therapy was the second most prevalent intervention in both groups. Referral to inpatient psychiatry hospitalization was low in both groups.

Discussion

Consultation rate

While hospital characteristics have remained the same, the rate of consultation has almost doubled over the five-year period (from 2.55 in 1998 to 4.70 in 2003). It has steadily increased ever since the establishment of the CLP department in 1989⁶⁻⁸. Recent studies indicate the range of the rate of psychiatric consultation to be 0.5–9.1⁹⁻¹¹. Worldwide reports concerning the rate of consultation indicate a range of 0.74%–5.8%^{9,12-20}, depending on the research population, hospital and the country.

The average annual rate of consultation rate in Europe is reported to be 1.4%^{21,22}. Rigatelli and Ferrari²² have reported a consultation rate of 3% (from 1.48 in 1989 to 3.6 in 2002). Studies in this regard generally report a more static rate over time. Creed et al²³ reported a 36% increase in rate of consultation. In a 10-year longitudinal observational study, Diefenbacher and Strain²⁴ re-

TABLE 1
DEMOGRAPHIC CHARACTERISTICS

	1998 (N=888)		2003 (N=1609)		Δ (%)	p
	N	%	N	%		
Gender						
Women	476	53.6	775	48.2	25.4	$p < 0.01^*$
Men	412	46.4	834	51.8	+5.4	$p < 0.01^*$
Age						
Young (<40)	343	38.6	451	28.0	-10.6	$p < 0.001^*$
Middle-aged (40–60)	310	34.9	562	34.9	0	$p > 0.05$
Old (>60)	235	26.5	596	37.0	+10.5	$p < 0.001^*$
Age, X±SD (range)	47.33 ± 18.36 (17–100)		53.08 ± 18.18 (17–98)			

*significant

TABLE 2
THE DISTRIBUTION OF THE CLINICS THAT REQUESTED CONSULTATION

Referring Departments	1998 (N = 888)		2003 (N = 1609)		Δ (%)	p
	N	%	N	%		
Internal medicine	212	23.9	529	32.6	+8.7	p<0.001*
General surgery	143	16.1	285	17.7	+1.6	p>0.05
Oncology - Hematology	90	10.1	14	0.9	-9.2	p<0.001*
Orthopedics	75	8.4	107	6.7	-1.7	p>0.05
Emergency surgery	70	7.9	71	4.4	-3.5	p<0.001*
Physical rehabilitation clinic	54	6.1	121	7.5	+1.4	p>0.05
Respiratory disease clinic	53	6.0	61	3.8	-2.2	p<0.05*
Neurology-Neurosurgery	52	5.9	88	5.5	-0.4	p>0.05
Cardiology	37	4.2	106	6.6	+2.4	p<0.05*
Medical emergency unit	33	3.7	66	4.1	+0.4	p>0.05
Intensive care unit	29	3.3	34	2.1	-1.2	p>0.05
Algology	12	1.4	40	2.5	+1.1	p<0.05*
Urology	12	1.4	38	2.4	+1.0	p>0.05
Obstetrics-gynaecology	8	0.9	35	2.2	+1.3	p<0.05*
Dermatology	8	0.9	19	1.2	+0.3	p>0.05

* significant

TABLE 3
COMPARISON OF THE TWO GROUPS WITH REGARD TO REASONS FOR CONSULTATION REQUESTS

Reason for request	1998 (N=888)		2003 (N=1609)		Δ	(%) p
	N	%	N	%		
Group 1	611	68.8	1170	72.7	+3.9	p<0.05*
Group 2	66	7.4	161	10.0	+2.6	p<0.05*
Group 3	101	11.4	122	7.6	-3.8	p<0.01*
Group 4	59	6.6	88	5.5	-1.1	p>0.05
Group 5	6	0.7	8	0.5	-0.2	p>0.05
Group 6	8	0.9	33	2.1	+1.2	p<0.05*
Group 7	37	4.2	27	1.7	-2.5	p<0.001*

* significant. Group 1 – encompasses psychiatric consequences of physical disorder, mainly differential diagnosis and management of disturbed behavior associated with delirium and dementia. Group 2 – includes physical and psychiatric disorders occurring together by chance, e.g., patient referred to the C-L service simply because a past history of schizophrenia came to light through routine history taking. Group 3 – comprises psychiatric disorder presenting with physical symptoms, for instance, somatoform and anxiety disorders. Group 4 – refers to physical complications of psychiatric disorder, for example, factitious disorders, alcohol and drug abuse, parasuicide. Group 5 – concerns psychosomatic disorders, such as ulcerative colitis, asthma, peptic ulcer. Group 6 – report medico-legal request with mental health examination. Group 7 – pre-op assessment.

ported a static consultation rate (1.2%) over time. The most distinctive finding of our study is the gradual and persistent increase in the rate of consultation, with its 2003 level being the highest reported in Europe.

The factors that play a role in the rate of consultation differ depending on countries and institutions; societal attitude towards psychiatry; communication and collaboration between psychiatrists and other physicians; and the existence and availability of consultation-liaison and psychosomatic service²⁵. In our study, hospital and social factors remained the same. It was the establishment and recognition of CLP as a separate and special unit, the training of non-psychiatric physicians to recognize psychiatric problems in the medical setting and the ongoing liaison connections that had a major role to play in the increased rate of psychiatric consultations.

Demographic characteristics

The demographic profile of the referred patients in terms of gender and age displayed changed over the five-year period, with more male patients being referred for psychiatric consultations. The literature reports a preponderance of female patients^{9,12,16,19,26}. Our study was in sharp contrast to this, a point that needs to be investigated further by taking into consideration the changes in the value system in society. Over time, the average age of referred patients increased. This has much to do with the change in the general age distribution of the population in general. The increase in psychiatric consultations from among the old age (>60) group is a reflection of this. These two findings, the changes of the demographic characteristic of patients in terms of gender and

TABLE 4
PSYCHIATRIC DIAGNOSES ACCORDING TO THE CATEGORIES OF DSM-IV

Psychiatric Diagnosis	1998 (N=888)		2003 (N=1609)		Δ	(%) p
	N	%	N	%		
Depressive disorders	184	20.7	329	20.4	-0.3	p>0.05
Adjustment disorders	182	20.5	516	32.1	+11.6	p<0.001*
No psychiatric diagnosis	157	17.7	270	16.8	-0.9	p>0.05
Delirium	127	14.3	181	11.2	-3.1	p<0.05
Anxiety disorders	101	11.4	97	6.0	5.4	p<0.001*
Somatoform disorders	36	4.1	47	2.9	-1.2	p>0.05
Psychotic disorders	30	3.4	48	3.0	-0.4	p>0.05
Sleep disorders	28	3.2	29	1.8	-1.4	p<0.05*
Dementia	13	1.5	41	2.5	+1.0	p<0.05*
Alcohol and substance use disorders	13	1.5	22	1.4	-0.1	p>0.05
Personality disorders	5	0.6	15	0.9	+0.3	p>0.05
Dissociative disorders	5	0.6	4	0.2	-0.4	p>0.05
Bereavement**	4	0.5	4	0.2	-0.3	p>0.05
Mental Retardation	2	0.2	3	0.2	0	p>0.05
Factitious disorders	1	0.1	1	0.1	0	p>0.05
Eating disorders	0	0	1	0.1	+0.1	p>0.05
Impulse control disorder	0	0	1	0.1	+0.1	p>0.05

*significant, **under the category of situations that may be of clinical interest

age are different from European and USA studies^{12,27}. Most authors¹⁷ generally report that the demographic features of patients do not change meaningfully over the years.

Referring clinics

In both groups, the largest number of consultations is from the department of internal medicine. While since 1989 the highest proportion of referrals has always been from the department of internal medicine^{6,7}, the percentage has increased over time.

Over the years, more and more consultations have been requested by departments of general surgery, physical rehabilitation clinic, cardiology, algology, emergency department, urology and obstetrics-gynaecology. As liaison psychiatry becomes increasingly accepted by physicians within non-psychiatric in-patient units of the hospital, it has begun to be used with greater frequency. Exceptions to this trend, include oncology, where the percentage of requests has decreased. But this is because there already exists a specific department of psychooncology that provides psychiatric service within the department of oncology. The requests included in this study are, hence, those that are made during »off hours« on an emergency basis.

In most accounts in the literature^{6,14,24,27-29} the highest proportion of referrals comes from the department of internal medicine. In one review⁹, a range of 41.7% – 90% was reported. The overall range of percentages in the literature for referrals is 10%–90%^{9,11}. The second greatest source of referrals is the department of general surgery, which is the case in most general hospi-

tal consultation settings^{6,24,29}. In a review conducted by Hengeveld et al⁶, referrals from such settings ranged between 7% and 34.7%. A more recent study³⁰ reports a figure of 25.5%. Grant and Meller²⁹ reported that it was the intensive care unit that was the second-most-likely department to consult psychiatry. Generally, a static source of pattern of referrals is reported^{27,29}. Those who reported change in the source referrals attribute these to various factors, including liaison activities, the approach of the physicians and specific health system variables^{12,17,24}.

The finding of low consultation referral rates from obstetric and gynecology department was quite similar to results obtained in other studies^{12,31}. The rate of consultation from obstetric-gynaecology moderately increased between 1998 and 2003, most likely a reflection of the collaborative studies and education programs conducted during that time. In the past, the consultations from obstetric and gynecology department were limited to patients presenting major behavioral outbursts or those with psychiatric history⁷.

The change in source referrals over the years stems from the development of our service and liaison connections.

Patterns of reasons for referral

The percentage of Group 1 in total consultation requests is the highest in both our groups. It is even higher in the 2003 group. This percentage is higher than other studies reported in the literature^{12,23,32}. Our finding that psychiatric consequences of physical disorder is the most frequent reason for consultation coincides with what is

found in the literature^{9,12,23,32}. In our 1989–1991 study⁶, the most frequent reasons for psychiatric requests were the referring physician's difficulty in making a diagnosis and previous psychiatric illness in the patient. Altogether, it reflects the improvement in the understanding that physical and psychological disorders co-exist and psychiatric consultations are not limited to »diagnostic evaluation« or to case presenting a past history of psychiatric disorder and suicide, which basically had been the case until the establishment of CLP service.

In general, the second-most-frequent reason for consultation is »psychiatric disorders presenting with physical symptoms^{12,23,32}. In our study, the increase in Group 2 over time is noteworthy. As for Group 4 (physical complications of psychiatric disorder), there were considerable differences compared to what has been reported in the literature. In both our groups, the percentage was lower (6.6%–5.5%) than other studies^{12,23}. Suicides, parasuicide, and deliberate self-harm are meaningfully low in our country due to cultural and religious factors. At the same time, alcohol and, more specifically, drug abuse are also still quite low. Therefore, a psychiatric consultation due to physical complications of psychiatric disorder is low in our study.

Diagnostic characteristics

There is a general trend in both groups towards depressive disorder, adjustment disorder, no major psychiatric diagnosis, and delirium. These comprise approximately 2/3 of the cases. In the 2003 study, the percentage of adjustment disorders reported is higher than it was in 1998. In our study, the determination of »no psychiatric diagnosis« means that the patient has been referred for psychiatric consultation but the assessment did not indicate the presence of a psychiatric disorder. The distribution of the most frequent diagnostic characteristics of patients in European and American studies indicate depressive disorder, adjustment disorder, delirium, somatoform disorder, anxiety disorder and alcohol and substance disorder, in differing ranks^{12,29,30–39}. Adjustment disorders and depression are the most frequently encountered disorders among hospital psychiatry cases in both the national and international literature^{13,33–39}. Depression of various kinds and subtypes constitute the most prevalent psychiatric diagnosis in inpatient psychiatric referrals^{13,37}.

The percentage of delirium is comparatively less in our groups, compared with other studies^{9,12,17,23,27,41}. Organic brain syndrome had been reported to be between 12%–27%^{33,41–43}. The decrease in the incidence of delirium can be explained by the fact that we have established ongoing liaison collaboration with the departments of oncology, intensive care unit, transplantation, hemodialysis, which means that routine psychiatric service is given. The increase in dementia can be explained by the increase in the age groups overall. A more recent study, covering a period of more than 10 years, by Diefenbacher and Strain²⁴ reports that organic mental disorders (delirium, dementia and substance-induced organic mental disorders) account for the majority of cases. This is fol-

lowed by depressive disorder (including adjustment disorders) and by substance-use disorders, respectively. In our study, problems related to alcohol, and especially substance abuse, are extremely rare since these kinds of problems are uncommon in Turkey. The very low percentage of substance abuse in our referrals is a reflection of the general situation in the country.

Past psychiatric history

To our knowledge, no relationship between a request for consultation and past psychiatric history has been established. Up until the department of CLP was established, the majority of requests were for »functional« syndromes or for those with psychiatric history. During the first few years, more than half of the consultation requests were for the differential diagnosis »organic-functional« and for those defining past psychiatric treatment⁶. However, the results of both of our study groups reflect that the vast majority of referred cases defined no past psychiatric treatment. This reflects an improvement in CL services in that the concept of co-morbidity has developed in physicians of the hospital and psychiatric care of medical patients is becoming more of an issue.

Psychiatric intervention

The most likely employed treatment modalities are the use of psychotropics and follow-up. The most frequently administered drugs are, in order of frequency, SSRIs, anxiolytics and low-dosage antipsychotics. The potential and the safety of the new psychotropics in medical patients compared to previous conventional medications is obvious⁴⁴. In liaison settings, long-term psychotherapy mostly cognitive-oriented psychotherapy becomes the second modality. Psychological support and crisis intervention therapy is the third most prevalent treatment modality. There has been an increase in the administration of psychotropic drugs – a phenomenon attested to by studies published since 1990–1991^{12,27}. Overall, psychopharmatics and education and psychological support are the most prevalent bedside psychiatric help. Education here includes informing the patient and explaining to the physician the nature of the patient's situation. Brief crisis intervention psychotherapy is included here.

In our study, the referral rate to psychiatry inpatient service is low in both groups. Only a tiny percentage of patients (most of which were psychotic cases) were referred for psychiatric hospitalization. The literature, on the other hand, reports a higher percentage of patients recommended for psychiatric hospitalization (a range of between 8%¹⁴–12.6%⁹). The lower rate we found is primarily due to the relatively few cases of substance abuse and suicide, which may need emergency hospitalization. Nevertheless, in daily practice, the actual number of patients in need of psychiatric hospitalization was actually higher. There are many patients who cannot or should not be hospitalized in medical clinics due to situational or structural factors. In addition, inpatient psychiatry departments experience a number of difficulties in receiv-

ing certain kinds of patients – for example, pregnant patients with psychosis, cancer patients with melancholic and suicidal features, and MI patients with severe panic attacks. Therefore, we believe that it is clinically necessary to establish medical psychiatric inpatient liaison service.

An evaluation of the patterns of referrals reflects a consistent and gradual increase in consultation rate, an increase in consultation request from departments where training programs are carried out and in the pattern of referral favoring co-morbidity.

Controlling for hospital characteristics, the increase in rate, nature and context of psychiatric inpatient referrals can be attributed to the effects of service delivery, characteristics and composition of CLP service and educational programs, which were the major changes and developments that occurred during the period of the study.

The establishment of CLP as a separate unit, the change in the composition and the increase in the size of the CLP team, whereby it became a larger multidisciplinary model; the training of non-psychiatric physicians in the recognition of psychiatric problems in the medical setting; and the establishment of liaison connec-

tion all played a role in the recognition of psychological disorders in the medically ill, generating a willingness for collaboration, producing an increase in requests for consultation and rational utilization of psychiatric services. While the factors behind this development cannot be substantiated 100%, it can be argued that the aforementioned reasons and developments are major factors relevant to our experience and observation. Benefits and potential of »active« approach cannot be overlooked²⁵.

This is the first study of its kind from Turkey. Our experience in establishing and developing a CLP service that has a larger multidisciplinary team and liaison connections and large scale of educational programs will contribute to the establishment of CLP in general hospitals in the country.

Our experience has also suggested that a larger multidisciplinary CLP service⁴⁵ and liaison work will contribute to the improvement of psychiatric referrals, hence enabling more patients with a wider variety of clinical problems⁴⁶ to be seen. In addition, the quality of both service given and psychiatric request made will improve since physicians will be able to detect psychiatric morbidity in their patients earlier on and obtain more effective collaboration.

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PROCJENA RAZVOJA PSIHOSOMATSKE MEDICINE U VELIKOJ SVEUČILIŠNOJ BOLNICI U TURSKOJ

S A Ž E T A K

Cilj ovog istraživanja bio je procijeniti razvoj psihosomatske medicine na sveučilišnoj bolnici u Istanbulu, s kapacitetom za 3 000 pacijenata. Promjene u načinima korištenja psihijatrijske djelatnosti analizirane su tijekom dva jednogodišnja istraživanja s razmakom od 5 godina (1998, n=888) – (2003, n=1609). Pacijenti upućeni na psihijatriju analizirani su u odnosu na učestalost konzultacija, demografske karakteristike, odjele koji su ih uputili na psihijatriju, razloge upućivanja, psihijatrijske dijagnoze i obrasce psihijatrijskih intervencija. Korišteni su upitnici za psihijatrijsku konzultaciju, psihijatrijski odgovor na konzultaciju i upitnik za psihijatrijski pregled. Kod procjene rezultata vidljivo je da se učestalost konzultacija udvostručila tijekom petogodišnjeg razdoblja. Značajne promjene također su uočene kod demografskih obilježja pacijenata (npr., više muškaraca, starija dobna skupina). Najučestaliji poremećaji u obje grupe bile su depresija i poremećaji prilagodbe. Zlouporaba alkohola i opojnih droga zadržala se u maloj grupi ispitanika. Postupno povećanje korištenja psihijatrijske djelatnosti može se pripisati unapređenju psihijatrijske djelatnosti i edukacijskim čimbenicima.

Connection between Classroom Abuse and Manifest Aggressiveness, Anxiety and Altruism

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ABSTRACT

The objective of this paper is to find out if the students exposed to abuse differ in their level of anxiety, aggressiveness, and altruism from other students, and to test if the pattern of these differences differs depending on whether the abuse they suffer is emotional or physical. The research was carried out on a sample of 127 senior elementary school students. The data was gathered at the end of the 2003/04 school year, and obtained through the respondents' self-statements in questionnaires about childhood abuse and by the scales of manifest aggressiveness, anxiety and altruism. The frequency analysis has shown that various forms of emotional abuse are more common in schools than physical abuse, and that they are reaching disturbing proportions. For example, more than half of the participants in the study reported facing intimidation and threats in school, and over a third of them have been yelled at. Although less commonplace, physical abuse in school can by no means be ignored. Those students who suffer from frequent physical abuse are more dissatisfied with school ($r=0.174$, $p<0.05$), display more aggressiveness ($r=0.441$, $p<0.001$), and are more often boys ($r=0.324$, $p<0.01$). Those students who are frequently emotionally abused are more anxious ($r=0.281$, $p<0.01$), dissatisfied with school ($r=0.237$, $p<0.01$), and display more manifest aggressiveness ($r=0.398$, $p<0.01$). The discriminant analysis has shown that the bullied students can be differentiated from their non-abused schoolmates as they are manifestly more anxious and aggressive, regardless of whether they suffer physical or emotional abuse. Instances of different forms of emotional and physical classroom abuse have increased alarmingly. Such traumatic experiences affect children's health and functioning in school, as well as in their private lives. The interdisciplinary studies of this phenomenon and the education of all those who work with young people emerge as the top priority in the prevention of this kind of abuse.

Key words: *abuse, anxiety, aggressiveness, altruism, school dissatisfaction, school performance*

Introduction

In this day and age, when the phenomenon of violence and abuse has generally escalated, it has suddenly become of great interest to scientists, doctors, teachers, parents, and the general public. The phenomenon of violence and abuse of children has become a current topic of anthropological, psychological, and pedagogical discussions.

Although peer violence is an age-old phenomenon, and the subject of numerous works of art, books, films, and personal memories of many adults, it became an object of serious scientific interest and study only in the 1970s¹.

Statistical indicators have given rise to concern about the ominous upsurge of classroom abuse. For example, 14% of Norwegian schoolchildren are bullied in school every day, 15% in Japan, 16% in the USA, 17% in Australia and Spain, and 19% in England².

According to the research carried out in 2003 by the Medical Centre for the Protection of the Children of the City of Zagreb, 27% of Croatian schoolchildren are victims of some form of classroom abuse: 19% are passive victims (they suffer violence), while 8% are provocative victims (both the victims and the victimizers). The same research shows that 16% of them regularly abuse other children².

Abuse occurs when one student or a group of students are repeatedly or perpetually exposed to intentional obnoxious acts by one student or a group of them. When discussing classroom abuse, we cannot ignore the fact that teachers may also be classroom abusers.

For the purposes of this paper, the focus has been placed on the forms of emotional and physical bullying, both open and hidden.

Neglect and abuse of children in their families and in school leaves short-term and long-term effects on their functioning and development. The most usual problems that lead to disorders are emotional by nature. They are most often displayed through externalized disorders, i.e. through children's mental difficulties characterized by antisocial behaviour: confrontation, destructiveness, aggression, delinquency, or through internalized disorders which are characterized by withdrawal from others, anxiety, fear, and dissatisfaction. A well-known study of 2500 children, Achenbach et al. (1991) found that the patterns of externalized behaviour changed with age³. The older the children, the amount of aggressive behaviour decreased while the amount of delinquent forms of behaviour increased. Anxiety generally increased with age, and girls displayed more symptoms of anxiety than boys. The sample of school age adolescents from various Croatian regions studied by I. Begovac, V. Rudan, M. Skočić, O. Filipović, and L. Szivovicza showed matching results and corroborated the major behavioural and emotional problems listed by adolescents, while the students themselves proved to be the most important source of information about their own problems⁴. This encouraged us to test the connection between classroom abuse and manifest aggressiveness, anxiety, or even altruism. The objective of this paper is to find out if the students exposed to abuse differ in their level of anxiety, aggressiveness, and altruism from other students, and to test if the pattern of these differences differs depending on whether the abuse they suffer is emotional or physical.

The term *physical abuse* by peers and teachers implies repeated exposure to physical violence or intentional affliction of bodily harm. The consequences of such acts are often visible in the form of bruises, broken bones, injuries; the number of cases when medical assistance was required in such situations is on the increase.

Even when the injuries heal, the emotional scars remain. That is why physical abuse is at the same time a form of emotional abuse.

Emotional abuse in the classroom refers to a lasting and deep-seated attitude on a student's – sometimes teacher's – part that impedes the development of a child's positive self-image or their self-respect and social competence⁵. The forms of emotional abuse are: yelling, mocking and derision, cursing and swearing, intimidation and threatening, chastisement and insults, bans and restrictions, blaming, ignoring and ostracizing, lack of understanding, and unfair response to classroom performance.

It is important to distinguish between the *open abuse* – when the victim is openly bullied physically or emotionally – and the *hidden abuse*. We are going to focus on indirect or hidden abuse. Bullying behaviour in the classroom, namely, is often manifested in the form of bad language, offensive gestures, repeated usage of hideous nicknames, pointing out mistakes/errors, failures, imperfections, etc. Such »covert« acts may turn into »psychoterror«. The *victims* are mostly physically weaker, shy, insecure and unassuming children, who shun fight-

ing and do not know how to defend themselves. Exceptional students and overachievers are also victimized since other children envy them and have a need to belittle their achievements. Often, the victims are newcomers whose style, speech and behaviour deviate from the majority's accepted conduct. Children with some blemish or physical handicap are often mistreated as well. After a prolonged period of exposure to ridicule, hostility or humiliating stratagems, children respond with fear, confusion or open confrontation. Whatever the reaction, the abusers use it as a pretext for new aggression. Teachers do not show much sympathy when bullied children turn to them for help. Similarly, when they complain to their parents they get labelled as »sissies«, incapable of functioning among their peers.

The outcome of a prolonged exposure to peer abuse may be loss of self-confidence and profound feeling of guilt. Such children become exactly what their group wants them to be. They feel insecure, misunderstood and lonely. After a period of introspection and self-reproaching and some discreet searching for help, the pressure may become unbearable and then the children cry out for help in some outrageous way: a tantrum or a crisis. If their efforts to get protection fail, they resort to self-pity, come to the »something-is-deeply-wrong-with-me-indeed« conclusion and become resigned to their cruel fate. All this can seriously affect their future functioning.

Bullies can be individual students or a group, whose leader is usually a more aggressive student with a deficient sense of guilt and the need for domination. Such children are joined by others with a similar agenda or because they dislike another child, but also by some of those who fear rejection and who bury their individuality in a gang. There is always a group of children who do not want to interfere or take sides, who are neutral regarding the violence they are witnessing, and keep their distance from the problems their schoolmates have to cope with.

In the dynamics of hidden classroom abuse by a group, the most usual forms are combinations of ostracizing, exclusion, isolation, pressuring, and deliberate misleading. The forms of abuse are extremely perfidious, and the results are disastrous. It is absolutely essential to unmask these forms of hidden abuse that make many childhoods miserable in order to help the victims – and the abusers – to mend their ways and become healthy adults^{6,7}.

Undesirable developmental outcomes, internalized and externalized disorders – primarily *anxiety* and *aggressiveness*, ensue from such traumatic events.

Threatening experiences are usually related to anxiety, which has been corroborated by Keresteš as well, 2002⁸. According to the same source, the traumatic experiences characterized by violence and conflicts are usually connected with aggressiveness and antisocial behaviour.

Anxiety refers to a diffuse feeling similar to fear without a clearly threatening object, when the threat is not

obvious and its rational estimation not possible. The limits of the danger are not visible, and the situation is not fully understood: there is only a kind of floating fear.

Aggressiveness is usually defined as a set of various forms of behaviour which include physical or mental harming of other people, destructive behaviour aimed at punishing others or destroying property. More recent studies mention the reactive and the proactive aggressiveness. The reactive aggressive behaviour is a reaction to certain situational conditions, primarily threats, provocations or thwarting goals. The proactive aggressiveness, on the other hand, occurs when a person, in anticipation of a desirable goal which he/she presumes may be achieved only by violence, adopts – unprovoked – some form of aggressive behaviour. Most aggressive individuals manifest both types of aggressiveness, the so-called reactive-proactive chains of aggressiveness.

This paper looks into the connection between classroom abuse and the incidence of anxiety and aggressiveness. Also, we wanted to see whether the violence school children are subjected to encourages the bullied children not to behave in the same way towards other children, i.e. whether this can motivate them to pro-social behaviour, primarily altruism, and whether they practice unselfish, helping behaviour (verbally, or in the form of helping other children with schoolwork or coming to their aid in dangerous situations) solely for the benefit of others. *Altruism* presupposes genuinely selfless, voluntary behaviour and affection for other people without any personal and material benefits.

Materials and Methods

The survey was conducted on a sample of 127 senior elementary school students (69 or 54.3% girls, and 58 or 45.7% boys) from Zagreb. At the end of their formal elementary education they were asked to appraise whether they had ever been bullied in school.

The following instruments were used in the study:

The childhood abuse questionnaire

The questionnaire was adapted by A. Karlović from the questionnaire »The comprehensive child maltreatment scale for adults« (CCMS) (Higgins and McCabe, 2000, cit. acc.⁹). The questionnaire validation was done by Karlović, Buljan-Flander and Vranić (2001)⁹. The reliability of the questionnaire proved to be very high (Crombach $\alpha = 0.95$). The scales of emotional and physical maltreatment have been used in this paper with some additional questions regarding the frequency of abusive behaviour and who abusers are⁹.

The manifest aggressiveness scale

The manifest aggressiveness scale (P. Bezinović and S. Smojver-Ažić, 2000) comprises ten items in which the respondents state how often they behave aggressively. The responses to individual units range from 0 (»never«) to 4

(»very often«). The internal consistency coefficient for this scale (Crombach α) is 0.80¹⁰.

The anxiety scale for children

Wieczerkowski et al. (1973) (cit. acc.¹¹) constructed this scale, which was adapted by A. Vulić-Prtorić and I. Sorić. Three subscales were used in this paper: the subscale to test the tendency to anxious reactions in different situations (Crombach $\alpha=0.78$); the school dissatisfaction subscale (Crombach $\alpha=0.63$); and the subscale of children's tendency to behave in an adjusted and socially desirable way (Crombach $\alpha=0.81$)¹¹.

The altruism scale

The altruism scale was created by Z. Raboteg-Šarić (1995). Three criteria have been used for the operationalization of the term: that the behaviour in question is voluntary and unforced, that its purpose is to benefit other people, and that it excludes expectations of material or social rewards or avoidance of external inducements and punishment. The scale is quite reliable and internally consistent (Crombach $\alpha = 0.84$)¹².

All of the data were obtained by means of the students' self-statements regarding their behaviour in a variety of situations. The data were collected at the end of the 2003/04 school year, with the permission of the ethics committees and the school principals.

The respondents were given some general instructions about the purpose of the research, its general and particular benefits, and the possible risks. The confidentiality of the results and the anonymity were guaranteed. The participation was completely voluntary. The students were asked to be perfectly honest. Aware that the questionnaire may bring back some unpleasant memories, the children were provided with psychological support and counselling, if needed.

The analysis of the results was carried out through their description. The relationship between the variables was treated with Pearson correlation coefficient. The reliability and the validity of the tests used was checked by Crombach α . The difference between groups was treated with a canonical discriminant analysis. All statistical actions were undertaken with the assistance of the SPSS program package.

Results

The objective of this paper was to find out if the students exposed to abuse differ in their level of anxiety, aggressiveness, and altruism from other students, and to test if the pattern of these differences differs depending on whether the abuse they suffer is emotional or physical.

Table 1 shows the frequency of abusive behaviours to which the subjects of our study were subjected. According to the given data, emotional abuse is much more frequent in schools than physical, and it is reaching disturbing proportions. For example, more than half of the

TABLE 1
FORMS OF EMOTIONAL AND PHYSICAL CLASSROOM
ABUSE IN ELEMENTARY SCHOOLS (N=127)

Form of abuse	% of students exposed to abuse
Emotional abuse	
Intimidation and threatening	52.0
Yelling	34.6
Swearing and cursing	30.7
Harsh criticism	21.3
Mocking and derision	15.7
Physical abuse	
Throwing on the ground and pushing down the stairs	23.6
Punching or hitting with an object	7.9
Slaps and blows	6.3
Physical injuries that require medical assistance	5.5

participants in the study reported experiencing intimidation and threats in school, and more than a third of them have been yelled at. Although less commonplace, physical abuse in school can by no means be ignored. Almost a quarter of respondents have been thrown onto the floor or pushed down the stairs, and over 5% of the students were subjected to physical abuse which required medical assistance.

To test the correlation of emotional and psychological abuse in correlational analyses, we formed variables of total emotional and total physical abuse. These variables were formed as simple linear combinations of frequency of exposure to emotional and physical abuse.

Table 2 shows the Pearson coefficients of the correlation variables that were used in the study. What is of special interest to our study is the fact that the variables of abuse have the following pattern of connection with the predictor variables. Physical abuse is significantly re-

lated to gender ($r=0.324, p<0.01$), dissatisfaction with school ($r=0.174, p<0.05$), and manifest aggressiveness ($r=0.441, p<0.01$). Students who suffered more frequently physical abuse are more dissatisfied with school, display more aggressiveness, and are more often boys. Those students who suffered more frequently emotional abuse are more anxious ($r=0.281, p<0.01$), dissatisfied with school ($r=0.237, p<0.01$), and display more manifest aggressiveness ($r=0.398, p<0.01$). It is interesting to note that both types of abuse are in correlation: those students who are frequently abused physically, also suffer frequent emotional abuse ($r=0.503, p<0.01$).

In order to test the degree and nature of the differences between the abused students and those who have never been abused in the variables of anxiety, altruism, aggressiveness, gender, and school performance, we carried out two linear discriminant analyses. One of them tested the pattern of differences between physically abused students (N=34) and those students who were never abused (N=93) in the given variables. The other tested the structure of differences between emotionally abused children (N=45), and those who never suffered any emotional abuse (N=82). The groups mentioned were formed in such a way that the groups of physically and emotionally abused children included all those students who had been exposed to any form of physical / emotional abuse at least on one occasion.

The discriminant analysis of physically abused students and those who were never physically abused resulted in a considerable discriminant function ($\lambda=0.368$, Wilks $\Lambda=0.731$; $\chi^2=38.07, df=7, p<0.01$). The coefficient of the canonical correlation (R_c) of 0.519 points to a moderate connection of the results of the discriminant function and affiliation with the group of physically abused children or the ones who were not abused. Exactly 26.9% of the total variability in the discriminant variables can be explained through the differences between groups.

Table 3 contains correlations of manifest variables with the discriminant function. The analysis of these correlations leads to the conclusion that the variables that

TABLE 2
MEANS, STANDARD DEVIATIONS AND CORRELATIONS AMONG VARIABLES USED IN ANALYSIS (N=127)

Variables	X	SD	Coefficients of correlations									
			1	2	3	4	5	6	7	8	9	
1 Gender ^a	1.5	0.50	-									
2 School performance ^b	1.1	0.36	0.158	-								
3 Manifest anxiety	11.9	5.53	0.068	-0.253**	-							
4 School dissatisfaction	8.9	3.02	-0.188*	-0.015	0.235**	-						
5 Social desirability	7.0	3.52	0.041	-0.187*	0.259**	-0.052	-					
6 Manifest aggressiveness	18.6	3.02	-0.204*	0.127	-0.027	0.223*	-0.405**	-				
7 Altruism	37.5	13.03	0.130	-0.051	-0.080	-0.197*	0.408**	-0.262**	-			
8 Physical abuse	0.5	1.01	-0.324**	-0.004	0.138	0.174*	-0.084	0.441**	-0.026	-		
9 Emotional abuse	2.3	2.47	-0.205	-0.065	0.281**	0.237**	-0.054	0.398**	-0.038	0.503**	-	

6^a gender: 1 - male, 2 - female, ^b school performance: 1 - excellent, 2 - very good, 3 - good, 4 - bad, * $p<0.05$, ** $p<0.01$

TABLE 3
STRUCTURE OF THE DISCRIMINANT FUNCTION WHICH
DIFFERENTIATES THE PHYSICALLY ABUSED CHILDREN
FROM THOSE THAT WERE NOT ABUSED

Manifest variables	r
Manifest aggressiveness	0.639
Gender	-0.523
Manifest anxiety	0.410
School dissatisfaction	0.288
Grade	0.102
Social desirability	0.025
Total altruism	-0.022

TABLE 4
CENTROIDS OF PHYSICALLY ABUSED CHILDREN AND THOSE
WHO WERE NOT ABUSED ON A DISCRIMINANT FUNCTION

Groups	Centroids
Children who were not physically abused	-0.364
Children who were physically abused	0.995

best discriminate between the abused children and those who were never abused are: manifest aggressiveness, gender, and manifest anxiety. Statistically significant differences between the above mentioned groups on the uni-variant level (all $F > 7.51$, $df = 1/125$; $p < 0.01$) are found in these very variables.

Taking into account the groups' centroid signs and their values, we can conclude that physically abused students display more manifest aggressiveness and anxiety, and that they are more often boys than girls.

The second discriminant analysis tested the differences between emotionally abused children and those who suffered no such abuse. Significant discriminant function was gained ($\lambda = 0.218$, Wilks $\Lambda = 0.821$; $\chi^2 = 23.97$, $df = 7$, $p < 0.01$). The canonical correlation (R_c) of 0.423 points to the fact that the differences between the groups account for the 17.8 % difference in the above mentioned variables.

TABLE 5
STRUCTURE OF THE DISCRIMINANT FUNCTION THAT
DISCRIMINATES THE EMOTIONALLY ABUSED CHILDREN
FROM THOSE WHO WERE NOT ABUSED IN SUCH A WAY

Manifest variables	R
Manifest aggressiveness	0.632
Manifest anxiety	0.616
School dissatisfaction	0.384
Grade	0.293
Social desirability	-0.060
Total altruism	-0.040
Gender	-0.039

TABLE 6
CENTROIDS OF EMOTIONALLY ABUSED CHILDREN
AND OF THOSE WHO WERE NOT ABUSED ON
A DISCRIMINANT FUNCTION

Groups	Centroids
Children who were not emotionally abused	-0.625
Children who were emotionally abused	0.343

The matrix of the discriminant structure shows that the variables that define positive polarity of the discriminant function are, just like physical abuse, manifest aggressiveness and manifest anxiety, and, unlike physical abuse, school dissatisfaction.. These differences were also found with the above mentioned variables on a uni-variant level (all $F > 4.03$, $df = 1/125$; $p < 0.05$).

According to Tables 5 and 6, the emotionally abused children are manifestly more aggressive, more anxious, and more dissatisfied with school. Unlike physical abuse, gender does not appear as one of the discriminant variables.

In conclusion, the analyses that were carried out show that the abused children differ from those who were not abused by manifest anxiety and aggressiveness, regardless of whether they were abused physically or emotionally. When looking at gender and school dissatisfaction, the differences do depend on the type of abuse. Gender appears as a relevant discriminant variable only in cases of physical abuse (male students are more often exposed to physical abuse than female students), and school dissatisfaction in cases of emotional abuse (emotionally abused children are dissatisfied with school).

Discussion

On the basis of this research results it can be concluded that students are predominantly exposed to emotional abuse (intimidation and threatening, yelling, swearing and cursing, chastisement, mocking and derision, bans and restrictions, insults, ostracism, ignoring). This conclusion is in line with the observed situation in practice, outlined in the introductory part of this paper. Children are usually subjected to the mentioned forms of emotional abuse by a group of their peers. Since this is hidden mistreatment, which is hard to notice, adults rarely respond timely and appropriately. Emotional (especially indirect) abuse, albeit less tangible, is nevertheless devastating, and if recurrent or persistent it may start to dominate the child's life. The most typical consequences of emotional abuse are an impaired self-image, undermined self-respect and problems with social competence.

The forms of physical classroom abuse that schoolchildren are most often subjected to are throwing on the ground, being punched or hit with an object, and also occasional injuries requiring medical assistance. Physical abuse is usually a person-to-person affair. There are statistically significant differences regarding the frequency

and the exposure to physical abuse between boys and girls. According to this study, boys are more often mistreated in general, and also more often abused physically. Regarding emotional abuse, there are no statistically significant differences between the genders. Physical abuse is in a way emotional abuse as well, because even after the bruises have faded and wounds healed, the emotional scars of the abused children remain.

According to the findings of this study (though the sample is rather small), it is evident that bullying is becoming part of school life, encroaching upon a setting in which it is not supposed to exist. Schools should be safe environments for growing up, but the findings of the study show they are not. Not only is the schoolchildren's physical safety jeopardized, but due to a variety of forms of classroom emotional abuse and its consequences, children have problems in functioning emotionally and socially as adults. Also, schoolchildren are subjected to abuse by their teachers: teachers yell at them, berate them, and sometimes even slap them. It has already been said that such behaviour is unpardonable but – though this is not a justification – it must be mentioned that the teachers are also subjected to different forms of abuse by their students, their parents and often by their superiors, and they find it increasingly hard to cope with this extra stress. A study of teacher abuse would provide a more objective picture of the situation in Croatian schools.

Joint efforts and prevention of all forms of abuse require first of all proper education of teachers, general practitioners, and parents as well as instructing schoolchildren to develop more acceptable forms of behaviour. Children need instruction because this is a problem they know little about, and they need help and advice based on the research insights into this complex phenomenon. An early recognition and provision of even minimal assistance, encouragement and comfort, or referral to appropriate institutions, can be more helpful than even the most expert but belated assistance.

The first important conclusion of this research is that the abused schoolchildren differ from the ones who are not subjected to abuse by being manifestly more aggressive, more anxious, more dissatisfied with school, and their school performance is also somewhat poorer.

Also, the physically abused schoolchildren are more often manifestly more aggressive and anxious than their peers who are not abused, and are mostly male.

Also, the emotionally abused schoolchildren are more often manifestly more aggressive, anxious, and dissatisfied with school than their peers who do not suffer abuse.

The variables of altruism and social desirability have not proved to be significant in any analysis.

Manifest aggressiveness, thus, occurs in the physically and emotionally abused children; it also discriminates between the abused children and the children who are not abused. According to its very definition reactive aggression occurs as a reaction to certain situations, usually threat, provocation or thwarted goals⁸. The side-effects

of this basically hostile interpersonal behaviour are powerful emotions of anger accompanied with intense physiological agitation. It is only natural, therefore, that after a series of various forms of emotional and physical classroom abuse, children begin to behave in manifestly aggressive ways. Very often they are aware that such behaviour of theirs is defensive and stems from fear. Such forms of behaviour, the need for appreciation or domination, low frustration tolerance, may result from a lack of support, a painful awareness of existential insecurity, and a deep mistrust of one's social immediate surrounding, and are typical for bullied children. The results of this study corroborate different theories and are in line with some research of children's aggressive behaviour⁸. However, the studies of the behaviour of abused children are few, especially in Croatia.

The results of this study will be especially useful to schoolteachers and help them not to brand the manifestly aggressive students as »naughty«, but to be aware that there is something else underlying their behaviour, that their aggressiveness is but a symptom of a more serious problem i.e. abuse, family interactions, etc. Social factors, such as the behaviour of parents, teachers or peers determine an early adoption of patterns of aggressive behaviour. Today it is universally accepted that aggressive behaviour is transmitted by learning by reinforcement and by modelling. How can a child subjected to violence, or who witnesses violence daily, who is shaped by hopeless educational procedures, behave? It has often been said that a battered child beats other kids, since this is the only option he/she has ever known. Such children only apply familiar models of behaviour. Childhood exposure to violence is one of the most reliable predictors of subsequent delinquency⁸.

We have already mentioned the research conducted by Achenbach et al. (1991), which found that the quantity of aggressive behaviour decreases as the child ages, while the quantity of delinquent behaviour increases³.

This must be borne in mind particularly when dealing with the prevention of aggressiveness and the assistance to schoolchildren with such problems; the results of this research show that the exposure to physical and emotional classroom abuse is conducive to students' manifest aggressiveness, and for the future unfavourable outcomes.

Manifest anxiety has turned out to be the discriminant variable between the abused children and the ones who were not abused, in general. The emotionally and physically abused students are manifestly more anxious than their schoolmates who suffer no such abuse. Similar results have been obtained in a number of studies⁸. As has already been pointed out, going to school every day with a feeling that you are going to be – once again – ridiculed, humiliated, that instead of joy you will meet only unpleasantness and perhaps even be physically abused, and that you are helpless, in many children results in anxiety, apprehension, insecurity and tension, which affects their school performance and increases their school dissatisfaction. This is more often the case

with girls, which correlates with the results of the already mentioned research by Achenbach et al.³ An anxious schoolchild lives in constant fear of an impending disaster he/she is not able to define, accompanied with a strong feeling of uneasiness. Although this threat is vague, it presents, nevertheless, a danger for the child's psychological well-being. This state of trepidation, of pervasive anxiety and torment, is manifested psychologically as a feeling of tension, restlessness and fearfulness. The ensuing edginess and lack of concentration affect the ability of memorizing and school performance. Sleeping disorders and nightmares further intensify the tension and edginess, which consequently triggers off physiological changes. The problem of anxious children in schools is harder to notice and thus very often goes unrecognized, mostly due to teacher's unfamiliarity with it. And it is this anxiety that can make the time spent in school difficult and miserable for many children. Recognizing the symptoms of anxiety and the timely reaction to them, ought to be an important component of teacher training. The results of this research show the connection between students' manifest anxiety and classroom bullying, and further stress the need for such education and research, both about the abuse and the resulting anxiety, and about the ways they affect each other in different stages of education.

This research has also showed that the abused schoolchildren are much more *dissatisfied with school* than their schoolmates who are not abused. Uneasiness, uncertainty and threat they so often feel, makes their school dissatisfaction understandable and only too logical. All this affects their school performance which, consequently, increases further their school dissatisfaction and disenchantment in general and quite often determines the course of their adult lives. A number of studies have shown that the mistreated children manifest emotional and behavioural problems, are cognitively dysfunctional and are consequently underachievers in school¹³. It is well known that children bring to school from their homes the potential for aggression, fear and sorrow, familiar behavioural patterns; if this is further exacer-

bated by an unfavourable school setting – a lack of understanding or bullying – their psychological health may be seriously impaired. Naturally, school situations, particularly the extreme ones, affect the functioning and health of families and societies.

Conclusion

Abuse, especially various forms of peer abuse – emotional or physical, hidden or open – is becoming an everyday occurrence in schools. Also alarming is the frequency of teachers' outrageous forms of behaviour.

The consequences of traumatic classroom experiences have far-reaching effects on children's health and functioning both in school and in life.

Especially obvious is the connection between abuse and certain developmental outcomes, e.g. classroom abuse is connected with manifest aggressiveness and anxiety. Other significant variables are school dissatisfaction, poorer performance and gender. The variables of altruism and social desirability are not significant in any of the analyses.

Education of teachers that would enable them to recognize forms of abuse and to provide expert and opportune help emerges as a must – the most essential part of prevention. Apart from teachers and physicians, parents should also be educated, and recognized as partners in this joint and extremely important task. When working with schoolchildren, it is important not solely to create straight A-students but to have in mind the kind of persons these young people are eventually going to turn into and whether they are going to leave school unscathed and healthy. A child is shaped by the entire environment and its values. That is why it is important that all those who work with young people join hands in fighting violence as this is the only way in which we can make our communities more protective and schools safer and nicer settings for children's healthy development – all in the interest of a better future for all of us.

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POVEZANOST ZLOSTAVLJANJA U RAZREDU S MANIFESTNOM AGRESIVNOŠĆU, ANKSIOZNOŠĆU I ALTRUIZMOM

S A Ž E T A K

Cilj ovog rada je provjeriti razlikuju li se učenici izloženi zlostavljanju u anksioznosti, agresivnosti i altruizmu od ostalih učenika te ispitati je li obrazac razlika drugačiji ovisno o tome radi li se o emocionalnom ili tjelesnom zlostavljanju.

U istraživanju je sudjelovalo 127 učenika završnih razreda osnovnih škola. Prikupljanje podataka provedeno je krajem školske godine 2003/2004. Podaci su dobiveni samoiskazom sudionika na upitnicima o zlostavljanju u djetinjstvu te skalama manifestne agresivnosti, manifestne anksioznosti i altruizma. Frekvencijskom analizom utvrdili smo da su u školi dominantniji oblici emocionalnog zlostavljanja od tjelesnog i dosežu zabrinjavajuće razmjere, primjerice više od polovice sudionika izvještava o plašenju i prijetnjama koje doživljava u školi, a više od trećine doživjelo je da netko na njih viče. Premda je manje prisutno, tjelesno zlostavljanje u školi nije zanemarivo. Učenici koji su tjelesno češće zlostavljani nezadovoljniji su školom ($r=0.174$, $p<0.05$), iskazuju veću agresivnost ($r=0.441$, $p<0.001$) i češće su muškoga spola ($r=0.324$, $p<0.01$). Emocionalno češće zlostavljani učenici su anksiozniji ($r=0.281$, $p<0.01$), nezadovoljniji školom ($r=0.237$, $p<0.01$) i manifestno agresivniji ($r=0.398$, $p<0.01$). Diskriminacijskom analizom utvrdili smo da zlostavljanu djecu od nezlostavljane razlikuje iskazana manifestna anksioznost i agresivnost bez obzira radili se o tjelesnom ili emocionalnom zlostavljanju. Različiti oblici emocionalnog i fizičkog zlostavljanja poprimaju zabrinjavajuće razmjere u školskoj svakodnevici. Posljedice takvih traumatskih iskustava utječu na zdravlje i funkcioniranje djece u školi ali i životu. Interdisciplinarna istraživanja ove pojave i edukacija svih ljudi koji se bave mladima, nameću se kao neophodnost i prvi zadatak prevencije.

Serum Concentration of Nine Hormones in Aging Male Population and Association with Potency and Libido Problems

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ABSTRACT

Aim was to determine if a serum levels of free testosterone and selected eight hormones are in correlation with potency and libido problems in aging male. Male population older than 45 years of two Slavonian villages was called for voluntary examination. Every patient filled a questionnaire concerning medical history, operations and potency and libido problems. Based on answers we formed six groups of patients, but only three were analyzed further. Population with potency and libido problems is on average older. In group of patients with normal potency and libido (PNLN group) average levels of free testosterone are 46.01 pmol/L, LH 4.62 IU/L and FSH 6.20 IU/L. In group of patients with mild-damaged potency and normal libido (PMLN group) average levels of free testosterone are 44.61 pmol/L, LH 6.19 IU/L and FSH 8.18 IU/L. In PALA group of patients with absent potency and libido (PALA group) average levels of free testosterone are 41.89 pmol/L, LH 8.07 IU/L and FSH 11.27 IU/L. Significant higher levels of FSH and LH were found compared with the control group (for FSH $p < 0.0001$ and $p < 0.003$, and for LH $p < 0.003$ and $p < 0.021$). No significant difference in serum levels of free or binned testosterone were found between three groups. Even if average serum levels of free testosterone is found lower in patients with libido and potency problems, this difference is not significant and testosterone deficiency itself can not explain potency and libido problems in aging male.

Key words: hormones, aging men, potency, libido

Introduction

Aging men is relatively new term which tries to describe a set of changes both biological and psychological in older male population. A term andropause was made in attempt to summarize hormonal changes that occur, with relation to same equivalent hormonal changes as they appear in women¹⁻⁵. Male potency and libido are complex issues, and still to this date a certain failure mechanism in libido loss and interaction of both was not observed. Serum levels of testosterone, both free and binned, and dehydroandrostendione are declining with age, while serum levels of LH, FSH, prolactine, sex hormone binding globulin (SHBG) and estradiol are raising as men get older⁵⁻¹¹. For some of these hormones is shown that a change in serum level is associated with potency or libido problems of aging men¹²⁻¹⁶. Aim of our study was to investigate whether there is statistical difference in serum levels of mentioned hormones in older men with normal potency and libido when compared to those with potency and libido problems.

Subject and Methods

A male population of two villages, mainly agricultural, in Eastern Slavonia: Josipovac and Čepin, older than 45 years was called to do a voluntary screening test as part of »Prostate Cancer Screening Project«.

Every men was asked to fill a semi-constructed questionnaire concerning a general health, previous medical history, family medical history, specific symptoms of lower urinary tract, potency and libido issues. Questions concerning libido and potency issues were set in such way to deal with subjective overlook of each patient. The questionnaire was constructed in a way that was clear and concise, so that it can be filled out after explanation without intervention from the side by our team of researchers. After completion of the questionnaire, a physical examination was performed which included rectal examination by the same physician. Blood samples, prior to examination, were taken for the analysis of PSA, colon tumor markers, FSH, LH, prolactine, T3, TSH, cortisol, androstendione, dehydroandrostendione, free and binned

testosterone, estrogene, progesterone, sex hormone binding globulin and vitamin D. Nine of those hormones were selected for further analysis. All blood samples were taken only once and at the same hour of the day (4–5 pm).

A total of 1000 men were included in this study. For the purpose of this study all men with previous medical history of severe metabolic disorders, previous operations that can alter hormonal balance (egg. orchidectomy), drugs intake with hormonal affect (egg. therapy for prostate cancer) were dropped from the study, and final number of 557 subjects was achieved.

Based on the answers concerning potency and libido problems all patients were divided in to six subgroups:

1. PNLN (Potency Normal Libido Normal) numbering 103 subjects.
2. PMNL (Potency Mild-damaged Libido Normal) with 288 subjects.
3. PALA (Potency Absent Libido Absent) with 138 subjects.
4. PALN (Potency Absent Libido Normal) with 21 subjects.
5. PMLA (Potency Mild-damaged Libido Absent) with 6 subjects
6. PNLA (Potency Normal Libido Absent) with 1 subject.

A PNLN group (normal potency and libido) was a control group, and since number of subjects in groups PALN, PMLA and PNLA were limited, we dropped those groups out from the further statistical analysis. All blood samples were analyzed at the same laboratory and calculated values expressed in standardized units. Since first statistical analysis indicated that distribution of calculated values was not normal or logarithmic we used Kolmogorov-Smirnov test to check out for statistical significance between three major groups.

Results

The average age of patients in the control group was lower when compared to other two groups, while average age of subjects in PALA group is highest in all compared groups (Table 1). In the control group (PNLN) average serum level of free testosterone was highest and levels of FSH, LH, binned testosterone, estrogen and sex-hormone binding globulin were lower then in other groups

(Table 2). Serum levels of FSH, LH, estrogen and binned testosterone are highest in PALA group and level of free testosterone is the lowest. There is statistical significance in serum levels of FSH, LH and progesterone ($p < 0,05$) in groups PNLN and PMLN, also statistical significance was found ($p < 0,05$) in levels of FSH, LH, DHEAS and estrogen in groups PNLN and PALA. Since group PNLN, PMNL and PALA are statistically significantly different, a comparison of PMNL and PALA group was performed, and there was no difference between the groups, $p = 0,078$. But when we compared serum levels of selected hormones in group PMLN with group PALA we found a statistical difference ($p < 0,05$) in serum levels of DHEAS, binned testosterone, estrogen and progesterone (Table 3). Also we analyzed serum levels of free testosterone, FSH and LH through decades in all patients: level of free testosterone is declining with age at approximate 0,4 pmol/year while both levels of FSH and LH are rising in older men (Table 4 and Figure 1).

Discussion and Conclusion

Only few decades ago terms »aging male« or »andropause« were not in use, since average age expectancy of male population was under 70 years. Things have changed: better medical care, advances in medical sciences, growing economy and improved life conditions, all together have prolonged expected life of male population. Now days almost every developed country in the world is facing a growing need for treatment of erectile dysfunction or impotence in elderly men.

Clear mechanism in libido impairment is still not certain. Complex mechanism of hormonal regulation, with

TABLE 1
NUMBER OF SUBJECTS AND AVERAGE AGE IN THREE ANALYZED GROUPS

Group	Patients (No)	Average age	Max	Min	SD
PNLN	103	56.35	73	45	6.20
PMLN	288	61.21	84	50	6.23
PALA	138	68.62	95	50	9.09

PNLN – Potency Normal Libido Normal, PMNL – Potency Mild-damaged Libido Normal, PALA – Potency Absent Libido Absent

TABLE 2
AVERAGE SERUM LEVELS OF SELECTED HORMONES IN THREE MAJOR GROUPS

Group	FSH (IU/L)	LH (IU/L)	Prolactine (mIU/L)	DHEAS (nmol/L)	Free testosterone (pmol/L)	Binned testosterone (pmol/L)	Estrogene (pmol/L)	Progesterone (nmol/L)	SHBG (nmol/L)
PNLN	6.20	4.62	294.53	4.08	46.01	15.30	49.80	1.83	48.37
PMLN	8.18	6.19	278.85	3.31	44.61	15.77	54.50	1.94	54.89
PALA	11.27	8.07	312.47	7.15	41.89	16.75	68.65	1.73	56.49

PNLN – Potency Normal Libido Normal, PMNL – Potency Mild-damaged Libido Normal, PALA – Potency Absent Libido Absent

TABLE 3
STATISTICAL COMPARISON OF AVERAGE HORMONE SERUM LEVELS IN THREE GROUPS

Hormone	PNLN vs.	PNLN vs.	PMLN vs.
	PMLN	PALA	PALA
	<i>p value</i>		
FSH	0.0001*	0.003*	0.075
LH	0.003*	0.021*	0.214
Prolactine	0.740	0.724	0.341
DHEAS	0.135	0.002*	0.0001*
Free testosterone	0.928	0.289	0.446
Binned testosterone	0.221	0.561	0.034
Estrogene	0.798	0.042*	0.038*
Progesterone	0.0001*	0.933	0.0001*
SHBG	0.235	0.334	0.529

*p<0.05 is significant, Kolmogorov Smirnov test, PNLN – Potency Normal Libido Normal, PMLN – Potency Mild-damaged Libido Normal, PALA – Potency Absent Libido Absent

TABLE 4
AVERAGE SERUM LEVELS OF FREE TESTOSTERONE, FSH AND LH IN ALL PATIENTS THROUGH DECADES

Hormone	Decades				
	40-49	50-59	60-69	70-79	80-89
Free testosterone (pmol/l)	54.68	45.52	43.13	39.57	38.42
FSH (IU/l)	7.03	6.92	8.73	15.60	-
LH (IU/l)	6.26	5.51	6.84	7.64	-

extensive numbers of hormones, is now recognized and a fall in regulation or effect of any of this can lead to impotence or libido impairment. Changes in anatomy, neural network or metabolism can all contribute to the same problem.

Testosterone is main hormone involved in regulation of sexual function in men. All available data suggest that levels of free testosterone are declining with age¹⁻⁵. Lower levels of testosterone are found in men with sexual dysfunction, but level of free testosterone is not significant in determining a level of dysfunction¹⁶. It has been observed that even hypogonadal men can achieve some level of erection and presence of libido when exposed to erotic stimuli^{11,12}.

Levels of FSH, LH, SHBG and prolactine are expected to rise with age, if the hypothalamo-gonadal feedback is still active and uncorrupted¹⁻⁵. Estrogen is found, by some reports, in elevated levels in aging men and can lead to obesity, loss of libido and masculine body hair pattern¹¹. At the same time some reports suggest decline in levels of estrogen, but with relative rise when compared to decline of free testosterone⁵.

In our experience average age of male patients with observed potency and libido problems is higher than in

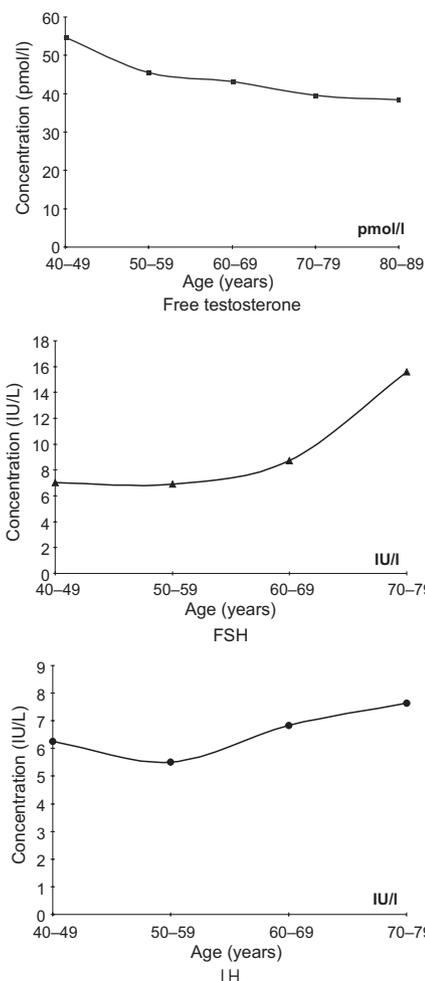


Fig. 1. Levels of free testosterone, FSH and LH in all patients through decades. FT- free testosterone.

normal subjects. Lower serum levels of free testosterone and higher levels of binned testosterone are expected and found in two groups of patients with potency/libido problems^{5,16}, and at the same time those groups are on average older than the control group, which correlates with so far known reports. Although lower values of free testosterone are observed, as mentioned, this observation is not statistically significant when compared to the control group. FSH and LH are lower in control group than in other two groups which is normal, since with age a deficiency in free testosterone occurs⁵, and still active gonado-pituitary regulation is as feedback increasing production of FSH and LH in order to match lower levels of free testosterone, and this difference is statistically significant in both PMLN and PALA group. Levels of estrogen and SHBG are higher in PMLN and PALA groups, but without significance in levels of SHBG. Elevated estrogen levels are found to be significant in PALA group when compared to the control group, and this observation is not as the one reported or expected^{5,11}.

Since all of the above mentioned data can be explained by the fact that patients in PMLN and PALA

groups are on average older than the control group, additional analysis of difference of serum hormone levels was performed between groups PALA and PMNL. Significant higher levels of DHEAS are found in group PALA, which was not expected^{2-5,11}, but can be explained by the higher FSH and LH as a final outcome resulting in the higher levels of DHEAS and estrogen.

In conclusion, a lower level of serum free testosterone is found in groups of patients with libido and potency problems, but this finding is not significant statistically.

However in average lower levels of free testosterone in coordination with other changes (circulatory deficiency, neural damage) could be responsible for overall effect of potency and libido impairment. Statistically higher values of serum FSH and LH are expected and are found in PMNL and PALA group, as contra measure of relative testosterone deficiency. We have found no evidence that serum levels of free testosterone alone are relevant and predictor to potency or libido problems in aging male.

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SERUMSKE RAZINE DEVET HORMONA U STARIJOJ MUŠKOJ POPULACIJI I POVEZANOST SA POREMEĆAJIMA LIBIDA I POTENCIJE

SAŽETAK

Cilj našeg istraživanja je bio utvrditi postoji li povezanost serumskih vrijednosti slobodnog testosterona i odabranih osam hormona sa poremećajima potencije i libida u muškaraca. Odabrana muška populacija starija od 45 godina u dva Slavenska sela je pozvana na dobrovoljni pregled. Svaki pristupnik je ispunio upitnik o prethodnim bolestima, operacijama i stanju potencije i libida. Na temelju odgovora formirano je šest skupina, ali zbog manjeg broja pristupnika, samo 3 skupine su uključene u studiju. Pristupnici sa poremećajima potencije i libida su u prosjeku stariji od ostalih grupa. U grupi pristupnika sa normalnom potencijom i libidom prosječna razina slobodnog testosterona je 46.01 pmol/L, LH 4.62 IU/L i FSH 6.20 IU/L. U grupi pristupnika sa umjerenim poremećajem potencije i normalnim libidom prosječna razina slobodnog testosterona je 44.61 pmol/L, LH 6.19 IU/L i FSH 8.18 IU/L. U grupi bolesnika sa težim poremećajima potencije i libida prosječna razina slobodnog testosterona je 41.89 pmol/L, LH 8.07 IU/L i FSH 11.27 IU/L. Statistički značajno više razine FSH i LH su nađene u tim grupama u odnosu na grupu pristupnika bez poremećaja (FSH $p < 0.0001$ i $p < 0.003$, a za LH $p < 0.003$ i $p < 0.021$). Nema statistički značajne razlike u vrijednostima slobodnog i vezanog testosterona za sve tri grupe. Iako je prosječna serumska razina slobodnog testosterona niža i potvrđena u pristupnika sa poremećajima potencije i libida, ta razlika nije statistički značajna i niže vrijednosti slobodnog testosterona same ne mogu objasniti poremećaje potencije i libida u starijih muškaraca.

The Role of Oral Glucose Intolerance Test in Reducing Pregnancy Complications

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ABSTRACT

The influence of glucose monitoring during pregnancy on newborn body weight, and complications during pregnancy and labor was assessed. We performed a retrospective analysis of macrosomal children, fetal growth, caesarean sections, malformations, still-births and the number of oral glucose tolerance test (OGTT) carried out in a five-year period. The proportion of women participating in OGTT tests increased from 20% to 40% ($p < 0.05$) between 2000 and 2004. Gestation diabetes mellitus (GDM) proportions among pregnant women seen at the Department of Obstetrics and Gynecology at Slavonski Brod General Hospital, Croatia increased from 1% to 6.7% ($p < 0.05$) during the observed period. Proportion of births identified as macrosomal decreased from 13.3% to 12.2% ($p < 0.05$). Additionally, infant mortality and stillbirths along with other fetal and maternal complications declined during the same period. These results suggest that regular measurements of glucose tolerance during pregnancy may prevent preterm birth, decrease the proportion of macrosomal newborns, lower mortality and decrease fetal and maternal complication incidence during pregnancy and delivery.

Key words: gestation, diabetes mellitus, oral glucose tolerance test, pregnancy, complications

Introduction

New data suggest that gestation impaired glucose tolerance (GIGT) has direct impact on beta cell dysfunction and insulin resistance^{1,2}. Excessive transfer of glucose from mother induces fetal hypoglycemia, leading to fetal pancreatic islet hypertrophy and beta cell hyperplasia with consequent rise in insulin secretion³.

Insulin secretion and resistance are some of the major contributing factors in appearance of macrosomal infants (defined as a birth weight at or above 4 kg, or when sex-specific birth weight for gestational age is above the 90th percentile of fetal growth curves), which increases the chance of birth trauma, preterm birth, stillbirths, shoulder dystocia, bone fracture or nerve palsy⁴. It has direct influence on the number of caesarean section, neonatal jaundice, respiratory complication and perinatal mortality⁵. Early detection of glucose intolerance can prevent all complications in maternal and fetal outcomes⁶.

Materials and Methods

At the Department of Obstetrics and Gynecology, Slavonski Brod General Hospital, Croatia, we performed a retrospective analysis of the number of OGTT performed in pregnant women and fetal characteristics during the years 2000 and 2004. Data on body mass index (BMI) of pregnant women, their age and familiar anamnesis of diabetes mellitus (DM) or GDM were collected. Fetal characteristics assessed in the study were the numbers of macrosomal children and their fetal growth, preterm birth, congenital malformations, stillbirths, and caesarean sections. According to the American Diabetes Association (ADA) criteria (fasting: 5.3 mmol/l, 1 h: 10 mmol/l, 2 h: 8.6 mmol/l, 3 h: 7.8 mmol/l) GDM was defined when more than two plasma glucose measurement were equal to or higher than the cut-off points.

Data were tested for conformity to the normal distribution using the Kolmogorov-Smirnov test. Statistical

significance was assessed using χ^2 test, and set to $p < 0.05$. All analyses were performed with the Statistical Package for Social Scientist 11.5.

Results

An increase of 20% in the number of performed OGGT was noted during the studied period. In addition, there was also an increase in the number of pathological OGTT in pregnant women, with no differences in BMI and family history (Table 1).

TABLE 1
WOMEN'S PARAMETERS OVER OBSERVED PERIOD

Parameter	Start year	End year
Age (X±SD, years)	33.7±10	35.2±8
BMI (X±SD, kg/m ²)	25.2±1.2	25.1±1.6
Weight increase (X±SD, kg)	7±3.5	6.5±4
Performed OGGT	20%	40%
Pathological OGTT	1%	6.7%
Familiar anamnesis of DM	21%	22%

OGTT – oral glucose tolerance test, BMI – body mass index

During the same period positive changes in all observed fetal characteristics were noted. Numbers of macrosomic infants and infants with faster growth decreased significantly (χ^2 , $p < 0.05$, Table 2) over the observed period. There were also less preterm births in the last observed year, although this difference was not significant (Table 2). Number of observed fetal malformations decreased over the observed period, but not significantly. Lower fetal mortality was also noted in the last studied year, but that difference was not statistically significant (Table 2).

Discussion

The small number of performed OGTT during the first year of observation (year 2000) had a strong influ-

TABLE 2
NEWBORN PARAMETERS OVER OBSERVED PERIOD

Parameter	Number (%)	
	Start year N=1615	End year N=1165
Macrosomic	215 (13.3%)	131 (11.2%)*
Faster grow up	209 (13%)	131 (9%)*
Preterm birth	315 (19.5)	215 (18.5%)
Malformations	11 (0.79%)	9 (0.73%)
Mortality	14 (0.95%)	9 (0.73%)

* Significantly lower than in the start year (χ^2 , $p < 0.05$)

ence on preterm birth rate and accelerated fetal grow up, but during the year 2004 the number of performed OGTT increased almost twofold.

In the first studied year there were only 1% of women with GDM, with a constant increase in numbers of women with GDM during the observational period. That is explained with more frequent controls of blood glucose during pregnancy, and increasing incidence of type 2 diabetes mellitus. Consequently, positive changes in all observed fetal characteristics were noted, with significant decrease in numbers of macrosomal infants, and infants with faster growth. The continued observation during pregnancy can improve fetal and maternal state and diminishing fatal and unwanted outcome during and after delivery^{7,8}.

In comparison to other countries, the prevalence of GDM in Croatia is between 2% and 6%. According to ADA criteria, the prevalence of GDM in Spain is 11, 6% (Toronto TRI-Hospital Gestational Diabetes Project). A preliminary review showed a prevalence of GDM to be around 4%.

Prevalence of GDM in Croatia is similar to other countries, but the main problem is the raising incidence and prevalence of diabetes mellitus type 2, since it raises the possibility of GDM. For that reason we conclude that laboratory screening for glucose intolerance should be a standard element of the prenatal evaluation⁹.

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RANO OTKRIVANJE INTOLERANCIJE GLUKOZE I KOMPLIKACIJE U TRUDNOĆI

S A Ž E T A K

Istražen je utjecaj praćenja tolerancije glukoze tijekom trudnoće na fetalni rast, te na komplikacije u trudnoći i porodu. Provedena je retrospektivna analiza broja makrosomne novorođenčadi, carskih rezova, malformacija, mrtvorodne djece i fetalnog rasta, u odnosu na broj učinjenih testova oralnog opterećenja glukozom (OGTT) tijekom petogodišnjeg razdoblja. Postotak trudnica koje su bile podvrgnute OGTT značajno je porastao s 20% na 40% u razdoblju od 2000. do 2004. godine (χ^2 , $p < 0.05$). Udio trudnica s gestacijskim dijabetes melitusom (GDM) na Odjelu za porodništvo i ginekologiju Opće bolnice »dr. Josip Benčević« Slavonski Brod, Hrvatska u istom promatranom razdoblju porastao je značajno s 1% na 6,7% (χ^2 , $p < 0.05$). Istovremeno, udio novorođenčadi identificiranih kao makrosomna, značajno je smanjen s 13,3% na 12,2% (χ^2 , $p < 0.05$). Također je smanjena stopa mortaliteta novorođenčadi, zajedno sa smanjenjem udjela mrtvorodne djece, te drugih fetalnih i maternalnih komplikacija. Rezultati istraživanja pokazuju kako redovito praćenje tolerancije glukoze tijekom trudnoće može prevenirati prijevremeni porod, smanjiti udio makrosomne djece, te smanjiti incidenciju fetalnih i maternalnih komplikacija tijekom trudnoće i poroda.

Pregnancy Outcome after Treatment with Radioiodine for Differentiated Thyroid Carcinoma

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ABSTRACT

The aim of the study was to investigate the influence of radioiodine (RAI) therapy on pregnancies and the health status of children born to mothers who had received therapeutic doses of I-131 for differentiated thyroid carcinoma (DTC). Gestational histories of 76 women treated for DTC from 1971–2005 were retrospectively analyzed. The outcome of 49 pregnancies after RAI was: 35 children (72%), 5 (10%) miscarriages and 9 (18%) induced abortions. RAI did not adversely affect the rate of successful delivery and live birth demographics. Congenital malformation and first year mortality were not observed. The children's ages range from 1 month to 29 years ($X \pm SD = 8.0 \pm 8.4$). A higher therapeutic dose (>100 mCi) did not significantly alter the pregnancy outcome. There is no reason to discourage females treated with I-131 from becoming pregnant. Patients should avoid pregnancy after RAI administration for 1 year.

Key words: differentiated thyroid carcinoma, radioiodine, dose, pregnancy

Introduction

Radioactive iodine I-131 (RAI) has been used for decades in the diagnosis and treatment of well differentiated thyroid carcinoma (DTC). It is an effective treatment of DTC, both in preventing relapses and treating metastases^{1–7}. Following total thyroidectomy, patients are administered a diagnostic dose of 37–185 MBq I-131 and subsequently a therapeutic dose, varying from 1.85 to 3.70 GBq I-131 for the ablation of the thyroid remnant or even more for the therapy of the metastatic disease, if necessary.

The thyroid gland is an uncommon site of cancer, accounting for 0.6% and 1.6% of cancers among men and women, respectively^{8,9}. However, if the age distribution is analyzed, a considerable number of patients in their younger age are found. The peak age for developing papillary carcinoma is about 30 years of age, for follicular carcinoma 45 years of age and both types are about three times more frequent in women. Of all 1231 patients treated for well differentiated thyroid carcinoma from 1971–2005 at the Department of Oncology and Nuclear Medicine, University Hospital Sestre Milosrdnice in Zagreb, 956 (78%) were woman, of which 180 (19%) were younger than 35 years.

A large number of young female patients may be considered cured after thyroidectomy and radioiodine therapy and their desire to have a child is therefore normal. However, it is well known that radiation exposure induces genetic mutation, which can result in genetic abnormalities in the newborns. Therefore, beside positive effects of such therapy, great interest has been shown in the research of possible mutagenic effect on germ cells, which could result in adverse outcome of pregnancy (spontaneous abortions, congenital abnormalities, malignancies in offspring). During pregnancy, well-defined changes in thyroid hormone physiology reflect an increased demand for thyroid hormone production (in one-third of patients on L-thyroxin therapy a dosage increase is required), which can also affect the pregnancy outcome^{10–12}. It is difficult to assess the effect of RAI therapy due to a small number of patients (thyroid carcinoma is not a frequent disease) and their restrictive age. Also, the patients appear to be less willing to have children, particularly if they were already parents, because of their primary disease. Several studies addressing this problem did not find statistically significant associations between

previous RAI exposure and unfavorable pregnancy outcome except for miscarriages^{13–21}. In our previously published data, a slight increase of miscarriages was also observed¹⁹. The largest reported series by Schlumberger et al.²¹ revealed that the miscarriage rate increased from 11% to 20% after surgery for DTC, irrespective of the use of RAI. More miscarriages were observed among the women treated with RAI in the year immediately preceding conception (40%), but there was no other unfavorable pregnancy outcome. However, it is hard to estimate real radiation risk as clinical data are still insufficient to assess the low level of RAI risk, compared to other influences which have an impact on pregnancy outcome²².

The aim of our study was to further evaluate the influence of radioiodine therapy on pregnancies and the health status of children born to mothers who had received therapeutic doses of I-131 for DTC, at the Department of Oncology and Nuclear Medicine, University Hospital Sestre Milosrdnice.

Patients and Methods

A group of 76 female patients, who were less than 35 years at the time when they were treated for DTC, were evaluated. They were all referred to the Department of Oncology and Nuclear Medicine, University Hospital Sestre Milosrdnice in the period from 1971 to 2005 and received regular follow-up. During routine check-ups between January 2003 and April 2005 pregnancy history, outcome and physical and intellectual condition of their children were assessed.

According to histological type of cancer, 71 patients (93%) had papillary and 5 patients (7%) follicular carcinoma. All patients were treated according to the standard protocol. After total thyroidectomy an ablation dose of 1.85–3.70 giga Becquerel (GBq) I-131 (50–100 milli Curie [mCi]) was administered for the ablation of thyroid remnants. Radioiodine administration was repeated in 3, 6 or 12-month intervals until significant uptake in thyroid bed had completely disappeared. One patient had lung and bone metastases at presentation and one developed lung metastases during follow-up period. They were treated with additional RAI doses and RAI treatment resulted in complete remission in these two patients. The average dose for metastatic disease at our department was 5.55 GBq (150 mCi). The numbers of I-131 administrations were the following: 41 patients received a single dose, 28 patients received two, 5 patients received three, one patient received four and one six doses. Total doses varied from 1.85 to 28.86 GBq I-131 (50–780 mCi), $X \pm SD = 5.72 \pm 3.87$ GBq, median 3.70.

The age of patients at first radioiodine administration for therapeutical purposes ranged between 12 and 35 years ($X \pm SD = 25.9 \pm 5.6$ years, median 26 years). All patients were recommended to avoid pregnancy after each I-131 administration for 12 months.

The patients were followed up according to the following protocol: whole body scintigraphy was performed 72 hours after the administration of 37–185 mega Becquerel

(MBq) I-131 (1–5 mCi). Thyroxin substitution therapy was discontinued four weeks prior to whole body scintigraphy and TSH level confirmed to be >30 IU. Thyroglobulin level was measured and ultrasound of the neck performed, along with routine clinical examination and chest X-ray if indicated.

After the therapy, all patients were given thyroxin at doses capable of suppressing thyroid stimulating hormone (TSH). Serum TSH level was measured regularly, while suppression doses were adjusted individually according to the obtained results. This was done in order to obtain optimal suppression effect with the smallest amount of thyroxin and thus avoid possible iatrogenic hyperthyroidism. Later during the follow-up period, in the patients who were free of disease thyroxin dose was decreased until normal TSH values were obtained. In the patients who were planning pregnancy, the thyroxin dose was also decreased and serum TSH levels titrated to reach the normal range. During the first trimester of pregnancy, thyroxin dose was increased and TSH levels carefully monitored for dosage adjustment since there is increased demand for thyroid hormone production during that period. Separation of thyroxin ingestion by at least four hours from iron and calcium supplements was recommended.

Clinical data on pregnancies were obtained during a routine check-up of patients, including pregnancy details and outcome, live birth demographic data and the physical and intellectual condition of these children (the latest was assessed subjectively by their mothers by comparison with other siblings and peer group).

Statistical analysis

Numerical data were presented as mean, standard deviation, or median with range. Qualitative data were described by frequencies and percentages. Mann-Whitney test was used to compare numerical data of two independent groups, while qualitative data were compared by two-sided Fisher's exact test. For statistical analysis, the SAS System for Windows, Release 8.02, TS Level 02M0 (SAS Institute Inc., Cary, NC, USA) was used.

Results

The mean follow-up of these 76 patients was 9.4 years, $SD = 7.0$, median 7.7, range 1–32 years.

Seventy-six women evaluated gave birth to a total of 91 children, of which 56 were born before any treatment for DTC.

After surgery for thyroid carcinoma and administration of therapeutical activities of RAI, 45 women (58%) had no wish to become pregnant and of the remaining 31 women who declared that they were not avoiding pregnancies, 24 women (32%) gave birth, 2 (3%) had 1 miscarriage each and 5 (7%) did not conceive (Figure 1).

The outcome of 49 total pregnancies that occurred after radioiodine therapy was: 35 children (72%) were born, 5 (10%) miscarriages were observed and 9 (18%)

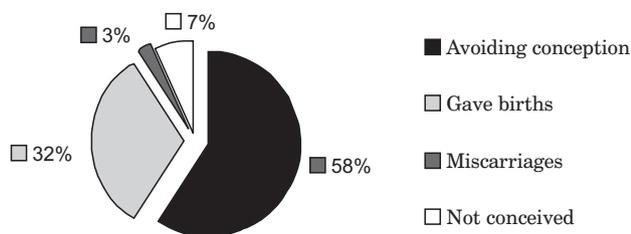


Fig. 1. Distribution of patients according to the intention to have children.

pregnancies ended by induced abortion. Three of these nine induced abortions were performed because they occurred during the period of 6 months after the RAI therapy, four pregnancies were terminated to prevent a feared negative outcome and reasons for other two abortions were not specified. After exclusion of pregnancies terminated by induced abortion, 5 miscarriages (12.5%) were observed in the 40 remaining pregnancies. Twenty-four women gave birth to a total of 35 children (22 males and 13 females). Nulliparous women easily decided to become, which is evident from data that 16 of these women gave birth to total of 27 children. Eight women who previously had children gave birth to one more each.

Median activity of I-131 prior to pregnancy was 3.7 GBq (100 mCi), range 2.96–28.86 GBq (80–780 mCi). The largest single dose administered was 5.55 GBq (150 mCi). Mean number of applications was 1.6 (SD=1.2, range 1–6). Patient's ages at the time of pregnancy ranged 18 to 40 years, $X \pm SD = 28.9 \pm 5.1$, median 30.0 years. The interval between the last administration of I-131 and conception ranged 0.4 to 19 years, $X \pm SD = 6.0 \pm 5.1$, median 4.0 years.

We also studied whether a higher total RAI dose had any association with adverse pregnancy outcome. Pregnancies occurred after the RAI therapies were classified according to I-131 dose before each pregnancy into two groups: Group A ≤ 100 mCi and Group B > 100 mCi (Figure 2).

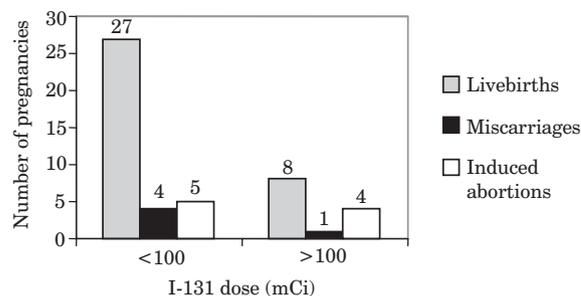


Fig. 2. Outcome of pregnancies as a function of total I-131 administration.

The clinical characteristics of patients, pregnancy outcomes and children in each group are presented in Table 1 and Table 2.

There was no difference between the groups for the age when DTC was diagnosed ($p=0.643$), follow-up period ($p=0.227$), the maternal age at pregnancy ($p=0.953$) or the interval between RAI and pregnancy ($p=0.267$).

Stillbirths, congenital abnormalities or first year neonatal mortality were not recorded. We did not find any difference in the adverse effects on outcome between low and high total I-131 dose groups. The proportion of live births was similar and after excluding pregnancies ended by elective abortion, the incidence of miscarriage was not different between the RAI groups (13% vs. 11%, $p=0.999$). Also, no difference was noted in the gender distribution ($p=0.680$), or children's birth weight ($p=0.723$) between the groups with different total I-131 dose administration. To the present, the children's ages' range from 1 month to 29 years, $X \pm SD = 7.8 \pm 8.1$, median 6.0 years. All children had normal growth and have not been afflicted with severe disease.

Discussion

The diagnostic and therapeutic use of I-131 for the evaluation and management of thyroid remnants and regional and distant metastases of differentiated thyroid

TABLE 1
CLINICAL CHARACTERISTICS OF PATIENTS ACCORDING TO PRECONCEPTION I-131 ADMINISTRATION

Characteristics	Group A (≤ 100 mCi)		Group B (> 100 mCi)		p
	Median	Range	Median	Range	
I-131 Dose (mCi)	91	30	200	672	$<0.001^*$
Fractions (n)	1	1	2	5	$<0.001^*$
Maternal age at DTC* diagnosis (y)	23	16	26	20	0.643
Interval before pregnancy (y)	4	17	6.5	16	0.267
Maternal age at pregnancy (y)	29	22	30	10	0.953
Patient's follow-up (y)	11.6	30.5	9	18	0.227
Children's birth weight (kg)	3.4	1.6	3.3	0.7	0.723
Age of child at last follow-up (y)	8	28.9	1.5	4.8	0.006*

* $p < 0.05$, Group A ≤ 100 mCi I-131, Group B > 100 mCi I-131, *DTC-Differentiated Thyroid Carcinoma

TABLE 2
CLINICAL CHARACTERISTICS OF PATIENTS ACCORDING
TO PRECONCEPTION I-131 ADMINISTRATION

Characteristics	Group A (≤100 mCi)		Group B (>100 mCi)		p
	N	%	N	%	
Pregnancies after I-131	36	100	13	100	
Pregnancy outcome					0.999
Live births	27	75	8	61	
Miscarriages	4	11	1	8	
Induced abortions	5	14	4	31	
(Therapeutic)	0		3		
(Social/psychological)	3		1		
(Unspecified)	2		0		
Live birth – gender					0.680
Female	11	41	2	25	
Male	16	59	6	75	

Group A≤100 mCi I-131, Group B>100 mCi I-131

carcinoma (DTC) have been routine for decades. Long-term studies^{1,5,23} reported that I-131 ablation of residual thyroid tissue after surgery decreased the risk of recurrence and cancer-specific mortality rates were significantly lower than in those who did not undergo remnant ablation. Since all diagnostic and therapeutic modalities should be assessed carefully for the relative benefits and hazards, the purpose of this study was to evaluate, in a clinical setting, the impact of I-131 therapy on pregnancy outcomes and the health status of their children born.

Knowledge that radiation is mutagenic and may affect gonads (thereby resulting in genetic damage to offspring) has raised concern regarding the use of radioiodine in patients during their reproductive years. Virtually every patient treated with any dose of I-131 is exposed to some potential risk. The potential hazards that have the greatest impacts on the decision to utilize this modality are the induction of second tumors^{18,24,25} and genetic and chromosomal damage^{16–21,26–30}. Also, a large individual variation in the reaction to RAI exposure was observed due to differences in each individual's features and environment.

Genetic risk of ionizing radiation in humans has been estimated in the offspring of survivors of the atomic bomb explosion^{31,32}, in populations living in areas of high background radiation levels³³ and in the descendants of persons exposed to radiation either on the job³⁴, or through diagnostic and therapeutic procedures³⁵. Studies on survivors of the atomic bomb explosion in Japan^{31,32} and of childhood and adolescent cancer survivors who had received radiation to the abdomen or pelvis³⁶ have failed to provide any clear evidence of increased germ cell mutation subsequent to exposure, but some studies have suggested an increased risk of congenital abnormalities³³ and leukemia³⁷ in children born to occupationally exposed men.

It has been estimated that the radiation dose delivered to the ovary is approximately 0.14 cGy after administration of 37 MBq (1 mCi) of radioiodine³⁸, which correlates well with in vivo measured doses³⁹. Since the rate of congenital anomalies due to RAI exposure is low compared to the other influences that have an impact on pregnancies outcome, it is hard to estimate the risk. The rate of spontaneous birth anomalies is 800 per 100.000 pregnancies and if all 100.000 women received 370 MBq (10 mCi) I-131 before they became pregnant, the rate of congenital anomalies would increase to 803 (only 3 new congenital anomalies on 100.000 women exposed with 10 mCi I-131)²². In addition, there are genetic diseases that are either easily recognized but uncommon, or more frequent but difficult to detect.

So far, studies about the impact of I-131 therapy on pregnancies included small number of patients and failed to reveal any significant I-131 related effect^{13–21,28}. Even in the largest study reported²¹, with data on 2113 pregnancies evaluated, 272 pregnancies presented referred to the period after the surgery for thyroid cancer and only 206 to the period after the RAI administration.

Our data show that all children born to mothers who had received therapeutic activities of I-131 for DTC had birth weight similar to the birth weight of healthy newborns from the Zagreb County, Croatia⁴⁰ and were in good health. The only untoward outcome of pregnancy was 5 (12.5%) miscarriages. One miscarriage occurred when the patient was off the substitution therapy with thyroxin and in two others, miscarriages occurred within 6 month following thyroidectomy and radioiodine therapy (subsequently these women had healthy children). Therefore, the contribution of other factors, as an inadequate control of the thyroid hormonal status, cannot be excluded (both hyperthyroidism and hypothyroidism have significant effects on estrogen metabolism, fertility and pregnancy outcome). Many factors capable of interfering with the hypothalamic-pituitary-thyroid axis may induce changes in TSH level and affect the pregnancy, so thyroid hormonal status should be carefully followed-up.

In the general population, the incidence of miscarriages in clinical recognized pregnancies is about 10%⁴¹, but in prospective studies, when healthy women attempting to conceive were under medical supervision, the incidence of miscarriages was significantly higher. In the study of 221 healthy women attempting to conceive, early pregnancy loss (1 to 91 days after the implantation) was 31%⁴². In similar studies the incidence ranges from 18%⁴³ up to even 63%⁴⁴. In the general population a great deal of early pregnancy loss remains clinically unrecognized. Patients with a diagnosis of DTC are under more strict medical control because of their primary disease and their pregnancies are usually planned and more carefully supervised.

In our group of patients, worst pregnancy outcome was not associated with higher total RAI therapy dose. Although our data do not establish that no risk exists, they indicate low level of RAI ablation risk and emphasize the importance of individual differences due to non-

uniform distribution of internally deposited radionuclides and different level of gene activity and chromosome repair in each patient. However, in the recently published Erselcan study²⁹ the connection between the dose and the chromosomal damage has been established. The authors evaluated acute and late chromosomal damage in the peripheral lymphocytes of 15 patients who received various doses of I-131. Acute and late effects were defined using the »damage ratio« (acute effect) and the »recovery ratio« (late effect), based on the basal, acute (3rd day) and late (6 months) data in patients treated for thyrotoxicosis or DTC. The »damage ratio« was not related to the dose administered, but a negative correlation was found between the I-131 dose and the »recovery ratio«. Results also suggested that part of the damaged lymphocytes disappear from the circulation in a dose dependent manner following I-131 treatment. These results, together with other studies indicate dose-effect relationship at the chromosomal level^{45,46}.

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TRUDNOĆE U ŽENA LIJEČENIH RADIOAKTIVNIM JODOM RADI DIFERENCIRANOG KARCINOMA ŠTITNJAJE

S A Ž E T A K

Cilj studije bio je utvrditi utjecaj radioaktivnog joda (RAI) na trudnoće, kao i na zdravlje djece bolesnica koje su primale terapijske doze I-131 radi diferenciranog karcinoma štitnjače (DTC). Trudnoće u 76 bolesnica liječenih radi DTC-a u periodu od 1971.–2005. g. su retrospektivno analizirane. Ishod 49 trudnoća koje su uslijedile nakon terapije RAI bio je: 35 djece (72%), 5 spontanih pobačaja (10%) i 9 arteficialnih pobačaja (18%). Terapija radioaktivnim jodom nije negativno utjecala na stopu uspješnih poroda, niti na demografske osobine novorođenčadi. Kongenitalne malformacije i mortalitet u prvoj godini života nisu registrirani. Srednja dob djece bila je 8 godina (raspon 1 mjesec do 29 godina, SD=8.4). U bolesnica koje su primile veće terapijske doze radioaktivnog joda (>100 mCi) nije registriran lošiji ishod. Bolesnicama liječenim radioaktivnim jodom radi diferenciranog karcinoma štitnjače nije potrebno preporučivati izbjegavanje trudnoće. Preporuča se izbjegavati trudnoću u godini neposredno nakon primanja terapije I-131.

A Study on Blood Pressures between the Tibet Born and India Born Tibetans Who are Permanently Residing in Northern India

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ABSTRACT

Both systolic and diastolic blood pressures are generally believed to vary in relation to various factors like sex, age, body build, altitude, socio-economic condition, chronic diseases, etc. but the evidences are not always unequivocal. This paper aims to study the effect of intergenerational change, age, sex, and body mass index on blood pressures and to see the effects of migration on blood pressures among the two generations of the Tibetan immigrants in Northern India. Findings from research on 1st and 2nd generation adult Tibetans indicate that there are no significant differences in blood pressures between two generations in both the sexes which may have contributed to their food habits.

Key words: blood pressures, food habits, Tibetans, India

Introduction

Blood pressures are generally believed to vary in relation to various factors like sex, age, anthropometric measures of body build, altitude, socio-economic condition, ethnic groups, etc.^{1,2-3,4-5} but the evidences are not unequivocal^{6,7}. Most studies on blood pressure reported an increase of both systolic and diastolic blood pressures with age have been done on many populations. It has also been suggested that age related increase of blood pressure is not a necessary part of individual's aging process⁸, socio-cultural differentials among populations have been responsible to a great extent for varying levels of blood pressures among them. Here modernization provides additional evidence that rapid sociocultural change is associated with increased prevalence of hypertension⁹.

Several studies have found that elevated blood pressure, which is associated with increased morbidity and mortality, is known to be related to several aspects of the modern lifestyle, including the western diet, lack of exercise, and increased psychological stress^{10,11}. It is evident that cultural change has an influence on blood pressures in social groups migrating to new settings¹².

In the present study, we have considered the 1st (Tibet born Tibetans who migrated to India) and 2nd (India born Tibetans) generation Tibetan immigrants of Northern

India to see the effect of intergenerational change (based on place of birth), age, sex, and BMI on their blood pressures and to see the changes associated with the migration to alien environment i.e., psychological trauma due to displacement, changes in socio-cultural milieu as well as living in more urbanized setting compared to their original homeland or in other word changes in life style and their impact on blood pressures.

Materials and Methods

Area and population

The present study has been conducted among the Tibetans of northern India. Two groups of Tibetan populations – one Tibet born Tibetans (TBT) and the other India born Tibetans (IBT) – have been selected as 1st and 2nd generation Tibetan immigrants from two different places of Northern India; one from Deradun district of Uttar Pradesh (now under Uttaranchal State of India) situated at 640 meters above sea level and other is from Dharamsala of Kangra district of Himachal Pradesh situated at a height of about 1,500 meters from the mean sea level (the town extends up to about 1,900 meters). The Ti-

betans of these two settlements are descended from the same Tibetan stock and they have lived in India more than 40 years (migrated in India around 1960's). Most of the parameters (for example, mean age at menarche, mean age at marriage, average live birth/woman, educational status) between the settlements are not significantly different. Therefore, the Tibetan populations of these two settlements were combined and presented as a representative homogeneous sample of the Tibetans of North India.

The Tibet born Tibetans are high altitude native of Tibet (average elevation of 4,500 meter above sea level from where they came to India), a section of which migrated to lower altitudes in India in the 1960s. The India born Tibetans (IBT) are originally Tibetans whose ancestors lived in Tibet but they are born and brought up at lower altitudes in India and at present share similar geographical locations with the TBT who migrated to India. It would be difficult to measure the effect of migration on blood pressures among them, since there is no data available on when during their life the TBT migrated to India. Therefore, in the present study a comparison between TBT and IBT has been made as a proxy between 1st and 2nd generation Tibetan immigrants, where an attempt has been made to see the intergenerational effects of migration on blood pressure.

The adult male and female Tibetans were selected from both the subgroups for the present study. Of the 317 individuals examined, 256 were included in the present analysis (since there were no individuals found above the age of 45 years among the India born Tibetans (IBT), the analysis was done up to 45 years age among both the groups). These 256 subjects range in age from 20–45 years and were fairly evenly distributed between the sexes and their places of birth (40, 73 TBT and 46, 87 IBT; males and females, respectively). Difficulties were encountered in the assessment of age as only a few households kept written records of dates of birth. They follow Tibetan animal element calendar instead of Gregorian calendar which was converted to nearest date of Gregorian calendar. The age was estimated by reference of some important local events. These were further cross-checked from a number of elderly individuals on subsequent visits.

Anthropometric and blood pressure measurements

The data on systolic and diastolic blood pressures, and two anthropometric measurements (height and weight) were utilised in the present report. Anthropometric measurements were taken by author using standard anthropometric techniques¹³. Height and weight were measured to the nearest 0.1 cm and 0.5 kg using Martin's anthropometer and weighing scale, respectively. Both for height and weight, subjects were requested to remove their shoes prior to taking measurements. Body mass index (BMI) was computed following the standard formula¹⁴;

$$\text{BMI (kg/m}^2\text{)} = \text{Weight (kg)} / \text{Height}^2 \text{(m}^2\text{)}.$$

Blood pressures of the subjects were taken after about a 15 minutes' rest period using an inflatable blood pressure cuff, sphygmomanometer and a stethoscope by employing auscultatory method. The subject was asked to sit on a stool or a flat raised platform, resting left arm on a table or on a flat place at heart level.

Statistical analyses

Technical errors of measurements (TEM) were computed following the standard method recommended by Ulijaszek and Kerr¹⁵. Since TEM's were found to be within acceptable limits, they were not incorporated in statistical analyses.

In the present analysis, two generations of the Tibetans (TBT and IBT) have been pooled as one large sample to measure the effect of intergenerational change (change in place of birth), age, sex, and BMI on blood pressures. For this, a multiple regression procedure was employed to measure blood pressure change with place of birth, age, sex, and BMI as the independent variables in the regression model. In a further analysis, two generations have been considered as two separate samples. A multivariate multiple regression approach was used in which separate regression model was developed for each generation and the multivariate relationships of blood pressures with age, sex, and BMI were compared between them. Statistical significance in these models was set at $p < 0.05$. Data were entered and analyzed using SPSS/PC with Windows®.

Results

The mean and standard deviations of age, height, weight, body mass index (BMI) and blood pressures for both the generations of the Tibetans have been presented in Table 1. In males, both the systolic (SBP) and diastolic blood pressures (DBP) and BMI tend to be higher among the IBT than the TBT. In contrast, in females, SBP and DBP and BMI are higher among the TBT than the IBT but the differences are not significant in both the cases. Thus, blood pressures have been found to be higher in both the generations with increasing BMI (Table 1).

The results of multiple regression analysis (Table 2) show that age, sex, and BMI are associated with both the blood pressures (SBP and DBP) among all Tibetans, while intergenerational change (based on their place of birth) shows a significant association only with diastolic blood pressure among them.

Regression results by generation for blood pressures indicate that BMI is significantly associated with both blood pressures in both the generations (Table 3). Results further demonstrate that age is associated with blood pressures (SBP and DBP) only in TBT, while sex has a significant relation with blood pressures only in IBT.

Discussion

Despite difference in place of birth, blood pressures between the two generations of the Tibetan population in India show some similarities. No significant differences have been found in blood pressures between two samples in both the sexes though blood pressures tend to be higher among females in the 1st generation than the 2nd generation. Additionally, in both the samples, BMI is significantly correlated with blood pressures.

In the light of the above findings it may be conjectured that there exist some effects of migration on blood pressures, including differences in food habits¹⁶, and stress associated with displacement¹⁷. The effect of BMI on blood pressures^{18,19} and the effects of differential levels of physical activity pattern on blood pressures^{20,21} may also have some impact.

The nature of dietary and stress differences, and their impact on blood pressure were not investigated as part of this study. However, it can be noted that diet of the TBT in Northern India consisted of high protein and fat. The salt intake among the TBT is comparatively low than the native Tibetans in high altitude, where the Tibetans were reported to consume a large amount of salt, chiefly in the form of salt-flavoured tea²². The dietary characteristic of the Tibetans was found to be the cause of high prevalence of hypertension among the high altitude Tibetans in Tibet²². In India, salt intake is comparatively

TABLE 1
MEAN AND STANDARD DEVIATIONS OF AGE, BMI AND BLOOD PRESSURES: TWO GROUPS (GENERATIONS ARE USED) OF THE TIBETANS

	Adult Males	
	TBT (Migrated from Tibet to India)	IBT (Offspring of the TBT)
	N=40 X±SD	N=46 X±SD
Age	35.33±5.71	32.80±5.70
Height	167.83±6.65	165.88±6.88
Weight	65.74±11.34	65.19±11.39
BMI	23.25±3.19	23.65±3.56
Systolic	110.13±14.06	114.35±10.82
Diastolic	69.43±12.66	74.11±9.90

	Adult Females	
	N=73	N=87
	Age	34.12±6.07
Height	155.70±5.54	153.63±4.54
Weight	59.83±10.47	55.37±8.86
BMI	24.64±3.82	23.56±3.64
Systolic	106.78±14.32	103.30±10.35
Diastolic	67.86±11.75	66.36±10.23

BMI – body mass index

TABLE 2
REGRESSION ANALYSES FOR BLOOD PRESSURES OF ALL TIBETANS

Predictor Variables	Systolic (SBP)				Diastolic (DBP)			
	B	SeB	Beta	t	B	SeB	Beta	t
Generations of a Population	1.279	1.514	0.049	0.844	2.766	1.339	0.122	2.065*
Age	0.423	0.129	0.197	3.277**	-4.624	1.368	0.246	4.052***
Sex	-7.390	1.547	-0.273	-4.778***	0.463	0.114	-0.195	-3.380**
BMI	1.167	0.205	0.327	5.703***	1.009	0.181	0.323	5.571***

B – sample regression coefficient, SeB – standard error of B, Beta – estimated population regression coefficient, BMI – body mass index
Sex coded as 1 for male and 2 for female, generations coded as 3 for TBT and 4 for IBT
Significant *** p<0.0005, ** p<0.002, *p<0.05

TABLE 3
REGRESSION COMPARISONS OF SYSTOLIC AND DIASTOLIC BLOOD PRESSURES BY GENERATION

Predictor Variables	Systolic (SBP)							
	TBT (N=113)				IBT (N=133)			
	B	SeB	Beta	t	B	SeB	Beta	t
Age	0.612	0.200	0.255	3.062**	0.223	0.161	0.108	1.386
Sex	-4.803	2.491	-0.162	-1.928	-10.434	1.880	-0.425	-5.551***
BMI	1.580	0.329	0.405	4.797***	0.763	0.248	0.234	3.074**

Predictor Variables	Diastolic (DBP)							
	B	SeB	Beta	t	B	SeB	Beta	t
	Age	0.608	0.165	0.301	3.686***	0.309	0.155	0.163
Sex	-2.736	2.058	-0.109	-1.330	-6.937	1.805	-0.308	-3.844***
BMI	1.373	0.272	0.417	5.046***	0.659	0.238	0.221	2.765**

Sex coded as 1 for male and 2 for female, BMI – body mass index
Significant *** p<0.0005; ** p<0.002; *p<0.05

low among the TBT, since they also like to take sugar-flavoured tea with salt-flavoured tea like the IBT. Thus, a low intake of sodium among the TBT in India may play a role in relatively low blood pressures among them. That there is no significant difference in blood pressures found between the TBT and IBT, may be contributed to the similar food habits among them.

Our results however show a tendency of higher blood pressures among the TBT than the IBT only in females. An increased prevalence of over weight among the women in the TBT may be the leading cause of higher blood pressures in the TBT females. A significant influence of BMI on blood pressures is the probable explanation behind it. Increased levels of physical inactivity^{20,21} as well as higher intake of calorie that are too high in relation to physical activity levels are possible causes for the higher prevalence of overweight²³. Higher intake of protein and

fat rich food with sedentary nature of work leads to obesity which may in turn leads to higher blood pressures among the TBT in India.

Additionally psychological stress due to displacement may have some effect on differentials in blood pressures. Further studies are needed to unravel the above causes in differentials in blood pressures.

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ISTRAŽIVANJA KROVOG TLAKA TIBETANACA ROĐENIH NA TIBETU I U INDIJI SA PREBIVALIŠTEM U SJEVERNOJ INDIJI

SAŽETAK

Opće je prihvaćeno da sistolički i dijastolički krvni tlak variraju ovisno o različitim čimbenicima kao što su spol, dob, tjelesna građa, socio-ekonomski status, kronične bolesti i sl., ali dokazi nisu uvijek nedvosmisleni. Cilj ovog rada bio je ustanoviti utjecaj međugeneracijske promjene, dobi, spola i indeksa tjelesne mase na vrijednosti krvnog tlaka i ustanoviti utjecaj migracija na razlike među vrijednostima krvnih tlakova između dvije generacije tibetanskih imigranata u sjevernu Indiju. Rezultati istraživanja na prvoj i drugoj generaciji odraslih Tibetanaca pokazuju da ne postoje značajne razlike u vrijednostima krvnih tlakova između dvije generacije kod oba spola, što upućuje na utjecaj prehrambenih navika.

Tracking of Anthropometric Parameters and Bioelectrical Impedance in Pubertal Boys and Girls

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ABSTRACT

The aim of this study was to investigate the anthropometric parameters and body impedance once per year during four years of the pubertal period in Estonian children. In total, 81 boys and 86 girls aged 10–11 years at the beginning of the study were investigated. Pubertal status was self-assessed by sexual maturation stages according to Tanner¹⁸ and physical activity index (PAI) according to Telama et al.¹⁷. Body height and weight were measured and body mass index (BMI) calculated. In total, 9 skinfolds, 13 girths, 8 lengths and 8 breadths/lengths were measured according to the protocol of the International Society for the Advancement of Kinanthropometry²¹. Somatotype components were estimated according to the method of Carter and Heath⁹. Body impedance was measured using Multiscan 5000 (Bodystat, UK) and the impedance index ($\text{height}^2/\text{impedance}$) was calculated. The tracking of body height, weight, BMI, skinfolds, girths, lengths, breadth/lengths and body impedance was high (as a rule $r \geq 0.9$). By increasing the time period, the correlation slightly decreased. In contrast, tracking correlations for PAI and Tanner stages were significant but quite low. Increase in mean body height was highest between 12–13 years of age (6.9 cm per year) in boys and in girls between 11–12 years of age (6.3 cm per year). In boys and girls, the peak increase in body weight was between 11 and 12 years of age, 5.7 kg and 5.2 kg, respectively. With the increasing age, body impedance decreased and impedance index increased. In conclusion, our results indicate that during puberty the detailed anthropometric parameters and body impedance tracked highly. However, the tracking of PAI and Tanner stages was significant but relatively low.

Key words: anthropometry, somatotype, bioelectrical impedance, sexual maturation, tracking

Introduction

Understanding and quantifying changes in anthropometric measures and body composition during pubertal period, and associated factors, would facilitate the early recognition of children with aberrant changes and/or unusual levels of body composition. There is a tremendous number of studies where the different anthropometric parameters and/or body composition have been investigated in children using different measurement methods. However, there are a few data about the tracking of these parameters during the pubertal period and the effect of the rate of biological maturation on anthropometry and body composition.

Such body composition measures as body mass index (BMI), sum of skinfolds, and relative weight have been found to track significantly from childhood to adult-

hood^{1,2}. However, tracking coefficients have been found to be higher over shorter time intervals and for BMI compared with those for skinfolds and relative weight measures. For example, the tracking correlations for BMI have ranged from $r=0.77$ to $r=0.89$ over 4- to 8-year time intervals³ and from $r=0.44$ to $r=0.84$ over 13- to 15-year time intervals⁴. Several authors^{5,6} have found that skinfolds tracked significantly from approximately 9 to 16 years of age. The tracking correlations between 9 and 13 years and 9 and 16 years of age ranged from $r=0.68$ to $r=0.76$ and $r=0.64$ to $r=0.72$, respectively^{5,6}. The Fels Longitudinal Study⁷ indicated that changes in childhood BMI were related to adult overweight and adiposity more in females than males. In a 3-yr longitudinal study (ages 9 to 12), the results suggest that BMI and skinfold thick-

nesses are more likely to track during early adolescence⁸. Relative weight during childhood is a poor correlate of relative weight in adulthood². However, it is well known that tracking correlations have been found to be higher over shorter time intervals. There is a lack of information about the tracking of other anthropometric parameters (girths, lengths, breadths/lengths) in children of different nationalities during puberty.

The somatotype which is a quantification of the present shape and composition of the human body has shown that both individual and group somatotypes change with age⁹. As a rule, during puberty, the somatotypes change dramatically and often reverse component dominance more than once^{10,11}. However, Claessens et al.¹² have observed a relatively high degree of constancy in body build during the growth period despite marked fluctuations in body dimensions.

Bioelectrical impedance analysis (BIA) is a frequently used method for estimating body composition in children^{13,14,15}. Several equations have been developed for the prediction of different parameters of body composition. Little is known about the longitudinal changes of bioelectrical impedance during pubertal time. However, using group-specific BIA equations, Phillips et al.¹⁶ recently concluded that BIA provided accurate estimates of the change in both fat free mass and body fat% over time in adolescent girls.

We hypothesized that different anthropometric parameters (skinfolds, girths, lengths, breadths/lengths) change differently during puberty. It was predicted that the somatotype components will track better than single anthropometric parameters. The aim of this study was to investigate the anthropometric parameters and body impedance once per year during four years over puberty in Estonian public secondary school boys and girls.

Materials and Methods

In total, 81 boys and 86 girls of the age 10–11 years at the beginning of the longitudinal study have been investigated. They were studied once a year in January and February for four consecutive years. All children participated at all four measurement time-points. The subjects were studying at several public secondary schools of Tartu (Estonia) and all children were of Estonian origin. The children were healthy and non-obese. Their school physical education consisted of two obligatory physical education (PE) classes per week. The physical activity index (PAI) of children was estimated by self-administration at school during a PE class time according to the questionnaire of Telama et al.¹⁷. The filling in of the questionnaire took about 15 minutes. All children, parents and teachers were thoroughly informed of the purposes and contents of the study and written informed consent was obtained from the parents or the adult subjects before participation. This study was approved by the Medical Ethics Committee of the University of Tartu (Estonia).

Measurements were performed at school in the morning. All children had a light traditional breakfast. The children did not exercise before being tested. The pubertal status of the subjects was assessed according to the descriptions of the stages given by Tanner¹⁸. The self-assessment method for the evaluation of pubic hair was used. Each subject was asked to observe photographs^{19,20} of the stages of secondary sex characteristics and also to read the descriptions of stages. Measurements on each child were made in the same day.

Body height was measured using a Martin metal anthropometer in cm (± 0.1 cm) and body weight with medical scales in kg (± 0.05 kg) and body mass index (BMI, kg/m²) was calculated. In total, nine skinfolds (triceps, subscapular, biceps, iliac crest, supraspinale, abdominal, front thigh, medial calf, mid-axilla), 13 girths (head, neck, arm relaxed, arm flexed and tensed, forearm, wrist, chest, waist, gluteal, thigh I, thigh II, calf, ankle), eight lengths (acromiale-radiale, radiale-styilion, midstyliion-dactyliion, iliospinale box height, trochanterion, trochanterion-tibiale laterale, tibiale laterale to floor, tibiale mediale-spy-tibiale) and eight breadths/lengths (biacromial, biiliocristal, foot length, sitting height, transverse chest, A-P chest depth, humerus, femur) were measured according to the protocol recommended by the International Society for the Advancement of Kinanthropometry (ISAK)²¹. Skinfold thicknesses were measured in triplicate using Holtain (Crymmych, UK) skinfold calipers. For each skinfold, the mean of all three trials was taken as the final measurement. The CENTURION KIT instrumentation (Rosscraft, Surrey, BC, Canada) was used for girth, length and breadth/length measurements. Calibration of all equipment was conducted prior to and at regular intervals during the data collection period. The three series of anthropometric measurements were taken by the same (A. L.) well-trained anthropometrist (Level 1 ISAK anthropometrist). Technical errors were for skinfolds between 0.9 mm and 1.6 mm and for length and girth values <10 mm. Somatotype components – endomorphy, mesomorphy and ectomorphy were calculated according to the Carter and Heath⁹ protocol.

Body impedance was measured on the right side of the body using a multiple-frequency impedance device (Multiscan 5000, Bodystat, UK) at standard conduction current of 800 μ A and 50 KHz and the impedance index was calculated (height²/impedance). The accuracy of the equipment was checked before the measurements with a 500 Ω resistor supplied by the manufacturer. Children were placed in a supine position with limbs slightly abducted. Skin current electrodes were placed on the dorsal surface on the hand and foot at the metacarpals and metatarsals. Skin was cleaned with 70% alcohol and a small drop of ECG cream was used to improve current conduction between the electrode and skin. Because of the fact that there are no specific regression equations available for Estonian children, body fat% and fat-full mass were not calculated. The hydration state of the children was not well controlled. They were tested in school on the mornings after a light breakfast at home. The

children did not exercise before testing. High intraclass correlation coefficients ($ICC > 0.979$) demonstrated excellent test-retest (between week) measurement reliability for BIA method.

Data analysis was performed using SPSS 10.0 for Windows (Chicago, IL). Standard statistical methods were used to calculate mean and standard deviation ($X \pm SD$). The interperiod Spearman correlations were used as tracking coefficients. All time points correlated with the baseline measure and additionally, between the second, third and fourth, and the third and fourth measurements. Significance was set at $p < 0.05$.

Results

Basic anthropometric parameters, somatotype components, PAI and Tanner stage results are presented in Table 1. Body height and weight increased significantly ($p < 0.05$ – 0.01) during each year both in boys and girls. However, increases in BMI of the girls between the third and fourth measurement was not significant ($p > 0.05$). From the somatotype components, ectomorphy did not change significantly during the study period. In boys, the endomorphy was lowest at the first measurement ($p < 0.05$ – 0.01). In girls, the changes were not significant ($p > 0.05$). Mesomorphy in boys did not change significantly; in girls, the index was significantly higher during the first measurement. The PAI did not change significantly during the pubertal period. The assessment of Tanner stage increased significantly ($p < 0.01$ – 0.001) every year.

All the measured skinfold thicknesses increased significantly between the first and second measurement (data not presented). At the end of puberty, especially in boys, some skinfold thicknesses decreased. Except that of the head, as a rule, other girths increased significantly. There were more significant increases in length and breadth/length parameters at the end of puberty (between the second and third, and the third and fourth measurements).

Tracking of the body height (in boys $r = 0.938$ – 0.986 ; in girls $r = 0.912$ – 0.987), body weight (in boys $r = 0.905$ – 0.957 ; in girls $r = 0.906$ – 0.979) and BMI (in boys $r = 0.828$ – 0.943 ; in girls $r = 0.814$ – 0.926) was very high (Table 2). The tracking coefficients for somatotype components were also relatively high: ectomorphy (in boys $r = 0.817$ – 0.933 ; in girls $r = 0.861$ – 0.970), endomorphy (in boys $r = 0.693$ – 0.901 ; in girls $r = 0.866$ – 0.947) and mesomorphy (in boys $r = 0.780$ – 0.882 ; in girls $r = 0.819$ – 0.912). The tracking of the PAI was relatively low (in boys $r = 0.272$ – 0.446 ; in girls $r = 0.252$ – 0.581). Compared with PAI, the tracking of Tanner stages was slightly higher (in boys $r = 0.314$ – 0.683 ; in girls $r = 0.354$ – 0.722).

The interperiod Spearman correlation coefficients of skinfold thicknesses were relatively high (Table 3). However, by increasing the time intervals between the measurements, the tracking coefficients decreased rapidly. As a rule, over four years (from 10 to 13), the tracking coefficients decreased about 0.2 units (Table 3). The tracking of the girth was quite high and the differences between the years were relatively low (Table 4). Normally, the changes in four years were relatively stable. Similar changes were observed for the length parameters (Table

TABLE 1
ANTHROPOMETRIC PARAMETERS, SOMATOTYPE COMPONENTS, PAI (PHYSICAL ACTIVITY INDEX) AND TANNER^{19, 20} STAGES DURING FOUR YEARS (UPPER LINE BOYS AND LOWER LINE GIRLS, $X \pm SD$)

	First measurement (10-year-old)	Second measurement (11-year-old)	Third measurement (12-year-old)	Fourth measurement (13-year-old)
Age (yrs)	10.0±0.8 9.9±0.7	10.9±0.8 10.8±0.8	12.0±0.8 11.9±0.8	12.9±0.8 12.8±0.8
Height (cm)	142.8±7.3 141.7±7.4	148.5±7.8 147.5±8.2	155.1±8.9 153.8±8.0	162.0±10.2 159.0±7.6
Weight (kg)	34.8±5.6 33.4±6.6	39.3±7.2 37.2±7.9	45.0±9.2 42.4±9.2	50.2±10.8 46.4±9.6
BMI (kg/m ²)	17.0±1.8 16.5±2.2	17.7±2.1 17.0±2.4	18.5±2.7 17.9±3.0	19.0±2.8 18.2±2.8
Endomorphy	2.1±0.9 2.6±1.3	2.7±1.2 2.8±1.6	2.6±1.5 2.8±1.5	2.5±1.4 2.9±1.5
Mesomorphy	4.2±0.9 3.8±0.9	4.1±1.1 3.5±0.9	4.3±1.1 3.5±1.0	4.3±1.2 3.3±1.0
Ectomorphy	3.5±1.1 3.8±1.3	3.5±1.3 4.0±1.4	3.6±1.4 3.9±1.4	3.8±1.4 4.0±1.4
PAI	11.7±1.4 11.6±1.8	11.7±1.5 11.8±1.4	11.8±1.3 11.9±1.4	11.8±1.5 11.8±1.6
Tanner stage	1–2 1–3	1–3 2–4	2–4 2–4	2–4 2–4

PAI – physical activity index, BMI – body mass index

TABLE 2
 INTERPERIOD SPEARMAN CORRELATION COEFFICIENTS OF SIMPLE ANTHROPOMETRIC PARAMETERS, SOMATOTYPE COMPONENTS, PAI AND TANNER STAGES (UPPER LINE BOYS AND LOWER LINE GIRLS)

	10 vs. 11 yrs	10 vs. 12 yrs	10 vs. 13 yrs	11 vs. 12 yrs	11 vs. 13 yrs	12 vs. 13 yrs
Height	0.986 0.987	0.951 0.957	0.938 0.912	0.972 0.974	0.956 0.931	0.962 0.978
Weight	0.952 0.966	0.905 0.940	0.912 0.906	0.954 0.966	0.940 0.941	0.957 0.979
BMI	0.886 0.926	0.828 0.814	0.847 0.861	0.928 0.838	0.913 0.915	0.943 0.847
Endomorphy	0.851 0.947	0.769 0.889	0.693 0.894	0.863 0.914	0.758 0.866	0.901 0.890
Mesomorphy	0.810 0.845	0.790 0.873	0.780 0.829	0.867 0.854	0.860 0.819	0.882 0.912
Ectomorphy	0.885 0.892	0.817 0.861	0.845 0.970	0.922 0.917	0.910 0.874	0.933 0.943
PAI	0.272 0.581	0.381 0.252	0.294 0.406	0.446 0.495	0.352 0.491	0.434 0.505
Tanner stage	0.490 0.636	0.508 0.365	0.314 0.354	0.662 0.722	0.484 0.607	0.683 0.632

p at least <0.05, PAI – physical activity index, BMI – body mass index

5). From the detailed measured anthropometric parameters, the tracking was lowest on the breadth/length values (Table 6).

In boys, the changes in body impedance were significant between four measurement years (Table 7). As a rule, the impedance decreased except between the second and third measurements. In girls, the increase in imped-

ance between the second and third measurement was not pronounced. Both in boys and girls, the impedance index significantly increased every year ($p < 0.05-0.001$) (Table 7). The tracking of both body impedance and impedance index was high and decreased slightly with increasing the time interval between the measurements (Table 7).

TABLE 3
 INTERPERIOD SPEARMAN CORRELATION COEFFICIENTS OF SKINFOLD THICKNESSES AT FOUR TIMEPOINTS (UPPER LINE BOYS AND LOWER LINE GIRLS)

	10 vs. 11 yrs	10 vs. 12 yrs	10 vs. 13 yrs	11 vs. 12 yrs	11 vs. 13 yrs	12 vs. 13 yrs
Triceps	0.829 0.923	0.698 0.848	0.646 0.860	0.819 0.883	0.743 0.815	0.842 0.859
Subscapular	0.822 0.950	0.760 0.873	0.653 0.916	0.861 0.910	0.747 0.901	0.915 0.911
Biceps	0.759 0.852	0.633 0.832	0.585 0.794	0.781 0.893	0.709 0.810	0.822 0.833
Iliac crest	0.830 0.946	0.725 0.892	0.592 0.867	0.830 0.910	0.693 0.844	0.884 0.907
Supraspinale	0.789 0.884	0.691 0.848	0.691 0.858	0.782 0.889	0.688 0.780	0.824 0.816
Abdominal	0.854 0.923	0.697 0.912	0.591 0.847	0.847 0.898	0.746 0.852	0.878 0.904
Front thigh	0.858 0.897	0.771 0.793	0.695 0.811	0.884 0.906	0.712 0.893	0.860 0.886
Medial calf	0.850 0.907	0.821 0.877	0.709 0.817	0.844 0.905	0.733 0.847	0.829 0.878
Mid-axilla	0.848 0.948	0.698 0.918	0.630 0.878	0.815 0.931	0.645 0.866	0.819 0.931

p at least <0.05

TABLE 4
INTERPERIOD SPEARMAN CORRELATION COEFFICIENTS OF GIRTHS AT FOUR TIME POINTS
(UPPER LINE BOYS AND LOWER LINE GIRLS)

	10 vs. 11 yrs	10 vs. 12 yrs	10 vs. 13 yrs	11 vs. 12 yrs	11 vs. 13 yrs	12 vs. 13 yrs
Head	0.755	0.846	0.813	0.843	0.796	0.889
	0.863	0.884	0.847	0.887	0.913	0.883
Neck	0.863	0.745	0.804	0.797	0.871	0.791
	0.908	0.846	0.833	0.904	0.853	0.876
Arm relaxed	0.889	0.877	0.848	0.905	0.866	0.946
	0.944	0.908	0.878	0.946	0.905	0.952
Arm flexed and tensed	0.921	0.881	0.860	0.933	0.905	0.944
	0.942	0.899	0.881	0.931	0.901	0.953
Forearm	0.844	0.906	0.889	0.812	0.921	0.949
	0.944	0.844	0.877	0.878	0.921	0.876
Wrist	0.889	0.849	0.810	0.932	0.893	0.934
	0.914	0.858	0.870	0.922	0.905	0.941
Chest	0.911	0.653	0.896	0.758	0.911	0.683
	0.950	0.911	0.839	0.951	0.896	0.935
Waist	0.923	0.855	0.859	0.879	0.837	0.907
	0.957	0.885	0.887	0.928	0.914	0.919
Gluteal	0.954	0.896	0.919	0.883	0.921	0.860
	0.971	0.822	0.905	0.833	0.922	0.831
Thigh I	0.924	0.827	0.905	0.838	0.919	0.812
	0.967	0.909	0.908	0.925	0.925	0.921
Thigh II	0.918	0.857	0.841	0.889	0.877	0.872
	0.951	0.813	0.892	0.841	0.916	0.852
Calf	0.959	0.788	0.909	0.833	0.935	0.831
	0.955	0.913	0.906	0.940	0.939	0.949
Ankle	0.926	0.893	0.866	0.931	0.862	0.910
	0.914	0.855	0.842	0.870	0.850	0.865

p at least <0.05

TABLE 5
INTERPERIOD SPEARMAN CORRELATION COEFFICIENTS OF LENGTH AT FOUR TIME POINTS
(UPPER LINE BOYS AND LOWER LINE GIRLS)

	10 vs. 11 yrs	10 vs. 12 yrs	10 vs. 13 yrs	11 vs. 12 yrs	11 vs. 13 yrs	12 vs. 13 yrs
Acromiale-radiale	0.961	0.886	0.588	0.934	0.613	0.585
	0.824	0.819	0.601	0.685	0.570	0.553
Radiale-styilion	0.875	0.709	0.851	0.836	0.948	0.817
	0.971	0.910	0.799	0.927	0.798	0.829
Midstyilion-dactyilion	0.781	0.347	0.595	0.445	0.643	0.552
	0.432	0.457	0.426	0.844	0.749	0.650
Iliospinale box height	0.950	0.915	0.923	0.974	0.964	0.958
	0.968	0.925	0.266	0.967	0.263	0.320
Trochanterion	0.947	0.914	0.920	0.940	0.942	0.948
	0.892	0.815	0.809	0.918	0.900	0.898
Trochanterion-tibiale-laterale	0.814	0.801	0.792	0.848	0.823	0.866
	0.901	0.828	0.477	0.870	0.486	0.515
Tibiale-laterale to floor	0.809	0.880	0.852	0.815	0.844	0.900
	0.842	0.838	0.787	0.865	0.839	0.865
Tibiale mediale spy. Tibiale	0.835	0.734	0.807	0.846	0.935	0.830
	0.900	0.847	0.754	0.886	0.789	0.807

p at least <0.05

TABLE 6
 INTERPERIOD SPEARMAN CORRELATION COEFFICIENTS OF BREADTHS/LENGTHS AT FOUR TIME POINTS
 (UPPER LINE BOYS AND LOWER LINE GIRLS)

	10 vs. 11 yrs	10 vs. 12 yrs	10 vs. 13 yrs	11 vs. 12 yrs	11 vs. 13 yrs	12 vs. 13 yrs
Biacromial	0.717 0.803	0.794 0.815	0.860 0.789	0.664 0.678	0.714 0.622	0.791 0.961
Biiliocrystal	0.828 0.883	0.766 0.758	0.707 0.691	0.887 0.881	0.854 0.776	0.923 0.829
Foot length	0.972 0.982	0.949 0.924	0.798 0.836	0.978 0.959	0.783 0.868	0.803 0.893
Sitting height	0.896 0.961	0.813 0.918	0.857 0.903	0.849 0.945	0.848 0.917	0.927 0.958
Transverse chest	0.468 0.945	0.486 0.899	0.376 0.880	0.838 0.955	0.820 0.929	0.821 0.936
A-P chest depth	0.804 0.916	0.788 0.869	0.700 0.591	0.867 0.928	0.387 0.561	0.897 0.599
Humerus	0.948 0.942	0.905 0.868	0.909 0.876	0.911 0.902	0.897 0.890	0.911 0.874
Femur	0.968 0.959	0.909 0.894	0.845 0.879	0.929 0.911	0.868 0.887	0.876 0.929

p at least <0.05

TABLE 7
 BODY IMPEDANCE AND IMPEDANCE INDEXES (X±SD) AND INTERPERIOD SPEARMAN
 CORRELATION COEFFICIENTS IN BOYS AND GIRLS.

	X±SD	Interperiod correlations		
		First measurement (10-year-old)	Second measurement (11-year-old)	Third measurement (12-year-old)
Boys (n=81)				
Body impedance (Ω)				
First measurement	576.7±57.6			
Second measurement	553.0±64.5	0.904		
Third measurement	557.8±71.0	0.900	0.911	
Fourth measurement	543.2±78.1	0.841	0.877	0.942
Impedance index (height ² /resistance)				
First measurement	35.9±5.7			
Second measurement	40.6±7.1	0.926		
Third measurement	45.3±9.4	0.904	0.916	
Fourth measurement	50.0±12.2	0.887	0.871	0.915
Girls (n=86)				
Body impedance (Ω)				
First measurement	627.9±58.2			
Second measurement	589.1±55.0	0.951		
Third measurement	623.7±68.1	0.874	0.921	
Fourth measurement	608.8±61.5	0.795	0.886	0.914
Impedance index (height ² /resistance)				
First measurement	32.5±5.1			
Second measurement	37.5±6.2	0.914		
Third measurement	38.7±6.8	0.864	0.907	
Fourth measurement	42.1±6.6	0.777	0.874	0.900

p at least <0.05

Discussion

The results of the present investigation confirm those of previous studies that during puberty, the changes in anthropometry are very quick and on the other hand there is a significant interindividual variation in the timing and tempo of puberty. The tracking coefficients during four years were, as a rule, high. The results did not confirm our hypothesis that the somatotype components are better predictors of anthropometry than other often used single anthropometric parameters. During puberty, the body impedance decreased and impedance index increased and the tracking was high.

According to Tanner et al.²², the onset of puberty corresponds to a skeletal age of approximately 11 years in girls and 13 years in boys. The peak height velocity in girls occurs at age 12 with an average height increase of 9 cm/year¹⁹ and in boys approximately 2 years later with a height increase for 10.3 cm/year²⁰. In our study, the data about the individual peak height velocities were not available. However, the mean body height increase in boys was highest between 12 and 13 years of age (third and fourth measurement, mean increase 6.9 cm per year) and in girls between 11 and 12 years of age (second and third measurement, mean increase 6.3 cm). One of the weaknesses of the study is probably the fact that we did not measure the children for at least one more year, at least the boys. However, the additional measurement was impossible because several children changed schools.

Previous investigations confirm that in boys, the peak weight velocity is at about the age of 14 and at an average of 9 kg/year. In girls, the peak weight gain lies behind peak height velocity by approximately 6 months and reaches 8.3 kg/year at the age of about 12.5 years^{23,24}. In our study, the mean peak increase in body weight in boys and girls was between 11 and 12 years of age, 5.7 and 5.2 kg, respectively (see Table 1). At that time the tracking correlations were also very high ($r=0.954$ in boys; $r=0.966$ in girls).

A previous study has indicated that the relationship between adult and childhood skinfold ratio measurements is weak in boys and slightly better in girls²⁵. In our study, during puberty, the development of skinfold thicknesses is different between trunk and extremities and there are sex-specific differences. Similarly to Tanner and Whitehouse²⁶ cross-sectional study, the skinfold thicknesses on the extremities decreased and trunk thickness continued to increase with increasing age in our investigation. However, in girls, both arm and trunk

skinfold thicknesses increased. Probably, the decline in skinfold thicknesses in boys reflects the regional growth of the fat-free mass.

Carter et al.¹¹ emphasized that in studies of children and adolescent growth, the measurement of somatotype is particularly important because it recognizes that individual somatotypes change over time. In our study, during puberty, endomorphy increased especially rapidly in boys at the beginning of puberty (see Table 1). However, compared with other somatotype components, the tracking coefficients were relatively low (see Table 2). This increase is connected with the increase of most of the skinfold thicknesses. This finding is in agreement with other studies⁹. Mesomorphy was stable during puberty in boys and decreased in girls (see Table 1). With increasing of the time interval, the tracking coefficients decreased rapidly (see Table 2). We agree with the results of several cross-sectional studies that the changes in somatotype components are sometimes contradictory²² and also depend on sex^{10,27}.

Several BIA equations have been presented for calculating different body composition parameters in children^{28,29}. However, all the equations are very group-specific and developed cross-sectionally against reference measures. Secondly, body composition is subject to very rapid changes during puberty, and this may explain why there is no longer a clear difference between the methods in that period. Accordingly, it is probably better to use only body impedance and calculated impedance index. However, the disadvantages of BIA measurements include the insensitivity of the method for detecting small changes in body composition in individuals followed over time, dependency of the estimates on the relative amounts of extra- and intracellular water, and the potential distortion of values due to body configuration, as in abdominal obesity³⁰. In our study, as a rule, the impedance decreased every year. It is well known that the hydration level of the fat-free body is higher in prepubertal children than at later ages and especially in boys fat decreased at the end of puberty. In contrast, impedance index increased with age, which is in accordance with other investigations³¹.

In conclusion, our results indicate that during puberty, the detailed anthropometric parameters and body impedance tracked highly. However, the tracking of PAI and Tanner stages was significant but relatively low.

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PRAČENJE ANTROPOMETRIJSKIH PARAMETARA I BIOELEKTRIČNE IMPEDANCIJE KOD DJEČAKA I DJEVOJAKA U PUBERTETU

SAŽETAK

Cilj ove studije bio je istražiti antropometrijske parametre i tjelesnu impedanciju estonske djece u pubertetu. Mjerenja su provedena jednom godišnje kroz period od četiri godine. Ukupno su istraženi 81 dječak i 86 djevojaka, koji su na početku studije bili u dobi od 10–11 godina. Pubertetski status mjeren je fazama seksualnog dozrijevanja prema Tanner-u¹⁸ i indeksom tjelesnih aktivnosti (PAI) prema Telami i dr.¹⁷. Mjerene su visina i težina te je izračunavat BMI. Ukupno je izmjereno 9 kožnih nabora, 13 opsega, 8 dužina i 8 širina/dužina prema protokolu internacionalnog društva za naprednu kinantropometriju. Komponente somatotipa procjenjivane su metodom prema Carter i Heath⁹. Tjelesna impedancija mjerena je uređajem »Multiscan 500« (Bodystat, UK) te je izračunavat indeks impedancije (visina²/impedancija). Korelacija tjelesne visine, težine, BMI, kožnih nabora, opsega, dužina, širina/dužina i tjelesne impedancije je bila viska (kao pravilo $r \geq 0.9$), a kroz duži period, korelacije su se smanjivale. Korelacije između određivanih parametara prema PAI i Tanner-u bile su značajne, ali prilično niske. Povećanje tjelesne visine bilo je najviše u dobi od 12–13 godina kod dječaka (6.9 cm po godini) te u dobi od 11–12 godina kod djevojaka (6.3 cm po godini). Respektabilno mršavljenje javlja se kod dječaka i djevojaka u dobi od 11–12 godina, 5.7 kg odnosno 5.,2 kg. Sa godinama tjelesna impedancija se smanjuje, a indeks impedancije raste. U zaključku možemo kazati da su određivani detaljni antropometrijski parametri i tjelesna impedancija tijekom puberteta bili visoki. Određivanje PAI i Tanner stadija bilo je značajno ali relativno nisko.

Calcaneous Ultrasonographic Assessment of Bone Mineral Density in the Roma Minority Population of Croatia – Preliminary Report

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ABSTRACT

A multidisciplinary anthropological and epidemiological pilot field study of the Bayash population living in 6 villages of the eastern Croatian region of Baranya has been performed in 2005/06. The Bayash (or Boyash) belong to the Roma minority population speaking a distinct archaic dialect of the Romanian language. Since the bone mineral density values in the Roma have not been explored so far and the prevalence of osteoporosis is unknown for this ethnic minority group a screening by means of the »Sahara« Hologic clinical bone sonometer has been performed on 232 voluntary participants (73 males and 159 females). The prevalence of osteoporosis (T-score < -2.5) in the Bayash aged 50 and older is found to be 9.1% in males and 34.4% in females, which is substantially higher than in the general population of Croatia. The prevalence of T-values ranging from -1 to -2.5 indicating osteopenia is found to be 63.6% in males and 45.3% in females, while T-values within the normal range are found only in 27.3% males and 20.3% females. In addition to the low bone mass in older subjects, the mean estimated bone mineral density in all age groups of Bayash men and women was lower in comparison to the manufacturer's reference ranges for European population of the same age. Since body size effects could not be declined, the reference values that would be appropriate for the Roma population should be further explored. The high estimated prevalence of developed osteoporosis calls for attention and the survey should also be extended to exploring the association of low bone mineral density with particular life style and reproductive factors present in this semi-sedentary Roma population.

Key words: Roma minority, Bayash, bone mineral density, osteoporosis, Croatia

Introduction

Low bone mineral density (BMD) has been established as an important predictor of future fracture risk. In recent years, a large number of techniques have been developed to estimate the bone mineral density for the diagnosis of osteoporosis.

Quantitative ultrasound (QUS) is a widely used screening tool for estimating the prevalence of low bone mineral density. There are some evidence that show that QUS is as good a predictor as DXA for the osteoporotic fractures (even independently from the bone mineral density)^{1,2}.

Although genetic factors are known to account for a major proportion of variation of BMD³⁻⁷, diet, smoking, physical activity are usually highlighted as most relevant

environmental risk factors for the development of osteoporosis^{8,9}. Recently, there is an increasing number of studies reporting the socio-economic status, particularly social deprivation, as a potentially important risk factor for osteoporosis¹⁰⁻¹².

From the genetic-epidemiological perspective, Roma population might be considered as a particularly vulnerable group for developing osteoporosis and other chronic common diseases¹³. Namely, with the increase in life expectancy, the prevalence of chronic disease in Roma population may rise even at a higher rate than in other populations since bearing additional risks, both genetic and environmental. Those risks are connected with the following characteristics of Roma populations:

- a) Low socio-economic level and cultural specificities that shape lifestyle patterns compromising health-related behaviors, including the nomadic life style obstructing the possibility of permanent employment and continuity of income;
- b) High degree of reproductive isolation from the surrounding population that enhances the possibility of a specific genetic makeup (e.g. increased frequencies of population-specific alleles and increased frequencies of homozygous genotypes as the consequences of drift and inbreeding).

Since the bone mineral density values in Roma have not been explored so far and the prevalence of osteoporosis is unknown for this ethnic minority group, the present study reports the preliminary results on bone mineral density and the prevalence of osteoporosis as assessed by the sonographic measurements of the calcaneus.

Materials and Methods

Population

In order to assess health status and health-related lifestyle attributes of the Roma minority population living in Croatia, a multidisciplinary anthropological and epidemiological community-based study was designed by the research team of the Institute for Anthropological Research, Zagreb. The pilot study was conducted in the fall of 2005 and spring of 2006 and involved 232 voluntary participants of the Bayash population (73 males and 159 females) aged 18–84 yrs, living in 6 villages and small towns of Baranya, eastern Croatia.

The Bayash (or Boyash) belong to the Roma minority population speaking a distinct archaic dialect of the Romanian language. This semi-nomadic group has arrived to Croatia most likely in the 19th century after the abolition of slavery in Romania and has kept traditional trades until recently. According to the 2001 census in the region of Baranya about 1000 Bayash residents are settled.

Study protocol

The study protocol was approved by the Scientific Board and Ethical Committee of the Institute for Anthropological Research in Zagreb and written consent was obtained from each individual at the onset of the study.

The protocol included the interview, ultrasound heel measurement, anthropometry, blood pressure reading and blood samples collection. Each participant completed a questionnaire developed by the research group that covered the following domains: lifestyle factors (smoking status, nutritional habits including alcohol and caffeine consumption), health history, medication usage (western and complementary), self-rated health, menstrual and reproductive history, migration history, demographics, education, economic status, housing, employment, medical insurance status, social support.

Bone densitometry

The »Sahara« Hologic clinical bone sonometer was used to estimate bone mineral density (BMD) of the calcaneus. The »Sahara« measures the broadband ultrasound attenuation (BUA, in dB/MHz) and speed of sound (SOS, in m/sec) of an ultrasound beam passed through the heel. The BUA and SOS are combined to yield a quantitative ultrasound or »stiffness« index (SI, % of age-matched controls), which is then used to estimate calcaneal BMD (in g/cm²). The manufacturer's reference values for female and male Caucasian populations were used to determine T- and Z-scores.

For quality control and the evaluation of precision, a single machine was used for the study and one member of the research team was assigned to take all the measurements. A phantom supplied by the manufacturer was used to calibrate the machine before each screening session. The measurements were carried in a temperature-controlled environment (room temperature about 18–24°C).

Data analysis

The sample was stratified by sex and age as follow:

1. 50+ year old adults (for the prevalence of osteoporosis);
2. seven 10-year age groups (for estimated average BMD values and percentages of expected BMD as compared with manufacturer's reference data).

Descriptive statistics included sample sizes, mean values, standard deviations and minimal and maximal values. The Student t-test was used to test for significant differences.

The proportion of osteopenic (T-score ≤ -1.0) and osteoporotic (T-score ≤ -2.5) persons were determined using cut-off BMD T-values recommended by WHO (1994).¹⁴

Results and Discussion

The sample size and sex-specific descriptive statistics for age and bone densitometry parameters in Bayash population are shown in Table 1.

According to the WHO criteria¹⁴, osteoporosis (T-score less than or equal to -2.5) is observed in 9.1% males and 34.4% females aged 50 and older (Figure 1). T-values ranging from -1 to -2.5 indicating osteopenia are found in 63.6% males and 45.3% females, while T-values within the normal range are found only in 27.3% males and 20.3% females. When the T-score cut-off value is raised to -1.8 , a recently recommended threshold in quantitative ultrasonography of calcaneus¹⁵, the osteoporosis prevalence increases to 50% in males and 56.3% in females aged 50 and older (Figure 2). The sex difference using later criteria disappears indicating that a considerable proportion of Bayash males and females are characterized by low bone mass. The prevalence of osteoporosis found in the Bayash population is substantially higher than those reported for other populations^{16–19} in-

TABLE 1
DESCRIPTIVE STATISTICS FOR AGE AND BONE DENSITOMETRY PARAMETERS IN BAYASH MALES AND FEMALES. SIGNIFICANT DIFFERENCES AMONG THE SEXES (T-TEST) ARE DENOTED BOLD

	Males					Females				
	N	X	Min.	Max.	SD	N	X	Min.	Max.	SD
Age	73	42.2	18	77	15.6	159	45.6	18	84	15.3
T-score	73	-0.9	-3.3	1.5	1.1	159	-1.1	-3.6	2.0	1.1
Z-score	62	-0.4	-2.9	2.3	1.0	149	-0.8	-2.8	1.8	1.0
BMD (g/cm ²)	73	510.9	249	769	112.1	159	453.0	180	802	127.5
BMD (% expected)*	62	92.2	44	146	20.4	149	82.5	39	135	21.2

* Percentage of expected by age and sex matched values

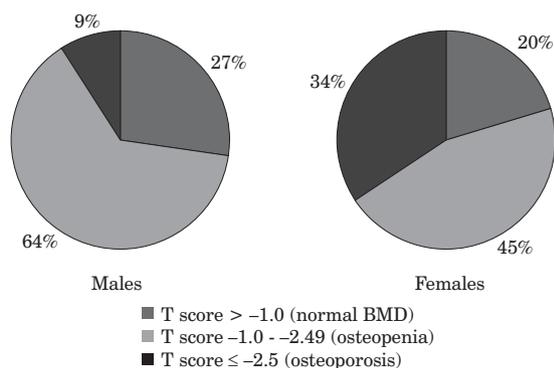


Fig. 1. Prevalence of osteopenia (T score ≤ -1.0) and osteoporosis (T score ≤ -2.5) in Bayash population aged 50 and over.

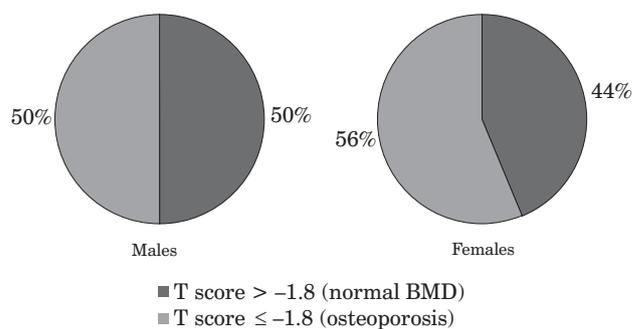


Fig. 2. Prevalence of osteoporosis (T score ≤ -1.8) in Bayash population aged 50 and over.

cluding the general population of Croatia. Respective prevalence for Croatian men are 5.8% (T-score ≤ -2.5) and 16.2% (T-score ≤ -1.8)²⁰.

The age related decline of BMD that is especially pronounced in postmenopausal females (type I osteoporosis) is an extensively documented physiological phenomenon^{21,22}. The same age-related trend is found in the Bayash population (Table 2, Figures 3 and 4). Figure 3 presents estimated BMD values (mean ± 0.95 CI) for Bayash males and females of different ages (7 age groups). The size of

the male-female difference in means and a large confidence interval in the male age group of 55–64 yrs provide explanation for the huge sex difference in the estimated prevalence of osteoporosis after the age of 50 yrs. This also explains the observed difference in the prevalence of osteoporosis in men when the two criteria, T-score ≤ -2.5 and ≤ -1.8, are applied.

Table 2 and Figure 4 show BMD values in Bayash population expressed as the percentages of expected BMD in respective sex and age groups. It is evident that the

TABLE 2
DESCRIPTIVE STATISTICS FOR BMD OF THE BAYASH BY SEX AND AGE. BMD IS EXPRESSED AS PERCENTAGE OF EXPECTED VALUE IN RESPECTIVE SEX AND AGE GROUP

Age group	Males					Females				
	N	X	Min.	Max.	SD	N	X	Min.	Max.	SD
25–34	15	94.0	69	112	12.7	32	83.9	53	125	16.6
35–44	19	93.0	44	126	21.8	36	93.5	58	132	17.6
45–54	11	94.1	64	131	23.0	33	85.2	51	135	23.7
55–64	9	100.0	73	146	24.4	26	72.0	41	113	20.4
65+	8	75.9	53	107	15.9	22	70.6	39	116	19.9

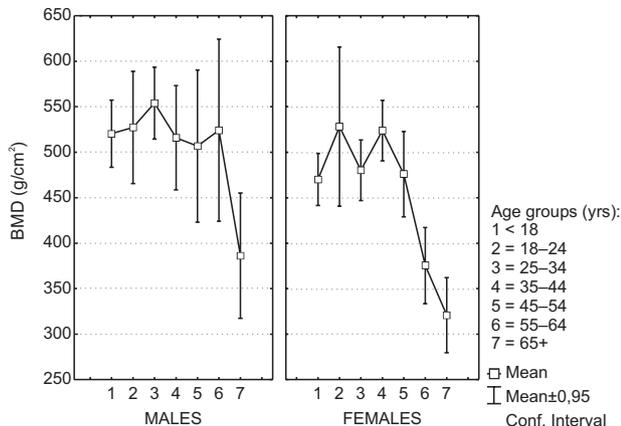


Fig. 3. BMD means and confidence intervals by sex and age in the Bayash population.

mean estimated bone mineral density is substantially lower in comparison to the manufacturer's reference ranges in all age groups and in both sexes (with exception of 55–64 year old males). Low bone mass observed among the Bayash can arise as a result of the impaired development of the peak bone mass (e.g. due to delayed puberty or undernutrition)²³ but it may also partially be due to the diminutive body size of the studied Roma²⁴ compared to the European population.

Conclusion

The high prevalence of developed osteoporosis observed among the Bayash of eastern Croatia calls for attention and the survey should be extended to exploring the association of low bone mineral density with particular life style and reproductive factors present in this semi-sedentary Roma population. The findings also indicate that, in addition to exceptionally high prevalence of osteoporosis found in older subjects, the Bayash popula-

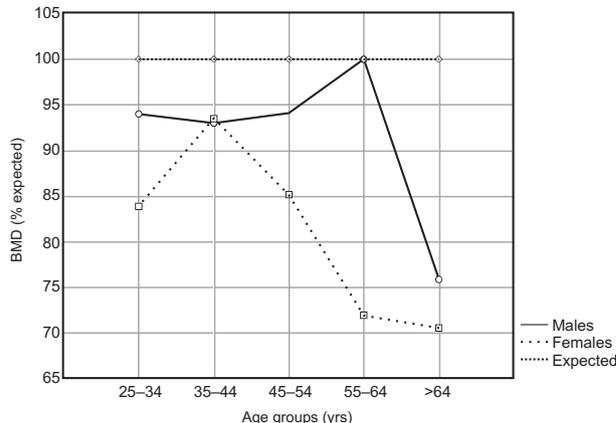


Fig. 4. BMD means by sex and age in the Bayash population. BMD expressed as percentage of expected value (i.e. 100%) in respective sex and age group.

tion is characterized by low bone mineral density in all adult ages. Since body-size effects can not be overseen, the normative values that would be appropriate for the Roma population are necessary to be further explored.

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ULTRAZVUČNA PROCJENA MINERALNE GUSTOĆE KOSTIJU U MANJINSKOJ POPULACIJI ROMA U HRVATSKOJ – PRELIMINARNO IZVIJEŠĆE

S A Ž E T A K

Tijekom 2005/06 godine provedena je multidisciplinarna antropološka i epidemiološka terenska pilot studija populacije Bajaša – stanovnika 6 Baranjskih sela (istočna Hrvatska). Bajaši (ili Bojaši) pripadaju Romskom manjinskom stanovništvu koje govori specifičan arhaičan dijalekt romskog jezika. Budući da vrijednosti mineralne gustoće kostiju Roma još nisu istražene i prevalencija osteoporoze nije poznata za ovu manjinsku etničku skupinu, na 232 dobrovoljaca (73 muškarca i 159 žena) provedeno je probirno istraživanje korištenjem ultrazvučnog denzitometra »Sahara« (Hologic). Prevalencija osteoporoze ($T \leq -2.5$) u Bajaša starih 50 i više godina iznosi 9.1% kod muškaraca i 34.4% kod žena, što je višestruko veća proporcija od prevalencije u općoj populaciji Hrvatske. Prevalencija T-vrijednosti u rasponu od -1 do -2.5 koje indiciraju osteopeniju iznosi 63.6% kod muškaraca i 45.3% kod žena, dok T-vrijednosti veće od -1 (odnosno, normalne vrijednosti gustoće kostiju) ima tek 27.3% muškaraca i 20.3% žena. Osim što nižu koštanu masu imaju starije osobe, srednja vrijednost mineralne gustoće kostiju niža je u svim dobnim skupinama Bajaških muškaraca i žena u usporedbi s referentnim vrijednostima proizvođača za europsku populaciju iste dobi. Kako se učinci veličine i građe tijela ne mogu isključiti, bilo bi potrebno ustanoviti referentne vrijednosti koje bi bile prikladne za Romsku populaciju. Visoka procijenjena prevalencija razvijene osteoporoze upućuje na daljnja istraživanja koja bi trebala obuhvatiti ispitivanja asocijacije niske mineralne gustoće kostiju i čimbenika povezanih s posebnim životnim stilom i reproduktivnim karakteristikama koji su prisutni u ovoj polu-sjedilačkoj Romskoj populaciji.

Effects of Raloxifene on Changes in Bone Density

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ABSTRACT

Raloxifene hydrochloride therapy effectiveness in bone mineral density (BMD) changes compared to calcium and vitamin D3 therapy over a 2-year period. Case-control study: a group of 254 women was prescribed raloxifene (raloxifene hydrochloride) together with calcium and vitamin D3 while other group of 254 women used calcium and vitamin D3 therapy. BMD was measured at the hip, spine and forearm at the beginning and at the end of the 2-year period. Treatment with raloxifene resulted in a 3.7% increase in BMD at the spine in 98% of examinees. A 1.2% BMD increase was shown in 75% of examinees at the hip. A 1.2% decrease in BMD at forearm shown in 93% of examinees using raloxifene. The calcium and vitamin D3 therapy led to an increase in BMD in 58% examinees at the spine, in 56% at the hip and in 38% at the forearm, which was significantly lower than in women using raloxifene. Among women using calcium and vitamin D alone an average BMD decrease of 1.2% was registered on 42% of examinees at the spine, 2.6% decrease on 46% of examinees at the hip and 4.2% decrease on 35% of examinees at the forearm. Treatment with raloxifene resulted in a significant increase in BMD at the spine with odds ratio (OR 5.85, $p < 0.05$) compared with calcium and vitamin D3 alone. There was no statistically proven increase in BMD at either the hip (OR 0.015) or forearm (OR 0.122).

Key words: bone mineral density, osteoporosis, raloxifene, vitamin D3, calcium

Introduction

One of the most represented metabolic diseases of the bone in the world is osteoporosis; its incidence is equal as that of cardiovascular and malignant diseases. It is known that risk of developing osteoporosis and severe complications as fractures increase with age. World population structure is getting older and by that the risk of fractures tends to increase. Fractures of the spine, forearm and the hip are the most frequent. Commonly induced by postmenopausal osteoporosis fractures of the spine are frequent in middle-aged women. Fractures of the hip, which are commonly induced by senile osteoporosis, are linked with: high mortality (25%), high long-term loss of function (25%) and lesser quality of life (50%)¹⁻³.

Society is trying to deal and cope with osteoporosis with raising awareness and education, acquiring and developing diagnostic tools, as well as developing new drugs and treatments. Currently on the market there is a variety of medications to prevent osteoporotic fractures.

Raloxifene is a first drug from the group of selective estrogen receptor modulators that has proven its effi-

ciency in prevention and treatment of postmenopausal osteoporosis^{4,5}. Its effects on 76% decrease of breast cancer incidence⁶ and reduction of the overall cardiovascular risk is well known⁷⁻¹³. It is confirmed that raloxifene significantly decreases vertebral and non-vertebral fracturing^{14,15}.

Aim of this scientific paper is to determine changes in the bone mineral density in a case control study of group postmenopausal women who were prescribed raloxifene, calcium and vitamin D3 and a group of those who were prescribed only calcium and vitamin D3. Both groups of women were compatible by age, BMD, BMI and age of starting menopause. Study was done in a 2-yrs period.

Subjects and Methods

All examinees were split into two statistically equal groups – for further details see Table 1.

TABLE 1
CLINICAL CHARACTERISTICS OF THE STUDY GROUPS

Variables	N (%) of postmenopausal women	
	Study group (n=254)	Control group (n=254)
Age, X±SD	56.1±7.9	56.1±7.9
Menarche, X±SD	14.7±2	14.4±1.7
Menopause, X±SD	47.8±5.7	47.9±5.2
Family history of osteoporosis	4 (1.57)	5 (1.96)
Immobility	7 (2.7)	8 (3.1)
Body mass index (BMI), X±SD	29.3±4.5	28.3±4.1
Smoking	30 (11.8)	32 (12.6)
Milk consumption < 500 ml	60 (23.6)	58 (22.8)
Serum Calcium, X±SD	2.7±2.4	2.4±0.2
Serum Phosphorus, X ±SD	1.1±0.3	1.1±0.6
Serum Alkaline Phosphatase, X±SD	69.2±22	73.5±26.4
Urine Calcium, X±SD	5.7±3.2	5.7±3.6
Urine Phosphorus, X±SD	24.4±15.4	23.9±10.1
Total Cholesterol, X±SD	6.6±1.5	6.1±1.2
Tryglicerids, X±SD	1.9±1.6	1.8±0.8

1. Study group was consisted of 254 women who had taken 60 mg of raloxifene (Eli Lilly Co. Ltd., Basingstoke, England), 1000 mg of calcium and 800 units of vitamin D3 per day over a 2-yr period.
2. Control group was consisted of 254 women who received only 1000 mg of calcium and 800 units of vitamin D3 per day throughout the same period.
3. The average age in both groups was 57 years. We monitored the effects on new vertebral and non-vertebral fractures. All women had been informed and had given their consent to the research by signing an informed consent form. Ethics Committee of the General hospital »Dr. Josip Benčević«, Sl.Brod approved this study Questionnaire handed out to examinees asked for information concerning daily intake of milk products, first and last menstrual period, family history of osteoporosis, tobacco intake and physical inactivity longer than 3 months. Before starting with the study/treatment examination of the BMD at the hip, vertebra and the forearm were conducted. Levels of calcium and phosphorus in the serum and 24h urine, alkaline-phosphatase, total cholesterol and tryglicerides were collected.

There was no statistically significant difference detected between of the control and study group after pre-treatment examinations. In both groups there was no vertebral fractures; during the 2-yr period every vertebral and non-vertebral fracture was reported. In the study group there were 9 fractures of the forearm, while in the control group 10 forearm fractures were detected. In both groups, during 2-yr period wasn't fractures of the hip and the vertebra detected.

BMD was measured by dual-energy X-ray absorptiometry with QDR 4500 densitometer (Hologic, Beresford,

MA, USA) and the results were expressed in g/cm²; for both groups at the beginning and end of the 2-year period.

Mean percentage changes in BMD at the both groups were determined and odds ratio (OR) was calculated. The statistical significance was tested using the chi square test and set to p<0.05. All statistics were performed using Statistica for Windows software, Version 6.0 (Tulsa, OK, USA).

Results

Raloxifene improved the spinal bone density in 98% of examinees. Hip BMD increased in 75% of cases whereas a decrease in density occurred at the forearm in 93% of examinees. The spine BMD increased from 0.807 g/cm² (±0.103) to 0.836 (±0.298), which was a 3.7% increase. BMD increased at the hip, from 0.813 (±0.125) to 0.821 (±0.09) that represented a 1.2% increase. However, the forearm BMD decreased from 0.615 (±0.071) to 0.541 (±0.073) that was a 1.2% decrease.

Calcium and vitamin D3 therapy led to an increase in BMD in 58% of examinees at the spine from 0.791 (±0.025) to 0.800 (±0.011) which is an 1.2% increase.

In 56% of examinees increase at the hip is 0,9% – from 0.783 (±0.025) to 0.790 (±0.014). In the forearm increase is an 0.8% in 38% of examinees- from 0.601 (±0.021) to 0.605 (±0.006).

A decrease in BMD was manifested in 42% of examinees at the spine area, 46% at the hip, and 35% at the forearm area. A decrease in BMD at the spine, hip and forearm was 1.6%, 2.6% and 4.2%, respectively (Table 2).

There were significantly more examinees with significant increase in bone density of the spine among raloxi-

TABLE 2
EFFECTS OF RALOXIFENE THERAPY AND CALCIUM AND VITAMIN D THERAPY ON BMD IN THE HIP, SPINE AND FOREARM THROUGHOUT A 2-YEAR PERIOD

Site of measuring	Time	Study group BMD (g/cm ²) X±SD	Control group BMD (g/cm ²) X±SD
Spine	In the beginning	0.807±0.103	0.791±0.025
	After 2-yrs	0.836±0.298	0.800±0.011
Hip	In the beginning	0.813±0.125	0.783±0.025
	After 2-yrs	0.821±0.821	0.790±0.014
Forearm	In the beginning	0.615±0.615	0.580±0.501
	After 2-yrs	0.541±0.073	0.556±0.451

fene group than among women using calcium and vitamin D3 alone, while in hip and the forearm the difference was not significant. Chi-square values of 24.67 ($p < 0.05$) for spinal BMD with OR 5.58 and CI 95% (1.535–53.967) were calculated. For the hips the χ^2 value was 43.14 with OR 0.0105 and CI 95% (0.0160–0.896), for the forearm the χ^2 value was 27.86 with OR 0.122 and CI 95% (0.0021–0.0568).

Discussion

Raloxifene increases total bone density, especially at the spine. Some authors indicate increase of the bone density at the hip as well^{13–16}. Results of our research were similar to those of earlier conducted research. We detected a slight improvement of the BMD at the hip and forearm. During the period of the study we detected no fractures of the hip. In the study group we have registered 9 and in the control group 10 fractures of the forearm, which difference was not statistically significant. In this age group of our examinees we found no fractures of the spine, probably due to raloxifene therapy. Monitoring the effects of these therapies in a longer period of time probably would have shown significant difference between vertebral and non-vertebral fractures. Significant differences of the efficiency of preventing fractures weren't and could not have been detected due to the younger age of women in study group.

Raloxifene improved significantly spine bone density. A 2.4% increase in BMD at the lumbar spine and hip and

a 2% increase in total bone mass following 2 years of therapy was previously reported¹⁴. Etinger et al.¹⁵ found a 2.6% increase in BMD at the spine and a 2.1% increase in BMD at the hips following 36 months of therapy. Zheng et al.¹⁶ found an increase of 2.3% in BMD at the spine and 2.5% at the hip area. Our results were consistent with most of these previously conducted studies on Caucasian or Asian women.

Our study showed insignificant positive effects of vitamin D and calcium therapy on the spine only, while a decrease in bone density at hip and forearm was revealed. However, the BMD decrease at forearm was lower among this group of women. Evidence by other researchers has shown how intake of calcium and vitamin D prevented the risk of bone density decrease at all areas¹⁷.

Our study had several limitations, the most important of them being the case-control design of the study. More relevant data, with possibility of definite conclusion could be yielded through a prospective randomized study. However, our place of work lacks organization and financial potentials for that kind of investigation. It would also be interesting to prospectively investigate the risk of fractures in these two groups of women.

Irrespective of age, raloxifene has advantages in patients who have a higher risk of breast and endometrial cancer, as well as those with medium hypercholesterolemia¹⁰. Raloxifene therapy is the good solution for both treatment and prevention of early postmenopausal osteoporosis.

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UTJECAJ RALOKSIFENA NA PROMJENE KOŠTANE GUSTOĆE

SAŽETAK

Kroz dvije godine je uspoređen utjecaj raloksifen hidroklorida (raloksifena) na promjenu koštane gustoće (BMD) u odnosu na kalcij i vitamin D3. »Case control« studija je obuhvaćala dvije skupine po 254 žene, od kojih su jedne kroz 2. godine uzimale raloksifen, kalcij i vitamin D3, a druge samo kalcij i vitamin D3. Koštana gustoća je mjerena na kuku, kralješnici i podlaktici na početku i nakon 2. godine terapije. Tretman raloksifenom je povećao koštanu gustoću za 3,7% na kralješnici kod 98% ispitanica. Povećanje gustoće na kuku je bilo 1,2% kod 75% ispitanica. Pad koštane gustoće je bio za 1,2% na podlaktici kod 93% ispitanica uz raloksifen. Terapija kalcijem i vitaminom D3 je povećala koštanu gustoću kod 58% ispitanica na kralješnici, 56% na kuku i 38% na podlaktici što je značajno niže u odnosu na skupinu liječenu raloksifenom. U skupini liječenoj kalcijem i vitaminom D3 zabilježen je prosječni pad koštane gustoće na kralješnici za 1,2% kod 42% ispitanica, na kuku za 2,6% kod 46% ispitanica te za 4,2% na podlaktici kod 35% ispitanica. Terapija raloksifenom značajno povećava koštanu gustoću na kralješnici (OR 5.85, $p < 0.05$) u odnosu na terapiju samo kalcijem i vitaminom D3. Nema statističke značajnosti u povećanju koštane gustoće na kuku (OR 0.015) ni podlaktici (OR 0,122).

Age Variations in Anthropometric and Body Composition Characteristics and Underweight Among Male Bathudis – A Tribal Population of Keonjhar District, Orissa, India

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ABSTRACT

A cross-sectional study of 226 male Bathudis, a tribal population of Keonjhar District, Orissa, India, was undertaken to investigate age variations in anthropometric and body composition characteristics and the frequency of underweight. The subjects were categorized into three age groups: Group I: < 30 years, Group II: 30–49 years, Group III: ≥ 50 years. Height, weight, circumferences and skinfolds data were collected. Body mass index (BMI) and several body composition variables and indices were derived using standard equations. Results revealed that there existed significant negative age variations for most of the anthropometric and body composition variables and indices. Correlation studies of age with these variables and indices revealed significant negative correlations. Linear regression analyses revealed that for all these variables, age had a significant negative impact. It was also observed that with increasing age, there was an increase in the frequency of underweight individuals. In conclusion, this study showed that among Bathudi men, age was significantly negatively related with anthropometric and body composition variables and indices. Furthermore, with increasing age, the frequency of underweight individuals increased.

Key words: India, Bathudis, age variations, anthropometry, body composition, underweight

Introduction

Several recent studies worldwide have focused on age variations in anthropometric characteristics and nutritional status of among men of different ethnic groups^{1–3}. Most studies on age variations in anthropometric and body composition parameters from India tend to focus on non-tribal populations^{4–5}. Very scanty data are available on anthropometric and body composition characteristics and the frequency of underweight of tribal populations of India⁶.

The tribes of India comprise about 8% of the total population of the country having probably the largest number of tribal communities in the world⁷. Bathudis are one such tribe whose mother tongue is Panchapargania, an Indo-Aryan language. They are inhabitants of three eastern provinces of India: Orissa, Bihar and Jharkhand. Majority of the Bathudis are found in three districts of Orissa, namely, Keonjhar, Mayurbhanj and

Sundargarh⁶. Information on Bathudis is very limited^{6,8}. The importance of the present study is that there is no published data dealing with age variations in anthropometric and body composition characteristics and the frequency of underweight among adult Bathudi males⁶.

The present study was undertaken to examine age variations in anthropometric and body composition indicators and the frequency of underweight individuals among male adult Bathudi tribals of Keonjhar District, Orissa, India. It presents unique data that can be used for comparative studies with other tribal and non-tribal ethnic minorities, both from India as well as abroad. It must be stressed here that the study design was cross-sectional and thus this report deals with age variations and not age changes which can be studied only by longitudinal investigations.

Materials and Methods

Area of study and subjects

The present investigation was conducted in collaboration of Associated Social Service Agency (ASSA), a non-governmental organization based at Sailongchhak, Anandapur, Keonjhar District, Orissa, India. Prior permission and ethical approval was obtained from local community leaders as well as relevant authorities before commencement of the study. Information on ethnicity, age, occupation and educational status were obtained from all subjects with the help of a questionnaire. The data were collected from three villages, Gahira, Kalora Gadira and Pathurkundi in Anandapur region of Keonjhar district of Orissa, India. These villages are located approximately 150 kms from Bhubaneswar, the provincial capital of Orissa. The residents of all houses (number of houses = 152) in the three villages were contacted and a total of 226 adult (>18 years) women were included in the study. The response (participation) rate was 76 %. The vast majority of the subjects were illiterate and very low-wage earning manual labourers. Thus, they belonged to the low socio-economic class. Subjects were grouped into three age groups: Group I: < 30 years (n = 72); Group II: 30–49 years (n = 104) and Group III: ≥ 50 years (n = 50).

Anthropometric measurements

Trained investigators, using internationally accepted standard protocol⁹, made anthropometric measurements. Anthropometric variables included height, weight, sitting height (STHT), mid upper arm circumference (MUAC), and biceps (BSF), triceps (TSF), subscapular (SUBSF) and suprailiac (SUPSF) skinfold thicknesses. Technical errors of measurements (TEM) were computed and they were found to be within acceptable limits¹⁰. Thus, TEM was not incorporated in statistical analyses. Body mass index (BMI) was computed using the following standard equation:

$$\text{BMI} = \text{Weight (kg)} / \text{height (m}^2\text{)}.$$

Underweight was evaluated using internationally accepted World Health Organization BMI guidelines¹¹. The following cut-off points were used:

Underweight: BMI < 18.5

Normal: BMI = 18.5–24.9

Overweight: BMI ≥ 25.0.

Body composition

Percent body fat (PBF) was calculated using Siri's equation¹². The equation is:

$$\text{PBF} = (4.95/\text{density} - 4.50) \times 100.$$

Density was derived following Durnin & Womersley's equation¹³ using the sum of BSF, TSF, SUBSF and SUPSF.

Fat mass (FM), fat free mass (FFM), fat mass index (FMI) and fat free mass index (FFMI) were computed using following standard equations:

$$\text{FM (kg)} = (\text{PBF}/100) \times \text{Weight (kg)}$$

$$\text{FFM (kg)} = \text{Weight (kg)} - \text{FM (kg)}$$

$$\text{FMI (kg/m}^2\text{)} = \text{FM (kg)} / \text{height}^2 \text{ (m}^2\text{)}$$

$$\text{FFMI (kg/m}^2\text{)} = \text{FFM (kg)} / \text{height}^2 \text{ (m}^2\text{)}.$$

Total body water (TBW) was calculated using Humes-Weyers formula¹⁴.

$$\text{TBW} = (0.194789 \times \text{Height}) + (0.296785 \times \text{Weight}) - 14.012934$$

where height in is cm and weight is in kg.

The distributions of anthropometric and body composition variables were not significantly skewed. Oneway ANOVA (Scheffes' post-hoc Procedure) was performed to test for age group differences in mean anthropometric and body composition characteristics. Pearson correlation coefficients (r) were utilised to study the association of age with these characteristics. Linear regression analyses were used to study the impact of age on these anthropometric and body composition characteristics. In linear regression analyses, age was used as an independent variable. All statistical analyses were undertaken using the Statistical Package for Social Sciences (SPSS) Package. Statistical significance was set at p < 0.05.

Results

The mean age of the subjects was 38.0 years (SD=14.4 years) years. Table 1 presents the age group differences in mean anthropometric and body composition characteristics. There were significant age group differences in means of most of the variables and indices. Among anthropometric variables, there existed significant age group differences in mean height (F=3.6943, p<0.05), weight (F=6.9869, p<0.005), BMI (F=3.5032, p<0.05), STHT (F=10.9306, p<0.005), BSF (F=6.1170, p<0.005), TSF (F=3.4375, p<0.05) and SUBSF (F=3.4375, p<0.05). There was a trend of decreasing mean from Group I to Group III. In all instances, Group I had the highest mean while Group III had the lowest mean. Among body composition variables and indices, there existed significant age group differences in mean PBF (F=6.250, p<0.005), FFM (F=5.151, p<0.05), FM (F=5.899, p<0.005), FMI (F=5.050, p<0.05) and TBW (6.834, p<0.005) with Group I having the highest mean while Group III had the lowest mean. There was a trend of decreasing mean from Group I to Group III.

Correlation studies of age with these anthropometric and body composition variables and indices were undertaken and results (results not shown) revealed that age was significantly negatively correlated with them.

Linear regression analyses were undertaken with age as the independent variable. Results revealed that (Table 2), for all these variables, age had a significant negative impact. A significant negative impact existed for height (t=-3.197), weight (t=-3.516), BMI (t=-2.000), STHT (t=-5.435), BSF (t=-3.627), TSF (t=-2.708), PBF (t=-3.240), FFM (t=-2.966), FM (t=-3.162), FMI (t=-2.947) and TBW (t=-3.781). The amount of variation explained by age ranged from 1.3% (BMI) to 11.2 % (STHT).

TABLE 1
AGE VARIATIONS IN MEAN ANTHROPOMETRIC
CHARACTERISTICS OF BATHUDI MEN OF KEONJHAR
DISTRICT, ORISSA, INDIA

Variable	< 30 years (n = 72)	30-49 years (n = 104)	≥ 50 years (n = 50)	F
Height (cm)	160.2 (6.3)	159.4 (5.8)	157.4 (7.3)	3.694*
Weight (kg)	48.2 (6.2)	47.4 (6.3)	44.2 (5.7)	6.987**
BMI (kg/m ²)	18.7 (1.6)	18.6 (2.2)	17.8 (1.7)	3.503*
STHT (cm)	81.2 (3.7)	79.9 (3.2)	77.3 (7.3)	10.931**
MUAC (cm)	23.8 (3.2)	23.6 (2.3)	22.9 (2.9)	1.907
Skin fold thickness (mm):				
BSF	4.1 (1.4)	3.5 (1.7)	3.1 (1.5)	6.117**
TSF	6.2 (1.8)	5.5 (2.3)	5.2 (2.3)	3.438*
SUBSF	8.4 (2.2)	7.9 (2.9)	7.0 (2.3)	4.455*
SUPSF	7.5 (2.8)	7.4 (3.2)	6.7 (2.7)	1.222
Body composition:				
PBF (%)	11.8 (3.4)	10.4 (4.6)	9.0 (4.8)	6.250**
FFM (kg)	42.5 (4.9)	42.2 (4.3)	40.0 (4.1)	5.151*
FFMI (kg/m ²)	16.4 (1.2)	16.6 (1.4)	16.2 (1.3)	1.709
FM (kg)	5.8 (2.2)	5.1 (2.3)	4.1 (2.6)	5.899**
FMI (kg/m ²)	2.2 (0.8)	2.0 (1.1)	1.7 (0.9)	5.050*
TBW (kg)	31.6 (2.9)	31.1 (2.6)	29.7 (2.8)	6.834 **

Standard deviations are presented in parentheses. * $p < 0.05$, ** $p < 0.005$, BMI – Body mass index, STHT – Sitting height, MUAC – Mid upper arm circumference, BSF – Biceps skinfold, TSF – Triceps skinfold, SUBSF – Subscapular skinfold, SUPSF – Suprailiac skinfold, PBF – Percent body fat, FFM – Fat free mass, FFMI – Fat free mass index, FM – Fat mass, FMI – Fat mass index, TBW – Total body water

Studies on the underweight status of Bathudi men revealed that (Figure 1) there was a consistent increasing frequency of underweight (BMI < 18.5) from Group I (45.8 %) to Group III (64.0 %). Bathudi men in Group II had intermediate frequency (52.4 %) of underweight.

Discussion

Anthropometry has also been extremely useful in identifying changes in body size and composition that occur with old age^{3-5,15-17}. Anthropometric measurements provide an indirect assessment of body composition and are easy and economical to undertake making them ideally suited for field surveys^{3,5,11}. Many studies worldwide¹⁻³ have already reported on the effects of age on anthropometry and body composition from different parts of the world. However, only a few studies from India⁴⁻⁵ have dealt with age variations in anthropometric and body composition characteristics. Moreover, to date, no detailed investigation has been undertaken on to study age variations in anthropometric and body composition characteristics among any tribal population of India. The present study provides unique data on age variations of

TABLE 2
LINEAR REGRESSION ANALYSES OF AGE WITH
ANTHROPOMETRIC AND BODY COMPOSITION
CHARACTERISTICS AMONG BATHUDI MEN

Variable	B	seB	Beta	Adj. R ²	T
Height	-0.0939	0.029	-0.209	0.039	- 3.197**
Weight	-0.1001	0.028	-0.229	0.048	- 3.516**
BMI	-0.0179	0.009	-0.013	0.013	- 2.000*
STHT	-0.1130	0.021	-0.341	0.112	- 5.435**
Skin fold thickness:					
BSF	-0.0258	0.007	-0.236	0.051	- 3.627**
TSF	-0.0268	0.010	-0.178	0.027	- 2.708*
SUBSF	-0.0295	0.012	-0.161	0.021	- 2.438*
Body composition:					
PBF	-0.0671	0.020	-0.212	0.041	- 3.240**
FFM	-0.0618	0.021	-0.194	0.033	- 2.966**
FM	-0.0382	0.012	-0.207	0.038	- 3.162**
FMI	-0.0135	0.005	-0.193	0.033	- 2.947**
TBW	-0.0478	0.013	-0.245	0.056	- 3.781**

* $p < 0.05$, ** $p < 0.005$, BMI – Body mass index, STHT – Sitting height, MUAC – Mid upper arm circumference, BSF – Biceps skinfold, TSF – Triceps skinfold, SUBSF – Subscapular skinfold, PBF – Percent body fat, FFM – Fat free mass, FM – Fat mass, FMI – Fat mass index, TBW – Total body water

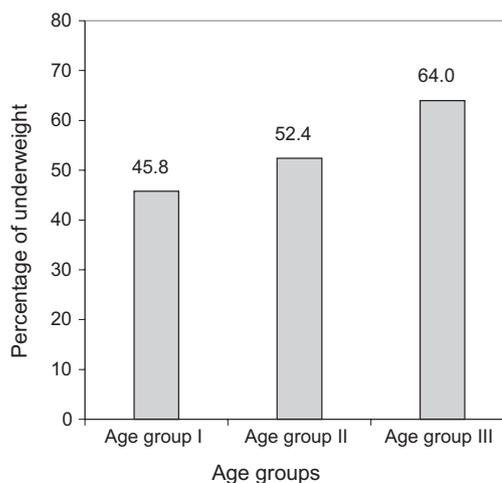


Fig. 1. Percentage of underweight among Bathudi men by age group category.

anthropometric and body composition profile and the prevalence of underweight of adult male Bathudis, a tribal population of Orissa, Eastern India.

The results of the present study demonstrated that a significant decreasing age variation existed in anthropometric and body composition variables among Bathudi males. These results are in concordance with studies from other parts of the world on different ethnic groups¹⁸⁻²⁰ which have also reported a similar inverse age varia-

tion in various anthropometric and body composition characteristics. In the Indian context, the significant negative age variation in anthropometric and body composition profile of Bathudi males was similar to that reported among older and elderly Bengalee Hindus by two recent studies^{5,21} from Kolkata, India.

It has been documented that undernutrition is more common in elderly persons than in younger adults²². Older people, especially those residing in rural areas, are at a greater risk of undernutrition²³. High prevalence of underweight among older Bathudi men is the noteworthy feature of the present study. According to WHO classification¹¹ based on BMI values, the prevalence of underweight was 45.8 %, 52.4 % and 64.0%, in Group I, Group II and Group III, respectively. This clearly indicated that there was a consistent increase in the frequency of underweight with increasing age. These rates of underweight were much higher than those reported among other rural populations in developing countries^{16, 19, 23–24} including India⁵. These results clearly suggested that underweight is a serious problem among Bathudi men that becomes amplified with age.

In conclusion, the two key points of this study were:

- 1) Among Bathudi tribal men, age was significantly inversely related with anthropometric and body composition variables and indices.
- 2) Underweight (BMI < 18.5 kg/m²) was a serious problem among this group, especially among the older individuals.

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Moreover, it should be noted that since prevention of underweight among Bathudi males, especially among the elderly, is essential, the roles of nutritional screening and assessment are of paramount importance. Since undernutrition in elderly people is a consequence of somatic, psychic or social problems²², the interrelationships between these factors should be further investigated among this ethnic group. Furthermore, it is essential that older and elderly Bathudi men be included in nutrition and health programmes and policy. Since underweight is serious problem in this group, recognition of social and health factors associated with the poor nutrition status will allow appropriate intervention to enhance the quality of the life, particularly among older and elderly males.

A recent study from India²⁵ has suggested that there exists ethnic variation in age-related anthropometric and body composition variations. It should also be pointed out that India is a home to a very large number of tribal populations²⁶. Therefore, studies similar to the present one should be undertaken among various tribes in India so as to highlight ethnic variations in the ageing process.

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**DOBNE PROMJENE ANTROPOMETRIJSKIH MJERA, SASTAVA TIJELA I PODHRANJENOST
KOD MUŠKE POPULACIJE BATHUDIS: PLEMENSKE POPULACIJE KEONJHARSKE REGIJE,
ORISSA, INDIA**

S A Ž E T A K

Cilj presječne studije koja je napravljena na uzorku od 226 muških Bathudisa, plemenske populacije Keonjharske regije, Orissa, India, bio je istražiti dobne promjene antropometrijskih mjera, sastava tijela te odrediti frekvenciju podhranjenosti. Ispitanici su bili kategorizirani u tri grupe; grupa I: < 30 godina, grupa II: 30–49 godina, grupa III: > 50 godina. Bila je vidljiva korelacija između visine, težine, obujma i kožnih nabora. BMI i nekoliko varijabli tjelesne kompozicije dobivene su upotrebom standardnih jednadžbi. Rezultati su pokazali postojanje značajnih negativnih razlika u dobi između varijabla i indeksa antropometrijskih mjera i tjelesne kompozicije. Uzajamna zavisnost godina sa varijablama i indeksima pokazuje značajno negativan međuodnos. Analiza linearne regresije pokazuje da za sve varijable, godine imaju značajno negativan učinak. Također je ustanovljeno da starenjem raste frekvencija podhranjenosti individua. Zaključak ove studije ukazuje na značajno negativnu relaciju antropometrijskih varijabli i indeksa sa varijablama i indeksima sastava tijela.

Nutrition, Body Weight and Deterioration of Familial Combined Hyperlipidemia

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ABSTRACT

Lipid and apolipoprotein serum levels as a consequence of excessive nutrition in the overweight individuals with familial combined hyperlipidemia (FCHL) in comparison with the obese ones are studied only sporadically. In this study, the effect of overweight and obesity in subjects with FCHL on serum lipids and apolipoproteins was investigated. The participants were 36 overweight and 10 obese men. 17 normolipidemic healthy men served as the control group. The mean age of all subjects included was 49 ± 9 years. Lipid and apolipoprotein serum levels were determined by standard methods. The increased body weight in overweight men with FCHL correlates with increased cholesterol and triacylglycerol serum levels ($p < 0.001$), atherogenous ratio values, apolipoprotein serum levels – apo C-III, apo C-II and apo B₁₀₀ ($p < 0.001$) as well as decreased HDL cholesterol serum levels ($p < 0.05$). Lipid metabolism in men with FCHL is deteriorated by a high energy intake and its low output. The overweight and not only obesity, in association with FCHL, is an important risk factor for premature development of ischemic events.

Key words: overweight, obesity, hypercholesterolemia, hypertriacylglycerolemia, apolipoproteins, lipids

Introduction

Familial combined hyperlipidemia is a common familial lipid disorder characterized by a variable pattern of elevated levels of plasma cholesterol and/or triacylglycerols and some apolipoproteins (e.g. apo B₁₀₀)^{1,2}. It is present in 10% to 20% of patients with premature coronary artery disease. The genetic etiology of the disorder, including the number of genes involved and the magnitude of their effects is unknown. Goldstein et al. gave the designation »familial combined hyperlipidemia« to the most common genetic form of hyperlipidemia identified in a study of myocardial infarction survivors. The affected individuals characteristically showed an elevation in both cholesterol and triacylglycerols in the blood. The combined disorder was shown to be distinct from familial hypercholesterolemia (Fredrickson type II), and from familial hypertriacylglycerolemia^{1,3}.

In this study, we investigated the extent of changes in lipid and apolipoprotein (apo) serum levels as well as atherogenous ratios in overweight men with familial combined hyperlipidemia (FCHL) and with behavioral pattern of excessive nutrition and low energy output. These parameters are only rarely studied in both the overweight and obese subjects with FCHL due to a low incidence of the disease. Therefore, we compared lipid

and apolipoprotein serum levels observed in overweight and obese subjects with those observed in the control group. In the overweight subjects, apolipoprotein B₁₀₀ (apo B₁₀₀), apolipoprotein C-III (apo C-III) and apolipoprotein C-II (apo C-II) serum levels are evaluated only very rarely.

Material and Methods

Forty-six subjects with FCHL were included in the present study. These subjects (6.5%) were selected from a group 710 re-screened adult clerks. Thirty-six of them were overweight (5.1%) (OW) and 10 were obese (1.4%) (OB). The control group included 17 men (2.4%) (CONTR) with normal body weight and not suffering from any genetic lipid disorder. The lipid and apolipoprotein serum levels were assessed after overnight fasting. The genetic causes of FCHL were not analyzed because of an exclusively epidemiological character of the present study. The presence of a hereditary disorder was concluded on the basis of positive history of FCHL in the first line family relatives using genealogical analysis. The criteria for FCHL included serum levels of triacylglycerols higher

than 2.3mmol/l and the total cholesterol higher than 6.2mmol/l after overnight fasting. Furthermore, LDL cholesterol and the atherogenous ratios were also calculated. Daily food consumption was estimated on the basis of questionnaires.

From the anthropometrical variables, the following ones were determined: the body weight, body height, surface of the body and body mass index (BMI kg/m²). BMI we used as a criterion to distinguish among normal weight, overweight and obesity⁴. Peripheral blood was collected after overnight fasting (12 hour).

The total cholesterol (TC), triacylglycerols (TG) and HDL-cholesterol (HDL-C) serum levels were assessed by using standard Pliva-Lachema sets (Brno, Czech Republic). The LDL cholesterol (LDL-C) and non-HDL cholesterol (non-HDL-C) serum levels were calculated using the Friedewald's⁵ and De Backer's⁶ formula, respectively. Vitamin C serum levels were assayed colorimetrically according to the method of Roe and Kuether⁷.

Apolipoprotein C-II and C-III serum levels were determined by the method of radial immunoassay according to Mancini⁸. Antibodies and standards purchased from Daiichi Company (Tokyo, Japan) were used for their determination. Serum levels of apo B₁₀₀ were detected by the electroimmunoassay according to the method of Curry⁹ using the standards and antibodies from Behringwerke Company (Marburg, Germany).

Statistics

The data were analyzed using the univariate and bivariate analysis, χ^2 -test, Pearson correlation test as well as partial correlation including canonical correlation and McNemar's test. The relative risk according to Sato's computation was also calculated. The standard deviation, median, percentile distribution, including the determination of 95% confidence limit (CL95%), and confidence interval (CI 5–95%) were calculated using the descriptive statistics (p value <0.05 was accepted as significant).

Results

The mean body weight and its CL95% were increased by 2.43 kg/m² and 3.1 kg/m², respectively, in overweight subjects, in comparison with the control group (11.8%). The mean body mass index in the overweight subjects was 27.8±1.4 kg/m². The food intake in overweight and obese subjects was compared to the control group and is described in Table 1. The p value was calculated for OW versus CONTR.

In overweight vs. control subjects, the mean serum levels of TC, LDL-C, TG and non-HDL-C were significantly increased by: 71.7%; 91.8%; 283.5%, and 104.2% (p<0.001, Table 2), respectively. Serum levels of HDL-C were significantly decreased by -5.8% (p<0.05). The mean values of TC/HDL-C, LDL-C/HDL-C, and high TG/low HDL-C known as the atherogenous ratios were significantly increased by 82.4%; 103.8%; and 308.1%

TABLE 1
THE FOOD INTAKE AND NUTRITION STATE IN HEALTHY MEN (CONTROL GROUP) AND OVERWEIGHT AND OBESE SUBJECTS WITH FCHL

Food intake	CONTR	OW	OB	p
Glycides (%)	51	50–54	52–47	<0.05
Fat (%)	25	30–28	35–42	<0.01
Proteins (%)	24	20–18	13–11	<0.01
Fiber (g)	25–28	15–18	8–11	<0.01

CONTR – control group, OW – overweight subjects, OB – obese subjects, p – probability

(p<0.001), respectively. The mean value of the TG/apo C-II atherogenous ratio was increased by 96.6% (p<0.003). The mean value of the HDL-C/LDL-C atherogenous ratio was significantly decreased by -50.8% (p<0.01, Table 2).

The mean serum levels of apo B₁₀₀ were significantly increased not only the obese but also in overweight subjects by 63.9% (p<0.001) and 58.5% (p<0.01), respectively. In overweight subjects the apo C-II serum level was increased by 93.3% and apo C-III by 161.8% (p<0.001, Table 2).

In Table 3 we summarize the confidence intervals (CI 5–95%) of all variables studied. In the overweight subjects and not only in the obese ones, differences of the TC serum levels significantly increased by 69.8% (p<0.01). Differences of the LDL-C, TG and non-HDL-C serum levels in CL95% also significantly increased by 100.7%, 357.7% and 108.1% (p<0.001). Significant differences in extent of 82.4% we detected in the TC/HDL-C ratio. Significant differences in extent of 116.7% in CL95% we detected in the LDL-C/HDL-C ratio.

In the overweight and obese men with FCHL we found in CL95% against control group significantly highly increased apo B₁₀₀ (p<0.01), apo C-II (p<0.03; p<0.01) and extremely high apo C-III serum levels (p<0.001).

The significant increases in triacylglycerol serum levels after overnight fasting in overweight subjects are associated with increases in the BMI mean value (27.8±1.4 kg/m²). Even more pronounced increases in TG serum levels were observed in obese men. Elevation in triacylglycerol serum levels is also associated with a significant decrease in the mean, median and CL95% values of HDL-C serum levels (p<0.05). The mean HDL-C/LDL-C ratio significantly decreased in overweight subjects (p<0.01). The differences in TG/HDL-C ratio values between the overweight and obese subjects versus the control group were extremely high (p<0.001).

Consistently with the changes in serum lipid levels, apolipoprotein serum levels were also more significantly elevated in obese men than in overweight subjects. In 8.7% of overweight subjects we detected significantly increased apo B₁₀₀ serum levels (p<0.01) but only a moderate increase in TC. This finding (after overnight fasting) is consistent with a picture of the hereditary hyperapobetalipoproteinemia¹⁰.

TABLE 2
DISTRIBUTION OF THE LIPID AND APOLIPOPROTEIN SERUM LEVELS IN OVERWEIGHT AND OBESE SUBJECTS VERSUS CONTROLS

Biological variables	OW		OB		CONTR		OW vs. CONTR		OB vs. CONTR	
	X±2SD	MD	X±2SD	MD	X±2SD	MD	Δ%	p	Δ%	p
BMI (kg/m ²)	27.8±1.38	28.50	32.80±2.81	32.80	25.37±0.64	25.10	9.58	<0.05	29.29	<0.05
TC (mmol/l)	6.80±1.25	7.40	6.76±0.92	6.70	3.96±0.46	3.72	71.72	<0.001	70.71	<0.001
LDL-C (mmol/l)	4.20±1.27	5.10	3.86±1.06	3.94	2.19±0.26	2.04	91.78	<0.001	76.26	<0.001
HDL-C (mmol/l)	1.29±0.14	1.32	1.27±0.24	1.25	1.37±0.23	1.35	-5.84	<0.05	-7.30	<0.05
TG (mmol/l)	3.26±1.41	2.51	4.21±2.15	3.60	0.85±0.21	0.94	283.53	<0.001	395.29	<0.001
non-HDL-C (mmol/l)	5.41±1.27	6.10	5.49±0.87	5.49	2.65±0.29	2.50	104.15	<0.001	107.17	<0.001
TC/HDL-C	5.27±0.36	5.61	5.32±0.23	5.36	2.89±0.13	2.42	82.35	<0.001	84.08	<0.001
LDL-C/HDL-C	3.26±0.57	4.62	3.04±0.22	3.15	1.60±0.07	1.51	103.75	<0.001	90.00	<0.001
HDL-C/LDL-C	0.31±0.05	0.22	0.33±0.02	0.32	0.63±0.02	0.66	-50.79	<0.01	-47.62	<0.01
TG/HDL-C	2.53±0.74	1.90	3.31±0.90	2.88	0.62±0.04	0.70	308.06	<0.001	433.87	<0.001
TG/apo C-II	0.57±0.03	0.48	0.56±0.12	0.46	0.29±0.05	0.31	96.55	<0.003	93.10	<0.003
Apo B ₁₀₀ / LDL-C	25.40±3.79	17.05	28.58±2.26	28.43	30.73±0.65	30.88	-17.34	<0.05	-7.00	ns
apo B ₁₀₀ (mg/dl)	106.7±11.5	104.00	110.3±19.20	112.00	67.30±9.59	63.00	58.54	<0.01	63.89	<0.001
apo C-II (mg/dl)	5.74±2.00	5.20	7.58±1.75	7.80	2.97±0.18	3.00	93.27	<0.001	155.22	<0.001
apo C-II (mg/dl)	11.52±4.58	10.50	16.34±4.96	15.80	4.40±1.01	4.00	161.82	<0.001	271.36	<0.002
Vitamin C (μmol/l)	48.30±19.80	43.90	43.40±17.7	42.80	50.60±9.06	49.40	-4.55	ns	-14.23	<0.05

OW – overweight subjects, OB – obese subjects, CONTR – control group, X – mean, SD – standard deviation, MD – median, Δ% – percentage differences, p – probability, ns – not significant

TABLE 3
DISTRIBUTION OF THE VARIABLES IN CI 5–95% INCLUDING DIFFERENCES IN THE CI95% IN THE OVERWEIGHT AND OBESE VERSUS CONTROL GROUP

Biological variables	OW	OB	CONTR	OW vs.	P	OB vs.	P
	(CI 5–95%)	(CI 5–95%)	(CI 5–95%)	CONTR Δ%		CONTR Δ%	
BMI (kg/m ²)	25.10–29.40	30.09–36.80	24.58–26.30	11.79	<0.05	39.92	<0.02
TC (mmol/l)	4.77–7.93	5.45–8.52	3.44–4.67	69.81	<0.01	82.44	<0.01
LDL-C (mmol/l)	2.14–5.40	2.20–5.54	2.01–2.69	100.74	<0.001	105.95	<0.001
HDL-C (mmol/l)	1.22–1.60	0.98–1.64	1.08–1.72	-6.98	<0.05	-4.65	ns
TG (mmol/l)	2.31–5.63	2.37–6.22	0.62–1.23	357.72	<0.001	405.69	<0.001
Non-HDL-C (mmol/l)	3.31–6.45	4.21–7.18	2.36–3.10	108.06	<0.001	131.61	<0.001
TC/HDL-C	3.91–4.96	5.56–5.20	3.19–2.72	82.35	<0.01	91.18	<0.01
LDL-C/HDL-C	1.75–3.38	2.24–3.38	1.86–1.56	116.34	<0.001	116.54	<0.001
HDL-C/LDL-C	0.57–0.30	0.45–0.30	0.54–0.64	-53.13	<0.01	-53.13	<0.01
TG/HDL-C	1.89–3.52	2.42–3.79	0.57–0.72	388.89	<0.001	426.39	<0.001
TG/apo C-II	0.68–0.69	0.46–0.64	0.11–0.19	263.16	<0.003	236.84	<0.003
Apo B ₁₀₀ / LDL-C	44.09–23.00	38.18–25.54	29.45–31.71	-27.47	<0.05	-19.46	<0.05
Apo B ₁₀₀ (mg/dl)	94.35–124.20	84.0–141.50	59.20–85.30	45.60	<0.01	65.89	<0.01
Apo C-II (mg/dl)	3.45–8.21	5.18–9.75	5.40–6.46	27.09	<0.03	50.93	<0.01
Apo C-III (mg/dl)	6.27–17.44	8.93–24.00	3.17–6.03	189.22	<0.001	298.01	<0.001
Vitamin C (μmol/l)	23.50–76.68	20.20–74.58	36.86–67.70	13.26	<0.05	10.16	<0.05
N	10	36	16				

OW – overweight subjects, OB – obese subjects, CONTR – control group, CI 5–95% – confidence interval, Δ% – differences in CL95%, p – probability, N – sample size, ns – not significant

Not only in obese subjects but also in overweight men we found significant correlation between the mean serum levels of apo C-II and TG ($r=0.33$, $p<0.04$). This is a sign of a tight metabolic relationship between the two variables. Significant changes were also detected in the TG/apo C-II ratio. Its value in CL95% was nearly three-times higher in obese and overweight subjects than in the control group ($p<0.003$, Table 3).

The TG/HDL-C ratio shows a significant inverse correlation with body mass index: $r=-0.48$ ($p<0.002$) in overweight adults. In addition, the apo B₁₀₀/LDL-C ratio directly correlates with body mass index: $r=0.47$ ($p<0.002$).

In Figure 1, a regression curve between apo C-II and apo C-III is shown. This regression confirms a close relationship ($p<0.01$) between both variables in overweight subjects. The correlation coefficient has the value: $r=0.41$ ($p<0.01$) at the t-value: 2.63.

In Figure 2, a regression curve is shown between apo C-III and HDL-C serum levels in overweight subjects. We

found an inverse relationship ($p<0.01$) between both variables: $r=-0.41$ at the t-value: 6.367.

Discussion

Traditionally, in our geographic region, moderate body weight excess is accepted as a sign of good health. This misleading view, together with low social status and behavioural patterns of excessive eating are the main causes of consuming a diet rich in fat (mainly saturated) and poor in fruits and vegetables what is associated with a body weight gain. Usually, the frequency of FCHL is relatively low but is increased in overweight and obese subjects^{11,12}.

Over-nutrition deteriorates genetically caused diseases of the lipid metabolism and this is evidently notable on the mean and CL95% of the lipids and apolipoproteins serum levels in overweight and obese subjects.

FCHL, as expressed by the changes in lipid and apolipoprotein serum levels, is besides genetic causes deteriorated also by an undesirable overeating. Significant elevation in LDL-C and TG serum levels and raised TG/HDL ratio after overnight fasting in overweight and obese men demonstrates this relationship.

The HTG after overnight fasting, as a dominant finding, is significantly expressed in overweight and obese subjects by significant elevation of apo C-III serum levels. This is an indirect signal of increased production of triglyceride rich-lipoprotein (TG-RLPs) in the liver. Elevated apo C-III serum levels are a reliable indirect sign of the production of these large lipoprotein particles. The TG-rich lipoprotein particles usually display a delayed catabolism that is among other reasons also caused by a direct inhibitory effect of apo C-III on the lipoprotein lipase activity^{13,14}. The TG-RLPs remnants are delayed in circulation. Therefore they are more susceptible to oxidation. Simultaneously with their increased uptake to by macrophages, they are deposits into the arterial wall. This explains why these large lipoprotein particles are atherogenous. The storage of TG in the adipocytes of subcutaneous fat mass and predominantly viscerally is the main cause of the body weight gain. However, accumulation of TG associates with significant increase in the apo C-III serum levels and their increased production has a malicious impact on the catabolism of TG-RLPs. Significant elevation of these variables potentiates the risk of premature development of atherosclerosis leading to an increased frequency of ischemic events in the heart, brain and peripheral arteries¹⁵. Our data suggest that not only obesity but also overweight, particularly in combination with genetic causation of the FCHL, is associated with serious changes in lipid and apolipoprotein serum levels^{2,12}.

High TG-RLPs serum levels are an indirect marker of endothelial dysfunction and through this pathway, they amplify the global risk of ischemic events¹². Therefore, they play a significant role in triggering the early phases of atherosclerosis and mainly atherothrombosis^{16,17,18} through the pathways of plaque destabilization. Usually, HTG as-

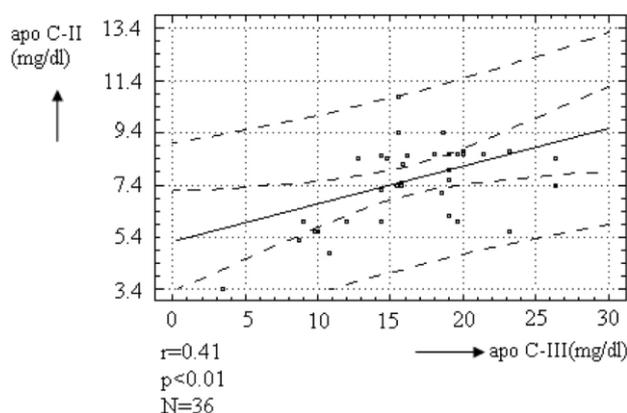


Fig. 1. Regression of the apo C-II and apo C-III in the overweight (OW) subjects. r – correlation coefficient, p – probability, N – sample size.

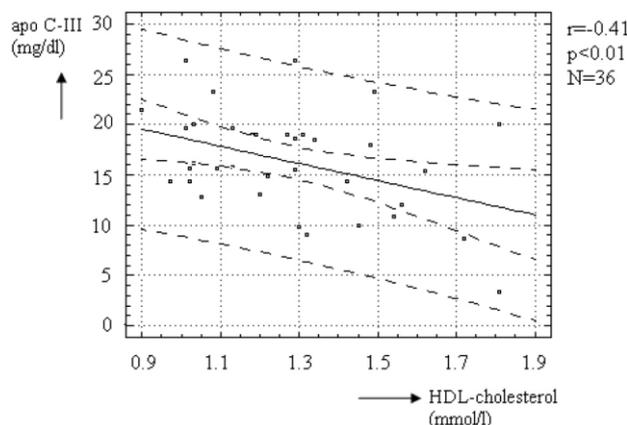


Fig. 2. Regression of the apo C-III and HDL-C in the overweight (OW) subjects. r – correlation coefficient, p – probability, N – sample size.

sociates with low serum levels of HDL-C and is an important clinical sign for dyslipidemia. The mechanism of this type of disorder is sophisticated. In overweight and obese subjects it is also potentiated by physical inactivity and an intake of high amounts of fat, mainly saturated and trans-fatty acids (Table 1). We assume that the significantly increased value of TG/apo C-II ratio in CL95% may be used as a useful indirect sign for delayed catabolism of TG-RLPs (Table 3). It seems to be evident that there is a significant disproportion between apo C-II and triacylglycerol serum levels in subjects with overweight. Nearly three times more increased apo C-III serum levels against the control group means that activation of the lipoprotein lipase by apo C-II is not satisfactorily efficient in the lipolytic pathway of lipoprotein particles in spite of its increased serum levels. With high probability we may exclude genetic defects and apo C-II deficit in the overweight and obese subjects as one of the FCHL causes. Generally, genetic defect in apo C-II is a very rare event because mostly it is not compatible with survival.

Inhibitory effect of apo C-III on the activity of lipoprotein lipase and lipolysis of TG-RLPs is also experimentally proven in transgenic mice^{19–21}. Increased apo C-III serum levels generally associate with production of TG-RLPs via inhibition of their clearance²².

The high serum levels of apo C-III, as a marker of premature acute ischemic events, show a 3.4-times higher ability to predict ischemic heart disease (IHD). Apo C-III has a specific effect in patients with HTG because it decreases apo E-mediated remnant removal by apo E displacement from VLDL particles²³. This mechanism contributes to HTG via blocking the binding of VLDL or IDL particles on the LDLR-1, LDLR-2, eventually also VLDLR and SR-BI receptor.

Not only obesity but also overweight closely associates with development of metabolic syndrome and has a close relationship to the development of insulin resistance^{24,25}. This explains why also the overweight subjects with genetically caused FCHL, increased blood pressure and low HDL cholesterol, are threatened with premature development of diabetes mellitus type 2, IHD, stroke and atherosclerosis of the peripheral arteries.

We propose to introduce to the clinical practice the calculation not only the LDL-C/HDL-C ratio in CL95% but also the high TG/low HDL-C ratio for its high potential to predict premature development of IHD and of stroke^{26,27}.

HTG detected after overnight fasting in the overweight subjects with FCHL associates with a significant increase in the apo B₁₀₀ serum level. Significant elevation in the apoB₁₀₀/LDL-C ratio value is a valuable pathogenic marker of a disproportion between apo B₁₀₀ and LDL-C serum levels, and an indirect sign of the LDL particles type B production, as well as a reliable predictor of the premature development of IHD and myocardial in-

farction^{11,28,29}. The TG serum levels equal or over 1.7 mmol/l in the overweight and obese subjects serve as an indirect predictor of atherogenic LDL particles production (ALP) and hence a cause of endothelial dysfunction^{16,30}.

Significant depletion of HDL-C has a negative influence on its anti-inflammatory and anti-atherosclerotic effects, from which thus the overweight subjects cannot benefit³¹.

Very tight correlation between HTG (after overnight fasting) and BMI in the overweight, as well as obese persons indicates a very close metabolic association between with deposition of TG into adipose tissue and over-nutrition, high-energy intake and mainly low energy output. It is the typical »acquired« behavioral pattern of the people with sedentary lifestyle and low physical activity^{2,32,33}.

Significant increase in the TG-RLPs in the obese and overweight subjects frequently associates with serious disorders in platelet aggregability. HTG triggers hyperactivity of the platelets with a tendency to hypercoagulation states. In addition, significant increase in the plasma apo B₁₀₀ levels predicts platelet-dependent thrombosis in patients with coronary artery disease³⁴.

Significant depletion of vitamin C serum levels in the HTG state in the overweight and obese subjects is an important sign of decreased anti-oxidative activity. Decreased vitamin C serum level is generally accepted as a predisposition for higher oxidative risk^{17,35–37}.

These changes detected in the overweight subjects with FCHL indicate that not only obesity but also the overweight is not a benign and tolerable biological, socio-cultural and socio-educational marker due to the above mentioned reasons.

Conclusion

The FCHL as a genetically caused disease is deteriorated by a high-energy intake and sedentary lifestyle because of serious changes in the lipid and apolipoprotein serum levels. In both the overweight and obese subjects, the HTG detected after overnight fasting, is associated with a significant increase in serum levels of apo C-III, apo C-II, apo B₁₀₀ and TG, as well as the HDL-C depletion. Surprising is the finding of the increased apo C-II serum levels because it is in contradiction to the traditional view. It might be a compensatory attempt to decrease high serum levels of triacylglycerols. Increased apo C-III serum levels in association with HTG (after overnight fasting) are a sign of TG-RLPs overproduction. This is associated with delayed catabolism of lipoproteins and triggering oxidation of lipoprotein particles and production of small LDL atherogenic particles. Decreased HDL-C serum levels indicate a lipid disorder what can be extremely hazardous mainly in adults with FCHL and increased body weight.

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PREHRANA, TJELESNA TEŽINA I POGORŠAVANJE OBITELJSKI NASLJEDNE HIPERLIPIDEMIJE

SAŽETAK

Razina lipida i apolipoproteina u serumu kao posljedica pretjeranog unosa hrane kod osoba s prekomjernom tjelesnom težinom s obiteljski nasljednom hiperlipidemijom (FCHL) u usporedbi s pretilim osobama proučava se sporadično. U ovome istraživanju proučavao se utjecaj prekomjerne tjelesne težine i pretilosti kod osoba s FCHL na razinu serumskih lipida i apolipoproteina. Učesnici u ovom istraživanju bili su 36 muškaraca s prekomjernom tjelesnom težinom i 10 pretilih muškaraca. 17 zdravih muškaraca s normalnom razinom lipida činili su kontrolnu skupinu. Srednja starosna dob svih ispitanika bila je 49 ± 9 godina. Razine lipida i apolipoproteina u serumu određivane su upotrebom standardiziranih metoda. Tjelesna težina i kod muškaraca s prekomjernom tjelesnom težinom s FCHL, a ne samo kod pretilih muškaraca, povezana je s povišenom razinom kolesterola i triglicerida u serumu ($p < 0.001$), aterogenim promjenama, razinom apolipoproteina u serumu – apo C-III, apo C-II i apo B₁₀₀ ($p < 0.001$) te sa smanjenom razinom HDL kolesterola u serumu ($p < 0.05$). Metabolizam lipida kod muškaraca FCHL pogoršava se s visokim unosom energije u odnosu na malu potrošnju. Prekomjerna tjelesna težina, a ne isključivo pretilost povezana s FCHL, značajan je rizični faktor za prerani nastanak ishemijske bolesti.

Body Mass Index and Nutritional Status of the Bayash Roma from Eastern Croatia

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ABSTRACT

This study examines anthropometrically assessed nutritional status of the Bayash, the Roma population from the eastern Croatian region of Baranya, and compares it to the non-Roma general population of eastern Croatia. The analysis of nutritional status and diets is a segment of multidisciplinary anthropological and epidemiological survey of the Roma minority population in Croatia began in 2005. The Bayash are an ethnic group that arrived to Croatia from Romania most likely in the 19th century and speaks a distinct archaic dialect of the Romanian language. The Roma population of Baranya approximates 1,000 according to the 2001 census. The Bayash sample comprised 227 adults aged 18–65 yrs. The women fall below the Croatian 10th percentile for stature and men track about the 10th percentile. Both sexes approximate the 25th percentile for body weight. Despite their diminutive size, the Bayash appear to have adequate nutritional status until the age of 35 yrs after which their average BMI exceeds the value of 25 kg/m² and falls in the overweight category. However, 8% of Bayash are underweight (BMI < 18.5) in contrast to 1% of the majority population in the region. Underweight rates are especially high in women (11%) compared to men (4%). The prevalence of overweight (BMI 25.0 to 29.9) of 30% is considerably lower than in the majority population (42%) while the prevalence of obesity (BMI ≥ 30.0) of 23% is approximately equal. Overall unsatisfactory nutritional status of the Bayash merits attention. It appears to be the product of unhealthy dietary habits and their socio-economic deprivation that resulted from their poor education and extremely high unemployment.

Key words: Roma minority, Bayash, nutritional status, BMI, Croatia

Introduction

Roma, an ethnic minority of northern Indian origin, live in many countries throughout the world and are well known for remarkably preserved traditions and resistance to assimilation¹. They are most often marginalized economically, spatially, politically and in terms of culture². Croatia has a substantial Roma population, the exact size of which is uncertain. In the 2001 Census, only 9,463 people declared being Roma (0.21% of the total population of Croatia). Another official estimate puts the Roma population at 30,000 to 40,000 (about 1% of the total population of Croatia)³. Whilst it is widely believed that the health of Roma people is often poorer than that of the majority population⁴, these inequalities remain largely unresearched.

Beginning in 2005, multidisciplinary anthropological and epidemiological survey of the Roma minority population has been undertaken in Croatia. One segment of this survey relates to their health status and includes the analyses of nutritional status and diets. The first phase of the survey focused on the Roma population in the eastern Croatian region of Baranya, approximating 1,000 according to the 2001 census. It is a Bayash ethnic group that arrived to Croatia from Romania most likely in the 19th century and speaks a distinct archaic dialect of the Romanian language¹.

Nutritional status of the majority population in the region of eastern Croatia is well documented but little is

known about the Bayash population. The purpose of this report is to present the initial information on nutritional status of the adult Bayash from Baranya assessed by the body mass index (BMI) and to compare it with the general population of eastern Croatia.

Subjects and Methods

The data reported here are a subset of the extensive material collected in the Bayash population which is settled in six villages and small towns of the eastern Croatian region of Baranya (Figure 1). Subjects, aged 18 to 65 yrs (mean: 40.5±13.4 men, 42.4±12.2 women), were all volunteers. The sample comprised 227 subjects (80 men and 147 women). Age and sex distributions of the sample are shown in Table 1.

Weight and stature were measured following standard International Biological Programme Protocol⁵. Weight was determined to the nearest 0.5 kg using a portable scale. Stature was measured to the nearest mm using a fixed stadiometer. The body mass index (BMI) was calculated as $\text{weight}[\text{kg}]/\text{stature}[\text{m}]^2$. In order to define the categories of nutritional status based on BMI, the WHO⁶ cutoff points were used. For comparative purposes, the representative sample of the general population from the region of eastern Croatia aged 18–65 yrs was utilised⁷. Numerical descriptions of the distributions of body measurements include means, standard deviations and selected percentiles.

Results

The age- and sex-specific descriptive statistical parameters for stature, weight and BMI are reported in Table 1. In general, the Bayash show low values of both primary anthropometric dimensions. This point is particularly evident in Figure 2, showing stature for Bayash



Fig. 1. Geographical map showing the location of Baranya in eastern Croatia.

men and women relative to the Croatian 5th and 15th percentiles. As shown, Bayash women fall below the Croatian 10th percentile and men track about the 10th percentile. Both sexes approximate the 25th percentile for body weight.

Despite their diminutive size, the Bayash appear to have adequate nutritional status until the age of 35 yrs after which their average BMI exceeds the value of 25 kg/m² and falls in the overweight category (Table 1). Their BMI ranges between the Croatian 50th and 75th percentile (Figure 3). The obtained prevalence within WHO nutritional status categories raises concern. The prevalence by sex and the combined prevalence are presented in Table 2. As much as 8% of Bayash are under-

TABLE 1.
AGE-SPECIFIC MEANS AND STANDARD DEVIATIONS OF ANTHROPOMETRIC DIMENSIONS FOR BAYASH MEN AND WOMEN

Age (y)	N	Stature (m)		Weight (kg)		BMI (kg/m ²)	
		X	SD	X	SD	X	SD
Men	80						
18–24	12	1.690	0.078	65.9	14.2	23.0	4.3
25–34	16	1.705	0.061	72.6	17.0	23.9	4.7
35–44	25	1.670	0.060	80.7	20.5	28.5	6.5
45–54	14	1.679	0.046	77.5	13.5	27.5	4.7
55–65	13	1.626	0.051	71.0	11.2	26.9	4.5
Women	147						
18–24	9	1.595	0.089	56.2	12.7	22.0	4.1
25–34	32	1.564	0.055	56.1	10.2	22.9	4.1
35–44	39	1.534	0.054	63.3	16.2	26.8	6.4
45–54	35	1.528	0.048	60.3	13.1	26.0	6.5
55–65	32	1.524	0.067	68.6	18.6	29.4	7.7

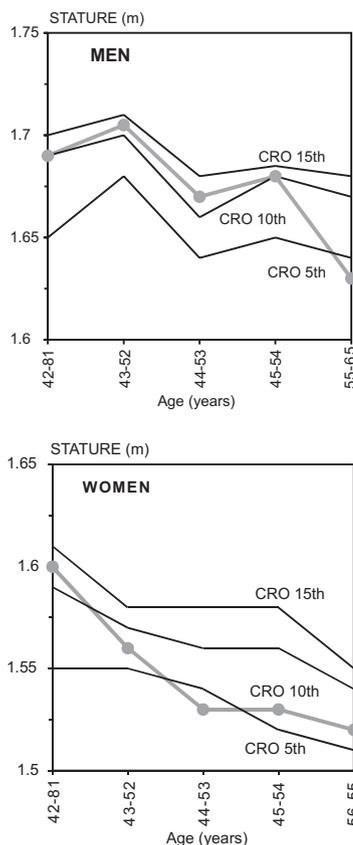


Fig. 2. The Bayash population means (—●—) and the selected Croatian percentiles (—) for stature.

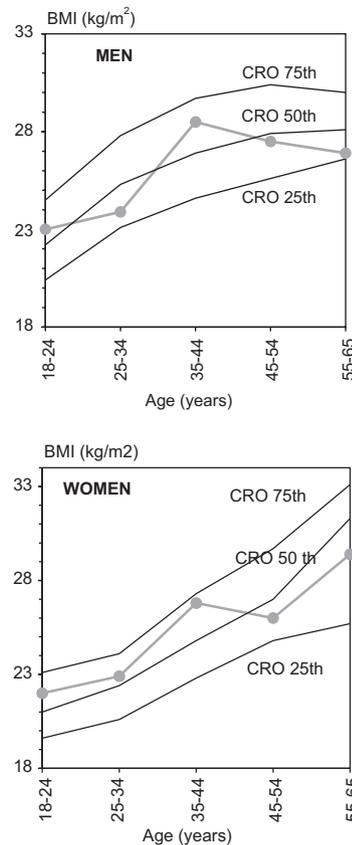


Fig. 3. The Bayash population means (—●—) and the selected Croatian percentiles (—) for BMI.

weight in contrast to 1% of the majority population in the region. Underweight rates are especially high in women (11%) compared to men (4%). The prevalence of overweight of 30% is considerably lower than in the majority population (42%) but the rate is markedly higher in men compared to women (40% vs. 24%). The prevalence of obesity among the Bayash approximates the rate in the Croatian majority population.

Discussion

The research on the nutritional status and dietary habits among the Roma is limited and difficult to access. Findings for the Bayash Roma from eastern Croatia re-

vealed greater occurrence of underweight compared to the local majority population but also a relatively high prevalence of obesity, and suggest inadequate nutrition. The problem of underweight is striking among women.

Traditionally the eating habits of Roma have been conditioned by their nomadic way of life and their diet has consisted largely of what was readily available⁸. As the Roma have gradually settled, their eating habits have gradually conformed to those of the majority population in the region of their residence. The anthropological and epidemiological survey of the Roma population from Baranya included examination of its nutritional habits⁹. The collection of these data presented many difficulties because this kind of information is believed to be very in-

TABLE 2.
PREVALENCE BY NUTRITIONAL STATUS CATEGORIES IN THE BAYASH AND THE MAJORITY POPULATION IN THE REGION

BMI	Bayash			Croatian
	Men	Women	Both sexes	Both sexes
<18.5 (underweight)	4%	11%	8%	1%
18.5 – 24.9 (normal)	36%	40%	39%	35%
25.0 – 29.9 (overweight)	40%	24%	30%	42%
≥ 30.0 (obesity)	20%	25%	23%	22%

timate. However, it pointed to the unhealthy dietary habits. Their main characteristics are irregular meals, high consumption of animal fat but low consumption of meat and milk products, low consumption of fresh vegetables and fruit, and high consumption of coffee and alcohol. It seems that the Bayash people in Baranya experience nutritional problems similar to other Roma groups in Central European countries^{10–13}.

There are many reasons to suppose that the unsatisfactory nutritional status and unhealthy dietary habits observed among the Bayash people can be associated with poverty. The Bayash minority has, with very few exceptions, a low socio-economic status resulting from prevailing unemployment (registered 79%), low levels of education (35% without any formal education), and the majority living from social welfare (69%)⁹. It seems that these problems are shared by the Roma living in transitional as well as in industrialized countries^{4,14}.

In conclusion, the findings of nutritional status of the Bayash Roma merit attention and reflect their socio-economic deprivation. It is partly due to their poor education and extremely high unemployment as well as to the

economic and social difficulties the region of Baranya is going through in the process of transition. Overall, the results highlight the importance of further work on determining body composition and sex differences in lifestyle correlates of nutritional status of the Bayash.

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INDEKS TJELESNE MASE I PREHRAMBENO STANJE ROMA BAJAŠA IZ ISTOČNE HRVATSKE

SAŽETAK

U radu je ispitivano antropometrijski procijenjeno prehrambeno stanje Roma Bajaša iz Baranje te uspoređeno s prehrambenim stanjem opće populacije istočne Hrvatske. Analiza prehrambenog stanja i prehrambenih navika Bajaša sastavni je dio multidisciplinarnih antropoloških i epidemioloških istraživanja romskog manjinskog stanovništva Hrvatske koja su započeta 2005. godine. Bajaši su doselili u 19. stoljeću iz Rumunjske i govore specifičnim arhaičnim dijalektom rumunjskog jezika. Procjenjuje se da je veličina današnje romske populacije u Baranji oko 1000 osoba. Uzorač je obuhvatio 227 odraslih Bajaša u dobi od 18–65 godina života. Visina tijela žena kreće ispod 10. percentila opće populacije istočne Hrvatske, a visina muškaraca kreće se oko 10. percentila. Težina tijela kreće se oko 25. percentila u oba spola. Prehrambeno stanje Bajaša, procijenjeno na temelju indeksa mase tijela (BMI), zadovoljavajuće je do dobi od

35 godina, nakon čega prosječne vrijednosti BMI premašuju vrijednost od 25 kg/m² i ukazuju na prekomjernu težinu. Utvrđeno je, međutim, da 8% bajaške populacije ima sniženu tjelesnu težinu (BMI<18.5) u usporedbi s 1% u općoj populaciji istočne Hrvatske. Smanjena tjelesna težina znatno je učestalija kod žena (11%) nego kod muškaraca (4%). Učestalost prekomjerne težine (BMI 25.0 to 29.9) od 30% kod Bajaša znatno je niža nego što je to slučaj u općoj populaciji regije (42%), dok je učestalost pretilosti (BMI≥30.0) od 23% podjednaka u obje populacije. Prehrambeno stanje Bajaša općenito nije zadovoljavajuće i posljedica je nezdravih prehrambenih navika i lošeg socioekonomskog statusa čiji su primarni uzroci nedostatan obrazovanje i visoka stopa nezaposlenosti pripadnika ove romske populacije.

Vaccination Coverage in Hard to Reach Roma Children in Slovenia

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ABSTRACT

The results of the retrospective analysis of data on vaccination coverage in the preschool-aged and school-aged Roma children (436 preschool and 551 schoolchildren) in three geographical regions of Slovenia were analyzed to establish the differences concerning coverage for specific vaccinations: poliomyelitis, diphtheria, tetanus, pertussis, measles, mumps and rubella between the two generation. The data were obtained from health records, immunization records (Vaccination booklet) and National Computerized Immunization System (CEPI 2000®). Vaccination coverage was calculated by comparing the number of children eligible for immunization with the number of vaccinated children. This article performs the log-rank statistical test, also known as the Mantel-Haenszel test. Log rang test is comparing survival curves for two generations. Preschool-aged Roma children showed higher vaccination coverage than the school-aged Roma generation. There was no significance difference in the generations of preschool aged and school aged Roma children fully vaccinated against poliomyelitis, diphtheria, tetanus and pertussis. Rubella vaccination was significantly lower in the school aged Roma generation. Only 33% of school aged Roma population received two doses of measles, mumps and rubella vaccine. Vaccination coverage of preschool Roma children in Slovenia against poliomyelitis, diphtheria, tetanus, pertussis and MMR (measles, mumps, rubella) were significantly lower then the national vaccination coverage for preschool aged Slovenia children. Many joint efforts will have to be made to improve the vaccination coverage in Roma communities.

Key words: Roma people, vaccination coverage, immunization, communicable diseases, Slovenia

Introduction

In 2004, Roma population in Slovenia numbered 8.000 to 10.000. Roma people live in settlements isolated from the rest of the population, mainly in the northeastern and southeastern parts of the country and in the surroundings of Ljubljana¹. Except in the northwestern region of Slovenia where they have been fully assimilated into the society, lifestyle of the Roma people in Slovenia differs markedly from that of other population. In Slovenia, like elsewhere in the world, a large proportion of Roma are poorly educated; and very few have a regular job². Roma families are usually large and the income per family member is low^{3,4}.

Their way of life, contemporary society and its rules are directed by culture, habits, customs and tradition, which were shaped by centuries-long nomadic way of life, isolation and cultural influence of different societies

where they were stationed for longer periods. Their health culture was shaped the same way and is reflected in their relation toward health, diseases, life, death and health service in general. The health service relations are conditional with the remains of traditional relations towards illness and healing. It is distinctive for Roma that they do not like to visit health services¹. The Roma seek medical attention only when they are severely ill or injured⁵. They practice various purification rites and other rituals to protect themselves against disease⁶. Although, Roma have settled down and are more or less civilized, accepted habits of surroundings and with that, changed their relation toward health and diseases, they still stayed strongly connected with their tradition. This is confirmed by the fact that living conditions play crucial part in Roma health situation. Because of their social

isolation they face a number of problems⁷. Because of unsatisfying knowledge about health services they do not attend systematic examinations and regular vaccinations for children, and because of that Roma children are recovering infectious children diseases on untypical manner. Many of them are recovered when they grow up and some of them are never recovered after all. As a result, such children have poor immunity and are not so physically and mentally resistant but are more susceptible for other diseases¹. Outbreaks of infectious diseases and small-scale family epidemics occur more frequently among Roma people than in the general population^{8,9}. Ten years ago, an outbreak of measles in Slovenia occurred in a Roma settlement¹⁰.

The Slovene health care network is well-developed, and health services are easily accessible to all who need them¹¹. Immunization programs for preschool and school children are financed with state budget funds, and they are conducted in compliance with the Infectious Diseases Act and Immunoprophylaxis and Chemoprophylaxis program. At birth, children are vaccinated against tuberculosis, and by the time they complete primary school they receive vaccinations against diphtheria, tetanus, pertussis, *Haemophilus influenzae type b*, poliomyelitis, measles, mumps, rubella and hepatitis B¹³. The health status of Roma people is an important indicator of health efficiency in the respective country. At asserting their health protection, Roma have same rights as the rest of citizens; they are treated under the same conditions. They have same health insurance like uninsured citizens under the 15. article of Health Care and Health Insurance Act. Because of poor knowledge, isolation and cultural conditioned points of view, they do not use all rights and possibilities and mostly do not even know the procedures for how to administer them. Therefore they look up for health services much less than other citizens. It is distinctive that they do not use preventional means of health care, such as systematical examinations, consulting and vaccinations.

The aim of this retrospective data analyses was to assess the level of vaccination coverage in Roma children in Slovenia, to determine possible differences in the coverage between preschool-aged (6 years of age) and school-aged (16 years of age) Roma children and to compare this results with the average national coverage level. The proportion of Roma children fully vaccinated against childhood diseases was expected to be lower than the national average which is 87% to 98%. The vaccination coverage in preschool-aged Roma children was expected to be higher than in the school-aged Roma generation. Another issue investigated was further implementation of the prescribed course of vaccinations in school children¹².

Our aim was to use these data to make proposals for improving the existing situation, and to put forward effective measures for raising the level of vaccination coverage in Roma children. In addition, we were interested in whether the vaccination coverage for poliomyelitis, diphtheria, tetanus, pertussis, mumps, measles and rubella has been increasing.

Materials and Methods

Population

This retrospective data analyses involved a preschool cohort of 436 six year old Roma children and a school aged group comprising 551 Roma children 16 years of age. We compared two generations of Roma children, who were preschool and school aged in year 2001.

The standard of vaccination defined for preschool children generation represented four applications of diphtheria, tetanus, pertussis and poliomyelitis vaccine and one application of measles, mumps and rubella vaccine given before two years of age; and the standard for school aged children generation at 16 years of age comprised four applications of vaccine against pertussis, six applications of vaccine against diphtheria, tetanus and poliomyelitis and two applications of measles, mumps and rubella vaccine. Vaccination programs against hepatitis B and *Haemophilus influenzae type b*, initiated in 1998 and 2000, were not considered in the data analyses.

Data collection

Data on childhood immunization were collected in two ways: in some areas, data on vaccination-eligible children were obtained from the lists of Roma children living in the region studied, and in other areas, information on eligible children and their vaccination status was provided by primary healthcare givers in health services, and by private doctors and social services knowledgeable about the situation. For each child, the vaccination status was assessed by direct measurement using either the medical record, vaccination record, Vaccination booklet, list of vaccinated school children or database of the National Computerized Immunization System (CEPI 2000[®]), which supports the national vaccination registry combined with computerized Central population register.

Methods

This article performs the log-rank statistical test. It is also known as the Mantel-Cox (Mantel-Haenszel) test. Log rank test is comparing survival curves for two populations. In , a log-rank test by creating a sequence of $k \times 2$ (k survival functions by event observed/event not observed at that time) one at each observed event time, and calculating a statistic based on the observed and expected values for these contingency tables. Survival methods are often used for other end points, like our example. Survival time here is time to end applications of vaccine.

Log-rank test calculates a p-value testing the null hypothesis that the survival curves are identical in the two populations. If that assumption is true, the p-value is the probability of randomly selecting subjects whose survival curves are as different as they were actually observed¹³.

The calculations of the log-rank test are tedious and best left to computer (R 2.1.1). For each time interval, compare the observed number of drop out in each group with the expected number of drop out if the null hypothesis were true. Combine all the observed and expected val-

ues into one χ^2 statistic and determine the p-value from that. For large samples, this statistic has an approximate χ^2 distribution with 1 degree of freedom.

Results

For the calculation of vaccination coverage for specific childhood diseases, a formula was used with the denominator representing the total number of vaccination-eligible children, and the numerator indicating the number of children who had received the prescribed doses of vaccine. Four applications of poliomyelitis vaccine were given to 52% of preschool-aged Roma children and to 51% of school-aged Roma children eligible for poliomyelitis immunization. Four applications of diphtheria and tetanus immunization course was completed in 51% of preschool-aged and to 48% of school-aged Roma children. Four applications of pertussis vaccine were given to 50% of preschool-aged Roma children and in only 38% of school-aged Roma children (Figure 1). No significant difference was found for the initial three doses of vaccine against poliomyelitis, diphtheria, tetanus and pertussis.

Furthermore we use the Mantel-Haenszel procedure to give the log-rank test for our data. First step of that

method is calculate the Kaplan-Meier estimate of the survival function, separately for each group and then graphical display of the Kaplan-Meier estimates on the same plot will give an initial indication of whether there is a difference in survival experience for the two groups. The Kaplan Meier survival curve gives us a good estimate of the survival probabilities for each group we are studying. (Figure 2).

In graphs (Figure 2) the differences in survival curves was small.

Second step is formal statistical hypothesis test of whether there is a difference in survival experience for the two groups. Second step be based on the log-rank statistic. The simplest formal test that compares two survival curves is the log rank test.

In all represent vaccination coverage, the p is more then 0.05, hence accept the null hypothesis that the survival curves are identical in the two populations of preschool and school Roma children (Table 1).

One application vaccination against measles and mumps was given to 72% of preschool aged and to 66% of school aged Roma children. In both examples, the p is borderline, indicating a possible trend, but not quite achieving statistical significance. At rubella, 72% of pre-

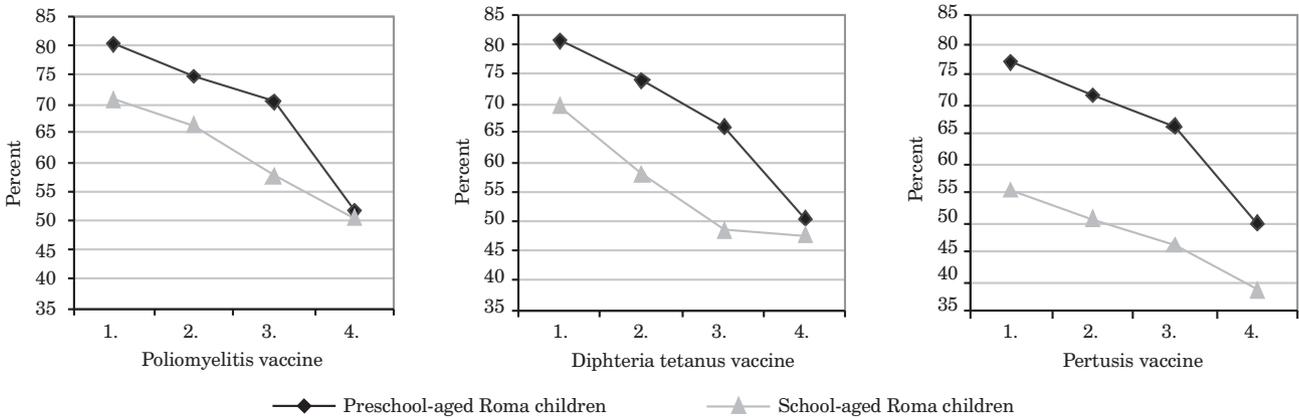


Fig. 1. The percent of preschool aged and school aged Roma children in Slovenia vaccinated against poliomyelitis, diphtheria, tetanus and pertussis, by number of applications given.

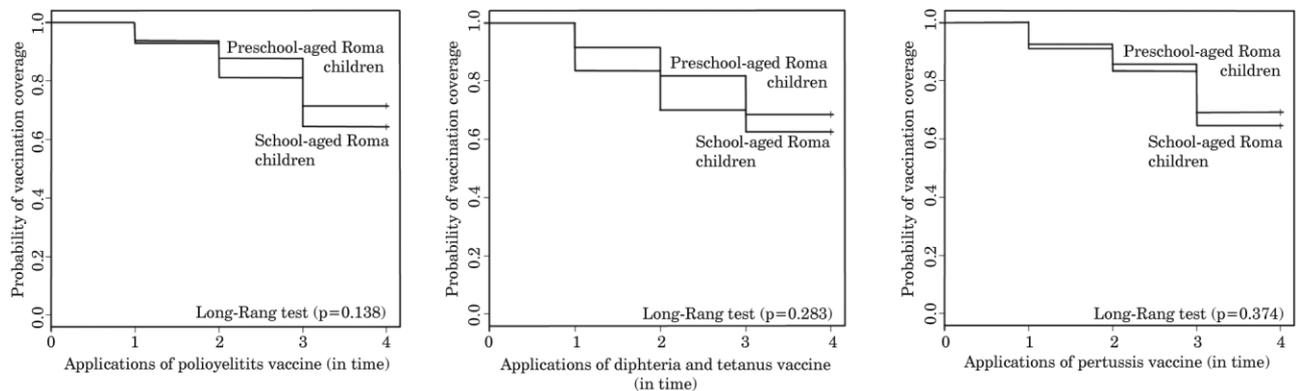


Fig. 2. Compare two survival curves to compare two generations – preschool aged Roma children and school aged Roma children in Slovenia vaccinated against poliomyelitis, diphtheria and tetanus and pertussis, by number of applications given.

TABLE 1
LOG RANK STATISTICAL TEST (MANTEL-HAENSZEL) FOR SPECIFIC VACCINATIONS: POLIOMYELITIS, DIPHTHERIA, TETANUS AND PERTUSSIS

Log rank test	Poliomyelitis	Di-Te	Pertussis
χ^2	2.2	1.2	0.8
p	0.138	0.283	0.374

Di-Te – diphtheria, tetanus

school and 64% of school aged Roma children were given one application of vaccine. Rubella vaccination was significantly lower in the school aged Roma generation ($p=0.024$) (Table 2). The only 33% of school-aged Roma population received two applications of measles, mumps and rubella vaccine.

In Slovenia, the national vaccination coverage for preschool aged children immunized against diphtheria, tetanus and pertussis was 92%, and against poliomyelitis was 93%. The national coverage for eligible preschool aged children immunized against measles, mumps and rubella was 94% (Table 3). Vaccination coverage of preschool Roma children in Slovenia against poliomyelitis, diphtheria, tetanus, pertussis and MMR were significantly lower than the national vaccination coverage for preschool aged Slovenia children ($p=0.000$).

Discussion

The paper presents the level of poliomyelitis, diphtheria, tetanus, pertussis, measles, mumps and rubella vaccination coverage in two generations of Roma children. Data collection was hindered by lack of immunization records, which was due to specific lifestyles of the Roma population.

Analysis of data on immunization status of preschool and school aged Roma children showed that the proportion of fully vaccinated children was low. Preschool aged children showed higher vaccination coverage than the school aged generation. No significant difference was found for the initial three doses of vaccine against poliomyelitis, diphtheria, tetanus and pertussis. The percent of school aged children vaccinated against measles, mumps and rubella was found to be by 6% lower than that of the preschool aged generation. The percent of Roma children vaccinated against childhood diseases was significantly lower than the national average¹². This finding suggests a relatively high drop out rate in Roma children, and the resulting increase in the proportion of susceptible to infectious diseases in the school aged generation, whose immunization course is practically completed.

Low vaccination coverage levels among hard to reach groups such as Roma children have been documented elsewhere in Europe and in the world^{14–21}. Vaccination coverage declining with age was reported in the Spanish study which compared three age groups of children (0–4 years, 5–9 years and 10–16 years) in terms of the prescribed vaccinations received. In Spain, 41% of Roma children were vaccinated against poliomyelitis, diphtheria and tetanus, 24% were immunized against pertussis, and 36% against measles, rubella and mumps. In the oldest age group, only 30 % of children were fully vaccinated¹⁸.

This data analyses confirmed differences in vaccination coverage between the preschool aged and school aged generations of Roma children. The data analyses also point to that the current level of vaccination coverage for specific diseases is not high enough to ensure adequate herd immunity against infectious diseases. Because of poor immunization status of preschool aged and school aged children, Roma settlements represent poten-

TABLE 2
COMPARATIVE VACCINATION RATE, 2001: PERCENT OF PRESCHOOL AND SCHOOL AGED ROMA CHILDREN IN SLOVENIA VACCINATED AGAINST MEASLES, RUBELLA AND MUMPS

Children	Mumps (%) ($p=0.060$)	Measles (%) ($p=0.069$)	Rubella (%) ($p=0.024^*$)
Preschool-aged Roma children	71.6	71.6	71.6
School-aged Roma children	65.6	65.8	64.3

*Statistical significance

TABLE 3
COMPARATIVE VACCINATION TEST RATE, 2001: PERCENT OF PRESCHOOL AGED ROMA CHILDREN IN SLOVENIA AND PRESCHOOL AGED SLOVENIAN CHILDREN VACCINATED AGAINST POLIOMYELITIS, DIPHTHERIA, TETANUS, PERTUSSIS, MEASLES, MUMPS AND RUBELLA

Children	Poliomyelitis (%) ($p=0.000^*$)	Di-Te (%) ($p=0.000^*$)	Pertussis (%) ($p=0.000^*$)	MMR (%) ($p=0.000^*$)
Preschool-aged Slovenia children	92.6	92.4	92.3	94.0
Preschool-aged Roma children	70.3	66.1	66.1	71.6

*Statistical significance, Di-Te – diphtheria, tetanus, MMR – measles, mumps and rubella

tial sources of infectious diseases. Once a specific disease is transmitted to a Roma settlement, the conditions there favor the spread of the infection to the rest of the population, which was the case in the measles epidemics of 1995. The established low coverage for tetanus vaccination among preschool and school aged Roma children, including girls, suggests that many women of child-bearing age will not be protected against this disease and that this will put their newborn babies at risk for developing neonatal tetanus.

In the recent years, the immunization status of preschool aged Roma children has markedly improved relative to previous generations. Higher coverage levels in preschool Roma children indicate improved health awareness of their parents. They are more knowledgeable about the importance of vaccination than the previous generations of Roma parents, and they take care that their children receive the prescribed vaccinations. An important role is played by nominated national and regional immunization coordinators and by the CEPI 2000[®] database on vaccination eligible children. If this trend continues, the likelihood of outbreaks of infectious diseases among the Roma population and in the larger community will be largely reduced.

Many joint efforts will have to be made to improve the vaccination status in dislocated Roma communities. The vaccination coverage among Roma children will have to be improved by reaching out to Roma communities and by adapting immunization schedule to fit their way of life and to respect the fundamental nature of human rights²². Effective health care actions and educational activities have succeeded in raising vaccination coverage rate in Spain by an average of 17% in one year²⁴. To improve health awareness of Roma parents, broader participation of all Roma communities will have to be encouraged, and appropriate written material comprehensible to Roma people will have to be prepared. Vaccination clinics should be set up closer to Roma settlements, and invitation to attend for immunization should be adapted to meet the specific needs of this community.

In Croatia, in particular case study, the epidemiologists at the Medimurje Country Public Health Institute had a difficult job in implementing the vaccination program among Roma people. The beginning of the Commu-

nication Initiative was, when the epidemiologists and the other members of the team started to learn the language of their Roma patients. This achieved a success rate of over 95% in children up to 15 years old – the very group whose vaccinations had previously been impeded by the traditional attitudes and fears of their parents. When the Communication Initiative started, the attitudes of the older generation still prevailed and the influence of traditional behavior continued to be strong. Actions such as the Communication Initiative should be a way of changing attitudes, as well as building trust between the community and health workers. The door to this Initiative was opened when a team of doctors first went into the Roma villages and convinced the people there to vaccinate their children. They did it successfully by learning the language of their patients, recognizing it as a way to overcome barriers to health service delivery. The success rate achieved in immunization during 1997 continues, with Medimurje Country still reporting higher vaccination rates than the national average. A specific intervention helped to ease some of the cultural barriers at the local level, increasing access to essential health services in poor and often isolated communities²³.

Public health programs could be modified to include essential interventions. Health services have a leading role to play in preventing and promoting health, by cooperation with municipal authorities and other social structures that influence health, such as school, social care, public service and nongovernmental organizations. These are especially important among the vulnerable communities, such as those of the Roma children. All of this points the need for special programs, which would inform, educate and approach health care to Roma population. Appropriate programs should be made by professional team from health department and social care on long and direct knowledge over Roma life and their cultural characteristics.

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OBUHVAĆENOST CIJEPLJENJEM TEŠKO DOSTUPNE ROMSKE DJECE U SLOVENIJI

S A Ž E T A K

Rezultati retrospektivne analize podataka o obuhvaćenosti cijepljenja kod romske djece pretškolske i školske dobi (436 pretškolske i 551 školske djece) na tri geografska područja Slovenije, analizirani su kako bi se utvrdile različitosti koje se odnose na obuhvaćenost specifičnih cijepljenja: protiv dječje paralize, difterije, tetanusa, pertusisa, ospica, zaušnjaka i rubeole između dviju generacija. Podaci su prikupljeni iz zdravstvenih kartona, podataka o cijepljenju (knjižice cijepljenja) i Nacionalnog kompjuteriziranog imunizacijskog sustava (CEPI 2000®). Obuhvaćenost cijepljenjem bila je izračunata usporedbom broja djece koja su pogodna za imunizaciju s brojem djece koja su cijepljena. Ovaj rad je načinio log-rank statistički test, također poznat kao Mantel-Haenszel test. Log rang test je usporedba krivulja preživljenja dviju generacija. Romska djeca pretškolske dobi pokazuju veću obuhvaćenost cijepljenjem, od djece školske dobi. Nije bilo značajne razlike kod romske djece pretškolske i školske dobi u cijepljenju protiv dječje paralize, difterije, tetanusa i pertusisa. Cijepljenje protiv rubeole bilo je značajno manje zastupljeno u generaciji romske školske djece. Svega 33% djece školske dobi primilo je dvije doze cijepiva protiv ospica, zaušnjaka i rubeole. Obuhvaćenost cijepljenjem pretškolske romske djece u Sloveniji protiv dječje paralize, difterije, tetanusa, pertusisa, ospica, mumpsa i rubeole značajno je manje nego kod slovenske pretškolske djece. Morat će se poduzeti mnogobrojni zajednički napori kako bi se obuhvaćenost cijepljenjem u romskim zajednicama poboljšala.

Analysis of Human Skeletal Remains from Nadin Iron Age Burial Mound

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ABSTRACT

This analysis attempts to reconstruct health, disease and life conditions of the population buried in Nadin, a burial mound, situated in central Dalmatia, Croatia. The analyzed skeletal material belongs to Liburnian culture and could be dated to early Iron Age, from 9th to 6th century B.C. The sample consists of a minimum number of 37 individuals, 7 children and 30 adults. The frequency of all the observed conditions is relatively low. Cribra orbitalia was observed only in females, the frequency of periosteal reaction on the tibiae is 26.1%. Two cases of cranial trauma were observed. Analyzed teeth exhibit low prevalence of carious lesions, ante mortem tooth loss and linear enamel hypoplasia. The case of hyperostosis frontalis interna on the endocranial surface of the frontal bone was observed. The affected skull belongs to the older adult female.

Key words: *life conditions, Nadin, Iron Age, Croatia, burial mound, skeletal remains*

Introduction

The importance of human skeletal remains in answering questions on the demography, health and behavior of past human populations has already been widely acknowledged¹.

Single skeleton offers much information about the life of the individual represented. Large number of skeletons from well documented archaeological context provides information about the population they represent. Bio-archaeological researches focus on both individual, and population level to gather information in order to get a wider picture of the analyzed population¹.

When studying archaeological samples, one faces various problems. These mostly result from the nature of the material studied i.e. skeletal remains. In the ideal scenario, the skeletal sample is of representative size and well preserved. However, this is not always the case and very often we lack some of the information about the studied population. Despite the limitations, studies of such material can provide some useful information on health and lifestyle of populations. This is especially important for the periods and populations for which we have limited data.

One of such cases is the sample analyzed in this work. Although relatively small in size and rather fragmented and poorly preserved, we believe that its analysis will

shed some light on characteristics of the Croatian Iron Age population. Iron age period in Croatia is archaeologically very well documented, but until now, only one skeletal analysis was published.

The analyzed skeletal sample from »Vinkovci-Nama« site is rather small (n=11)². Even though, its analysis provided the first available data about health, disease and stress levels in this sample of Iron Age continental Croatian population². Due to the small sample size, the interpretation of the published results should be taken with caution. Thus, our study will add to the overall understanding of the lifestyle of Croatian Iron Age population.

The skeletal sample analyzed in this work comes from the site located in the coastal part of the Croatia. According to the burial characteristics, ceramics and grave goods they belong to Liburnian culture dated from 9th to 6th century B.C.³

The Liburnians were the ancient inhabitants of Liburnia, a region along the Adriatic coast between the rivers Titius (now Krk) in Dalmatia and Arsia (now Raša) in Istria. Their territory also included islands of the Kvarner bay (Krk, Rab, Cres, Lošinj and Pag)⁴. Their neighbors were Illyrian tribes Histri in Istria, Dalmatae in Dalmatia and Japodes in the hinterland^{4,5}.

The earliest mention of the Liburnians in a classical source is by Hecateus in the 6th century B.C., who placed them on the Adriatic coast. In the *Periplus of Pseudo Scylax* from the 4th century, the whole chapter describes Liburnians located between Ilyrians and Histri⁵.

The Liburnians were known as seafarers along the Adriatic sea and Romans described them as pirates. In the 8th century B.C. they have controlled the sea down to the Corfu, but in 734 B.C. following the fight with the Greek navy, they were pushed back to their original borders^{4,5}.

Liburnian material culture has been recovered from their cemeteries and settlements. The majority of the settlements were located on the hilltops, like Radovin and Bribir that were later fortified with walls. Other lowland sites on the coast, such as Zadar and Nin, later become well known as Roman cities⁶. The preferred burial rite among Liburnians is inhumation in flat cemeteries, although burial mounds, like the one we analyze here were recorded. The deceased were in a flex position with graves located in the circles⁵. Grave goods were mostly jewelry, parts of dress, and personal things. The pottery is relatively little known as it rarely occurs in graves, but some quantities of imported wares, such as Daunian from south Italy, Hellenistic and small quantities of classical Greek were found^{5,7}.

By the middle of the 1st century B.C. Liburnians started losing their territory to their Illyrian neighbors, the Delmatae and Ardiaei. In the year 35 B.C. the Romans conquered the Liburnians and incorporated their territory into the province of Dalmatia⁴.

Material and Methods

The skeletal material for this study comes from the burial mound 13 in Nadin. Archaeological site of Nadin is located about 30km from the town of Zadar, in central Dalmatia, Croatia.

The mound excavated in 2004 is a part of the group of 12 mounds that surround the hillfort from the northeast. During the excavation 19 damaged graves were revealed³. They were positioned in three concentric circles with three graves in its center. Majority of the graves were in the middle circle, with only six in the outer^{3,8}.

Three burial types were identified: inhumation in flexed position, inhumation in extended position, and cremation. Majority of the deceased (n=11) were in flexed position, in graves situated in the middle of the circle. Two cremated individuals were buried in urns^{3,8}. Their burials, along with those of the deceased in the extended position were located in two outer circles. Although multiple burials were present, the majority were single inhumations^{3,8}.

The grave goods mostly consisted of jewelry, such as needles, fibulae and iron bracelets. Based on the burial characteristics, ceramic and grave good typology, the site can be dated to the early Iron age, from 9th to 6th century B.C.^{3,8}.

The mound 13 is important as it offers a series of new data on the Liburnian culture. This is the site with the highest number of graves found in a single mound^{3,8}. One

specific of the mound is the use of the three different burial types, as well as their location in three concentric circles. Furthermore, this is the first documented example of cremation burials after the Bronze age in the region. Their existence confirms that incineration was also one of the Liburnian funerary rites^{3,8}.

The preservation and the completeness of the analyzed bones varied from generally good, to fragmented and poorly preserved. The reason for this state of the preservation is the position of the graves, which is very close to the modern ground surface.

The demographic parameters of the population (minimum number of individuals – MNI, sex, age) were determined first, as this provided the basis for all further analyses. All other indicators were then scored for each individual. Data collecting followed the procedures described by Buikstra and Ubelaker⁹.

Due to the fragmentary state of the bones and presence of the multiple burials the minimum number of individuals present in the sample was determined. In order to determine MNI, the procedure described by White was used¹⁰. After all the skeletal remains were identified and separated according to elements and side of the body, the MNI within each category was counted. The frequency of the most prevalent bone category was used as a MNI in analyzed sample.

The age and sex of each individual was determined using the standard morphological criteria. Sex was determined following the methods based on cranial and pelvic morphology discussed in Buikstra and Ubelaker⁹, White¹⁰ and Bass¹¹. There was no attempt to sex the subadult remains. In the cases when the skeletal material was not sufficient to make sex determination, the category indeterminate was used.

The age of the adults was estimated using Meindl and Lovejoy¹² method for ectocranial suture closure, Lovejoy¹³ and Brothwell¹⁴ method for the dental wear. The methods by Ubelaker¹⁵ and Smith¹⁶ for dental formation and eruption were used for the subadults. In the cases where the skeletal material was too fragmentary for age determination, categories adult or subadult were used.

Due to the fragmentary state of the skeletal remains, adults were assigned to rather broad age categories: young adults (18–25 years), mature adults (26–45 years) and older adults (>45 years). Subadults were assigned to the following categories: <5 years, 5–9 years, 10–14 years and 15–17 years.

Data were also recorded for some diseases and stress conditions. These included cribra orbitalia, periosteal reactions, and trauma. Observations were made for linear enamel hypoplasia, caries, alveolar abscesses, and ante mortem tooth loss of permanent teeth as indices of oral and general health.

Results

The minimum number of 37 individuals was in the Nadin sample. Sample consisted of 7 (18.9%) subadults and 30 (81.1%) adults. Among the adults (over 18 years),

9 (30%) were males, 9 (30%) females, and 12 (40%) of undetermined sex. This clear under-representation of subadults can be explained by the position of the graves. Their position close to the modern surface resulted in the removal and destruction of the fragile bones. The best represented age category for age assessed remains was the 26–45 years (30%), though of course this is the broadest category. A small number of individuals (13.3%) appear to have survived over 45 years. In the young adults category were 23.3% of the individuals, of which majority were females (57.1%). Taking into consideration preservation problems and relatively low sample size, the age distribution cannot be considered as representative of living population.

Due to the fragmented preservation of the remains all parameters could not be used for all the individuals in the sample, that resulted in different sample size for each condition.

Cribra orbitalia

Cribra orbitalia as a skeletal reaction to iron deficiency affects the upper surface of the orbits¹⁷. Its porous appearance is caused by the expansion of diploë towards the outer table of the skull¹⁸. The frequency of the lesion is 40%, or 4 of 10 observable individuals. All of the ob-

served cases were in adult females. Lesions were not found within subadult category, because their orbits were not preserved. We believe that the prevalence of this lesion, especially among the children, might be higher considering the preservation of the cranial material available for the study.

Periostitis

Evidence of periostitis, inflammation of the periosteum, is visible as an abnormal bone deposition, or porosity, on the bone surface¹⁸. This condition is frequently observed in archaeological populations, and usually suggests presence of infection, but can also be related to a direct trauma to the soft tissue¹⁹. The frequency of the lesion is 26.1%, or 6 out of 23 preserved tibiae of both sex have the lesion.

Trauma

Skeletal fractures provide the direct evidence of both accidental and intentional human actions¹⁹. Only the evidence of cranial trauma was observed in the sample. Two out of 14 individuals were affected, making the frequency of 14.3%. An older adult male and an adult female both have small and circular cranial depressed fractures. The male had a fracture in the posterior portion of the left pa-

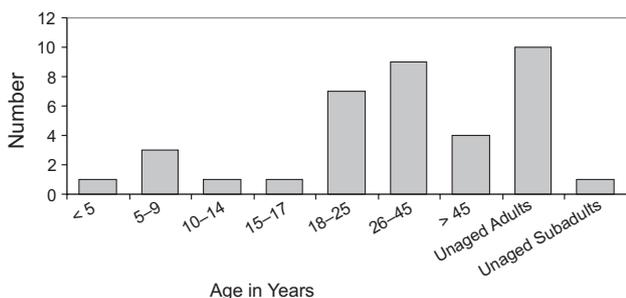


Fig. 1. Age at death for subadults and adults irrespective of sex.

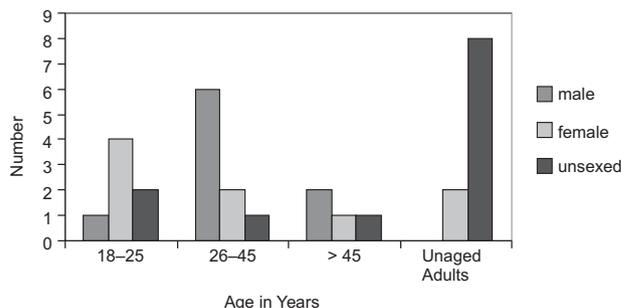


Fig. 2. Age at death for adults by sex.



Fig. 3. Fracture of left parietal bone.



Fig. 4. Endocranial view of Hyperostosis frontalis interna in an older female.

rietal bone and it was in the process of healing. Female had fracture in the posterior portion of the frontal bone that did not penetrate the inner table of the bone.

Dental pathologies

The available adult remains were scored for dental pathologies such as caries, alveolar abscesses, and ante mortem tooth loss. A total number of 292 permanent teeth were recovered from the site.

Cariou lesion occurs when oral bacteria metabolize fermentable carbohydrates present in mouth. The consequence of this process is demineralization of the tooth²⁰. The presence of lesions can be the result of the poor oral hygiene, but also indicative of a diet rich in carbohydrates, such as sugar^{21,22}. Dental caries was observed in 13 of 292 available permanent teeth (4.5%). Lesions were more common in mandibular (8.2%) than in maxillary teeth (0.7%). The highest caries frequency in the mandibular teeth is found on the molars (58.3%) and on maxillary dentition, the lesions were only present on molar teeth.

Ante mortem tooth loss is caused by several factors, such as caries, occlusal wear, trauma, tartar or periodontal disease that weakens the root-supporting ligament²². Ante mortem tooth loss was scored in both jaws by counting the closed and reabsorbed alveolar sockets. Of 359 observations for ante mortem tooth loss, 31 teeth or 8.6% were missing. Ante mortem tooth loss was more common on mandibular (14.3%) than maxillary teeth (1.3%). Majority of the lost teeth were molars (54.8%). The loss of other teeth was less frequent and observed only in mandible: 25.8% premolars and 16.1% of canines and incisors.

Linear enamel hypoplasia is the developmental defect characterized by linear grooves in the enamel of the tooth. This cessation in the enamel formation is caused by infections, malnutrition and metabolic diseases, as well as other types of stress²³. In this analysis, the linear enamel hypoplasia was scored on all available teeth. All the 292 permanent teeth were observed for evidence of dental hypoplasia. Prevalence is relatively low, only 4.8%, or 14 teeth were affected. The mandibular and maxillary teeth were equally (4.8%) affected.

Hyperostosis frontalis interna

In the examined skeletal material from Nadin, a case of a bilateral new bone formation on the endocranial surface of the frontal bone was observed, which I believe is a case of hyperostosis frontalis interna. The affected skull belongs to an older adult female and is consistent with the results from already published diagnosis¹⁸.

Hyperostosis frontalis interna is manifested as an irregular bony growth on the inner surface of the frontal bone²⁴. Generally, the lesion is bilateral and affects only the frontal bone, but some cases in which it extends to other bones, such as parietals, temporals and occipitals have been documented^{18,25}. The etiology of this condition is still ambiguous. It has been associated with Morgagni syndrome, a metabolic disorder affecting older women. It

can also result from hormonal disturbances such as prolonged estrogen stimulation, menstrual disorders, as well as from obesity or genetic factors^{25,26}. Until now, several cases of the hyperostosis were documented in the archaeological material. Clinical studies on modern populations as well as archaeological cases show that the condition is age and sex specific, more frequently affecting older, postmenopausal women^{18,25}.

Discussion

The relatively low sample size and the fact that the preservation and completeness of available skeletal material was sometimes rather poor, do not allow us to make interpretations without caution. Whenever it was possible comparison with the Vinkovci-Nama serie was made.

Analysis of the skeletal markers of health showed that the major health problem in this population identifiable on the skeletal remains are conditions causing cribra orbitalia (40%). The lesions were observed in 4 adults, all females. This apparent sex difference could maybe be explained by different iron demands between sexes. Women, as well as children, require the highest amount of iron as a consequence of periodical loss of blood, pregnancy, lactation and growth requirements²⁷. The lesions were not observed in the subadults while their orbits were not present. At the Vinkovci-Nama site, this type of lesion was found in two, out of three, subadults and in one adult male (out of five adults). Comparing to Vinkovci serie, where only two individuals (one subadult and one adult female) show evidence, the prevalence in the Nadin seems more realistic.

The evidence of periostitis is observed on 26.1% of the preserved tibiae of both sexes. In the Vinkovci-Nama serie only two individuals have evidence of periostitis on tibia. Both tibiae of subadult show new bone deposits, while female has localized periosteal new bone formation on distal third of the diaphysis of the left tibia.

In the Nadin sample, traumas were rare, and only two were observed on crania, in adult male and female. In the Vinkovci-Nama serie two individuals exhibit evidence of trauma, a healed fracture on the frontal bone of a male and a compression fracture of the first lumbar vertebra in a female.

Analysis of the dental status revealed low frequency of caries and ante mortem tooth loss, as well as linear enamel hypoplasia, indicating rather good overall dental health. The relatively low frequency of caries in the analyzed sample is indicative of low consumption of carbohydrates. Cariou lesions were more frequently on the molar teeth, which can be explained by the fact that plaque more easily accumulates on the broad occlusal surfaces. Food particles that are trapped in their fissures and pits cannot be easily removed by the natural mechanisms such as saliva, tongue and cheeks²⁸. Ante mortem tooth loss can be created by different causes, but I believe that in the analyzed sample, caries was the most likely explanation. The majority of the lost teeth (51.7%) are molars, the most susceptible to caries. The higher frequency in

mandibular dentition follows the distribution of caries, but is also a result of better preservation of mandibular alveolar bone. Low frequency of linear enamel hypoplasia suggests that individuals from Nadin were not under the strong systemic stress during their childhood. In the Vinkovci-Nama serie, 11.6% of the teeth had carious lesions and 26.2% of adult tooth sockets exhibit evidence of alveolar bone disease. The better dental health in Nadin could be explained by the fact that the site is located near the sea. Proximity of the sea and fact that Liburnians were famous sailors implicates that their diet was rich in sea food which is less cariogenic.

Only one case of hyperostosis frontalis interna was observed in the sample. The new bone formation was observed on the endocranial surface of the frontal bone of an older adult female.

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Conclusion

At this moment, the available skeletal data do not allow us to make conclusions about the demography, health and behavior for the Croatian Iron age population. A more detailed picture must await additional analyses of samples from both the continental and coastal Croatia. We hope that this analysis of the skeletal material from the Nadin burial mound, despite its limitations, is a contribution leading in that direction.

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ANALIZA LJUDSKIH SKELETNIH OSTATAKA IZ NADINA, ŽELJEZNODOBNOG TUMULA

SAŽETAK

U radu se pokušalo rekonstruirati zdravstveno stanje, bolesti i uvjeti života populacije pokopane u grobnom humku Nadin u Dalmaciji, Hrvatskoj. Analizirani skeletni materijal pripada liburnskoj kulturnoj grupi i može se datirati u rano željezno doba, od 9. do 6. stoljeća pr. K. Uzorak predstavlja minimalni broj od 37 individua, unutra kojih je prisutno sedmero djece i 30 odraslih osoba.. Učestalost uočenih stanja je relativno niska. Cribra orbitalia je uočena samo kod žena, dok je učestalost periostealnih reakcija na goljeničnim kostima 26.1%. Uočena su samo dva slučaja kranijalne traume. Analizirani zubi pokazuju nisku stopu karioznih lezija, gubitka zubi prije smrti i linearne hipoplazije. Kod starije individue ženskog spola uočen je slučaj hiperostoze frontalne kosti na endokranijalnoj površini čeonke kosti.

A Radiographic Study of Location of Mental Foramen in a Selected Turkish Population On Panoramic Radiograph

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ABSTRACT

Purpose of this study was to investigate the most common position of the mental foramen in a selected Turkish population. The study sample included three hundred and sixty one panoramic radiographs of selected Turkish population taken in Faculty of Dentistry, University of Gazi. The most common position of the mental foramen was between the first premolar and the second premolar (71.5%). The mental foramen was symmetrical in 90.4% of patients. In this study, the difference of the location of the mental foramen in different ethnics groups was discussed. Clinicians and anthropologists should expect to find the position of the mental foramen to be symmetrical and between the first premolar and the second premolar teeth.

Key words: mental foramen, anatomic location, panoramic radiography, mandible, implant

Introduction

The accurate clinical location of the mental foramen is usually a difficult procedure. Its position is generally described as being below the mandibular second premolar¹. However, individual variation could place the mental foramen anywhere from below the first premolar to between the roots of the first molar.

Knowledge of the position of the mental foramen is important both when administering regional anaesthesia, performing periapical surgery and dental implant surgery and endodontic treatments in the mandible. Although it is often possible to identify the mental foramen radiographically and by palpation, knowing the normal range of possible locations is essential².

The extra oral panoramic radiograph has gained popularity in the last four decades. The advantages of this technique over intra oral radiography include a greater area of third and soft tissue coverage, continuity of the visualized area, and rapidity with which the view is formed. The ability to view the entire body of the mandible should allow a more accurate location of the mental foramen in both a horizontal and vertical dimension¹.

Panoramic radiography is curved plane tomographic radiographic technique used to depict the body of the mandible, maxilla, and the lower one half of the maxillary sinuses on a single image. This modality is probably the most utilized diagnostic modality in implant dentistry³.

According to Yosue and Brooks^{4,5} the radiographic appearance of the mental foramen can be classified into four types: in the first the mental canal is continuous with the mandibular canal; the second is the separated type, where the foramen is distinctly separated from the mandibular canal; a third is said to be diffuse with a distinct border of the foramen, while the fourth group is the so-called »unidentified type«.

Neurovascular bundles of the supraorbital, infraorbital and mental foramina are important structures that need to be considered in local anesthesia and surgical procedures in the maxillofacial area. An understanding of the anatomy of the location of these block and avoiding injuries to the neurovascular bundles⁶.

The purpose of this study was to determine the location of mental foramen on panoramic radiographs in a selected Turkish population.

Material and Methods

In this study retrospectively panoramic radiograph taken from four hundred patients of the radiology department of Faculty of Dentistry at Gazi University was analyzed.

All radiographs were exposed at 66 kV and 16 mA for 17.6 s. with an Orthopantomograph OP100 (Trophy Instrumentarium Corp. FINLAND).

All panoramic radiographs were of dentate Turkish patients, with erupted first and second premolars and first molars. In addition, the films were free from radiolucent or radiopaque lesions in the lower arch and showed no exposure or processing artifacts. The youngest patient was 14 years-old and the oldest 57 years-old with a mean of 24.93 years. All panoramic radiographs which the mental foramen could not be identified were excluded from the study.

The panoramic radiographs of the 361 subjects were placed on a view box and a line was drawn on the radiographs with the longitudinal axis of a tooth and lying between two teeth.

Radiographs were investigated by one observer as twice. Observations were repeated with a random sample of 50 radiographs which were re-examined.

The position of the image of the mental foramen was recorded according to the categories by Jasser and Nwoku.⁷

Position 1: Situated anterior to the first premolar.

Position 2: In line with the first premolar.

Position 3: Between the first and second premolar.

Position 4: In line with the second premolar.

Position 5: Between the second premolar and first molar.

Position 6: In line with the first molar (Figure 1).

Statistical analysis was performed using a SPSS for windows release 7.5.1. Gamma test was used to deter-

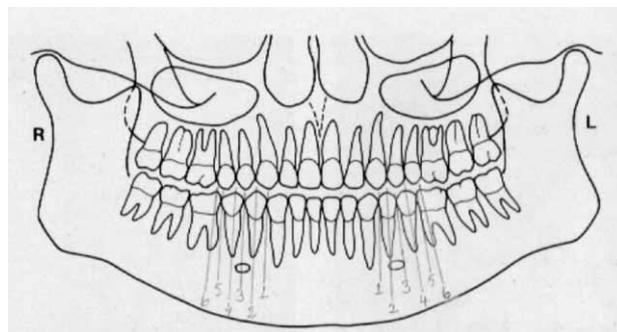


Fig. 1. Determining the position of the mental foramen.

mine the relationship between two ordinal variables (right positions and left positions of mental foramen). (Gamma statistic: 0.955, $p < 0.001$). A Chi-square-test was applied to explore the relation between two categorical variables.

Results

The appearance and location of mental foramina were determined on panoramic radiographs of 361 patients. Of these, 144 were male and 217 were female (Table 1–3).

In 71.5% of cases the mental foramen was in between the first and second premolars and in 22.4% in line with the second premolar. The position of the mental foramen was symmetric in 85.8% and asymmetric in 14.2% of cases.

In the asymmetric cases, on the right side on the mandibular, 50% of mental foramen was located between the second premolar and first molar; while on the left side 50% were located in line with the second premolar.

In addition 42.9% of the foramens on the right side were found to be in line with the first premolar and between the first and second premolar on the left side.

It is appeared decade and location distribution of mental foramen (Table 4).

TABLE 1
FREQUENCY OF LOCATION OF THE MENTAL FORAMEN IN RELATION TO THE APICES OF TEETH AND INTERDENTAL SPACES ON THE PANORAMIC RADIOGRAPHS OF 361 SELECTED TURKISH POPULATIONS AT 722 SIDES (LEFT AND RIGHT SIDES) OF THE MANDIBLE

Location	Male		Female		Total	
	Side frequency	%	Side frequency	%	Side frequency	%
1: Situated anterior to the first premolar	7	2.4	2	0.5	9	1.2
2: In line with the first premolar	6	2.1	17	3.9	23	3.2
3: Between the first and second premolar	192	66.7	324	74.7	516	71.5
4: In line with the second premolar	78	27.1	84	19.4	162	22.4
5: Between the second premolar and first molar	5	1.7	7	1.6	12	1.7
6: In line with first molar	0	0	0	0	0	0
Total	288	100.00	434	100.00	722	100.00

TABLE 2
DISTRIBUTION OF ASYMMETRICAL MENTAL FORAMINA IN
RELATION TO THE APICES OF THE TEETH AND INTERDENTAL
SPACES ON THE PANORAMIC RADIOGRAPHS OF 51 PATIENTS

Location of mental foramen	Number	Percentage
1: Situated anterior to the first premolar	0	0
2: In line with the first premolar	7	2
3: Between the first and second premolar	21	6
4: In line with the second premolar	20	5.4
5: Between the second premolar and first molar	3	0.8
6: In line with first molar	0	0
Total	51	14.2

TABLE 3
DISTRIBUTION OF SYMMETRICAL MENTAL FORAMINA IN
RELATION TO THE APICES OF THE TEETH AND INTERDENTAL
SPACES ON THE PANORAMIC RADIOGRAPHS OF 310 PATIENTS

Location of mental foramen	Number	Percentage
1: Situated anterior to the first premolar	3	.8
2: In line with the first premolar	7	1.9
3: Between the first and second premolar	235	65.1
4: In line with the second premolar	62	17.2
5: Between the second premolar and first molar	3	.8
6: In line with first molar	0	0
Total	310	85.8

TABLE 4
DECADE AND LOCATION DISTRIBUTION OF MENTAL FORAMEN

Decade	Right							Left						
	1	2	3	4	5	6	Total	1	2	3	4	5	6	Total
14–24	2	9	164	45	4	0	224	4	7	171	40	2	0	224
25–35	1	5	72	16	2	0	96	2	2	69	19	4	0	96
36–46	0	0	16	19	0	0	35	0	0	15	20	0	0	35
47–57	0	0	4	2	0	0	6	0	0	5	1	0	0	6
Total	3	14	256	82	6	0	361	6	9	260	80	6	0	361

Discussion

Radiography is the only available non-invasive method for diagnosis and treatment planning of major surgical procedures of the mandible. Panoramic radiographs are commonly used for screening, diagnosis, and selecting the best possible surgical approach⁸.

The location of the mental foramen could change during the development of the jaws⁹; therefore, panoramic radiographs taken from patients who had completed their development were evaluated in this study. In addition, patients having missing teeth were excluded from the study because the evaluation was made according to the present canines, premolars, and molars.

We utilized panoramic radiographs because they have certain advantages over intra-oral radiography. It includes a greater area of hard and soft tissues and also the visualized area in continuity, thus allowing for a more accurate location of the mental foramen in both the horizontal and vertical dimensions. On the other hand, periapical radiographs may not show several positions of the mental foramen if it is below the edge of the film¹⁰.

In the research literature the mental foramen is frequently described as situated in the region of the second premolar in the fully developed mandible, but individual variations may occur occasionally^{1,10,11}. In our analysis of 361 panoramic radiographs, we found the mental foramen

positioned anywhere between the long axis of the canine to that of the mesiobuccal root of the first molar. The result of our study was similar to the other studies.

According to the anatomy text by Sicher and DuBrul, the mental foramen lies midway between the free alveolar border and the border of the mandible or closer to the latter¹².

Studies done in other ethnic and racial groups were shown (Table 5)^{13, 14}.

Sample size of the studies above varied between 40 and 2000. In our study our sample size was 361 people. The weighted frequency of the location of the mental foramen in the above studies was in position 3. It was similar in our study.

Our study design was similar with Jasser's study design⁷. Our results and Jasser's studies results were shown (Table 6).

The location of the mental foramen most commonly was found along the longitudinal axis of the second premolar tooth in other studies, our study of Turkish patients placed the location of the mental foramen between the first premolar and the second premolar teeth.

Although our study is limited with a group of people; according to the analyze of the Table 5, our results are similar with the other countries. But also advanced studies are necessary to achieve clearer results.

TABLE 5
SUMMARY DATA ON THE POSITION OF THE MENTAL FORAMEN (GREEN 1987, SHANKLAND 1994)

Sample size	Distribution %						Population studied	Reference
	Average	1	2	3	4	5		
50	1.96	40	28	30	0	2	French	Olivier
150	2.30	20	27	48	3	0	Yugoslavian	Stosic
262	2.40	10	42	46	2	0	Russian	Gruber
150	2.40	6	48	46	0	0	German	Moral
60	2.44	8	45	42	5	0	German	Merkel
100	2.46	0	61	32	7	0	German	Hubner
108	2.52	8	39	46	6	0	Italian	Esposito
72	2.55	11	36	39	14	0	Meican(Ketchipauan)	Kay
100	2.57	12	22	63	3	0	Central European	Ashley-Montagu
1033	2.59	1	44	50	5	0	British	Kay
138	2.62	7	34	49	10	0	Whites	Simonton
1000	2.63	6	35	50	8	1	Italian	Toni and Favero
336	2.67	5	32	55	8	0	Mixed Amerindian	Simonton
55	2.69	6	26	62	6	0	German	Sachse
494	2.69	6	32	51	9	2	Italian	Martani and Stefanini
300	2.70	0	34	58	7	0	Russian	Kuznetsova
372	2.73	2	25	63	8	0	Brazilian	De Freitas
40	2.76	11	40	32	16	5	European	Esposito
75	2.79	3	38	40	20	0	Hindu	Miler
114	2.83	4	25	56	14	1	Eskimo	Simonton
898	2.88	1	29	54	9	6	Egyptian	Kay
250	2.89	1	13	78	7	0	Beijing Chinese	Zhang
60	2.90	0	23	63	13	0	Australasian(Sarawak)	Kay
150	2.92	2	20	64	13	1	Kentucky Indian	Simonton
272	2.92	0	22	64	14	0	Japanese	Akabori
76	2.99	0	31	43	23	3	Melanesian	Esposito
760	3.00	1	20	58	20	1	Shenyang Chinese	Zhang
100	3.01	2	23	49	24	2	American	Tebo and Telford
860	3.01	0	18	64	17	1	Chengdu Chinese	Wang
87	3.05	0	21	51	25	2	Hong Kong Chinese	Green
152	3.07	4	26	32	34	4	Egyptian(Sud Kerma)	Kay
58	3.07	0	21	59	14	7	African(Teita)	Kay
516	3.08	0	12	66	19	2	Kunming Chinese	Zhang
108	3.14	0	6	79	9	6	Arkansas Indian	Simonton
330	3.15	0	6	57	33	0	East African(Bantu)	Zivanovic
1100	3.15	0	10	66	23	1	Shanghai Chinese	Wang et al
302	3.16	0	13	61	23	3	Thai	Boonpiruk
159	3.16	3	19	47	25	7	Egyptian	Simonton
262	3.17	0	15	54	30	1	Japanese	Hori
192	3.21	0	15	51	32	2	African	Kay
2000	3.26	0	6	65	26	3	Chengdu Chinese	Zhang
58	3.34	2	10	41	41	5	Melanesian	Simonton
41	3.37	0	2	70	12	15	East African(Bantu)	Schultz
208	3.69	0	1	45	38	16	Australian aboriginal	Murphy
313	3.04	4	18	55	15	7	Singaprean Malasy and Indians	Neo
604	2.89	2	27	56	12	3	Nigerians	Kekere-Ekun
100	3.00	0	21	59	19	1	Chinese	Wang et al
150	3.01	0	18	63	19	0	Unknown	Phillips et al
138	3.16	0	6	75	12	4	Asian Indian	Shankland

TABLE 6
SUMMARY DATA ON THE POSITION OF THE MENTAL FORAMEN (JASSER AND NWOKU 1998, GUNGOR ET AL., PRESENT STUDY)

Reference	Population	Sample size	Distribution %					
			1	2	3	4	5	6
Jasser and Nwoku (1998)	Saudis	794	0.6	5.3	42.7	45.3	5.2	0.9
Present study	Turkish	722	1.2	3.2	71.5	22.4	1.7	0.0

In conclusion, according to our results the location of the mental foramen on the panoramic radiographs of selected Turkish population was most commonly in between the first and second premolars. In majority of cases there was bilateral symmetry in the position.

Clinicians and anthropologist should expect to find the positions of the mental foramen to be symmetrical and between the first premolar and the second premolar teeth.

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ISTRAŽIVANJE LOKACIJE MENTALNOG FORAMENA PANORAMSKIM RADIOGRAFIMA NA ODABRANOJ POPULACIJI U TURSKOJ

S A Ž E T A K

Cilj ove studije bio je istražiti najčešću lokaciju mentalnog foramena na odabranoj populaciji u Turskoj. Ova studija uključuje tristo šezdeset i jedan panoramski radiograf odabrane turske populacije uzete na stomatološkom fakultetu, sveučilišta u Gazi. Najčešći položaj mentalnog foramena bio je između prvog i drugog pretkutnjaka (71.5%), a simetričan je bio u 90.4% pacijenata. U studiji je raspravljano i o različitoj lokaciji mentalnog foramena kod raznih etničkih grupa. Kliničara i Antropolozi trebali bi očekivati simetričan položaj mentalnog foramena između prvog i drugog pretkutnjaka.

The Influence of Age on Tooth Root Colour Changes

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ABSTRACT

The purpose of this study was to examine the relationship between tooth root colour and age, and its possible application in age assessment. In this research altogether 100 tooth roots have been analysed. All teeth, that is their roots, were digitally recorded and the colorimetric treatment was made using Adobe Photoshop 7.0 computer program. Studies have shown no significant difference between RGB values analysed on the whole root surface or only on its central part, with certainty $p > 0.99$. It is also established that there is no statistically significant difference in colouration on four anatomical surfaces (buccal, mesial, lingual, distal) of tooth roots with certainty $p > 0.99$ for red, $p > 0.99$ for green and $p > 0.50$ for blue colouration component. Statistical data interpretation showed that there is a linear correlation between obtained RGB values and age, with $r = -0.994$, $p > 0.99$ for red component, $r = -0.972$, $p > 0.99$ for green and $r = -0.982$, $p > 0.95$ for blue colouration component. From the obtained results it is possible to conclude that analysing the above mentioned parameter we can easily establish dental age and this technique can be the basis of practical application in establishing chronological age of man.

Key words: forensic odontology, age determination, teeth, cementum, colouration

Introduction

The identification of human remains is a recurrent problem in both forensic and anthropological contexts. It understands analysis of three most important parameters: gender and heights identification and age estimation. The most difficult task is age estimation^{1–4}. Skeletal methods based on closure of sutures exist, but because of wide biological variations these are not very accurate. Consequently, the teeth present the alternative solution for age estimation^{5,6}. Although there are genetic, nutritious and external influences, the time and periods of teeth development are synchronised according to a very precise model^{7,8}. All teeth, therefore, develop through morphologically different stages that can be established using atlas approach or using scoring systems. After the growth and development is finished and with eruption of third molars, around the age of 24, it is no longer possible to establish the age by the help of this method. For this reason morphological⁹, histological¹⁰, and biochemical¹¹ methods based on degenerative changes in the teeth have been developed to establish chronological age in adults.

Because of their reliability, morphological changes in teeth form the basis of some of the most common methods to estimate age in forensic cases¹². The purpose of this study was the analysis of one of the morphological parameters that is believed to be one of the best for age estimation. In this research the colorimetric treatment of tooth roots with the most recent approach was performed and it was analysed whether it is possible to use the tooth colour criteria as biological marker for dental age estimation.

Materials and Methods

The sample was made of 100 teeth of known age and gender, grouped into five age groups (21–30, 31–40, 41–50, 51–60, 61–70), all extracted upon special indications by oral surgery, orthodontic and paradontology specialists. After extracting them, each tooth was rinsed with water and the tissue remains were removed with tweezers and scalpel from the root. After that the tooth was

disinfected with 5% Na-hypochlorite and the surface was smoothen with a small rubber stone. Material prepared in that way was closed into small plastic boxes. The colourimetric analysis was made using Adobe Photoshop 7.0 computer program. Each root was digitally recorded using a digital camera (Nikon D1X, with illumination of Multiblitz 400) and after that the digital photographs were transferred to the computer. The quantitative analysis of the tooth root colour was made through Red Green Blue system (RGB). In order to show possible differences in colour on the root surface and to choose safer determination of the average colour value, two measurements were made. In the first measurement the RGB values were analysed on the surface that covers all three root areas (cervical, middle and apical third). In the second measurement the RGB values were analysed only on the middle root third. The tooth root colour analysis i.e. red, green and blue component values were made on all four root sides: buccal, mesial, lingual and distal. In order to adjust the analysis better to this research, software was developed to calculate the average RGB values on the exact given surface.

Statistical analysis

The statistical equality of RGB values of two root surfaces and RGB values ratio on all four root sides were tested using the HI^2 test. The correlation between colour and age was examined using the Pearson test and the certainty by HI^2 test. All examinations were performed on the 99.5% level of certainty.

Results

This study has shown no difference whether the average RGB values are taken on the whole root surface or only on its central third, with certainty $p > 0.99$. The statistical data processing established also that the measurement results do not depend on the fact whether the samples were taken from buccal, lingual, mesial or distal root sides and that there is no significant difference in

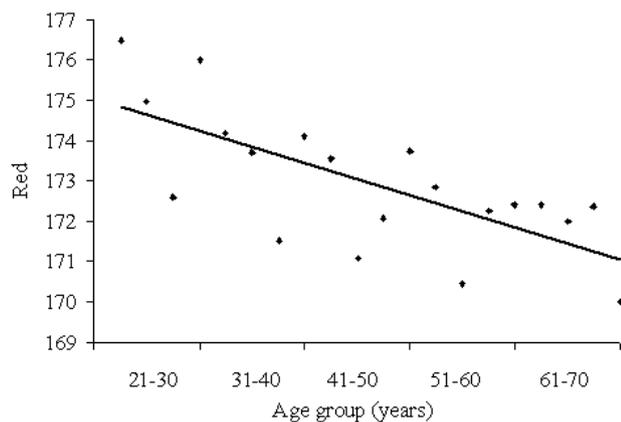


Fig. 1. Relationship between RED colour component and age groups.

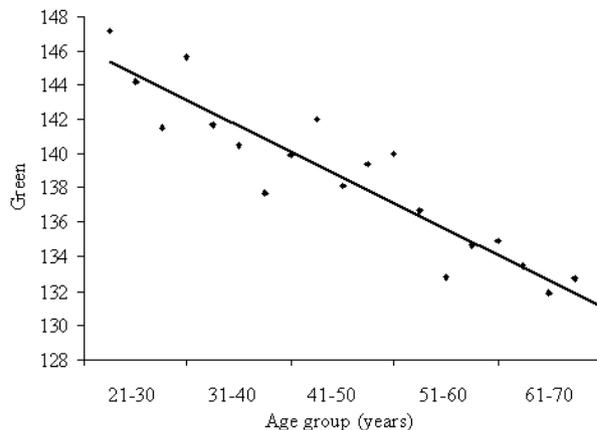


Fig. 2. Relationship between GREEN colour component and age groups.

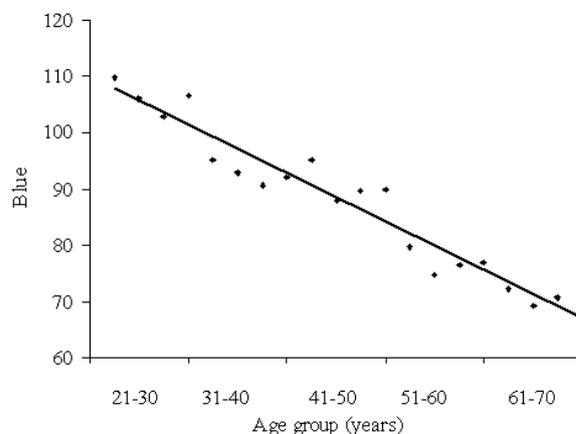


Fig. 3. Relationship between BLUE colour component and age groups.

colouration between mentioned root sides. This is completely valid only for red and green colour component ($p > 0.99$). For the blue component the HI^2 test value is quite high and results 17.48. For $k=19$ degrees of freedom for blue component the certainty lies between 0.50 and 0.75, still less than $HI^2=35.58$, what means that for this case we can also claim that the given hypothesis was correct with a little certainty ($p > 0.50$). The results of this research have confirmed the hypothesis that there is a correlation between tooth root colour and age. The correlation between the average RGB values of tooth root colour and age shows a linear trend with high correlation coefficients, i.e. $r = -0.994$ with certainty of $p > 0.99$ for red colour component, $r = -0.972$ with certainty of $p > 0.99$ for green and $r = -0.982$ with certainty of $p > 0.95$ for blue component. Figures 1, 2 and 3 show the visual control of the results.

Discussion

The two general methods commonly used to analyse the natural colour of teeth are visual comparison and in-

strumental measurements. Estimation of tooth root colour by comparison with dental shade guides has been used extensively by forensic odontologists, since changes in colour with aging have been described. Nevertheless the use of tooth colour for age estimation in forensic odontology is limited by difficulties with objective measurements^{12,13}. In any visual-colour matching procedure, questions that have to be answered constantly are just how great a colour difference exists between the samples to be matched and the standards and how great a difference is acceptable in given situation¹⁴. In an attempt to develop a more objective method scientific approach should be applied by utilizing a measuring instrument¹². Answers that scientists are always looking for are connected with new and objective enough methods that can be used in everyday routine practise. Several new studies have shown that digital camera can also be used in colour determination^{15–17}. In this study digital measurements have shown that there is a significant correlation between tooth root colour and age, in which all colour components (RGB) correlated well. The results obtained in this study are in agreement with previously published results¹⁸. This change in colour with age is due to continued cementum deposition through life¹⁹ or perhaps due to some undefined intrinsic change in the dentin²⁰. In the study which resembles the most to this one uneven colouration of four anatomic root surfaces was found.

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The percentage of yellow was similar on three of the four sides, and considerably less on the mesial side¹⁸, what was not the case in this research. Although there are assessments about small quantity of cementum found on mesial side comparing with the distal one²¹, the biological mechanisms of tooth root colour should be further examined^{13,18}. The advantage of the colourimetric analysis using digital camera and Adobe Photoshop 7.0 software used in this study, lies in the fact that this method does not require a long and expensive laboratory preparation and can be performed with a minimal knowledge of computer skills. According to the given results it is also objective enough for this kind of examination. The disadvantage of this method is the fact that it belongs to the invasive techniques what means that teeth need to be extracted. One of the problems for age calculation is also the time period when the teeth were found. Namely, some studies have shown that the postmortem interval could affect age-related morphological changes¹³, and that different methods of dental age estimation should be used depending on the time after death^{6,22}.

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UTJECAJ DOBI NA PROMJENU BOJE KORIJENA ZUBA

SAŽETAK

Svrha ove studije bila je ispitivanje povezanosti između boje korijena zuba i starosne dobi u čovjeka. Ispitivanje je napravljeno na uzorku od 100 zubi. Svi zubi, odnosno njihovi korijeni snimljeni su digitalnom kamerom, a kolorimetrijska obrada napravljena je računalno u programu Adobe Photoshop 7.0. Studija je pokazala da nema statistički zna-

čajne razlike između dobivenih RGB vrijednosti na cijeloj površini korijena, u odnosu na samo središnji dio korijena, uz pouzdanost $p > 0.99$. Također je utvrđeno da nema statistički značajne razlike u obojenosti na četiri anatomske površine korijena (bukalno, mezijalno, lingvalno, distalno), uz pouzdanost $p > 0.99$ za crvenu, $p > 0.99$ za zelenu i $p > 0.50$ za plavu komponentu boje. Statistička obrada podataka pokazala je da postoji linearna korelacija između dobivenih RGB vrijednosti i starosne dobi uz $r = -0.994$, $p > 0.99$ za crvenu komponentu, $r = -0.972$, $p > 0.99$ za zelenu i $r = -0.982$, $p > 0.95$ za plavu komponentu boje. Iz dobivenih rezultata može se zaključiti da se analizom promatranog parametra može izračunavati dentalna dob te da ova tehnika može poslužiti kao podloga za praktičnu primjenu u svrhu određivanja kronološke dobi čovjeka.

Antropological Measurement of the Sacroiliac Joint

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ABSTRACT

This study was done on 65 isolated pelvic bones. These cadavers belonged to adult people of mature and old age, and they had no pathological changes. These measurements were performed on osteological collection of Department of Anatomy Drago Perović. Many geometrical parameters of facies auricularis were measured, which we considered important for further studies, simulations of joint's action, transfer calculations of the forces from spine to hip joint. We used paper, which partially adapted to the surface of facies auricularis, so the values are closer to real ones than projection values. The results have shown that the average surface of facies auricularis is 13.46 cm². There was no statistical significance found between left and right cadavers. For easier orientation we divided surface of facies auricularis into two parts: vertical and horizontal. Height of the vertical part was 3.99 cm, while the width was 2.05 cm. Height of horizontal part was 2.07 cm and the width 3.62 cm.

Key words: *facies auricularis, sacroiliac joint, pelvic bone, os coxae*

Introduction

Research of the sacroiliac joint started in time of antique. Hippocrates was one of the earliest researchers. Researches continued during the next centuries, up till today, pausing during the middle age, because the section was prohibited. In the modern society with the use of today's imaging methods (like 3D computed tomography, high resolution CT, planar and SPECT bone scintigraphy, magnetic resonance) the knowledge has been especially improved. Despite all that, there are still many unknown facts about function and biomechanic of this joint.

Two bones, whose embryological development is very complex, take part in shaping sacroiliac joint. Os sacrum is developed from five sacral vertebrae, vertebrae sacrales I–V, which grow together into a single bone around age of 15. *Os coxae* is created from three parts: *os ilium*, *os ischii* and *os pubis*. Because of such development frequent variation of this area is not surprising. There are variations in vertical and anteroposterior direction, and in shape of the joint's surface^{1,2} (Figure 1 and 2).

This joint is extremely important for the transfer of the forces from the trunk to lower extremities. Authors used to deny movements in sacroiliac joint, while today, many studies showed that, movements in this joint, although relatively small, are very important^{1,3}, which is best seen in pregnancy and labor⁴. Possible movements, because of inadequacy of joint's surfaces and tight ligaments, are mostly sliding and rotation. Many authors consider rotation around transversal axis, which goes through second sacral segment as most important movement of this joint. This movement is important in flexion and extension of the spine¹. Still, movements of this joint are shadowed by the movements of the spine. Because of that this joint is mostly noticed only during diseases. Many pathological changes have been described, congenital anomalies, inflammatory diseases, traumatic lesions and neoplastic processes. The importance of this joint in clinical medicine can be seen in the fact that the sacroiliac joint is a source of pain in the lower back and but-



Fig. 1. Pelvic bone – *facies auricularis*.

tocks in approximately 15% of the population^{5,6}. This pain doesn't have to be caused by some pathological change in the joint itself. Still, there is no reliable clinical method that could define stability of this joint⁷. Many radiological methods for defining pathological changes of this joint have been already described. But X rays can be as hurtful as useful for the patient so it is very important to use them as rarely as possible. That is why it is necessary to know the basic anatomy and biomechanic of this joint. The microscopic anatomy of this joint has been well researched, but for understanding the transfer of the forces it is important to know precise size of the surface of the joint's contact area, which is the goal of this study. It is important for simulations of the walk, results of biomechanical analysis and calculations of the transfer of the forces from the spine to hip joint.

In our references there wasn't many information about surface of the sacroiliac joint, except at the begin-

ning of the last century⁸, and in some younger studies centered on something else, while the information about the surface is only signed⁹, but these studies do not represent systematic research of *facies auricularis* on larger number of bones. We haven't found any research that accurately measured surface of *facies auricularis*, especially not on this number of bones. Some researches have measured parameters on pictures of bones¹⁰, but they got projection values, which are quite different from real values because of curved *facies auricularis* in all three dimensions and irregular surface. Approximations done on these same bones^{11,12} are, by it's definition, larger than actual values. Punctual specification of difference between approximate and actual surface makes it possible for future authors to get more accurate values from approximate on their specimens, and punctual measurements show whether it is justified or not to use approximate values, which is important for future practice.

Materials and Methods

65 isolated pelvic bones of the osteological collection of department of Anatomy »Drago Perović« were included in this research. These cadavers belonged to adult people of mature and old age, and they had no pathological changes. But we have to point out that there was no data on bones gender, so we couldn't interpret results in that direction.

We measured 6 parameters, which define width, height and surface area of *facies auricularis* (Figure 3):

- a – width of vertical part
- b – height of vertical part
- c – height of horizontal part
- d – cranial width of horizontal part
- e – caudal width of horizontal part

It was necessary to take more parameters besides the surface area, because of relatively large variety of shapes, so just the value of surface was not enough to create an image of *facies auricularis*, and especially it didn't pro-

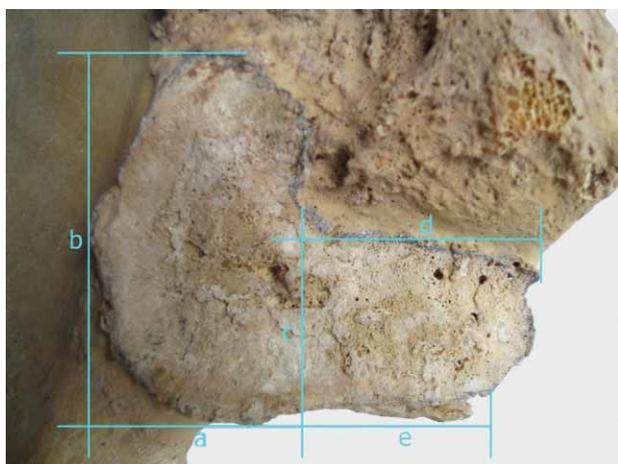


Fig. 2. *Facies auricularis* of the pelvic bone with measured parameters. a – width of vertical part, b – height of vertical part, c – height of horizontal part, d – cranial width of horizontal part, e – caudal width of horizontal part.

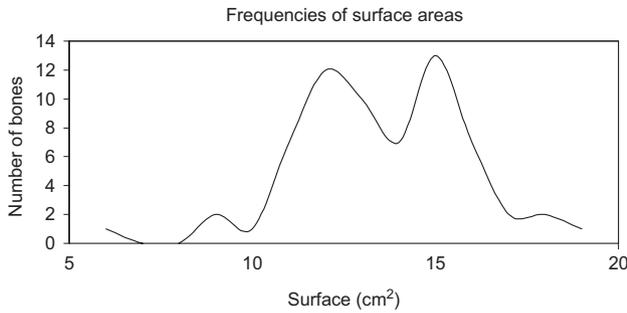


Fig. 3. Frequencies of surface areas of the *facies auricularis* of the pelvic bone. Most of the specimens had surface between 11 cm² and 17 cm², with noticeable exceptions. It can also be seen that there are two peaks, which would most likely refer to differences between male and female specimens.

vide more precise simulations of joint’s action and calculations of the forces that influence on different parts of joints cartilage and ligaments.

We decided to measure both widths of horizontal part of *facies auricularis* (d and e) because they are often different, and obviously slantwise connected, which is not the case with other parameters.

Surface area was measured with tracing paper, which we attached firmly to the surface, and then by marking the edges we shaped the surface. The paper partially adapted to the surface of *facies auricularis*, so the values are closer to real ones than projection values. All measurements were repeated, to insure the highest accuracy.

The data we got have been statistically analyzed, to get average value, standard deviation, metering error and coefficient of variability. Calculations were done using Microsoft Excel program.

Results

All data have been statistically analyzed, and they are as follow:

Surface (Figure 3): average value was 13.46±0.86 cm², standard deviation 2.32. Maximal value was 18.59 cm², minimal value 6.42 cm², coefficient of variability 17.24. Metering error was ±0.14 cm².

a – width of vertical part: average value was 2.0538±0.1452 cm, standard deviation 0.39. Maximal value was 2.8 cm, minimal value 0.9 cm, coefficient of variability 18.99. Metering error was ±0.05 cm.

b – height of vertical part: average value was 3.9938±0.1899 cm, standard deviation 0.51. Maximal value was 5.0 cm, minimal value 2.1 cm, coefficient of variability 12.77. Metering error was ±0.03 cm.

c – height of horizontal part: average value was 2.0692±0.1266 cm, standard deviation 0.34. Maximal value was 2.8 cm, minimal value 1.5 cm, coefficient of variability 16.43. Metering error was ±0.05 cm.

d – cranial width of horizontal part: average value was 3.6200±0.2457 cm, standard deviation 0.66. Maximal value was 4.8 cm, minimal value 2.0 cm, coefficient of variability 18.23. Metering error was ±0.02cm.

e – caudal width of horizontal part: average value was 3.3331±0.2328 cm, standard deviation 0.64. Maximal value was 4.6 cm, minimal value 1.6 cm, coefficient of variability 19.20. Metering error was ±0.02 cm.

We presented results in Table 1, for better view and more simple comparison.

Discussion

We tried to make the most accurate measurements of surface of *facies auricularis* of the pelvic bone. These results will make further researches about this joint easier, simulations of joint’s action, calculations of the forces from the spine to the hip joint.

Caudal width of the horizontal part of *facies auricularis* (e) has the largest coefficient of variability. Our predictions about variability of this parameter turned out to be true, and that is why we decided to measure it. Height of vertical part (b) has the smallest coefficient of variability.

Surface that we measured is 30% smaller than Brok’s results (1924.), and 20% larger than results which Ebraheim (2003.) got on 30 cadavers. These differences are statistically significant. They can be result from metering error, nonrepresentative sample, difference in regional constitution of people, which could be result from different way of walk and transfer of forces through this joint in different areas of the world.

TABLE 1
RESULTS OF MEASUREMENTS

	Surface	a	b	c	d	e
X	13.46 cm ²	2.0538 cm	3.9938 cm	2.0692 cm	3.6200 cm	3.3331 cm
Standard Error	0.2878	0.0484	0.0633	0.0422	0.0819	0.0794
SD	2.32	0.39	0.51	0.34	0.66	0.64
Maximal value	18.59 cm ²	2.8 cm	5.0 cm	2.8 cm	4.8 cm	4.6 cm
Minimal value	6.42 cm ²	0.9 cm	2.1 cm	1.5 cm	2.0 cm	1.6 cm
Coefficient of variability	17.24	18.99	12.77	16.43	18.23	19.20
Metering error	±0.14 cm ²	±0.05 cm	±0.03 cm	±0.05 cm	±0.02 cm	±0.02 cm

Results for surface are 14% smaller than approximations done on these same bones^{11,12}. Approximations were calculated by dividing surface into two rectangles, and by multiplying the length of the edges, which occupied more area, because of the irregularity of *facies auricularis*.

Although the difference between left and right *facies auricularis* of *os sacrum* has already been proved¹³, we couldn't prove it for *os coxae*. Difference between average values is not statistically significant, but since we couldn't make left and right pairs, we couldn't make two dependent groups. Size of the specimen probably influenced too. It would be interesting to do such a research, and prove results of more often using of the right hand in the most of the population in this region of the body as well. This influence is a consequence of the transfer of the forces from upper portion of the body to the contra lateral side of the lower, which brings to creation of bigger and stronger structures on that side of the lower body.

Conclusion

Despite many invasive approaches to sacroiliac joint, which are necessary to diagnose or treat different pathological states, there is relatively small number of studies, which are dealing with anatomy of this joint.

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With this study we helped building more precise definition of surface of this joint. In full definition of this joint there is a lack of studies about normal morphology of ligaments involved in this joint.

Using these measurements we showed that there are still many unknown things about anatomy of this joint. These results will make further researches about this joint possible, which are important for understanding and treating degenerative changes that effect more and more old people.

Based on measurements of 65 dry bones we conclude that surface of *facies auricularis* is 13.46 cm², and other parameters are as follows: a = 2.0538 cm, b = 3.9938 cm, c = 2.0692 cm, d = 3.6200 cm, e = 3.3331 cm. These results are statistically significantly different than results in our references.

Without understanding basic anatomy, we can not accurately interpret images produced by new methods, and that decreases value of the methods, increases costs of treatment because of the use of more expensive methods, and last but not the least, it is bad for the patients. Considering that it is of great importance to continue with researches like this one, so all the parameters of macroscopical anatomy would be accurately defined, what is very important especially in these days, when precision and evidence based medicine is especially stressed.

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ANTROPOLOŠKO MJERENJE SAKROILIJAKALNOG ZGLOBA

SAŽETAK

Ovo istraživanje je napravljeno na 65 izoliranih zdjelčnih kostiju. Uzorci su pripadali ljudima zrele i starije dobi, i nisu imali nikakvih patoloških promjena. Mjerenja su se vršila na osteološkoj zbirci Zavoda za anatomiju Drago Perović. Mjereno je više geometrijskih parametara *facies auricularis*, koje smo smatrali važnima za daljnja istraživanja, simulacije rada zgloba, proračune prijenosa sila sa kralježnice na zglob kuka. Sva mjerenja su ponavljana kako bi se osigurala što veća točnost. Rezultati su pokazali da prosječna površina *facies auricularis* iznosi 13.46 cm². Nije nađena statistički značajna razlika između desnih i lijevih primjeraka. Radi lakšeg snalaženja podijelili smo površinu *facies auricularis* na dva dijela: okomiti i vodoravni. Visina okomitog dijela bila je 3.99 cm, dok širina 2.05 cm. Visina vodoravnog dijela bila je 2.07 cm, a širina 3.62 cm.

Foot Anthropometry and Morphology Phenomena

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ABSTRACT

Foot structure description is important for many reasons. The foot anthropometric morphology phenomena are analyzed together with hidden biomechanical functionality in order to fully characterize foot structure and function. For younger Croatian population the scatter data of the individual foot variables were interpolated by multivariate statistics. Foot structure descriptors are influenced by many factors, as a style of life, race, climate, and things of the great importance in human society. Dominant descriptors are determined by principal component analysis. Some practical recommendation and conclusion for medical, sportswear and footwear practice are highlighted.

Key words: foot anthropometry, foot shape, foot biomechanics

Introduction

The human foot is a complex structure, playing an important role in the locomotion processes of the lower extremity. It is a part of the body that acts on external surface, providing support and balance during stance and gait. The foot structure description, beside geometrical anthropometric descriptors needs biomechanical factors such as muscle deformation, tissue stiffness, stress and strain distribution¹. The full morphological description of the foot for more than 26 anthropometric measures is desired. Foot dynamic anthropometry has a vital role in medical rehabilitation, sport science, and footwear design among others². Distribution of the internal foot structure for given population is an indicator of the foot deformity, aging, and body growth anomaly among other things. The human foot as a complex structure is under dynamic loads, producing elevated plantar pressure and stress evolution within and between its structural components. Several techniques have been developed to study the morphology, architecture and kinematics of the foot³. Integrated experimental technique is able to measure simultaneously both the kinematics and dynamic structural behaviours of the foot during gait, including development and validation of the 3D finite element model of the foot. The purpose of this study is to investigate the relationship between foot anthropometrical and biomechanical descriptors and derive usefulness regression

equations for Croatian population. Scatter data of the individual foot variables are interpolated by multivariate statistics. This is multiple regression analysis, in which various combinations of these variables were regressed against each other with physical explanation.

Foot Anthropometric Descriptors

Anthropometric variables such as foot length, joint girth, bottom width, are stochastic variables in geometrical description of the foot⁴. Probability distribution of these variables is determined by measurement for given population in determined geographic region in specified time interval. In the past decade there has been some remarkable advance achieved in morphometrics and multivariate statistics of the shape object⁵. In the footwear practice, manufacturers assume that most foot dimension follow multivariate n – dimensional normal distribution

$$f(x) = \frac{1}{(2\pi)^{n/2} |\Sigma|^{1/2}} \exp \left[-\frac{1}{2} \{x - \mu\}^T [\Sigma^{-1}] \{x - \mu\} \right] \quad (1)$$

Here $\{x\}^T = \{x_1, x_2, \dots, x_n\}$ is an n – dimensional vector, $\{\mu\}^T = \{\mu_1, \mu_2, \dots, \mu_n\}$ is a mean vector, and $[\Sigma]$ is $n \times n$ – covariance matrix. Traditional shoe size system has

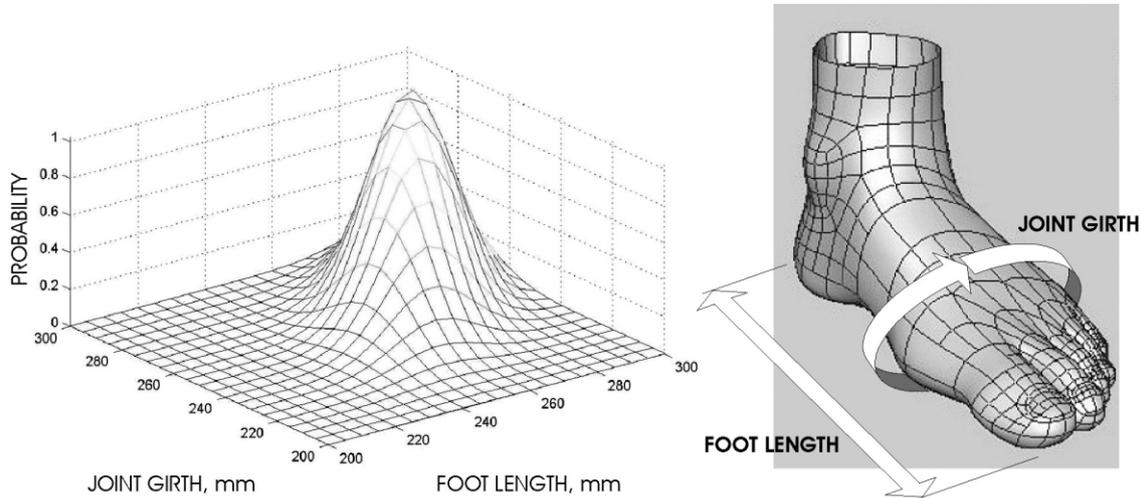


Fig. 1. Bivariate normal distribution of foot length and joint girth.

been constructed using bivariate normal distribution (Figure 1) with adequate classes and intervals for both foot length and joint girth.

In Japanese Industrial Standard⁶, shoe size is defined by three parameters; length of foot, joint girth and width of foot. The industrial footwear production can be adopted for given population by constructing their size distribution function according to the stochastic interpolation theory. These few measures are not sufficient for proper foot fit and comfort under shoe; hence a good description of the 3D foot shape is necessary. The anthropometric variables are ordered in a hierarchical manner based on the geometric and statistical relationships among the variables. Length and height variables measure the lengths of the foot segments. In contrast, width, depth and circumference variables measure the cross-sectional sizes of the foot.

Foot Shape Representation

A foot in CAD (Computer Aided Design) is represented as a set of digitized points on the surface that more closely approximate an average shaped foot⁷. The 3D foot surface can be represented using Bezier or B spline, as the closest interpolating surface across digitizing points. According to the shape searching techniques, we classify 3D shape representation into the following categories⁵; global feature-based, manufacturing feature recognition-based, graph-based, histogram-based, product information-based and 3D object recognition-based method. Global feature-based methods use global properties of the 3D model such as moments, invariants and Fourier descriptors. The foot surface A can be approximated using spherical harmonics, the three coordinate functions are decomposed and the surface $A(\phi, \vartheta) = \{x(\phi, \vartheta), y(\phi, \vartheta), z(\phi, \vartheta)\}$ had the following form

$$A(\phi, \vartheta) = \sum_{i=0}^{\infty} \sum_{m=-1}^i C_i^m \cdot Y_i^m(\phi, \vartheta) \quad (2)$$

where the coefficients C_i^m are 3D vectors, Y_i^m are spherical harmonic basic functions, and Φ and ϑ are polar angles. The spherical harmonics had been frequently used for visualization and comparison of human body objects in medical practice. Another accurate shape description relies on geometric moments up to the second order. A three-dimensional moment λ_{ijk} can be described by the following integral

$$\lambda_{ijk} = \iiint f(x, y, z) x^i y^j z^k dx dy dz \quad (3)$$

i, j, k = 1, 2

where $f(x,y,z)$ describes the object. Using this notion the mass m and inertia tensor I_{ij} can be expressed as

$$m = \lambda_{000} = \iiint f(x, y, z) dx dy dz \quad (4)$$

$$I_{ij} = \begin{bmatrix} \lambda_{020} + \lambda_{002} & -\lambda_{010} & -\lambda_{101} \\ -\lambda_{110} & \lambda_{200} + \lambda_{002} & -\lambda_{011} \\ -\lambda_{101} & -\lambda_{011} & \lambda_{200} + \lambda_{020} \end{bmatrix} \quad (5)$$

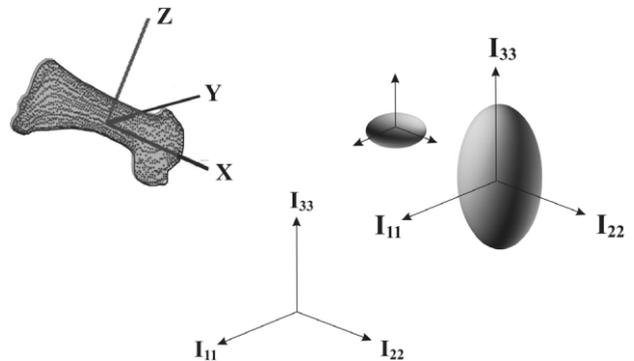


Fig. 2. Bone inertial tensor space.

The principal axes orientation is determined by the eigenvectors of the inertia tensor I_{ij} . The shape of each bone is characterized by eigenvalue of the inertial tensor defined for a coordinate system in bone centroid. Each bone is defined by the confidence ellipse in eigenvalue space. The separation between bone types is evident⁸, (Figure 2). The inertial tensor of the group bones can be useful morphological descriptor of the foot functionality.

Some 3D shape can be represented by graph-based techniques, as other shape representation possibilities. Topology is typically represented in the form of a relational data structure such as graphs and trees. The shape can be represented by boundary representation, spectral, Reeb or skeletal graph. One of the shape description methods is medial representation m-rep of the foot. The m-rep parameters are element of the Lie groups, and therefore all statistical calculations must be performed in tangent space.

Principal Component Analysis

The recent development of 3D laser scanner has provided another efficient way for surface registration and analysis. The scattered grid point data are fitted by small second-order polynomial surface patch. At each point we have calculated the principal curvatures, mean curvature, Koenderink shape index⁹, which are shape representing parameters. On the basis of parameter values at each point, potential landmark area could be detected. Now the shape descriptor becomes a $3n$ element vector (n – number points)

$$X_i = \{x_1, y_1, z_1, \dots, x_n, y_n, z_n\}^T \quad (6)$$

Each shape S_i is represented by a set of n landmarks (i.e. sampling points) X_i .

$$S_i = B \cdot X_i \quad (7)$$

where B is spline matrix. The mean shape \bar{X} for a group of N shapes can be calculated using

$$\bar{X} = \frac{1}{N} \sum_{i=1}^N X_i \quad (8)$$

where X_i is the landmark shape descriptor of the i -th shape. Principal component analysis is applied to reduce dimensionality, for example reduction 3D (X, Y, Z) to 2D ($PC1, PC2$) as shown by Figure 3. The global covariance matrix Ξ of the data

$$\Xi = \frac{1}{N-1} \sum_{i=1}^N (X_i - \bar{X}) \cdot (X_i - \bar{X})^T \quad (9)$$

$$\Xi = \Phi \cdot \Lambda \cdot \Phi^T \quad (10)$$

where the columns of Φ hold eigenvectors, and the diagonal matrix $\Lambda = \text{diag}(\lambda_1, \dots, \lambda_i, \lambda_j, \dots)$, $\lambda_i > \lambda_j$ holds eigenvalues of Ξ . The first few eigenvectors $m \leq 3n$ (with greatest eigenvalues) can explain most of the variance in the data. This means that the $3n$ dimensional space is approximated by the m dimensional space (Figure 3) Now any shape X in the data can be obtained by writing

$$X = \bar{X} + \Phi \cdot A \quad (11)$$

Where A is a vector containing the components of X in basic Φ , which are called principal components?

$$A = \Phi^T (X - \bar{X}) \quad (12)$$

The analysis for the foot structure⁷ has shown that the first principal component (PC) reflects foot size, while the second PC's define the shape parameter of the foot, and the third PC's define comfort.

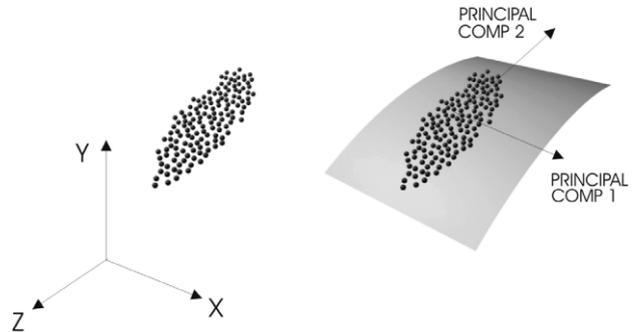


Fig. 3. Principal component analyses.

Comparison and Adjustment of the Foot and Shoes

Some known morphological measures (shape distance metric, segmentation, clustering) applied to fully 3D foot shape can explain many foot deformity phenomena in the best possible way. Proper fit is more than fit length and width; it requires a good understanding of the total 3D shape. The starting point is the geometric similarity between two feet and between foot and last. The basic idea is to compare the lasts which were used to manufacture the shoes and the scanned feet of the clients. The foot geometric similarity can be described by two-step procedures: the pose estimation and object comparison¹⁰. The appropriate pose estimation, which consists of computing the scaling, translation and rotation of the objects, so that their surfaces lay one on the other. The cost function had minimized the following least-squared distance metrics

$$\min_{R,T} \sum_i \|A_i - (R \circ B_i + T_i)\|^2 \quad (13)$$



Fig. 4. Comparison between last and foot.

Where A_i and B_i represents points on the objects A and B respectively. The goal is to find a rotation R and translation T matrix, which minimize the least-squared distance metric. By co-locating the centroids of two objects at the origin of the reference coordinate system 3 degrees of freedom for translation can be removed. The closest pose is derived by comparing the ray distances while one shape is virtually rotated with respect to the other¹¹.

After the pose estimation, we determine for each object how big portion of its volume is outside of the other object (Figure 4). This comparison problem is possible to be resolved by discrete 3D distance field the concept of which is described in¹⁰. We transform each triangle of the foot surface into the closest triangles on the last by spatial distortion. This comparison method is better replaced by Free Form Deformation (FFD) method because foot is not a simple deformable shape¹². The FFD technique smoothly transforms a shape of an object by setting control lattice points around the object and then moving these control lattice points.

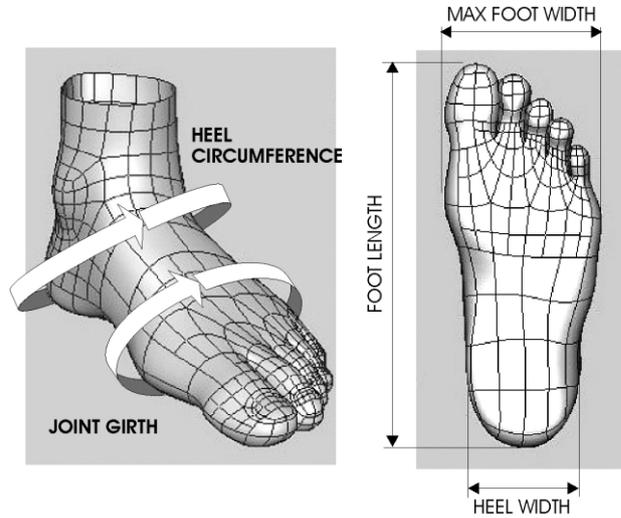


Fig. 5. Measured foot dimensions.

$$f(x) = \frac{1}{2\pi|\Sigma|^{1/2}} \exp\left(-\frac{1}{2} \{x - \mu\}^T [\Sigma^{-1}] \{x - \mu\}\right) \quad (14)$$

Material and Methods

A group of 103 normal adult males selected among student population in Croatia has participated in this study. Their stature height and weight were first recorded. All measurements were made under 'no-load' conditions. The age of participants was between 18–21 years. The five dimensions (Figure 5) on the left foot have been measured for each subject (foot length, joint girth, maximum foot width, heel width and circumference).

According to footwear practice, we assume that foot length and joint girth obey bivariate normal distribution

The scatter data (Figure 6) are interpolated by bivariate normal distribution. The calculated average foot length is $\mu_1=273.29$ mm (standard deviation is $\sigma_1= 12.01$ mm) and the average joint girth is $\mu_2=263.18$ mm (standard deviation is $\sigma_2=12.52$ mm). The correlation coefficient of foot length and joint girth is $\rho_{12}=0.665$ (Figure 6).

We calculated inter-variable correlation coefficients for the measured data displayed in the Table 1.

In order to study the influence of race on basic footwear dimensions, we compared bivariate normal distribution foot length – joint girth for Croatian population with some other nationality and race. We compared our

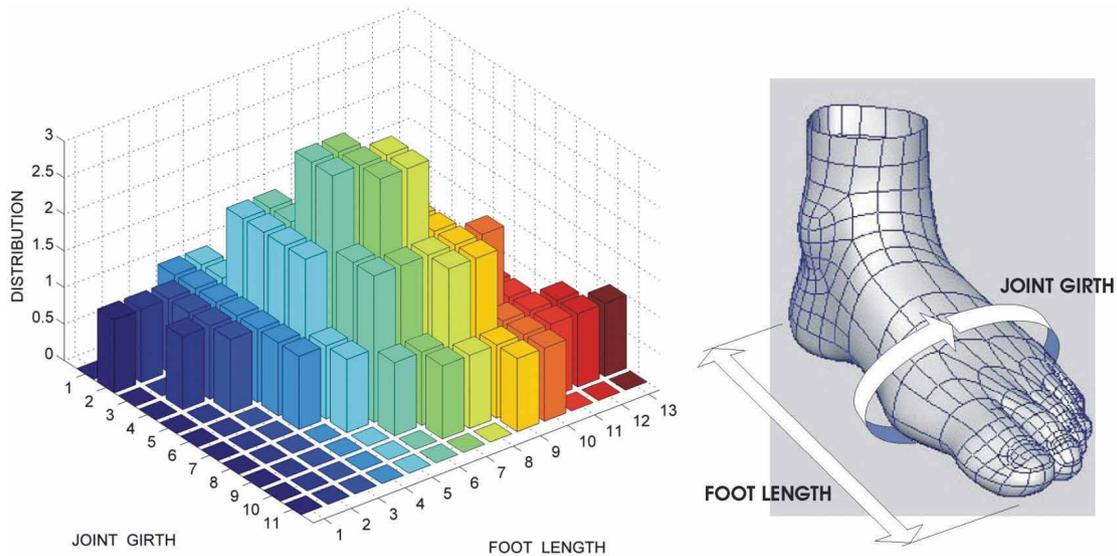


Fig. 6. Calculated normal distribution foot length-joint girth.

TABLE 1
INTER-VARIABLE CORRELATION COEFFICIENTS

	Foot Length	Joint Girth	Foot Width	Heel Circum.	Stature
Foot Length	1	0.665	0.560	0.410	0.930
Joint Girth		1	0.925	0.829	0.78
Foot Width			1	0.865	0.721
Heel Circum.				1	0.609
Stature					1

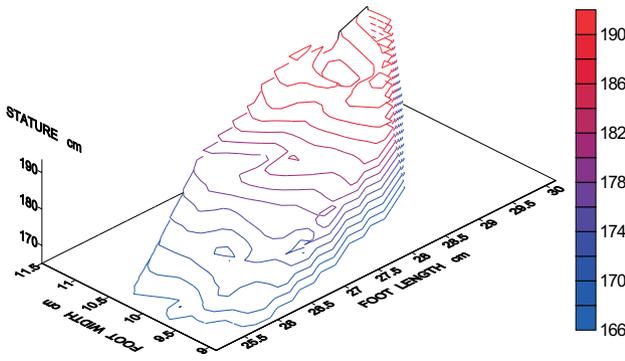


Fig. 7. The stature dependence on foot length and on maximum foot width.

data with literature data¹³, for urban population in Russia and four populations in East Asia, i.e. Chinese, Japanese, Korean and Taiwanese¹⁴. In terms of race, the people in the region of East Asia belong to the Mongolian race and they have significantly different foot shape than the Europeans. Ethnic diversity is a significant factor and affects foot shape, too. It is possible to conclude that the Croatian population has large feet and different cor-

relation coefficient. Therefore, establishing a national anthropometry database for the population is now inevitable. In medical and forensic practice the correlation between foot dimensions, stature height and body weight is recommended as shown on Figure 7.

One important structural characteristic is the height of the medial longitudinal arch above the ground plane during weight bearing activities¹⁵. The variations of the foot structure influence the shape of a footprint made by that foot. The measurement of the width or the contact area on the imprint is suggested to provide simple and objective means of foot classification. The arch index Θ is defined as the ratio of area of the middle third part area A_2 (Figure 8) of the footprint the entire footprint area ($A_1 + A_2 + A_3$), (excluding the toes) and it can be written as

$$\Theta = \frac{A_2}{A_1 + A_2 + A_3} \quad (15)$$

The dependence of the body mass index (BMI) on arch index (Figure 8) indicates that the sample population has got normally distributed foot structure. The subjects with a lower arch (higher arch index) appeared to have a greater BMI. We can conclude that both body mass and arch index contribute to increased foot pressures. The objective of classification of the foot according to arch type can be helpful for sporting people in searching proper performance, and useful for people with abnormal foot structure to prevent injury. Some of subjects have been selected to test comfort and fit. The most preferred wearing shoes out of 10 pairs of running shoes are compared. The appropriate shoe size is supplied by last. The main fit indicator was circumference allowance defined as follow

$$\lambda = \frac{(C_{last} - C_{foot})}{C_{foot}} \quad (16)$$

Where C is circumference? On Figure 9 constructed confidence region between foot circumference and allowance.

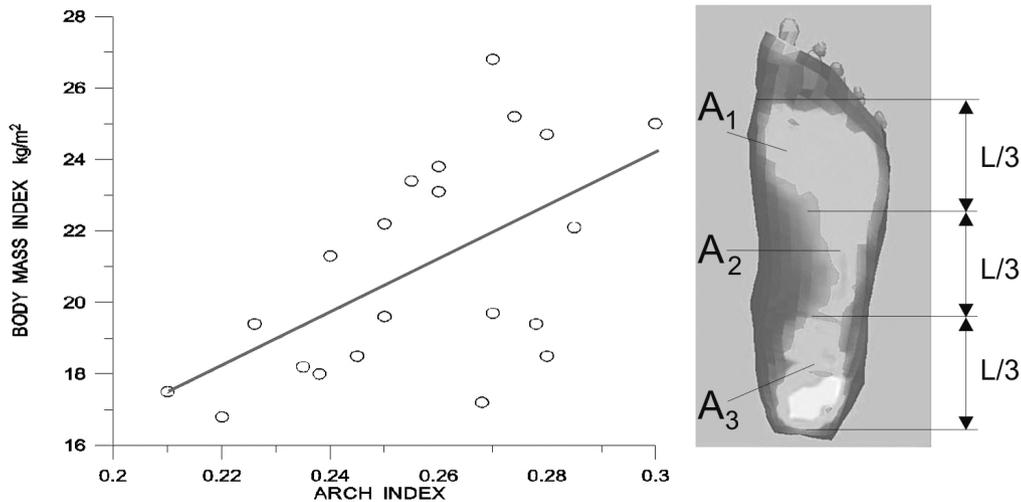


Fig. 8. Dependence of the body mass index on Arch index.

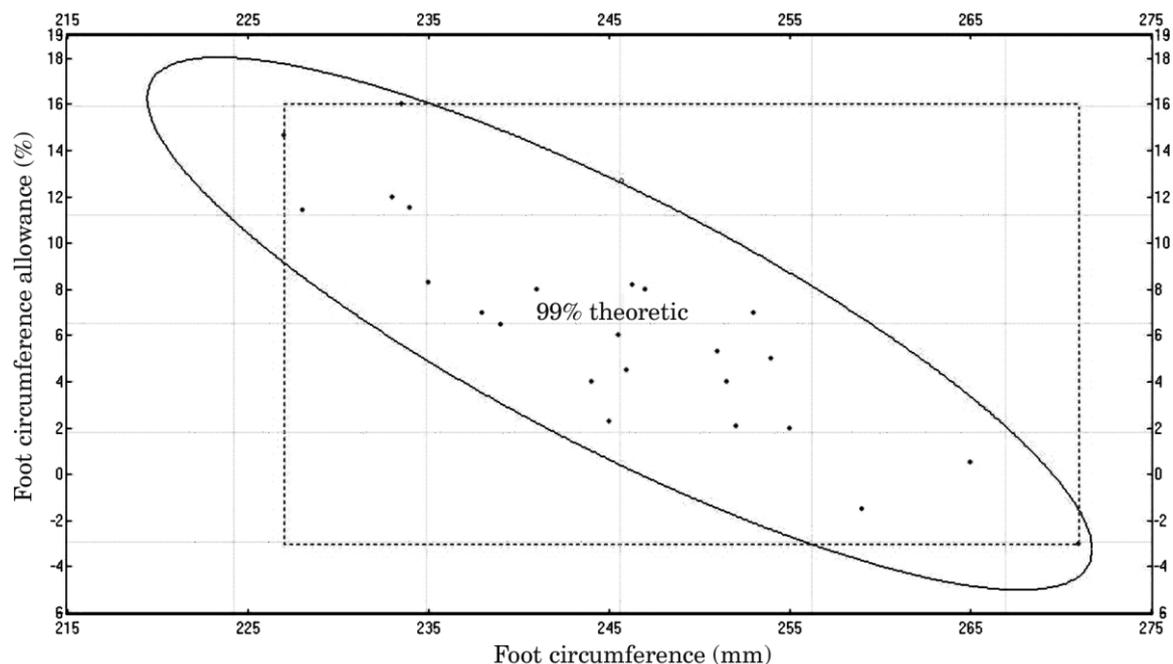


Fig. 9. Confidence regions for foot circumference-allowance.

The calculated average foot circumference is $\mu_1=245.57$ mm (standard deviation is $\sigma_1=10.69$ mm and the average circumference allowance is $\mu_2=6.52$ % (standard deviation is $\sigma_2=4.71$ %). There is evident negative correlation between comfort and foot circumference $\rho_{12}=-0.85$ (Figure 9).

According to collected data some well-known relationships for foot structure have been confirmed, for example that length dimensions are proportional with foot length. The influence of the candidate's birthplace and the style of life are indicative for some foot dimensions. The mean (virtual) shape is based on homologous modelling¹⁶. The shape distribution maps visualize the distance relationship between individuals calculated on the basis of the homologous shape model. According to the shape theory,

the distance metric could be established by using multi-dimensional scaling. After that, we can categorize a foot into sizes or types for target population.

Discussion and Conclusion

There are many factors that influence foot structure; therefore 3D foot shape descriptor has been supplemented by biomechanical structural and functional factors. Multivariate analysis between foot dimensions reveal how to precisely improve footwear fit and comfort. The future standardization decisions must be made to choose a few dominant descriptors needed for foot customization. The computational methods needed for comparison and adjustment must be adopted in shoemaking practice.

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ANTROPOMETRIJSKI I MORFOLOŠKI FENOMENI STOPALA

S A Ž E T A K

Strukturni deskriptori stope su važni iz mnogo razloga. Antropometrijski i morfološki fenomeni analizirani su zajedno s biomehaničkom funkcionalnošću s ciljem potpune karakterizacije funkcije i strukture stopala. Za hrvatsku studentsku populaciju izvršena je statistička obrada prikupljenih podataka. Funkcionalni i strukturni deskriptori stopala ovise o mnogo faktora kao napr. stilu života, klimi i nizu dominantnih faktora ljudske zajednice. Bitni faktori određeni su metodom glavnih komponenti. Neki bitni zaključci za proizvodnju medicinske i sportske obuće su komentirani.

Influence of Instability and Muscular Weakness in Ethiopathogenesis of Hip Fractures

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ABSTRACT

The aim of our study was to, in accordance with the presented theoretical presumptions, analyze the possible reasons for hip fractures of the older population in the north-eastern part of Croatia. A group of 2,696 persons (1,936 women and 760 men) with hip fractures has been analyzed during a 12 year period (from 1993. until 2005. year) in the Clinical Hospital Osijek. The date of admittance, age, gender and fracture location were recorded. In men, the incidence of total hip fracture number on the left side was greater 23.5% ($p < 0.01$) compared to the right side, while in women this difference does not exist. Men have a greater incidence of trochanteric fractures than fractures of femoral neck on both sides, while in women this difference could be shown on the right side only. In women, a 30.5% ($p < 0.001$) higher fracture incidence occurred in the winter compared to the summer. It has been concluded that in men the impaired neuromuscular function on the left body side caused the greater incidence of falls on this side. The more frequent multifragmental fractures of the trochanteric massive in men indicate the possible role of preserved pelvitrochanteric muscle tension in fracture characterization. Increased incidence of falls and fractures in the older female population can be interpreted with a more pronounced weakness of pelvitrochanteric muscles and consequent walking instability. Furthermore, a smaller incidence of hip fractures was noticed in the summer compared with winter. This is explained by a reduced exhaustion of pelvic muscles in summer (primarily pelvitrochanteric) and decrease in fall frequency.

Key words: bone, hip fractures, instability, osteoporosis, seasonal variations

Introduction

Upper femoral fractures are, and will always be, a distinctive part of trauma in older people^{1,2}. This kind of injury occurs more frequently in women than in men^{3,4}, and displays an exponential rise with age^{5,6}. The treatment and accommodation of these injuries imposes significant investments to the community^{7,8}. The epidemiological studies show an increase of people aged over 65 years⁹. In etiopathogenesis of hip fractures, as the outlined factors are cited osteoporotic and weak bones of older people, especially women^{10,11}. The bone loss with increasing age is partially the effect of the accompanying biochemical changes like: lowering of serum sexual hormones^{12,13} and of 25-OH-cholecalciferol levels¹⁴ and an increase of parathyroid hormone concentration¹⁵. Bone mass loss is more rapid in diabetics¹⁶, chronic alcoholics¹⁷, cigarette smokers¹⁸ and others.

Some authors indicate a low correlation between hip fractures and bone mass, and point out the critical role of instability in falls of older people^{19,20}. Frequent falls and walking instability of older people may be the consequence of decreased physical activity and weakness, as well as bone and muscle diseases^{21,22}. The fear of falling and of injury in older people may result in a further reduction of physical activity. Some articles indicate that more active older people experience a lesser incidence of lower extremity fractures^{23,24}, as well as decreased fall frequency^{21,25}.

Muscular weakness and polyneuropathic difficulties are clinically well known in diabetics and chronic alcoholics²⁶, which comprised a marked fraction of patients with hip fracture. It is also known that after recovery

from cerebrovascular insults, due to the remaining peripheral neurological defects, falls as well as hip fractures are more frequent, and that falls are located mainly on the body side with neurological defects^{27,28}. A certain number of works indicate the anatomical differences in the structure of the upper part of the femur as a possible reason for the more frequent fractures²⁹.

Materials and Methods

Patients

Clinical Hospital Osijek, with its Department of Traumatology, is located in the north-east part of Croatia, with typical continental climate. Injured persons with hip fractures comprise a significant number of patients, admitted each day. Only a small number are not hospitalized (those with femoral neck fractures and clear contra-indications for operation, or people who are judged, according to their general condition, as incapable to undergo the operation). This minority of injured patients are sent to home care after adequate plaster immobilization. The bulk of patients are treated operatively 2–3 days after reception, following preoperative care.

In our investigation, a group of 2,696 men and women with hip fractures, hospitalized during the 12 year period from 1993. to 2005. has been analyzed. Recorded were the date of admittance, age, gender, fracture location and accompanying chronic diseases like diabetes and peripheral neurological defects of the lower extremities. In our analysis those injured in traffic accidents, children, pathological fractures, and falls which were not in the level have not been included.

Our clinical experience shows that patients with hip fractures are mainly from the older population, and were injured while performing their usual everyday activities. In women with hip fractures, physical weakness, which is not compatible with age, is frequently noticed. Chronic diseases diagnosed in injured patients included diabetes, arterial hypertension, peripheral neurological disorders, chronic bronchitis, trouble with vision, and, rarely, hyperparathyroidism. In men with the same injury, in addition to the diseases already cited, chronic alcoholism is frequently found. Generally, our impression is that the population with hip fractures consists of people with a

lower living standard, some of them displaying the syndrome of *dementia senile*.

Trochanteric, subtrochanteric and neck lateral part fractures were treated by stabilization. In the cases of medial neck fractures, where healing could not be accomplished due to abruption of blood supply, we performed arthroplasty. Our operative techniques and indications do not differ from those found in the literature. Postoperative hospital treatment lasted slightly more than two weeks. Physical rehabilitation in old people was more successful in the patients with hip arthroplasty. In the postoperative course we experienced complications like heart decompensation, bronchopneumonia and, less frequently, pulmonary embolism. After hospital treatment the majority of patients continued with rehabilitation in adequate institutions. The healing of stabilized fractures was followed up in the out-patient clinic. A smaller number of patients with fracture stabilization were hospitalized two years later, for the purpose of extraction of osteosynthetic material. A portion of the operated old persons stopped attending the control inspections during the next year, and we lost the evidence about their actual health status.

Data elaboration

The age of the male and female patients is expressed by mean values. The significance of difference between hip frequencies was calculated by χ^2 -test. Differences between groups of interest are expressed in percents. The correlation between hip fracture frequencies and the number of sunny hours is expressed by R-value.

All calculations were performed by Microsoft program Excel.

Results

The mean age of the injured women was 75.5 and men 68.9 years. Total fracture number in women was 1,936, in men 760. In women, total number of hip fractures is similar on both body sides (985 vs. 951, ns) while in men the number of fractures is 23.5% higher on left body side (420 vs. 340, p<0.01).

Table 1. presents the frequency of hip fractures in hospitalized patients depending on gender, body side and fracture type. In females, the frequency of trochanteric

TABLE 1
HIP FRACTURE FREQUENCIES IN FEMALES AND MALES, ACCORDING TO THE BODY SIDE AND FRACTURE TYPE

Body side	Gender	Fracture Type			Difference	
		T	N	T + N	T > N (%)	p<
Right	Female	550	435	985	26.4	0.001
	Male	203	137	340	48.2	0.001
Left	Female	503	448	951	12.3	ns
	Male	234	186	420	25.8	0.05

T – trochanteric, N – femoral neck

fractures on the right body side was 26.4 % higher compared to femoral neck fractures ($p < 0.001$). Such difference could not be shown on the left body side. In males, a greater frequency of trochanteric compared to femoral neck fractures could be demonstrated on both body sides (on the left side 25.8%, $p < 0.05$, on the right side 48.2%, $p < 0.001$).

Among the chronic diseases that could influence the frequency of hip fractures, we noticed diabetes and peripheral defects of lower extremities like hemiparesis and hemiplegia (Table 2). Diabetes frequency in analyzed females was 7.2% and males 2.5%. In the group of patients with peripheral neurological defects of lower extremities, hip fracture was located on the affected body side in 81.3%.

TABLE 2
INCIDENCE OF DISEASES ACCOMPANYING HIP FRACTURES

Diabetes			
Females		Males	
Frequency	%	Frequency	%
139	7.2	19	2.5
Neurological defects of lower extremities			
On the fractured extremity		On the extremity without fracture	
Frequency	%	Frequency	%
122	81.3	28	18.7

The hip fracture frequency in each month of the 12 years period is compared with the number of sunny hours. The correlation of hip fracture frequency and number of sunny hours is outlined in Table 3. The nega-

TABLE 3
CORRELATION OF THE NUMBER OF SUNNY HOURS WITH HIP FRACTURE FREQUENCY IN WOMEN, MEN AND BOTH GENDERS TOGETHER, EXPRESSED BY COEFFICIENT OF CORRELATION, R, AND ITS STATISTICAL SIGNIFICANCE, p.

Patients	R	p <
Women + Men	-0.757	0.01
Women	-0.683	0.05
Men	-0.455	ns

TABLE 4
HIP FRACTURE FREQUENCY IN FEMALES AND MALES COMPARED WITH THE NUMBER OF SUNNY HOURS IN WINTER (NOVEMBER, DECEMBER, JANUARY AND FEBRUARY) AND SUMMER (JUNE, JULY, AUGUST AND SEPTEMBER) PERIODS

		Winter	Summer	Difference	
				%	p <
Hip fractures	Females	762	582	30.5	0.001
	Males	275	238	15.6	ns
Number of sunny hours		3,498	11,169	219	0.001

tive correlation is highest for all fractures in both gender ($R = -0.757$, $p < 0.01$), slightly smaller in females ($R = -0.6829$, $p < 0.05$), while in males no correlation can be proved ($R = -0.455$, ns).

Mean values of number of sunny hours and hip fracture frequency in each month of the time period analyzed are presented in Figure 1.

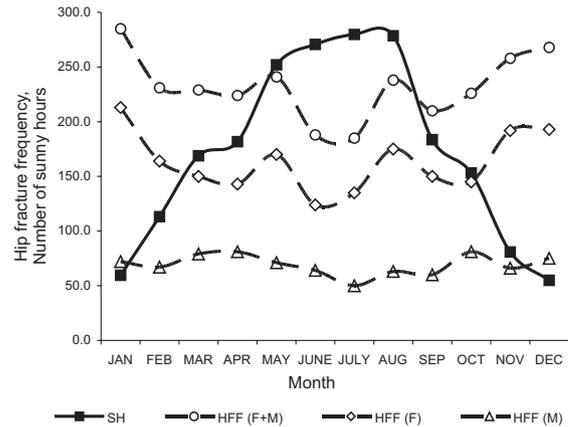


Fig. 1. Number of sunny hours (SH) and hip fracture frequencies (HFF) in females (F), males (M) and both gender (F+M) in each month of the analyzed period.

Further on, fracture frequency in men and women is calculated and compared between the winter (November, December, January and February) and summer (June, July, August and September) period (Table 4). In women, a 30.5% ($p < 0.001$) higher fracture incidence in winter is shown, while in men the difference was not significant.

Discussion

The strength of bone tissue is known to be proportional to the bone mass and bone mineral content^{30,31}. The loss of bone mass in women starts in postmenopausal period, while in men much later, and it increases almost proportionally with age³². The exponential rise of hip fractures with age is hardly possible to explain as a consequence of continuous bone mass loss. If the osteoporosis and weak bones were the only reason of hip fractures, affected women should be younger than affected men. However, we showed that the men with hip fractures are 6.6 years younger than affected women.

Moreover, if osteoporosis attacks equally both sides of pelvis and consequently both right and left femurs, similar frequency of hip fractures on both body sides should be anticipated. On the contrary, in our group of 760 males, the fractures of the left hip are more frequent (23.5%, $p < 0.01$).

To better discuss our results, it is necessary to point out some fundamentals of hip and pelvis biomechanics. Pelvic balance in horizontal position of a walking person is achieved by pelvotrochanteric muscles. The demands on these muscles of the walking old people are not small. Furthermore, in the most people, the more refined movements are always performed by the right (more skilled) foot. Also, great majority of people are right – handed (or »footed«). In most persons, the supporting foot is the left one (i.e. jumping persons in athletics). Of course, it does not mean that the left foot is weaker, or that the pelvotrochanteric muscles on the left side get more quickly tired. In other words, the more frequent fractures of the left hip in our males occur on the less skilled, and, the most probably, on the stronger foot. Our conclusion is that the more frequent left hip fractures in men, can be a consequence of the instability, caused by the weaker neuromuscular function on the left body side.

This presumption is additionally encouraged by our other observations. Out of 2,696 injured persons with hip fractures, 150 displayed peripheral neurological defects on the lower extremities. In 81% of them, the fracture was located on the side with the worse neuromuscular function (Table 2). In addition, several authors showed that persons with neurological defects have a greater incidence of falls, and, consequently, hip fractures, and that these fractures are predominantly located on the diseased side^{27,28}.

In women, the fracture frequency was similar on both body sides, which can be interpreted by a minor role of weaker neuromuscular function, but a greater exhaustion of pelvic muscles, and, consequently, greater fall incidence. We also demonstrated a high incidence of diabetes in women (7.2%) and men (2.5%) with hip fractures (Table 2). In the literature, the evidence about the quicker loss of bone mass¹⁶ and significant muscular weakness and polyneuropathic difficulties in diabetics²⁶ can be found.

In most patients (apart from the fractures on the left body side in women) we found the greater incidence of trochanteric fractures, compared to the neck femoral fractures. Such fractures are often multifragmental. It is

well known that trochanteric part of pelvis serves as the insertion of the strong pelvotrochanteric muscles. Multifragmental fractures of the trochanteric massive in the region of pelvotrochanteric muscle insertions indicate the possible role of their tension in the fracture characterization.

Analyzing the periodicity of hip fractures, we showed that women exhibit a 30.5% ($p < 0.001$) higher incidence of fracturing in winter, compared to summer period (Figure 1, Table 4). This difference could not be shown in men. Also, a negative correlation could be established between the number of sunny hours and hip fracture incidence in our female patients for the whole analyzed period ($R = -0.68288$, $p < 0.05$, Table 3), while such correlation could not be proved for male patients. Similar observations on the seasonal variations of hip fractures reported other authors¹⁹. It is evident that in the conditions of typical continental climate in our region, the number of sunny hours in summer months is more than three times as high, compared to winter months (Table 3). Consequently, in the summer period, with higher ultraviolet radiation, more intensive 7-dehydrocholesterole synthesis³³ and higher D-vitamin concentrations in old people³⁴, we observed less hip fracture incidence in old women, despite their more intensive physical activity in the summer. Well known is the efficiency of D-vitamin in the expression of muscle activity^{35,36}. Similarly, D-vitamin therapy provides successful prevention of falls in older people^{37,38}. We can conclude that during the summer period (when physical activity is generally more intensive) the pelvic muscles of old women have a lower tendency to get tired, and therefore in this part of the year, walking becomes more stabile. Seasonal variations of bone mass seem less likely to be the cause of the variations in hip fractures because seasonal bone mass changes do not exceed more than 1%. Similar observations, dealing with seasonal variations in hip fracture frequency, have been reported earlier³⁹.

Conclusion

General conclusion is that in our patients (independently of gender) hip fractures mainly occur as a consequence of increased instability.

In men, instability is predominantly caused by an impaired neuromuscular function on the left body side; in women, the main reason of instability seems to be weaker pelvotrochanteric muscles.

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ULOGA NESTABILNOSTI I MIŠIĆNE SLABOSTI U ETIOPATOGENEZI PRIJELOMA KUKA

S A Ž E T A K

Analizirana je grupa od 2,696 ozljeđenika (1,936 žena i 760 muškaraca) s prijelomom kuka koji su hospitalizirani u Kliničkoj bolnici u Osijeku tijekom posljednjih 12 godina. Navedeni su podaci o datumu prijeloma, dobi, spolu i mjestu prijeloma. U muškaraca je učestalost prijeloma lijevoga kuka češća za 23% ($p < 0,01$) u odnosu na desni, a takova razlika se nije našla u traumatiziranih žena sa istovrsnom ozljedom. U muškaraca je obostrano uočena veća učestalost prijeloma trohanteričnih masiva u odnosu na vrat bedrene kosti. U žena se takova razlika uočila samo na desnoj strani. U grupi analiziranih žena postoji veća učestalost prijeloma kuka u zimskom periodu za 30.5% ($p < 0,001$) u odnosu na ljetni period. Zaključili smo da su u muškaraca češći padovi i lomovi na strani tijela s lošijom neuromuskularnom funkcijom. Drugim riječima, ukazuje su na ulogu nestabilnosti u etiopatogenezi padova i prijeloma kuka muškaraca starije dobi. Česti višekomadni prijelomi trohanteričnog masiva u muškaraca ukazuju na moguću ulogu očuvanoga mišićnog vlaka pelvitrohanterne muskulature u ispoljavanju karakteristika prijeloma. U žena, povećana učestalost padova i prijeloma kuka starije životne dobi može se prije tumačiti izraženom slabošću pelvitrohanterne muskulature, a posljedično tome i nestabilnošću pri hodu. Također je uočena i izrazito visoka učestalost dijabetesa (7.2%) u grupi od 1,936 analiziranih žena. U klinici su poznate posljedice dijabetesa u ispoljavanju mišićne slabosti i polineuropatskih poteškoća. Nadalje, pokazana je manja učestalost prijeloma u žena tijekom ljeta u odnosu na zimski period. Manja učestalost prijeloma kuka ljeti u starijih žena može se tumačiti manjim zamorom mišića (u prvom redu pelvitrohanterne muskulature) i manjom učestalošću padova.

The Impact of Cognitive Processors and Conative Regulators on Specific Motor Abilities in Boxers

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ABSTRACT

The aim of the study was to analyze the relations of cognitive processors and conative regulators with specific motor abilities of elite boxers. Three sets of variables including 3 cognitive and 6 conative variables as predictors, and 6 specific motor (boxing) variables as criteria were used in a sample of 92 boxers. A series of regression analyses between the set of cognitive variables and particular criterion variables revealed a predominant impact of serial processor on specific motor abilities based primarily on specific speed (frequency of boxing technique performance). The series of regression analyses also showed a predominant negative impact of dysregulation of the organ function regulators from the set of conative variables on the manifestation of specific motor abilities in boxers. The data obtained in the study were used to develop an alternative model of the motor – cognitive – conative processes in boxing.

Key words: elite boxing, specific motor – cognitive – conative parameters, model of selection

Introduction

Previous studies have demonstrated the existence of positive correlation between intelligence and performance of complex motor tasks^{1–5}, and thus also with specific motor knowledge in various sports. This correlation is explained by the general speed of information flow as well as by the role of cognitive processes in motor activity. Cognitive processes and cognitive functioning are central mechanisms of cortical regulation. Central nervous system (CNS) has primarily an integrative function, ensuring reasonable and adaptive behavior in humans. Integration at the cortical level is of utmost importance because reasonable behavior is directly linked with the integrative function of the cerebral cortex. The integration also exists at the subcortical level, especially in the situations that require automatic reaction. Luria's study (1973)⁶ has shown that tertiary zones of cerebral cortex play the main role in ensuring simultaneous (spatial) syntheses, and include cortical segments of the visual, auditory, vestibular and tactile-kinesthetic analyzer. According to Semmes (1968)⁷, left hemisphere favors integration of similar units into information, whereas right hemisphere favors integration of dissimilar units. Mar-

teniuk (1976)⁸ reports that perceptive-motor skills include a major cognitive component which influences activities related to organization, direction and control of movements. Ismail and El-Naggar (1981)⁴ emphasize that both successive and simultaneous processes including left and right cerebral hemispheres are present on performing motor coordination tasks. In a study performed in high-school students, Katić (1977)³ found a high positive correlation of coordination, speed and explosive strength tests with the tests of visual spatialization (simultaneous processor) and perceptive reasoning (perceptive processor). Investigating the relations of motor abilities and knowledge in school subjects among high-school students, Katić (1988)⁵ concluded that the success in the subject of physical education significantly depended on the function of simultaneous (parallel) and perceptive (serial) processors in both sexes.

There are various theories (Schmidt, 1975; Ackerman, 1988)^{9,10} on what is necessary in the formation of the motor program. Task duration and structure definitely are the main characteristics that influence the

mode of the motor program formation. When a child is acquiring a motor program (motor knowledge or skill), it initially occurs at a cortical level, to continue at the subcortical level as the program is gradually being mastered^{1–5,11–13}.

Accordingly, cognitive functions that depend on integration of the mechanisms for the receipt, transmission and decoding of information in the CNS, are connected to the mechanisms of motor regulation of movement. As many motor behaviors are complex and include a variable component of cognitive behavior, it is presumed that the same and/or similar mechanisms are responsible for the motor and intellectual behavior in humans. Cognitive mechanisms will certainly influence performance in boxing; however, it should be assessed whether this performance is predominantly influenced by the perceptive and simultaneous (spatial) processor.

Behavioral modalities are more or less responsible for performance in any human activity. Therefore, kinesiology studies of relations between different conative factors and sports engagement and performance are of utmost importance. Some conative space characteristics are known to limit efficiency in various activities. In addition, the same conative characteristics may have a restricting role in some activities while stimulating efficiency in others, especially when implicated in the particular activity performance. Considering the fact that conative factors are not independent of the process of conditioning during one's lifetime, sports turns to be a very important activity because it provides an opportunity to almost naturally reduce the adaptively unwanted behavioral modalities while developing the individually and socially desirable ones. Being aware that other functions, along with motor and intellectual abilities, can also be upgraded through proper treatment or control of particular conative features, is highly relevant for both sports and social efficiency. Mraković and Katić (1989)¹⁴ analyzed association of some typical conative characteristics with the intensity of kinesiological activities in women. The relations thus obtained showed bipolar properties: kinesiological activities of women was found to depend on the negative effect of anxiety on the one hand, and on the positive impact of hypomania on the other hand, whereby the negative influence of anxiety greatly outweighed the positive impact of hypomania. In other words, anxiety limited kinesiological activity to a significantly greater extent than it was stimulated by hypomania. In male *versus* female subjects (high-school students), Katić (1977)¹⁵ defined canonic relation between psychomotor and pathologic conative factors¹⁶ by a general degree of equilibrium of psychomotor characteristics determined by pathologic conative characteristics, positively by elevated values of the sthenic and negatively by elevated values of the asthenic syndrome. The results obtained are explained as follows: anxious symptoms predominated by inhibition reduce, while sthenic symptoms (hypomania in particular) predominated by nervous system excitation improve motor efficiency (explosive strength in particular). The present study determined conative struc-

ture, i.e. conative features that determine results of tests of specific motor abilities in boxing.

Subjects and Methods

Subjects

Study sample included 92 boxers from Croatian boxing clubs (Rijeka, Pula, Split, Zagreb, Slavonski Brod, Osijek, Varaždin and Zadar). At the time of study measurements, the subjects were healthy, regularly performing their training activities, and free from pronounced morphological, motor and physiologic deviations.

According to boxing categories, there were three flyweight (≤ 50 kg), six bantam-weight (≤ 54 kg), eight featherweight (≤ 57 kg), ten lightweight (≤ 60 kg), 12 light-welterweight (≤ 64 kg), 14 welterweight (≤ 69 kg), 15 middleweight (≤ 75 kg), ten light-heavyweight (≤ 81 kg), eight heavyweight (≤ 91 kg) and six super-heavyweight (> 91 kg) boxers.

Variable sample

The variable sample consisted of 15 variables including 3 cognitive variables and 6 conative variables as predictor sets of variables, and 6 variables of specific motor abilities as a criterion set of variables.

Cognitive abilities were assessed by use of three representative tests for determination of the main cognitive processor function efficiency (Momirović et al., 1982)¹⁷:

- efficiency of perceptive processor – IT_1 (designed to measure perceptive ability which represents synthesis of the ability of perceptive analysis, perceptive structuring and perceptive identification. Test tasks are of the multi-choice type; the subject has to identify which of the four images is identical to the given image. The test consists of 39 tasks that have to be solved in 4 minutes, thus meeting the criteria of a speed test);
- efficiency of serial processor – AL_4 (designed for assessment of verbal comprehension and contains 40 tasks consisting of pairs of words; the subject has to determine whether the pair words have identical or opposite meaning. The time allowed for the test is 2 minutes, thus the test meets the criteria of a speed test); and
- efficiency of parallel processor – S_1 (designed for assessment of visual spatialization; the test consists of 30 tasks, each of them representing a three-dimensional image of a pile of bricks; the subject has to choose one of the four transverse projections of the brick pile which corresponds to the given image when observed from a particular angle; the time allowed for the test is 8 minutes).

Conative characteristics were assessed by use of measuring instruments designed on the cybernetic model of conative regulatory functions (Momirović, Horga and Bosnar, 1982)¹⁸. These tests estimate six conative regulatory mechanisms of the model:

- activity regulator – ϵ (one of the elementary and lowest subsystems in the hierarchy, which is responsible for

the activity and energy level at which other subsystems are functioning, including cognitive and motor processors);

- organ function regulator – χ (formed by correlated action of subcortical centers for the regulation of organ functions, mostly located in the hypothalamic region, and superior cortical systems responsible for the regulation and control);
- regulator of defense responses – α (located in the hypothetical center for the regulation of defense responses, in the limbic system; it modulates tonic excitation, probably on the basis of appropriate programs transmitted by genetic code or formed during the ontogenic development, as a rule due to conditioning);
- regulator of attack responses – σ (located in the hypothetical center for the regulation of attack responses, in the limbic system; likewise the center for regulation of defense responses, it also modulates primary tonic excitation, however, based on the program of destructive response, formed during the phylogenic or ontogenic development);
- system for coordination of regulatory functions – δ (coordinates functions of the subsystems that are functionally or hierarchically different, including the functions of cognitive processors; therefore, this system is functionally superior to the regulators of organ functions, regulators of attack responses and defense responses, and to a certain extent also to the activity regulator); and
- system for integration of regulatory functions – η (it is superior to all conative regulatory systems, it integrates conative processes within the psychological area structure, and the social area structure and its changes in particular, thus the level of socialization directly depending on this system).

Each of the six conative tests contains 30 statements, and the subject has to mark one of the five answers on Likert scale. The time to solve the test is not limited (some 30 minutes for the whole test battery), and each test score can range from 30 to 150 points.

The following variables were used to assess *specific motor abilities* of speed and speed-strength endurance in boxing:

- speed of performing 100 straight punches against punching bag (at an arm distance from the punching bag; at this distance, the subject assumes boxing guard, then at the timekeeper's sign alternately hits left and right straight punches against the punching bag at the chest level; the time needed for 100 straight punches is recorded);
- speed of performing combined punches against punching bag, i.e. two straight punches, two hooks and two uppercuts (at a 60-cm distance from training bag; at this distance, the subject assumes boxing guard and at the timekeeper's sign alternately hits punches against the punching bag at maximal speed in the following order: left and right straight punches, left and right

hooks, and right and left uppercut, 100 punches in total; the time needed for all 100 punches is recorded);

- jumps while performing left-right straight punches in 10 seconds (at the sign given by the timekeeper, the subject performs jumps and left-right straight punches at maximal speed; at 10 seconds it is interrupted and the number of properly performed straight punches is recorded as test result);
- jumps while performing left-right hooks in 10 seconds (at the sign given by the timekeeper, the subject performs jumps and left-right hooks at maximal speed; it is interrupted at 10 seconds and the number of properly performed jumps and hooks is recorded as test result);
- jumps while performing left-right uppercuts in 10 seconds (the subject performs jumps and left-right uppercuts in 10 seconds; the number of correctly performed jumps and uppercuts during the given period of time is recorded as test result); and
- defense from left straight punch and countering three straight punches – defense from right straight punch and countering three straight punches against coach's arms (the subject has to perform defense by avoiding the coach's left straight punch and to counter three straight punches (right-left-right) as quickly as possible, then defending from the coach's right straight punch and countering three straight punches (left-right-left); the time to perform this motor task is recorded as test result.

The first and second tests are predominated by the integration of specific speed, strength and endurance (speed-strength endurance), whereas the third, fourth, fifth and especially sixth tests are predominated by specific speed.

Data processing

The basic descriptive statistics parameters of arithmetic mean and standard deviation (SD) were calculated for each of the variables used in the study. A series of regression analyses were used to calculate the influence of the cognitive variable and conative variable systems (as sets of predictor variables) on particular variables of situation motor abilities (as a set of criterion variables). Regression coefficients of each standardized predictor variable upon a particular criterion variable (β), multiple correlation between the set of predictor variables and the criterion variable (ρ), and coefficient of determination, i.e. overall variance of the system of predictor variables and the criterion (ρ^2) were calculated.

Results

Descriptive statistics parameters of the cognitive, conative and specific motor space variables in elite Croatian boxers are presented in Table 1. In elite boxers, cognitive abilities are strongly pronounced, functioning of the serial processor (AL_4) in particular, followed by the perceptive processor function (IT_1) and parallel proces-

TABLE 1
DESCRIPTIVE STATISTICAL PARAMETERS (X, SD) OF
COGNITIVE, CONATIVE AND SPECIFIC MOTOR VARIABLES
IN ELITE BOXERS (n=92)

Variable	X	SD
Perceptive processor efficiency (IT ₁)	33.25	3.30
Serial processor efficiency (AL ₄)	35.97	2.55
Parallel processor efficiency (S ₁)	22.11	3.03
Activity regulator (ε)	129.65	7.38
Organ function regulator (γ)	38.94	4.60
Defense response regulator (α)	38.43	3.41
Attack response regulator (σ)	139.10	6.22
Coordination of regulatory functions (δ)	38.30	3.24
Integration of regulatory functions (η)	38.18	3.39
100 straight punches (100S)#	185.80	25.93
100 combined punches (100C)#	249.86	37.65
Jumps and straight punches in 10 s (JS)	18.96	2.43
Jumps and hooks in 10 s (JH)	17.95	2.45
Jumps and uppercuts in 10 s (JU)	17.48	2.75
Defense – counter straight punch (DCS)#	21.48	4.79

#variable with opposite metric orientation

processor function (S₁). It results from the selection and long-term training processes directed to achieving the goal, i.e. top result in boxing. The highest variability, i.e. between-boxer differences, was recorded in the function of parallel processor, followed by the perceptive processor function, and lowest in the serial processor function. The arithmetic means and standard deviations (SD) of cognitive variables suggested the performance in elite boxing to be determined by serial, perceptive and parallel processing of the nervous system information.

The basic statistical parameters of conative variables showed the values in the regulatory mechanisms of attack response (σ) and activity (ε) to be generally very high in elite boxers, which is quite conceivable considering that innate aggressiveness and ability of energy mobilization are major preconditions to engage in combat sports, boxing in particular. In contrast, rather low values were obtained in other conative regulators, i.e. integration of regulatory functions (η), coordination of regulatory functions (δ), defense response regulator (α) and organ function regulator (γ). It should be noted that the

function of these four regulators is inversely proportional to the value of the respective test result. As data processing yielded a high variability, i.e. differences among boxers, in these regulators, being highest for the organ function regulator (γ), it could be postulated that various conversions such as cardiovascular, respiratory, gastrointestinal, etc., would limit the level of performance in boxing.

The basic statistical parameters of the specific motor ability tests revealed differences in their duration. So, the first and second specific motor tests took a mean of 19 and 25 seconds, respectively, suggesting them to be saturated by the speed, strength and endurance. The mean length of the third, fourth and fifth specific motor tests was 10 seconds each, indicating them to be saturated by speed and strength. The sixth specific motor test took a mean of 2 seconds, thus being predominantly saturated by psychomotor speed. Individual boxers differed most in the test assessing specific speed in terms of punch defense and multi-punch counter (defense – counter straight punch, DCS), suggesting that this specific motor ability may serve as a major determinant of performance in elite boxing.

Correlations between cognitive variables and specific motor variables (Table 2) revealed marked complexity of specific motor abilities in the area of cognitive processors. All three cognitive processors showed significant correlation with criterion variables. There was a predominant correlation between serial processing of CNS information and the criteria, followed by the correlation of perceptive processing and parallel processing of CNS information with the criteria. Accordingly, the correlations between cognitive abilities and specific motor abilities followed the level of development of particular processing functions in elite boxers.

Table 3 indicates the set of cognitive predictor variables to have a statistically significant effect on all the criterion variables used in the study, i.e. speed of performing 100 straight punches against punching bag (100S), with multiple correlation coefficient of 0.54 and determination coefficient of 0.29; speed of performing combined punches against punching bag – two straight punches, two hooks and two uppercuts (100C), with multiple correlation coefficient of 0.53 and determination coefficient of 0.29; jumps while performing left-right straight punches in 10 seconds (JS), with multiple correlation coefficient of 0.57 and determination coefficient of 0.32;

TABLE 2
CORRELATIONS BETWEEN COGNITIVE VARIABLES AND VARIABLES OF SPECIFIC MOTOR ABILITIES IN ELITE BOXERS

Variable	100S#	100C#	JS	JH	JU	DCS#
	R	r	R	r	R	R
IT ₁	-0.37	-0.22	0.42	0.43	0.33	-0.39
AL ₄	-0.49	-0.39	0.53	0.55	0.48	-0.44
S ₁	-0.31	-0.19	0.41	0.42	0.33	-0.34

#variable with opposite metric orientation, r – correlation coefficient, IT₁ – perceptive processor efficiency, AL₄ – serial processor efficiency, S₁ – parallel processor efficiency, 100S – 100 straight punches, 100C – 100 combined punches, JS – jumps and straight punches in 10 s, JH – jumps and hooks in 10 s, JU – jumps and uppercuts in 10 s, DCS – defense – counter straight punch

TABLE 3
RESULTS OF REGRESSION ANALYSIS BETWEEN THE SET OF PREDICTOR COGNITIVE VARIABLES AND INDIVIDUAL CRITERION VARIABLES

Variable	100S [#]	100C [#]	JS	JH	JU	DCS [#]
	β	β	β	β	β	β
IT ₁	0.24	0.67 ^a	-0.45	-0.45	-0.59 ^a	-0.02
AL ₄	-1.00 ^b	-1.25 ^b	0.96 ^b	0.98 ^b	1.08 ^b	-0.58 ^b
S ₁	0.33	0.28	0.00	-0.01	-0.06	0.17
ρ	0.54 ^b	0.53 ^b	0.57 ^b	0.58 ^b	0.54 ^b	0.45 ^b
ρ^2	0.29 ^b	0.29 ^b	0.32 ^b	0.33 ^b	0.29 ^b	0.20 ^b

[#]variable with opposite metric orientation, ^ap<0.01, ^bp<0.001, β – regression coefficient, ρ – multiple correlation, ρ^2 – coefficient of determination, IT₁ – perceptive processor efficiency, AL₄ – serial processor efficiency, S₁ – parallel processor efficiency, 100S – 100 straight punches, 100C – 100 combined punches, JS – jumps and straight punches in 10 s, JH – jumps and hooks in 10 s, JU – jumps and uppercuts in 10 s, DCS – defense – counter straight punch

TABLE 4
CORRELATIONS BETWEEN CONATIVE VARIABLES AND VARIABLES OF SPECIFIC MOTOR ABILITIES IN ELITE BOXERS

Variable	100S [#]	100C [#]	JS	JH	JU	DCS [#]
	r	r	r	r	R	r
ε	-0.51	-0.44	0.51	0.52	0.43	-0.54
χ	0.66	0.65	-0.66	-0.67	-0.58	0.70
α	0.62	0.57	-0.59	-0.59	-0.55	0.51
σ	-0.45	-0.36	0.45	0.46	0.38	-0.43
δ	0.55	0.50	-0.56	-0.56	-0.50	0.49
η	0.55	0.53	-0.54	-0.54	-0.51	0.43

[#]variable with opposite metric orientation, r – correlation coefficient, ε – activity regulator, χ – organ function regulator, α – defense response regulator, σ – defense response regulator, δ – coordination of regulatory functions, η – integration of regulatory functions, 100S – 100 straight punches, 100C – 100 combined punches, JS – jumps and straight punches in 10 s, JH – jumps and hooks in 10 s, JU – jumps and uppercuts in 10 s, DCS – defense – counter straight punch

jumps while performing left-right hooks in 10 seconds (JH), with multiple correlation coefficient of 0.58 and determination coefficient of 0.33; jumps while performing left-right uppercuts in 10 seconds (JU), with multiple correlation coefficient of 0.54 and determination coefficient of 0.29; and defense from left straight punch while countering three straight punches – defense by avoiding right straight punch while countering three straight punches against the coach's arms (DCS), with multiple correlation coefficient of 0.45 and determination coefficient of 0.20.

In the set of cognitive predictor variables, only AL₄, i.e. efficiency of serial processor, elicited a statistically significant effect (regression coefficient, β) on all criterion variables. This means that subjects with better serial processor efficiency achieved better results in each criterion variable. However, there was a significant unfavorable impact of perceptive processor on the criterion variables of performing combined punches against punching bag (100C) and jumps while performing left-right uppercuts in 10 seconds (JU), indicating the inclusion of perceptive processor during the course of these specific tasks to be unnecessary.

The complexity of specific motor abilities in the area of conative regulators was very pronounced, as indicated by the correlations between these two sets of variables (Table 4). The activity regulator (ε) and attack response regulator (σ) yielded positive correlation with criterion variables, whereas dysregulation of the organ function regulator (χ), of defense response regulator (α), of the coordination mechanism of regulatory functions (η) showed negative correlation with the criteria. Considering the specificity of boxing, boxers obviously possess a specific conative structure, as suggested by both basic statistical parameters of conative variables and their correlations with the criteria.

Table 5 clearly shows the system of predictive conative variables to exert a statistically significant impact on all the criterion variables used in the study, i.e. speed of performing 100 straight punches against punching bag (100S), with multiple correlation coefficient of 0.68 and determination coefficient of 0.47; speed of performing combined punches against punching bag – two straight punches, two hooks and two uppercuts (100C), with multiple correlation coefficients of 0.69 and determination coefficient of 0.47; jumps while performing left-right

TABLE 5
RESULTS OF REGRESSION ANALYSIS BETWEEN THE SET OF PREDICTOR CONATIVE VARIABLES AND INDIVIDUAL CRITERION VARIABLES

Variable	100S [#]	100C [#]	JS	JH	JU	DCS [#]
	β	β	β	β	β	β
ε	0.00	0.10	0.02	0.01	-0.06	-0.13
χ	0.51 ^b	0.73 ^b	-0.52 ^b	-0.54 ^b	-0.46 ^b	0.85 ^b
α	0.26	0.18	-0.17	-0.17	-0.20	-0.01
σ	0.05	0.19	-0.06	-0.06	-0.07	0.16
δ	-0.08	-0.18	-0.00	-0.00	0.06	0.02
η	0.06	0.18	-0.03	-0.02	-0.15	-0.18
ρ	0.68 ^b	0.69 ^b	0.67 ^b	0.68 ^b	0.61 ^b	0.72 ^b
ρ^2	0.47 ^b	0.47 ^b	0.45 ^b	0.46 ^b	0.37 ^b	0.51 ^b

[#]variable with opposite metric orientation, ^b $p < 0.001$, β – regression coefficient, ρ – multiple correlation, ρ^2 – coefficient of determination, ε – activity regulator, χ – organ function regulator, α – defense response regulator, σ – attack response regulator, δ – coordination of regulatory functions, η – integration of regulatory functions, 100S – 100 straight punches, 100C – 100 combined punches, JS – jumps and straight punches in 10 s, JH – jumps and hooks in 10 s, JU – jumps and uppercuts in 10 s, DCS – defense – counter straight punch

straight punches in 10 seconds (JS), with multiple correlation coefficient of 0.67 and determination coefficient of 0.45; jumps while performing left-right hooks in 10 seconds (JH), with multiple correlation coefficient of 0.68 and determination coefficient of 0.46; jumps while performing left-right uppercuts in 10 seconds (JU), with multiple correlation coefficient of 0.61 and determination coefficient of 0.37; and defense from left straight punch while countering three straight punches – defense by avoiding right straight punch while countering three straight punches against the coach's arms (DCS), with multiple correlation coefficient of 0.72 and determination coefficient of 0.51.

According to regression coefficients (β), in the set of conative predictor variables only the variable assessing the factor responsible for organ function regulation (χ), formed by coordinated action of subcortical centers for organ function regulation, mostly located in the hypothalamic region, and superior cortical regulatory and control systems, had a statistically significant impact on all criterion variables. Thus, even low values of the organ function regulator dysregulation will have adverse impact on the performance of specific motor tasks in boxing, the efficiency of defense from punches and performance of counter punches in particular. Therefore, influencing a small part of the variability in dysregulation of the organ function regulators is of utmost importance for upgrading the boxer's adaptive ability. An increase in the level of control of the organ function regulator dysregulation symptoms, i.e. conversion responses, will improve not only motor functions but also other function that may be blocked by this dysregulation.

Discussion

Study results revealed the boxers to possess some specific motor abilities, cognitive abilities and conative properties, which taken together limit their engagement and

top performance in boxing. An appropriate motor, cognitive and conative structure determining superior performance in boxing is formed by the selection and training processes. The motor structure is predominated by specific speed of movement frequency (mostly punches), which is saturated by coordination and explosive strength. The ability of coordination manifests in the integration of successive-individual movements into a unique structure, i.e. performing a series of boxing techniques (primarily punches). The cognitive structure is predominated by serial processing of information, i.e. flow rate of successive CNS information, which is closely related to the speed of performing a series of identical or combined punches in boxing. The conative structure is predominated by the attack response regulator, underlain by the intensity of CNS excitation and intensity of energy mobilization, which is expressed by aggressive behavior. This is supported by the regulator of activity in terms of excitation to inhibition balance. In addition, the organ function regulator tends to eliminate or at least minimize various conversions, thus facilitating great strain and pain to endure, and urgent combat situations to master.

The basic motor, cognitive and conative properties of boxers are generally listed. However, the question is what anthropological properties determine performance in boxing. To answer this question completely, one has to have information on the results that the boxers achieve at contests. As no such information was available in the present study, only a hypothetical, alternative model of the motor – cognitive – conative processes in boxing is proposed.

Certain anthropological properties determine the inclusion of individuals in boxing training. This has been related to various selection procedures, while the long-term training in boxing also leads to the formation of an ideal anthropological structure needed for achievement of top results. The formation of an ideal anthropological structure assumes that primary selection is followed by

the action of training processes, primarily producing qualitative modifications within and across the subsegments of the anthropological structure (morphological, motor, cognitive and conative), with all these relations among the anthropological dimensions assessed brought to optimal position. Development of the relevant anthropological characteristics is paralleled by the stages of selection, characterized by more or less different predictors of boxing performance. Therefore, efficient selection in boxing cannot be performed exclusively on the basis of information obtained by the analysis of relations among some anthropological status dimensions and specific motor abilities in the definitive, ultimate state. Thus, a new, alternative, 3-stage (age 12–14, 15–17 and 18–20 years) model of selection in boxing is proposed on the basis of the results obtained in the present study, results of studies in elite athletes^{19–21}, and information on developmental processes of anthropological status dimensions in elementary school children^{11–13} and high-school children^{3,5,15}.

In stage one (age 12–14), the initial anthropological status of the children disposed to boxing training should be assessed and primary selection according to their health status performed, as all body functions should be optimally developed and free from impairments (e.g., the function of the vegetative nervous system, sensorimotor nervous system, transport system and locomotor system). Considering conative regulators involved in the vegetative nervous system, the individuals with above-average dysregulation of the defense response regulators, manifesting by pronounced symptoms of anxiety, phobia, obsession, depression, and predominant nervous system inhibition in general, should be eliminated from further training procedures by selection. Only the individuals with above-average functions of the attack response regulators and activity regulators, manifesting by innate controlled aggressiveness and hypomania, i.e. good energy mobilization and predominant nervous system excitation in general should be accepted.

At the same time, these individuals will show good function of the superior conative regulators and facilitated function of cognitive and motor processors. The function of all cognitive processors involved in the sensorimotor nervous system should be at an above-average level, with the simultaneous processor proved to be a better predictor of performance in boxing at this stage; it is responsible for simultaneous, i.e. parallel processing of information, which enables integration of different information for reasonable action in a particular situation, thus also facilitating motor learning, i.e. acquiring new motor programs. The adoption of new motor programs depends most on coordination, which is defined as the ability to integrate different movements or motor routines into a unique movement structure. Although simultaneous processor as well as cortical regulation of movement is expected to better predict performance in boxing than other cognitive processors at the beginning, selection should still primarily rely on the basic motor abilities of movement speed and coordination, and on cognitive processor for serial information processing because

these abilities, along with the mentioned conative regulators will ultimately limit performance in boxing. At this stage, the boys should acquire basic motor knowledge, i.e. basic techniques in boxing, while motor learning of this specific knowledge will take place from cortical through subcortical level, and from simultaneous and perceptive information processing through serial information processing. The interaction of speed, coordination and serial processor plays a major role at the end of this stage.

In stage two (age 15–17), all cognitive processors, i.e. perceptive, simultaneous and serial ones, should be included in motor learning and adoption of the greatest possible number of new motor programs, whilst a certain number of basic specific motor knowledge-techniques in boxing should reach full automatism through numerous repeats. Motor coordination has developed to the level that enables quality performance of basic techniques and simple yet efficient combinations, along with optimal use and regulation of strength, speed and muscle tone. Training processes increase in volume and intensity, influencing not only the development of explosive strength but also the development of specific endurance and improves conative regulation of organ functions. At this stage, the interaction of speed, strength, coordination, cognitive processors and stability of organ function regulators in particular is crucial, along with due control of aggressive responses.

In stage three (age 18–20), all relevant body functions should reach the highest possible level. Training processes are predominated by an extremely high work volume, with maximal and submaximal load. Specific motor abilities, cognitive processors and conative regulators should be in optimal interrelations. Appropriate mechanisms are activated on solving a particular combat task. As combat situations are diverse and complex, performance depends on the interaction of all relevant specific motor abilities, from specific speed (movement frequency), specific strength (power), specific endurance through coordination of all cognitive processors and proper functioning of all conative regulators, the regulators of organ functions (where even a minor dysregulation of the organ function regulators exerts unfavorable effect on combat performance) in particular. The present study demonstrated favorable impact of serial processor on the specific motor ability tests, where a series of punches should be performed as fast as possible, by nature of these tasks predominantly saturated by the speed of frequency and serial processing of information. However, many other combat situations also require inclusion of the other two processors, simultaneous information processing in particular. The combat situation has to be identified and the most efficient of a number of possible responses to the opponent's activity should be chosen. Deciding on the choice of response is a special aspect of coordination, representing simultaneous information processing. Accordingly, combat performance will greatly depend on the number and quality of the programs acquired, i.e. on the use of various combinations of boxing techniques, such

as efficient defense and efficient countering with a series of punches^{20,21}. In this stage, interaction of specific endurance and stability of organ function regulators plays a crucial role, manifested as endurance in delivering and receiving punches.

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UTJECAJ KOGNITIVNIH PROCESORA I KONATIVNIH REGULATORA NA SPECIFIČNE MOTORIČKE SPOSOBNOSTI BOKSAČA

SAŽETAK

Cilj istraživanja je bio analizirati relacije kognitivnih procesora i konativnih regulatora sa specifičnim motoričkim sposobnostima vrhunskih boksača. U tu svrhu na uzorku od 92 boksača primijenjena su tri skupa varijabli i to: 3 kognitivne i 6 konativnih kao prediktori i 6 specifičnih motoričkih (boksačkih) varijabli kao kriteriji. Serija regresijskih analiza između skupa kognitivnih varijabli i pojedine varijable iz kriterijskog skupa utvrdila je dominantni utjecaj serijalnog procesora na specifične motoričke sposobnosti u osnovi kojih je prvenstveno specifična brzina (frekvencija realizacije boksačkih tehnika). Serijom regresijskih analiza je također utvrđen dominantni negativni utjecaj disregulacije regulatora organskih funkcija iz konativnog skupa varijabli na manifestaciju specifičnih motoričkih sposobnosti boksača. Temeljem informacija ovog istraživanja izrađen je alternativni model: motoričkih – kognitivnih – konativnih procesa u boksu.

Monitoring of Cross-Country Skiers by Means of an Expert Model of Potential Performance

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ABSTRACT

On the basis of expert knowledge, an expert model of potential performance covering the motor, morphological, psychological, and sociological subspace was constructed (MMPS). The scores of variables were obtained by application of the computer program Sport Measurement Management System (SMMS). In the subjects included in measurements, trends of the obtained average scores of variables were established through various competition categories and age periods. The sample of subjects consisted of 48 cross-country skiers in three competition categories. Fluctuations in development in individual age periods are larger in the motor and morphological subspace. In the psychological subspace, an upward trend of average scores can be noticed, while the sociological subspace is not subjected to any essential changes in different age and competition categories. Monitoring of cross-country skiers across all three competition categories showed that in these age categories there are periods which owing to laws of development do not allow uniform progress. Therefore, the principle of individuality must be taken into account especially in planning the transformation process.

Key words: cross-country skiing, potential performance, expert modelling, longitudinal monitoring

Introduction

The basic goal of every top-level athlete is competition performance which manifests through good result. Top-level sport is becoming more and more an economic category which can hardly afford any larger slips. The work with the young is, of course, an even more tricky matter. Faults in these age categories are also questionable from the moral and ethical aspect; hence, many are already aware of possible detrimental consequences of introducing children into intensive sport competitions whose rules are written by adults. This is exactly why – or perhaps only why – the transformation process should not be merely a shortened programme according to which the grown-ups work.

Modern scientific findings say that a good quality process of the preparation of athletes can be guided only via a model. This model should be based on the actual athlete's competition result, as well as on the effects of all individual and interrelated dimensions of the psychosomatic status¹. The sense of modelling lies in the advance information on how the system would probably behave if the initially selected, limiting conditions of the model happened. Of course, models must be practical and expedient, and correspond to the reality².

Multiparameter modelling is understood as a process of evaluation. The theory of such decision-making offers a formal basis for the construction of a model, where the fundamental issue is the connection of scores by individual parameters into an overall score. To master these problems, expert modelling can be used.

The most desirable effects of the transformation process on the psychosomatic status or its part are achieved when there comes to agreement between the demands of »top-level quality« of the selected sport in a given age period and the nature of an individual athlete. It can be argued that performance of an athlete depends on the state of all model dimensions representing the linear combination of performance (equation of performance specification) in a given age period.

The subject of the present research was focused on the study and evaluation of competition performance in cross-country skiers by means of an expert model of potential performance in three competition categories (older boys, younger juniors, and older juniors). The selection of basic dimensions, which are systematically interconnected in the structure of the performance model, was

carried out in the motor and morphological subspace (primary potential dimensions) and in the psychological and sociological subspace (tertiary potential dimensions). In all selected subspaces, the knowledge base was written in the formalism which could be used for application in the SMMS program. The whole structure of elementary and derived variables was written in the form of a uniform hierarchically arranged tree.

Methods

Participants

The sample of measured subjects consisted of cross-country skiing competitors from three competition categories: older boys – STDKI (born in 1989 and 1990, n=17), younger juniors – MLMCI (born in 1987 and 1988, n=17) and older juniors – STMCI (born in 1985 and 1986, n=14 subjects). All subjects were included onto the final list of SLO_FIS points in the 2003/2004 season.

Instruments

In the potential model of competition performance (MMPS: motorics, morphology, psychology, sociology), 64 independent variables are included. A detailed description of the variables and the measurement protocol are available from the authors at the Faculty of Sport in Ljubljana.

Variables of the motor subspace: long jump from standing (MMENS DM), triple jump from standing (MTRSK), balance frontally (MSRKF), balance sagittally (MSRKS), tapping with hand (MTAPRO), Cooper’s test – 2400 m (MSCT), 20-m sprint – high start (MEMTEK), 60-m run (MMENS60), polygon backwards (MPON), eight with bending (MKAOSP), side steps (MKVS), hang with elbows bent (MSMIZT), trunk lifting on Swedish gymnastic bench (MSDTSK), jumps over Swedish gymnastic bench (MSPSK), bent hangs on parallel bars (MSSNB), bending forward on bench (MTPK), heavy ball throw (MEMMED).

Variables of the psychological subspace:

Special psychological abilities: fluid intelligence (FLUIDINT), function of encouragement (FUNVZPOD), function of control (FUNKONTR); motivation or dynamic component of personality: performance (success) based on work (USPEZDEL), performance (success) irrespective of work (USPNGDEL), motive of power (MOC), positive competition motivation (POZITIVN), negative competition motivation (NEGATIVN), self-motivation (SAMOMOT); personality traits: neuroticism (NEVROTIC), spontaneous aggressiveness (SPONTAGR), depressiveness (DEPRESIV), irritability (RAZDRAZL), sociability (DRUZABN), self-control (OVLADAN), reactive aggressiveness (REAKTAGR), inhibition (ZAVRTOST), sincerity (ODKRITO), extroversion (EKSTRAV), emotional lability (EMOCLAB), masculinity (MASKULIN), endurance (VZTRAJNO), competition anxiety (TEKMANKS), anxiety as personality trait (ANKOSLAS).

Variables of the morphological subspace: body height (ATV), body weight (ATT), length of upper limbs (ADZGO), length of lower limbs (ADSPO), circumference of relaxed upper arm (AON), chest circumference (AOPR), thigh circumference (AOS), elbow diameter (APKOM), shoulder width (ASR), pelvis width (ASM), knee diameter (APKOL), abdominal skinfold (AKGT).

Variables of the sociological subspace: education of mother (SIZOBRM), education of father (SIZOBRO), conditions for training (PDOBPOG), good expert work (PDOBSTDE), good organisation of club (PDOBORG), involvement of mother in sport (PSPAKTM), involvement of father in sport (PSPAKTO), function of mother in club (IKLFUNM), function of father in club (IKLFUNO), position of mother at work (IDELMSM), position of father at work (IDELMSO).

Procedure

Measurements were carried out in March 2004. Tests of motorics were carried out by the subjects in the sports hall and on the athletic running track. The data were processed with the SPPS software package and program Sport Measurement Management System (SMMS), developed at the Faculty of Sport in Ljubljana. In agreement with the objectives and hypotheses, the research was conducted in the following phases:

A model of potential competition performance of cross-country skiers (MMPS) in the form of a decision-tree was developed. The model covered motor, morphological, psychological and sociological subspaces of the psychosomatic status of competitors.

Normalisers for all elementary variables (tests) in the MMPS model were set up (positional configuration). They represent the points that determine the utility function v , which for a given measured (raw) result x on the base criterion determines its value or utility. The function is determined in such a way that in the variable for raw results, an arbitrary number of points is defined. The expert thus gives only the explicit, numerical and attribute value of the utility function for some points³, while for other points, the values are determined by computing the straight line between two points by means of interpolation. An example of normalisers for the MSCT variable – Cooper’s test 2400 m (see also Table 1: e.g. 504 : 8 means that time 504 s, achieved in this test, has received the numerical score 8 – very good).

TABLE 1
AN EXAMPLE OF SETTING UP THE NORMALISERS FOR THE MSCT VARIABLE

Value of variable	480	492	504	515	530	537	554	820
Score of variable	10	9	8	7	5	4	2	0

Numerical and descriptive values of scores: 0–1.99 – unsatisfactory, 2–3.99 – satisfactory, 4–6.99 – good, 7–8.99 – very good, 8.99–10.00 – excellent

In evaluating individual variables, experts have in mind a vision of top creativity in this sport (champion model) and at the same time, significant long-term development characteristics of an athlete. The expert's score becomes thus far-reachingly useful. In this way, »longitudinal« treatment of the athlete is attained and a corresponding universal model of potential performance is created as athletes go through various development, age and competition periods during their transformation process.

Decision rules for all nodes in the MMPS model were set up (dimensional configuration). This is the value of a hypothetical contribution (in %) of each individual variable to competition performance at the respective node of the MMPS model. It was determined according to the method applying dependent determination of weights. According to this method, the total contribution of the weights of all variables of lower order that constitute a variable of higher order is, in relative terms, 100 at any individual node. In absolute terms, however, the sum of the weights of all variables of lower order (tests) in the MMPS model yields the sum 100.

By the SMMS program, scores for all variables at all levels in the MMPS model were calculated for each subject measured. First, for elementary variables (tests) and then gradually for all composite variables at higher nodes, up to the highest node, the so-called prognostic

score of competition performance of the subject measured. The calculation was made according to the following formula: $Svr = (Snr_1 \times P) + (Snr_2 \times P) + \dots + (Snr_n \times P)$. Legend: Svr – normalised value of a higher-order variable, Snr – normalised value of a lower-order variable, P – weight of a lower-order variable (decision rule, weight).

To establish differences between competition categories of subjects as regards scores at the highest levels of the MMPS model, a T-test for independent samples was used.

Results

Construction of the MMPS model

Table 2 shows the structure of the MMPS model at the highest levels and as an example also part of the structure in the motor subspace (energy component of movement). Given is also an example of evaluating the potential competition performance of the subject at the shown levels of the MMPS model.

The analysis through various competition categories

By the SMMS program, numerical scores at the highest level were calculated for individual subspaces of the MMPS model for the subjects of three competition cate-

TABLE 2
PART OF THE STRUCTURE OF THE MMPS MODEL, WEIGHTS, NORMALISERS AND AN EXAMPLE OF EVALUATING THE POTENTIAL COMPETITION PERFORMANCE

Test code	Weights	Normalisers	Potential comp. performance Competitor »A«		
			RES	f(x)	SCORE
URMPU	100			7.13	very good
└ Morphology	24			6.53	good
└ Sociological characteristics	14			7.89	very good
└ Psychology	26			7.67	very good
└ Motor abilities	36			6.85	good
└ Energy comp. of movement	24			7.14	very good
└└ Excitation duration	16			8.35	very good
└└└ Endurance power	6.6			7.30	very good
└└└└ Repetitive power	5.2			6.83	good
└└└└└ MSPSK	1.9	8:0, 24:2, 26:4, 27:5, 29:7, 31:8, 33:9, 42:10	33	9.00	excellent
└└└└└ MSSNB	1.6	1:0, 10:2, 14:4, 16:5, 18:7, 20:8, 22:9, 25:10	12	3.00	satisfactory
└└└└└ MSDTSK	1.7	0:0, 12:2, 15:4, 17:7, 19:9, 21:10	18	8.00	very good
└└└└└ Static power	1.4			9.06	excellent
└└└└└ MSMIZT	1.4	0:0, 56:2, 65:4, 85:7, 102:9, 120:10	103	9.06	excellent
└└└ Running endurance	9.4			9.08	excellent
└└└└ MSCCT	9.4	480:10, 492:9, 515:7, 530:5, 537:4, 554:2, 820:0	491	9.08	excellent
└ Excitation intensity	8				

URMPU – universal reduced model of potential performance, RES – raw test results, f(x) – numerical score, SCORE – attribute score, Numerical and descriptive values of scores: 0–1.99 – unsatisfactory, 2–3.99 – satisfactory, 4–6.99 – good, 7–8.99 – very good, 8.99–10.00 – excellent

TABLE 3
ANALYSIS OF THE DIFFERENCES BETWEEN COMPETITION CATEGORIES IN THE SCORES AT THE HIGHEST LEVELS OF THE MODEL

	STDKI : MLMCI		MLMCI : STMCI		STDKI : STMCI	
	t	sig (t)	t	sig (t)	t	sig (t)
URMPU	-3.23	0.00**	-5.12	0.00**	8.53	0.00**
Motor abilities	-2.99	0.01**	-3.97	0.00**	6.73	0.00**
Morphology	-2.78	0.01**	-5.20	0.00**	8.44	0.00**
Psychology	-1.50	0.14	-1.78	0.09	3.10	0.00**
Sociological characteristics	0.69	0.49	-1.25	0.22	0.36	0.72

**p<0.01, URMPU – universal reduced model of potential performance, older boys – STDKI (born in 1989 and 1990, n=17), younger juniors – MLMCI (born in 1987 and 1988, n=17) and older juniors – STMCI (born in 1985 and 1986, n=14 subjects)

gories on the basis of dependent determination of weights. The differences in the obtained scores between the subjects of these three groups were established (Table 3).

In the score of the highest level (URMPU), all three competition categories differ between one another statistically significantly. Statistically significant differences between all categories are only in the score of the subspaces of motorics and morphology. In the psychological subspace, statistically significant differences can be noticed only between older boys and older juniors. Sociologically, all three competition categories are very homogenous, therefore there are no differences between them as expected.

The analysis through varying chronological age of competitors

In planning the training process from the aspect of laws of development, it is necessary to also take into account the specifics of the chronological age of competitors. They provide a more detailed picture of the status of an athlete. In the continuation, we use average numerical scores, which merely show upward and downward trends by individual age periods but not also the actual quality of the subjects. The latter can be expressed only after the transformation of the numerical score into the attribute score that must be determined for individual competition categories.

Table 4 shows arithmetic means and standard deviations of the numerical scores obtained on the most important elementary and aggregated variables of the MMPS model, for which on the basis of expert findings of individual science disciplines we are of the opinion that they are the carriers of the most important information in planning the performance.

Trend of the average scores at the highest levels of the MMPS model

The average final score (URMPU) shows an upward trend (Figure 1). A very similar trend can also be established in motor abilities. The most abrupt increase in the average URMPU scores and motorics can be noticed between the 14th and 15th year, and between the 16th and 17th year of age.

The average score of morphological suitability shows, generally, an upward trend. Between the 15th and 16th year, a slight fall-off trend is evident. Between the 16th and 17th year, a considerable jump in the average score of morphology can be noticed, which as a result means a rise in the competition performance in cross-country skiers.

The average score of the psychological subspace increases uniformly through all years selected. No dramatic increasing in the average score relative to the age period of the subjects can be noticed anywhere. The trend of the average final score of the sociological subspace is in accordance with the expectations. This score is not subject to age categories and is the highest among the scores of all studied subspaces.

Trend of the average scores of variables in individual subspaces of the MMPS model

The trend of individual average scores of the main components of motorics is in accordance with the trend of the score of the motor subspace itself. The average scores of the energy component of movement and the duration of excitation (ENKOGI and TRAEKS) have a similar trend as in motorics. Involved is a declining trend between the 13th and 14th year, and between the 15th and 16th year (Figure 2).

The information component of movement (INKOGI) shows, however, a constantly growing trend (Figure 3). Within the information component of movement, a different trend of the scores of the variables of coordination (KOORD) and regulation of synergists (REGSIN) can be noticed at the end of the period of younger juniors (16th year) in comparison with other periods.

In the psychological subspace, the scores of the both dimensions of general performance motivation (USPEZDEL, USPNGDEL) differ considerably (Figure 4). While the score on one motivational form decreases strongly, the score on the other one increases strongly. The average score USPEZDEL is rather too low for a favourable motivational basis of performance in cross-country skiing.

Up to the age of 16, the trend of the average score in the competitive motivational field (NEGATIV, POZITIV) is rather unstable (Figure 4). Competition motivation,

TABLE 4
TREND OF THE SCORES OF MODEL VARIABLES OF HIGHER RANK IN THE MMPS MODEL RELATIVE TO THE AGE OF COMPETITORS

Test Code	STDKI		MLMCI		STMCI	
	13 year X±SD	14 year X±SD	15 year X±SD	16 year X±SD	17 year X±SD	18 year X±SD
URMPU	3.8±0.4	4.1±0.6	4.6±0.8	4.8±0.7	6.0±0.6	6.4±1.1
Motor abilities	2.3±0.6	2.2±0.6	3.2±1.4	3.2±0.9	4.8±1.1	5.5±1.8
ENKOGI	2.2±0.6	1.8±0.5	2.9±1.5	2.7±0.8	4.6±1.0	5.7±2.1
TRAEKS	2.5±0.8	1.7±0.5	2.8±1.4	2.7±1.0	4.0±1.1	6.2±2.6
INTEKS	1.5±0.5	1.9±0.9	3.2±2.2	2.5±0.6	6.0±2.4	4.8±2.1
INKOGI	2.6±0.8	3.1±1.0	3.8±1.5	4.3±1.8	5.0±1.8	5.1±1.4
REGSIN	2.3±0.3	3.0±1.2	3.6±1.3	3.2±1.4	5.0±1.9	5.3±1.4
KOORD	2.8±1.1	3.1±1.4	3.9±2.0	5.0±2.2	5.0±1.9	4.9±1.7
Morphology	2.5±0.9	3.2±1.3	4.3±1.5	4.2±1.4	6.6±1.5	7.0±1.0
Psychology	5.0±0.7	5.6±1.1	5.7±0.9	6.2±0.9	6.5±0.8	6.6±1.1
MOTIVAC	5.4±1.6	5.8±1.6	5.3±1.0	6.3±1.9	5.9±1.2	6.5±1.5
SPLSTMOT	6.1±0.9	5.6±2.3	5.4±1.3	6.3±2.7	6.0±1.6	7.0±1.3
USPEZDEL	5.4±1.4	5.5±2.8	4.7±1.4	6.3±3.0	5.3±2.4	7.3±2.2
USPNGDEL	8.0±2.1	5.8±2.8	7.2±3.0	6.3±2.7	7.7±1.2	6.4±2.5
TEKSTMOT	6.0±1.7	5.9±1.8	5.2±1.5	6.2±2.2	5.5±1.4	6.3±1.8
POZITIV	5.5±2.3	5.3±2.9	4.2±2.3	5.7±2.7	5.8±2.1	6.8±2.4
NEGATIV	7.5±2.0	6.4±2.5	6.2±2.5	7.4±2.1	4.2±1.4	4.7±1.9
OSEBLAST	4.7±0.4	5.9±1.1	6.2±1.1	6.5±0.7	7.1±0.6	6.8±1.7
SPSTRLAS	4.7±0.9	6.0±1.4	6.3±1.3	7.0±0.9	7.4±0.3	6.8±1.9
SOCPSLAS	4.5±1.6	5.6±1.5	6.7±2.1	6.3±1.7	6.2±1.8	6.4±1.5
TEKMLAST	4.9±0.9	6.0±1.2	5.8±1.4	6.2±1.1	7.2±0.8	6.9±1.9
Sociology	7.3±0.9	7.4±1.0	6.8±0.4	7.6±0.8	7.4±0.6	7.5±0.7

ENKOGI – energy component of movement, TRAEKS – excitation duration, INTEKS – excitation intensity, INKOGI – information component of movement, REGSIN – regulation of synergists, KOORD – coordination, MOTIVAC – motivation, SPLSTMOT – general performance motivation, USPEZDEL – performance (success) based on work, USPNGDEL – performance (success) irrespective of work, TEKSTMOT – competition motivation, POZITIV – positive competition motivation, NEGATIV – negative competition motivation, OSEBLAST – personality traits, SPSTRLAS – special structural traits, SOCPSLAS – sociopsychological properties, TEKMLAST – competition properties, older boys – STDKI (born in 1989 and 1990, n=17), younger juniors – MLMCI (born in 1987 and 1988, n=17) and older juniors – STMCI (born in 1985 and 1986, n=14 subjects)

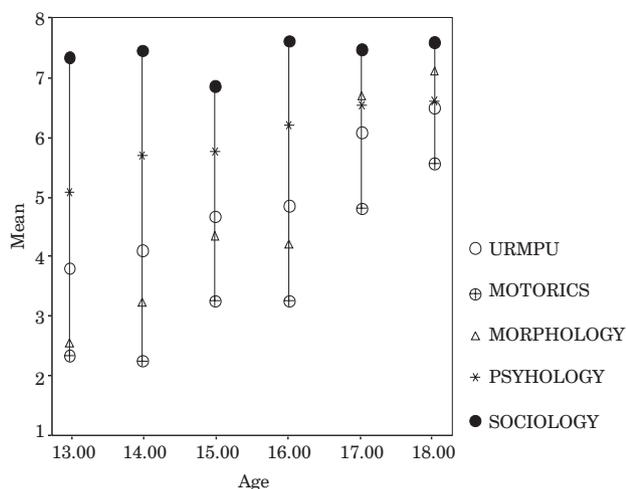


Fig. 1. Trend of the average scores of all studied subspaces and the final score of potential competition performance (URMPU).

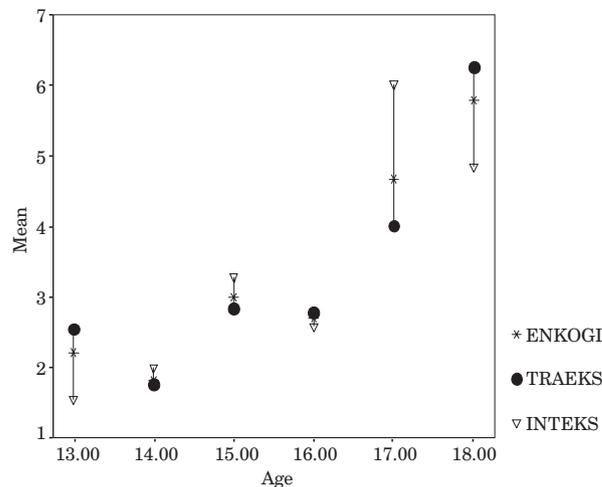


Fig. 2. Trend of the average scores of the energy component of movement and its variables. ENKOGI – energy component of movement, TRAEKS – excitation duration, INTEKS – excitation intensity.

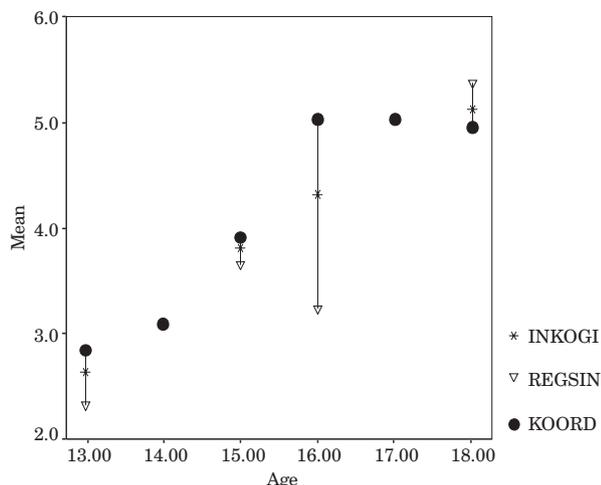


Fig. 3. Trend of the average scores of the information component of movement and its variables. INKOGI – information component of movement, REGSIN – regulation of synergists, KOORD – coordination.

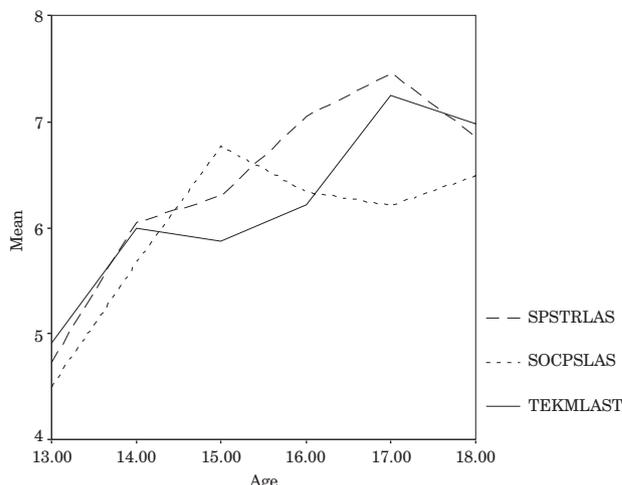


Fig. 5. Trend of the average scores of personality traits variables in the psychological subspace. SPSTRLAS – special structural traits, SOCPSLAS – sociopsychological properties, TEKMLAST – competition properties.

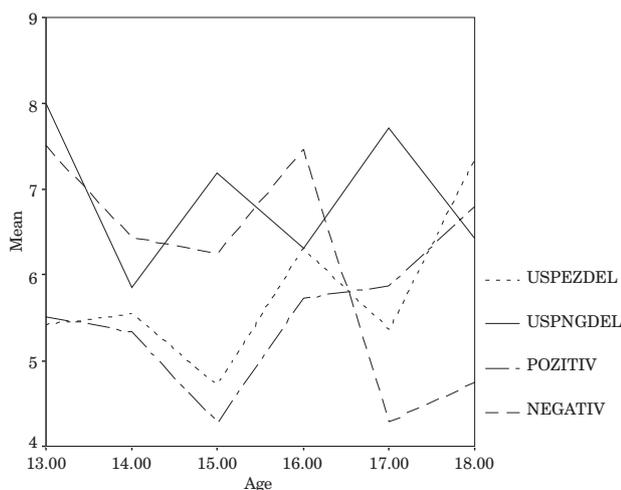


Fig. 4. Trend of the average scores of variables of the motivational component of the psychological subspace. USPEZDEL – performance (success) based on work, USPNGDEL – performance (success) irrespective of work, POZITIV – positive competition motivation, NEGATIV – negative competition motivation.

which is focused on the avoidance of failure and represents the negative component of competition motivation (NEGATIV), is at the age of 13 at first very high but afterwards it falls gradually. Then, between the 15th and 16th year, its rapid increase followed by a steep fall can be noticed. The score grows slightly again between the 17th and 18th year of age. The average score of positive motivation (POZITIV) shows a similar trend up the age of 16; later, however, the trend of this score is in accordance with expectations.

Generally, the average score of all three main dimensions of personality traits grows with years (Figure 5).

However, the direction of the trend of the scores of all three components is not the same. The trend of gradual increasing in the average scores is the most pronounced in the dimension of special structural properties (SPSTRLAS), which, however, falls slightly in the last year.

The universal (for all competition categories) reduced model of competition performance – MMPS – was elaborated for the needs of cross-country skiing. The positional configuration (normalisers) of the knowledge base was uniformly built. Thus, younger subjects obtained lower numerical scores in comparison with the older ones, which, however, considered developmentally, does not mean poorer suitability for this sport. On the basis of expert knowledge and findings of the present research, we can, at the end, round off the import of monitoring and give appropriate developmentally oriented attribute and numerical scores for individual competition categories (Table 5).

TABLE 5
SCORES OF COMPETITION POTENTIAL BY INDIVIDUAL COMPETITION CATEGORIES RELATIVE TO THE ATTAINED NUMERICAL SCORES IN THE MMPS MODEL

	STMCI	MLMCI	STDKI
Excellent	7.00–10.00	5.50–10.00	4.50–10.00
Very good	6.50–6.99	5.00–5.49	4.00–4.49
Good	6.00–6.49	4.50–4.99	3.50–3.99
Satisfactory	5.50–5.99	4.00–4.49	3.00–3.49

Numerical and descriptive values of scores: 0–1.99 = unsatisfactory, 2–3.99 = satisfactory, 4–6.99 = good, 7–8.99 = very good, 8.99–10.00 = excellent, older boys – STDKI (born in 1989 and 1990, n=17), younger juniors – MLMCI (born in 1987 and 1988, n=17) and older juniors – STMCI (born in 1985 and 1986, n=14 subjects)

Discussion

Coaches should know the current status of preparation of their competitors throughout their whole competition career and should direct the training process on the basis of that. It often happens that the patterns of training process are carried over also when competitors advance to a higher competition category and that only the loads change. Our analyses have shown that cross-country skiers differ statistically significantly among themselves relative to competition categories above all in the dimensions on which coaches have the largest influence (motorics) and indirectly on a rather large indivisible psychosomatic status represented by the final score of the model of potential performance (URMPU) in this analysis. The desired changes can, however, not be achieved by merely increasing the motor and cognitive loads.

Competition categories encompass cross-country skiers of different age categories (each consisting of two years); however, work in the categories normally takes place in a uniform way, irrespective of the chronological age of individuals. Despite an overall positive trend of gradual motor progress, a certain degree of stagnation of the motor score can, nevertheless, be observed between the 13th and 14th year, and the 15th and 16th year. The sample of subjects does, however, not allow any generalisations, but we may conclude that periods of accelerated physical development are involved owing to which, the learned motor patterns in the cortex break down and do not allow any larger progress in motor efficiency until new movement patterns are structured. This is the sign that loads are to be planned with a large degree of »psychological wisdom and caution«, and above all, selectively. In this period, giving up sport activities owing to mistakes of the grown-ups is very often. The reasons for the later abrupt progress (between the 16th and 17th year) can be sought in the fact that physical development is coming to an end, and partly also in larger demands of the environment since the final selection for admission to the top-level adult sport is involved.

If for the score of the energy component of movement (ENKOGI) we said that its trend is similar to the trend of the score of its superordinated component (OC MOTOR), then in the information component (INKOGI), constant increasing in the average score is involved, which means that despite development, the cognitive demands of movement can increase, while the energy potential is often limited in the period of adolescence. It is also necessary to mention that up to the very age of 17, the average score of the information component of movement is higher than the average score of the energy component of movement. After that year, a radical change is noticed for the first time; the reason for it must be sought in lower (higher) abilities of the subjects in the both mentioned variables. Motor cortical patterns have been re-established; further development or performance of a cross-country skier depends to a much larger extent on the energy potentials.

It often happens that an almost invisible trend of growth in psychological dimensions is the reason for errors in the psychological preparation of competitors. Roberts and Treasure⁴ say that the »folklore of training« (the same means of loading for all) increases mistakes in the motivational approach. We can probably agree with this statement and use it for the whole psychological subspace. Yet, it is also true that for correct reaction to information of psychological nature, proper psychological expertise – which in experts in practice is poor rather than good – is necessary.

In the dimension of general performance motivation it is not possible to give a common psychological developmental denominator which would encompass the whole scheme of falling and growing both of the motivation which is directed towards success attained with work (USPEZDEL), as well as the motivation focused on success irrespective of work (USPNGDEL). The confused situation in the competitive motivational field (POZITIV, NEGATIV) can be explained with an obvious inner insecurity and conflicts, which with the degree of involvement in sport even grow. Motives are in some way associated with the stimuli from the environment and arouse emotional states that lead to coming closer or avoiding the goal. With successes, cross-country skiers become more self-confident and the initial uncertainty changes to the motive for achieving success. In younger athletes, the experience of success is also associated with the fear how to succeed also in the future. Another reason for such a motivational development can be sought in the last year before the competitor advances from one into the next age category. As already found in motorics, the demands of the environment (coaches, parents) suddenly become larger to which fact competitors react very differently. Such a trend of competition motivation is, in fact, understandable, however it must not be an alibi for passive observation of the negative motivational developments.

Though between individual competition categories, an increase in the average scores of the socio-psychological properties (SOCPSLAS) and competition properties (TEKMLAST) can be noticed, it fluctuates within the categories relative to age. In the category of older boys (13 and 14 years), the both scores show an upward trend. The category of younger juniors (15 and 16 years) is marked by the search for own identity, hence also the resultant rise and fall in social and psychological properties. In the category of older juniors (17 and 18 years), the fall in competition properties can be attributed to the quality peak that has already structured in this category. This fluctuation is probably more a consequence of the characteristics of the sample than the consequence of laws of development. Nevertheless, the prediction of performance by means of personality traits is ungrateful since various tests and various methodologies are used for establishing personality traits. The opinions on the credibility of researches are divided⁵. However, as argued by Tušák and Tušák⁶, small but important relations between performance and personality traits can be noticed

if research work is carried out properly and correctly. Similar can be said for the trends of scores in individual competition categories and age periods.

In the sociological subspace, any other results could not be expected since both the positional and dimensional configuration do not allow any larger selectivity owing to smaller influence on performance in cross-country skiing. Interventions into the sociological sample of young competitors are, as a rule, not necessary, except by limited possibilities in extremely low score values.

Conclusion

Our objective was to qualitatively evaluate a cross-country skier through different age periods joined into competition categories. In the thus formulated positional configuration (normalisers), the main goal of the transformation process is »top level quality« in the national category. The conversion of numerical scores into attribute scores allows us to see »top-level quality« also in other (younger and older) competition categories and to compare the subjective score (given by the coach) with the score obtained on any model variable. In this way, a

competitor can be critically dealt with, while he himself can objectively follow the trend of his development. This is the method for fast and sufficiently good corrections of training programmes.

Monitoring and evaluating the performance of a cross-country skier should be the mission of every coach as this is the foundation for building the top-level quality in every sport. Each piece of feedback information must have the character of an immediate input into the system called the transformation process. By building the model(s) and evaluating the trend of scores through a given time cross-section, the sport profession is given a quality measuring instrument which ensures feedback and thus monitoring of the functioning of the whole system. The performance score pointed to the fact that in this age category there are periods which owing to development characteristics do not allow any major progress. Any exaggeration can lead to frustrating situations and can result in leaving cross-country skiing. By evaluating the numerical scores and tolerance in the dimensional and positional configuration, performance score was also admitted into the absolute category, while lower age categories were adequately descriptively evaluated.

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PRAĆENJE SKIJAŠKIH TRKAČA POMOĆU EKSPERTNOG MODELA POTENCIJALNE USPJEŠNOSTI

SAŽETAK

Na temelju stručnog znanja konstruiran je ekspertni model potencijalne uspješnosti koji uključuje motornu, morfološku, psihološku i sociološku razinu (MMPS). Ocjene za pojedine varijable dobivene su primjenom kompjuterskog programa Sport Measurement Management System (SMMS). Kod ispitanika uključenih u mjerenje, uspostavljeni su trendovi dobivenih prosječnih ocjena za pojedine varijable kroz različite natjecateljske odnosno dobne kategorije. Uzorak se sastojao od 48 skijaških trkača u tri natjecateljske kategorije. Fluktuacije u razvoju u pojedinim dobnim razdobljima veće su na motornoj i morfološkoj razini. Na psihološkoj razini, primijećen je rastući trend prosječnih ocjena, dok sociološka razina ne podliježe bitnim promjenama kod različitih dobnih odnosno natjecateljskih kategorija. Praćenje skijaških trkača kroz sve tri natjecateljske kategorije pokazalo je da u određenim dobnim kategorijama postoje razdoblja koja zbog zakonitosti razvoja ne omogućuju uniformno napredovanje. Stoga se u obzir mora uzimati individualni pristup, osobito prilikom planiranja procesa transformacije.

Biomotor Systems in Elite Junior Judoists

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ABSTRACT

The aim of the study was to assess the impact of motor abilities and morphological characteristics on junior judoka performance. A set of 14 morphological parameters and a set of 14 motor tests as predictor variables, and 3 variables evaluating judo performance as criteria were applied in a sample of 40 judoists aged 17 years \pm 6 months. Three factors were isolated by factor analysis in morphological area: factor of muscle mass and bone volume (muscle and bone mass – mesoectomorphy), factor of longitudinal skeleton dimensionality, and factor of subcutaneous adipose tissue (endomorph). Four factors were isolated by factor analysis in motor area: factor of coordination and strength (regulated force), factor of movement frequency (speed), factor of muscular and cardiovascular endurance (endurance), and factor of tonus regulation and synergy regulation (flexibility/balance). Canonical correlation analysis between latent morphological and motor variables, and variables for assessment of competitive performance of junior judoists yielded two linear combinations, i.e. two pairs of canonical factors. Correlation in the first pair of canonical factors was underlain by the favorable impact of coordination/strength, speed, flexibility and balance, along with above-average muscle mass and bone volume, and above-average skeleton longitudinality on performance in judo. Correlation in the second pair of canonical factors was based on positive determination of above-average endurance along with moderate coordination/strength and speed, and below-average muscle mass and bone volume and skeleton longitudinality upon judo performance as expressed by the fight winning score.

Key words: elite judo, morphological and motor structures, fighting efficiency

Introduction

Studies based on structural and biomechanical judo analysis¹⁻⁴ suggest that three motor-functional abilities, i.e. strength, coordination and aerobic-anaerobic endurance, may be crucial for judo performance. During judo fight, all strength types (i.e. maximal, repetitive, explosive and static strength) are employed, thus stronger judoists being at an advantage over their opponents of comparable technical skills. It is desirable that total body mass is predominated by muscle tissue. An excess of subcutaneous adipose tissue implies greater total body mass, leading to a higher category, which generally diminishes the chances for success at contest.

As current judo regulations impose a very high fight dynamics (the mean heart rate in fight is about 180 beats per minute), hard work performance is expected from judoists on training and at contest, which requires great psychophysical strain. This in turn entails an increased nervous and muscular adaptation to training load, re-

sulting in muscle hypertrophy, bone-ligament changes, cardiac muscle hypertrophy, and an increase in all vital function capacities⁵.

Gualdi-Russo and Graziani (1993)⁶ estimated morphological status in Italian elite judoists. They performed somatotyping according to Heath-Carter scale in 1593 male and female athletes from various sports. A predominance of the mesomorphic (muscle) component over endomorphic and ectomorphic components was found in gymnasts, rowers and judoists in particular. Krawczyk et al. (1997)⁷ conducted a large study determining Heath-Carter somatotypes in a sample of 300 athletes (66 of them judoists) from various sports (volleyball, rowing, wrestling, boxing, judo and karate). Like the Italian authors above, they also recorded a very high mesomorphic component in judoka, where muscle component was most pronounced, immediately following wrestlers. The mean somatotype in judoists was endomorphy 2.84, mesomorphy 6.07 and ectomorphy 1.51.

Relations among morphological, motor-functional and technical variables in elite judoists were observed by Franchini et al. (2001a)¹. They analyzed the number of attacks by a particular technique in fight and correlated it with the morphological and motor-functional characteristics. Their results indicated the judoists with a lower proportion of adipose tissue and high anaerobic capacity to have a higher rate of attempted use of various techniques in fight. They also conclude that arm techniques are more energy demanding than leg techniques.

Takeuchi et al. (1999)⁴ point to the role of basic physical preparation in judoists. They report on a higher level of basic motor abilities in the judoists of higher contest performance. Major differences between judoists of different quality were recorded on static strength testing in favor of higher quality judoists, according to weight categories. Monteiro et al. (2001)³ conducted a similar study in 18 judoists divided into two groups according to success at international contests. They used a battery of tests to assess morphological features, and basic and specific motor abilities. Statistically significant differences between the two groups were obtained in tests of specific endurance, forearm grabbing strength, and flexibility.

Significant differences between elite and non-elite fighters in specific judo-fitness test for endurance assessment were confirmed by Franchini et al. (2001b)². These authors also found significant between-group differences in some morphological measures, e.g., contracted triceps circumference and forearm circumference were significantly greater in elite judoists. In addition, elite judoists had higher stature relative to leg length.

Jagiello et al. (2004)⁸ investigated strength development in children and adolescents engaged in judo. The aim of the study was to compare the development of various strength types between the children without sports engagement and those on active judo training. Study results revealed significant differences in strength endurance between the children and adolescents engaged in judo training, and a control group without such a sports engagement. The older the children and the longer their engagement in judo training, the higher was the level of difference significance.

The main aim of the present study was to assess the impact of motor abilities and morphological characteristics on junior judoist performance. In order to recognize particular phases in acquiring comprehensive information on the biomotor systems in junior elite judoists, the following partial aims were set in line with the main one:

- to determine latent structure of the (a) morphological status and (b) motor abilities in a sample of currently top Croatian junior elite judoists; and
- to determine the association of latent morphological characteristics and motor abilities with the junior judoist performance as expressed by the manifest variables of fight efficiency.

Subjects and Methods

Subject sample

Study sample included 40 junior judoists, mean age 17 years \pm 6 months. Testing was performed in all seven regular judo junior weight categories (<60, 66, 73, 81, 90, 100 and >100 kg), attending a camp organized by the Croatian Judo Association (CJA) in Split. As judoists from all regions of Croatia attended the camp, the sample could be considered representative and relevant for the study objectives. The subjects had been actively training judo for 4 to 10 years, and none of them had a category lower than blue belt.

Variable sample

The set of variables used on assessment of morphological characteristics included 14 standard anthropometric measures evaluating the following four anthropometric dimensions^{9–13}:

- longitudinal skeleton dimensionality: body height, leg length, arm length, and biacromial breadth;
- transverse skeleton dimensionality: bitrochanter breadth, wrist breadth, and femur breadth;
- body mass and volume: body weight, forearm girth, calf girth, and chest girth; and
- subcutaneous adipose tissue: triceps skinfold, subscapular skinfold, and abdominal skinfold.

The relevant basic motor abilities of junior judoists were evaluated by use of 14 standard motor tests. Twelve of these tests were previously used on several occasions^{9, 14–16}, only the 6-min run was now used instead of 3-min run, and two new tests were introduced, i.e. push-ups and 60-m run. The following tests were chosen for:

- assessment of coordination: backward polygon and floor agility;
- assessment of flexibility and equilibrium: sit and reach flexibility test and astride standing on equilibrium bench (bench standing);
- assessment of movement frequency: hand tapping and foot tapping;
- assessment of explosive strength: small ball throw, standing long jump, 20-m run, and 60-m run;
- assessment of repetitive strength: trunk lifting with legs bent (sit-ups) and push-ups; and
- assessment of muscle and aerobic endurance: hang with elbows bent (bent arm hang) and 6-min run.

All junior judoists had previously participated in three criterion contests (according to CJA regulations), one of these being Croatia Championship. The contest performance of the study subjects was evaluated by three criterion variables, as follows:

- ranking score – score sum from three contests (ranking at each of the three criterion contests was allocated respective score: 5 points for first place, 4 points for second place, 3 points for third place, 2 points for fifth place, and 1 point for lower places. Points from all

three contests were summed up, yielding a score range of 3 to 15 points in all study subjects);

- number of wins – sum of wins from three contests (as a varying number of contestants were present in different weight categories, reaching finals or winning was determined by a variable number of fights. So, in some weight categories a series of three wins were required to win the contest, whereas in others the respective figure amounted to six wins. Therefore, another criterion variable of contest performance, i.e. total number of wins at three contests, had to be defined); and
- technical score – total technical points from three contests (the third criterion variable of contest performance included total score of all technical points from all three contests for each individual judoist. At judo contests, a fighter wins with one of the four possible results expressed in technical points, i.e. 3:0, 5:0, 7:0 or 10:0. Even score is not anticipated by judo rules).

By use of these three criterion variables, the possibility of error on assessing the real contest efficiency of study subjects was minimized.

The study was expected to produce model values of anthropometric and motor status of elite junior judoists. The results obtained in the study will be useful in upgrading objective selection of junior judoists as well as in subsequent monitoring their performance.

Statistical analysis

The following statistical methods were used on data analysis: factor analysis to determine factor structure in the samples of morphological variables and motor variables (calculations: V1... – significant varimax factors according to Guttman-Kaiser criterion of $\lambda > 1$; Lambda –

characteristic values; Variance % – percentage of variance explained by each latent dimension); and canonical correlation analysis to determine relations between latent morphological and motor variables, and the set of variables for assessment of contest performance (CAN – structure of canonical variable; Can R – canonical coefficient of correlation; Can R² – coefficient of canonical determination; p – level of significance).

Results and Discussion

Table 1 shows results of factor analysis (varimax rotation) of the variables assessing morphological characteristics of junior judoists, where V1, V2 and V3 = significant varimax factors according to Guttman-Kaiser criterion ($\lambda > 1$); Lambda = characteristic values; and Variance % = percentage of variance explained by each latent dimension. Factor analysis isolated three dimensions which taken together explained 82% of total variability of the system observed.

As illustrated in Table 1, the measures of body mass and volume, and of transverse skeleton dimensionality elicited highest projection upon the first varimax factor (V1). Two presumably independent latent dimensions were observed to have integrated into a single factor. This integration can be explained as follows. Large and massive bone surfaces generally are more tightly connected with muscle tendons and ligaments. In addition to reducing the potential lesions, these bonds enable work with greater external load in athletes, which eventually leads to better musculature development. This process is of a cause-and-effect character, i.e. muscles as well as the bone-ligament and tendon apparatus are strengthened by training. In other words, the judoists with greater

TABLE 1
DESCRIPTIVE STATISTICS (X±SD) AND RESULTS OF FACTOR ANALYSIS (VARIMAX ROTATION) OF VARIABLES ASSESSING MORPHOLOGICAL CHARACTERISTICS OF JUNIOR JUDOISTS

Variable	X±SD	V 1	V 2	V 3
Stature	180.60±7.08	0.37	0.86	0.13
Leg length	103.51±5.22	0.00	0.95	0.10
Total arm length	81.14±4.20	0.18	0.92	0.12
Biacromial breadth	40.69±2.27	0.49	0.08	0.54
Bitrochanter breadth	28.50±1.98	0.69	0.47	0.07
Wrist breadth	5.87±0.33	0.83	0.26	0.02
Femur breadth	9.97±0.52	0.67	0.54	0.35
Body mass	76.91±13.29	0.80	0.32	0.43
Forearm girth	95.81±7.26	0.72	0.16	0.50
Calf girth	27.33±2.09	0.89	-0.06	0.28
Chest girth	37.12±3.36	0.80	0.08	0.45
Triceps skinfold	7.57±2.05	-0.04	0.32	0.82
Subscapular skinfold	8.42±2.12	0.43	0.00	0.84
Abdomen skinfold	8.22±3.07	0.36	0.07	0.83
Lambda		4.91	3.33	3.24
Variance %		35.07	23.79	23.14

V – significant varimax factors, Lambda – characteristic values, Variance % – percentage of variance explained by a particular factor

TABLE 2
DESCRIPTIVE STATISTICS (X±SD) AND RESULTS OF FACTOR ANALYSIS (VARIMAX ROTATION) OF VARIABLES ASSESSING MOTOR ABILITIES IN JUNIOR JUDOISTS

Variable	X±SD	V 1	V 2	V 3	V 4
Polygon backward [#]	8.05±1.21	-0.77	-0.12	-0.34	-0.03
Floor agility [#]	9.16±1.40	-0.71	0.05	-0.46	-0.27
Bench standing	8.64±1.99	0.06	0.31	0.17	0.85
Sit and reach	72.98±11.21	0.04	0.00	-0.04	0.95
Hand tapping	39.28±3.91	0.09	0.88	0.17	0.29
Foot tapping	44.23±4.53	0.27	0.63	0.54	0.06
Ball throw	41.66±7.57	0.38	0.68	0.26	0.11
Standing long jump	252.58±15.63	0.72	0.32	0.11	0.29
20-m run [#]	3.28±0.16	-0.70	-0.59	0.15	0.06
60-m run [#]	8.41±0.50	-0.73	-0.55	0.11	0.07
Sit-ups	55.95±7.93	0.65	0.14	0.05	-0.01
Push-ups	41.48±11.68	0.72	0.10	0.35	-0.02
Bent arm hang	53.71±16.36	0.23	0.11	0.81	0.11
6-min run	1487.1±127.0	0.07	0.13	0.81	-0.01
Lambda		3.86	2.44	2.24	1.90
Variance %		27.57	17.43	16.00	13.57

[#]variable with opposite metric orientation, V – significant varimax factors, Lambda – characteristic values, Variance % – percentage of variance explained by a particular factor

transverse skeleton dimensionality measures had superior musculature development. Thus, the first varimax factor could be described as a factor of muscle mass and bone volume (muscle and bone mass).

The second varimax factor (V2) was mostly defined by the variables of longitudinal skeleton dimensionality, and could thus be described as a dimension of longitudinal skeleton dimensionality.

All skinfold variables showed significant projection upon the third varimax factor (V3). As these are ballast mass measures, the third varimax factor could be described as a dimension of subcutaneous adipose tissue.

Table 2 presents results of factor analysis (varimax rotation) of the variables assessing motor abilities of junior judoists, where V1, V2, V3 and V4 are significant varimax factors. As the four dimensions taken together explained 75% of total variability of the system observed, it was concluded that the variables chosen to describe motor abilities of junior judoists were properly selected.

The variables assessing coordination, explosive strength of the jump and sprint type, and relative repetitive strength of the trunk, arms and shoulder girdle elicited highest projection upon the first varimax factor (V1). Therefore, the first varimax factor could be described as a factor of coordination and strength (coordination/ strength). Other studies¹⁻⁴ indicate that strength and coordination of motor ability have greatest impact on the performance in judo fights. In the present study, the highest quality judoists must have stood out from other study subjects on strength and coordination testing, resulting in the respective structure of the first varimax factor.

The variables assessing movement frequency and explosive strength of throwing type showed significant cor-

relation with the second varimax factor (V2). The result of small ball (weighing 200 g) distant throw was predominantly determined by the speed of movement. Considering the second varimax factor structure, it could be described as a factor of movement frequency (speed).

The third varimax factor (V3) was primarily defined by high projections of the variables assessing aerobic endurance and strength endurance. The hang with elbows bent test requiring the longest possible isotonic contraction and cardiovascular endurance test showed correlation with the third varimax factor. The correlation of these two variables is justified, thus the third varimax factor could be described as a dimension of isotonic muscle and cardiovascular endurance (endurance).

The variables of astride trunk bending and astride standing on equilibrium bench showed significant projection upon the fourth varimax factor (V4), which could therefore be described as a factor of tonus and synergy regulation (flexibility/equilibrium).

Table 3 shows results of canonical correlation analysis of latent morphological and motor variables, and variables assessing contest performance of junior judoists. The results obtained by canonical correlation analysis indicated the correlation of latent morphological characteristics and motor abilities with criterion variables of judo performance to be defined by high coefficients of correlation, yielding two linear combinations, i.e. two pairs of canonical factors.

There was a significant correlation ($p < 0.001$) of the first pair of canonical factors with a high canonical correlation coefficient of 0.88, explaining 77% of the system variance. The first canonical factor of the predictor set of variables was predominantly defined by the very high

projection of the morphological latent variable of muscular and skeletal body mass, motor latent variables of coordination and strength, tonus regulation and synergy regulation, and movement frequency at one pole, and of longitudinal skeleton dimensionality at the opposite pole.

The structure of the canonical factor of the criterion set of variables was characterized by very high positive projection of all three criterion variables, i.e. number of technical points, followed by the number of ranking points and number of wins. These results indicate that the high values of primarily muscle mass and bone volume had a favorable effect on judo performance, generally expressed as total ranking at three contests and number of technical points collected from all three contests. It is concluded that the high values of coordination, strength and movement speed as well as of flexibility, equilibrium and movement frequency exerted favorable impact upon contest results of junior judoists.

The results obtained suggested that the high values of longitudinal dimensionality had unfavorable effect on judo performance, primarily expressed as the number of technical points from three contests. Favorable impact on judo performance was recorded in those junior judoists with high values of muscle mass and bone volume (muscle and bone mass). The judoists with lower longitudinal measures had a shorter stature than their opponents in the same weight category, facilitating them to assume a favorable position on applying throwing techniques (below the opponent's gravity center). On the other hand, on defense from the opponent's action, shorter judoists had greater dynamic stability because their gravity center was lower.

As judo is a very demanding sports activity, weight lifting plays a major role in training process. The methods of work and the volume of load used in training definitely influence the judoist morphological characteristics, as demonstrated by Gualdi-Russo and Graziani (1993)⁶, and Krawczyk et al. (1997)⁷. These authors showed elite judoists to be at the top of mesomorphic somatotypes in sports hierarchy. Thus, it is quite logical that performance in the study sample of junior judoists, in the area of morphological characteristics, was primarily dependent on the proportion of muscle-bone mass in total body mass.

Other studies¹⁻⁴ suggest that performance in judo is predominantly influenced by the motor abilities of strength and coordination. Elite judoka achieved significantly better results in the tests assessing strength and coordination than other subjects. Junior judoists with greater explosive strength were able to more quickly and properly draw the opponent off balance. Also, they could more readily apply the technique of judo throw (the final phase of opponent throwing in particular). Most probably they were more successful in defense from the hold catch, counter-throwing, and breaking the opponent's catch off. All these were the potential reasons for their superior performance at all three contests.

Another two latent motor variables, i.e. factors showed significant positive correlation with the criteria of judo performance used in the study. These factors were

tonus and synergy regulation, and movement frequency (speed). Those junior judoists who could more readily apply a technique or a combination of throwing techniques had the best chances to surprise or outwit the opponent, and thus to successfully apply the throwing technique. The importance of movement frequency in judo also manifests in defense by timely avoiding the opponent's actions.

The second pair of canonical factors with canonical correlation coefficient of 0.83 explained 69% of the system variance. In the morphological-motor area, the second canonical factor was defined with rather high, significant and positive factor projections for assessment of muscular and cardiovascular endurance, and moderate projections of the coordination/strength and speed factors, along with negative projections of morphological factors, the factor responsible for the muscle and bone mass volume in particular. In the area of situation efficiency, the second canonical dimension was defined by quite a high, significant and positive projection of the criterion variable of the number of wins, and to a lesser extent by the number of technical points and number of ranking points. The correlation of the second pair of canonical factors was underlain by positive determination of judo performance as expressed by the number of wins by the above-average endurance, along with below-average muscle mass and bone volume and skeleton longitudinality. This linear combination favors the judoists with such a morphological-motor system where motor abilities are more pronounced, with a predominance of endurance over muscle mass and bone volume development. The opposite pole is occupied by judoists with above-average development of muscle mass and bone volume and inadequately developed motor abilities, especially muscular and aerobic endurance, exerting an unfavorable effect on efficient judo performance.

Conclusion

Acquiring relevant information on the impact of morphological characteristics and motor abilities on performance in Croatian elite junior judoists was primarily limited by the small sample of study subjects. Therefore, latent variables were formed by use of factor analysis in the series of morphological variables and motor variables. Then, relations of latent morphological and latent motor variables as an integral set of predictor variables with the set of variables assessing performance in judo as a criterion were determined by use of canonical correlation analysis. Using this methodology, the morphological-motor mechanisms determining performance in junior judoists were identified.

Study results showed properly developed muscular and skeletal mass, i.e. mesoectomorphy, to be the predominant morphological characteristic of junior judoists (Table 1), whereas general motor efficiency was found to be defined by the first varimax factor (Table 2), which integrated coordination and (explosive and repetitive) strength factors. In the first canonical linear combina-

TABLE 3

RESULTS OF CANONICAL CORRELATION ANALYSIS BETWEEN LATENT VARIABLES OF MORPHOLOGICAL AND MOTOR AREAS AND VARIABLES OF FIGHT PERFORMANCE IN JUNIOR JUDOISTS

Variable	CAN 1	CAN 2
Muscle and bone mass	-0.72	-0.58
Skeleton longitudinality	0.89	-0.31
Adipose tissue	-0.22	-0.16
Coordination/Strength	-0.61	0.25
Speed	-0.38	0.21
Endurance	-0.05	0.62
Flexibility/Equilibrium	-0.49	0.05
Ranking points	-0.79	0.44
Number of wins	-0.68	0.71
Technical points	-0.80	0.58
Can R	0.88*	0.83*
Can R ²	0.77*	0.69*

* $p < 0.001$, CAN – canonical variable, Can R – canonical correlation, Can R² – coefficient of canonical determination

tion, the muscle-skeletal mass from the group of morphological factors and coordination/strength from the group of motor factors were those that predominantly determined performance in judo (Table 3). In this linear combination, the factor of tonus and synergy regulation and the factor of movement frequency (speed) also had significant effect on the manifest criterion variables. In junior judoists, tonus regulation and synergy regulation manifested in developed motor abilities of flexibility and equilibrium, which facilitated application of all judo techniques, both in upright position and on the floor. The factor of movement frequency manifested in the speed of applying particular technique and/or a combination of techniques. The speed of reaction and movement frequency are important, especially in kick sports such as boxing and karate¹⁷. Motor abilities of movement speed, equilibrium and flexibility precede the development of the factors of strength and motor coordination¹⁸. Judo training significantly influences the development of flexibility and equilibrium as early as in 7-year-old boys¹⁹, indicating that these abilities determine judo performance in the beginners. Judo training has no major influence on the development of psychomotor speed¹⁹, because this ability is mostly genetically determined. As the achievement of superior results in judo greatly depends on psychomotor speed, it should be included in the primary selection criteria.

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In the first linear combination, the factor of muscular and cardiovascular endurance was neutral relative to the criteria of judo performance, indicating the judoists characterized by this linear combination to have a satisfactory developmental level of motor abilities defining this factor, without major differences. However, the second linear combination of predictor and criterion sets of variables shows the importance of muscular and cardiovascular endurance for achievement of top results in judo. In this linear combination, muscular and cardiovascular endurance is opposed to muscle and skeletal mass. Accordingly, the judoists of outstanding endurance have below-average muscle and skeletal mass but are characterized by moderate above-average coordination/strength and speed, allowing them to achieve a higher win score at contests. These obviously include the judoists on intensive judo training for years, which has resulted in the formation of an appropriate morphological structure relying on the quality rather than quantity of muscular tissue. This in turn facilitates them the use of specific motor abilities and specific motor skills in judo. Therefore, training processes focused on strength development should be based on specific, situation operators-exercises while avoiding weight lifting exercises which simply increase muscle mass.

An increasing number of anthropologic system predictors relevant for judo performance have been included in the formation of elite judoists. Resolving the tasks and situations in judo fight is a complex issue that is closely related to the development of basic and specific motor abilities, as follows:

- phase one – approximately at age 7–9 years: development of psychomotor speed, equilibrium and flexibility;
- phase two – approximately at age 10–12 years: development of coordination and (explosive and repetitive) strength factors, ensuring proper learning and acquiring specific motor knowledge;
- phase three – approximately at age 13–15 years: integration of all these basic motor abilities for efficient use of judo techniques in series and combinations; and
- phase four – approximately at age 16–18: development of muscle and aerobic endurance, built upon the development of motor abilities attained in previous phases, eventually ensuring achievement of top results in judo.

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BIOMOTORIČKI SKLOPOVI ELITNIH JUDAŠA JUNIORA

S A Ž E T A K

Cilj ovoga istraživanja je bio utvrditi utjecaj motoričkih sposobnosti i morfoloških osobina na uspješnost u judu kod juniora. U tu svrhu na uzorku od 40 judaša starosne dobi od 17 godina ± 6 mjeseci primijenjen je skup od 14 morfoloških mjera i skup od 14 motoričkih testova kao varijabli prediktora i 3 varijable za procjenu uspjeha u judu kao kriterija. Faktorskom analizom u morfološkom prostoru izolirana su 3 faktora: faktor mišićne i koštane mase (MezoEktomorfija), faktor longitudinalne dimenzionalnosti skeleta, te faktor potkožnog masnog tkiva (Endomorfija). Faktorskom analizom u motoričkom prostoru izolirana su 4 faktora: faktor koordinacije i snage (regulirana sila), faktor brzine frekvencije pokreta (Brzina), faktor mišićne i kardiovaskularne izdržljivosti (Izdržljivost) i faktor regulacije tonusa i sinergijske regulacije (Fleksibilnost/Ravnoteža). Kanonička korelacijska analiza između latentnih morfoloških i motoričkih varijabli i varijabli za procjenu natjecateljske uspješnosti judaša juniora je utvrdila dvije linearne kombinacije, tj. dva para kanoničkih faktora. U osnovi povezanosti prvog para kanoničkih faktora je pozitivni utjecaj koordinacije/snage, brzine, fleksibilnosti i ravnoteže, uz iznadprosječnu mišićnu i koštanu masu i ispodprosječnu longitudinalnost skeleta, na uspjeh u judu. U osnovi povezanosti drugog para kanoničkih faktora je pozitivna determiniranost iznadprosječne izdržljivosti uz umjerenu koordinaciju/snagu i brzinu, a ispodprosječne mišićne i koštane mase i longitudinalnosti skeleta, na uspjeh u judo borbi izražen brojem pobjeda.

Anthropometric Evaluation of the Crèches Children Furniture in Turkey

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ABSTRACT

The dimensions of the living and working space and buildings, the types of material and different riggings should be designed to conform to the users' anthropometric measures. The first requirement to design an ergonomic system is to measure the human being who will work and live in that system. Because of this, anthropometric measures are the most frequently used ergonomic data during the design process. In this research paper, we attempt to organize a new data base of anthropometric data to use in the design of children's equipment and furniture used in crèches. A starting point for research on the proper dimensions of crèche furniture is to investigate how the dimensions of furniture reflect the body dimensions and the functional needs of the children using furniture. The anthropometric data of 3, 4 and 5 year-old-children in crèches was used. We report the results of the measurements of 18 anthropometric characteristics of children which constitute a set of basic data for the design of functional spaces and furniture.

Key words: anthropometry, crèches, design, Turkey

Introduction

Anthropometrics is a term used to describe the measurements of a »user« or »target« population for which a product is designed. Measurements are reported in terms of the range of body dimensions, of the target population. Having data available on the dimensions of a population takes the guesswork out of furniture and equipment design. With anthropometric measurements to hand, the designer can build equipment for a specific age group of children or to conform to a range of sizes of children. In fact, there are already considerable data available, gathered from taking measurements of large numbers of people in standard positions, which provide designers with the exact information they need.

It is necessary to know the body dimensions of the potential user for the proper design of product. This is important for service sectors such as schools, hotels and banks as well as in the production and manufacturing sectors. On the other hand, it has been found that even small changes in dimension of the work space can have considerable impact on worker productivity and may also impact occupational health and safety. Therefore, the user characteristics and specifically the structural anthropometric dimensions should be known for design of an effective workstation¹.

During the past decade, research in ergonomics has led to an increased interest in the technology of equipment and furniture design based on the biomechanics of the human body. The debate, building on early work in the field by Branton² and Keegan³, has been especially active in trying to determine guiding principles for the design of furniture in the workplace⁴. The design of furniture is generally not different from that of other industrial products. Thus, the functional uses of the furniture define the design of the final product. The design features that play significant roles in the design of a final product are: aesthetics, economics, functionality and originality. The functionality of furniture is based on its comfort, safety and usefulness. And these qualities of comfort, safety, and usefulness are related to the anthropometric characteristics of the user and the suitability of materials used in furniture design.

When a manufacturer or designer designs a product or products, he must know the body dimensions of the prospective user. Reasons for applying ergonomic design are that accidents (falls, strikes, injuries, etc.), reduced productivity, ineffectiveness, and user discomfort may

arise from incorrect product dimensions that do not match those of the user. Consequently, health problems such as musculoskeletal, visual, and circulatory⁵ problems may result from an improperly designed product. Mandal⁶ noted the importance of furniture specifically designed to conform to a child's body proportions and recommended different sitting postures for different activities⁷. For example, it has been noted that without proper design, sitting will require greater muscular force and body control to maintain stability and equilibrium. This, in turn, results in greater fatigue and discomfort and these are likely to lead to poor postural habits in the child as well as neck or back complaints⁴. On the other hand, good posture, which leads to improved lung expansion and reduces organ crowding and strain on soft bones, tendons, and muscles⁸, can be facilitated by proper ergonomic design. In the same way that industrial accidents and health problems may occur through badly designed equipment, so it⁹ may occur in school and crèches due to badly designed furniture such as tables, chairs, beds, TV stands and shoe cupboards. In this respect, many health problems and accidents appear to be increasing throughout the world. For example, eighty percent of the citizens of the U.S.A seek medical attention for back problems some time in their lives¹⁰. Contrary to what one might assume, back problems are not confined to the adult population. A surprising number of grade school children and adolescents are reported to have regular bouts of back, neck, and headache pain^{11,12}. Back and neck pain also have a substantial economic impact. In 1990, direct medical care costs for low back pain exceeded \$24 billion, and total costs increase substantially when the indirect costs of disability are included¹³. Given these statistics, the importance of prevention through proper product design is evident⁹.

However, surprisingly little interest has been shown in the ergonomic design of crèches. Crèche children are especially prone to suffer the adverse effects of badly designed and ill-fitting furniture owing to the prolonged periods of time they spend seated during crèches. In addition, it is in the crèches during their formative years where children acquire their permanent habits of sitting. For these reasons, public health concerns over the effects of bad posture need to be focused on the design of crèche furniture. However, studies that provide empirical evidence on the extent and the nature of a possible mismatch between crèche furniture and crèche children's bodily dimensions are rare⁴.

It is well known that there are serious ergonomic problems among the school-age children of Turkey. These problems have arisen through the non-implementation of the aforementioned design concepts in the schools of Turkey. The absence of reliable ergonomic and anthropometric data of school-age children, which measurements take into account the applications for which the children's furniture and equipment are designed as well as the dimensions of the children, can serve as examples of the national inattentiveness to design principles in crèches.

Crèche furniture from manufacturers is typically not designed to accommodate the dimensions of the individual user. Even among developed countries this problem is quite widespread and is not limited to less developed countries. Instead, for reasons of economy, a one-size-fits-all philosophy has been adopted in the manufacture of children's furniture. Such furniture is less costly to manufacture and easier to sell at a lower price. In addition, this practice reduces inventory problems for manufacturers and crèches. Today most companies base their designs on specifications from the American Furniture Manufacturers Association and the National Standards Board to decide »seat width, belly room, and prohibited combustible materials«. Existing designs have basically been unaltered for years⁴.

On the other hand, while it is known that manufacturing and inventory expenses are significant topics, it is also recognized that there are hidden costs associated with products that have not been designed using anthropometric data and according to ergonomic principles. These hidden costs are, of course, the previously mentioned health and safety problems and their attendant costs. At the same time, not surprisingly, observations and measurements indicate that furniture designed to accommodate a specific task and the individual's size is more acceptable to users than standardized styles.

It has been observed that a beginning has been made recently toward the consideration of ergonomic necessities in the design of products such as children's furniture intended for use in crèches. This growing trend is gaining speed especially in European countries like Denmark, Sweden, Germany, France and Switzerland⁵. For Turkey, it is known that, there are serious problems in this respect. It has not been so quick to adapt ergonomic principles in the design of furniture for school-age children. This situation resulted from both lack of anthropometric data as well as design and product problems. As a consequence, there are a lot of ergonomic problems in schools in Turkey and these problems could increase the number of health problems¹⁴⁻¹⁶ in the future.

In light of these problems and in the absence of data, this study was undertaken to meet the urgent need for anthropometric data from Turkey and to examine the possible mismatch between the individual body dimensions of children and the crèche furniture they use.

Methods

Sample and study design

The research area included crèches located in the centre of Trabzon. The potential data set, from which optimum furniture dimensions were to be calculated, included twenty crèches which were active during the years 2001–2002. Measurements were taken in 16 crèches that were randomly selected. The methods used for random selection have been cited in previous publications¹⁵. Measurements included the depths, breadths and heights of the furniture used in crèches. These measure-

ments were tabulated to compare them with the optimum furniture dimensions calculated according to children’s anthropometric dimensions (Table 1).

In order to calculate optimum furniture dimensions, anthropometric measures were taken from a total of 286 children attending crèches (154 male, 132 female) who were 3–5 years of age¹⁹. A total of 18 different measurements were made while the children were the in sitting and standing positions (Table 2).

The dimensions of existing furniture were measured. From these measurements, optimum values were calculated based on the anthropometric datum previously acquired¹⁹ (Table 2). In calculating the optimum dimensions of the furniture, dynamic or static anthropometric measures, minimum and maximum values, and also the function of the furniture were taken into consideration. All of the furniture was divided in to two groups according to reach and volumetric function based on the main criteria of anthropometric design. The formula for calculating the optimum furniture dimension is as follows:

Maximum values were calculated for volume measurements:

$$\text{Furniture dimension} = X + SDxZ$$

Minimum values were calculated for reach measurements:

$$\text{Furniture dimension} = X - SDxZ$$

Because some anthropometric values of females can be greater than those of values males, suitable male or female values were used in the calculating processes.

It is known that anthropometry tables give measurements of different body parts for men and women, and split into different nationalities, and age groups. Firstly, it is need to be known who you are designing for. The group you are designing for is called the user population. If an office chair is designed, it would be needed to consider dimensions for adults of working age and not those for children or the elderly. You also need to know whether you are designing for all potential users or just the ones of above or below average dimensions. This depends on what it is that you are designing. For instance, if you are designing a doorway using the height, shoulder width, hip width etc., of an average person, and then half the people using the doorway would be taller than the average, and half would be wider. Since the tallest people are not necessarily the widest, more than half the users would have to bend down or turn sideways to get through the doorway. Consequently, in this case you would need to design using dimensions of the widest and tallest people to ensure that everyone could walk through normally^{16,20}. At the same time, deciding whether to use the 5th, 50th or 95th percentiles of the potential users’ values depend on what you are designing and who you are designing it for.

TABLE 1
FURNITURE DIMENSIONS MEASURED IN CRÉCHES*

Crèches	Table depth	Table breadth	Table height	Chair depth	Chair breadth	Chair height	Bed depth	Bed length	Bed height	Bunk height	W.C. pan depth	W.C. pan Height	W.C. pan breadth	Washbasin depth	Washbasin height	Mirror height	TV height	Coathanger height	Shoe cupboard height
1	485	768	555	225	275	275	962	1,550	390	1,213	-	-	-	250	450	-	1,000	820	905
2	850	1,480	570	235	245	265	540	1,205	255	-	330	320	300	145	540	815	-	1,040	960
3	605	1,305	530	300	300	290	765	1,345	560	-	310	305	280	220	710	-	650	-	600
4	650	1,200	545	305	270	284	605	1,300	350	1,186	320	310	280	130	790	-	-	900	160
5	790	790	510	270	249	275	675	1,165	260	-	440	405	375	187	635	1,200	1,270	870	1,105
6	520	885	520	245	285	270	760	1,220	365	-	280	320	270	190	545	-	1,030	1,150	765
7	695	695	520	295	275	310	700	1,200	480	1,300	-	-	-	240	650	-	1,370	1,140	550
8	595	735	525	270	300	285	535	1,285	150	-	300	295	290	195	485	800	-	1,030	1,000
9	515	1,020	530	285	330	310	670	1,465	195	1,335	310	335	265	200	440	1,100	-	950	1,500
10	690	1,190	525	313	310	255	660	1,200	340	1,200	310	280	250	320	640	950	-	920	445
11	490	1,900	450	-	-	-	650	1,450	430	-	-	-	-	170	525	-	-	1,530	560
12	685	1,990	445	285	315	270	700	1,330	510	1,320	-	-	-	180	590	-	-	-	515
13	600	600	500	240	280	310	765	1,370	260	1,090	-	-	-	170	510	1,010	-	1,160	1,090
14	600	1,100	460	290	270	285	580	1,200	100	-	360	290	280	190	640	1,250	1,150	1,060	240
15	700	1,400	505	265	275	265	660	1,265	200	920	-	-	-	290	760	-	-	900	1,020
16	350	520	500	260	245	270	760	1,375	500	-	345	310	290	160	640	-	630	-	740
Total	16	16	16	15	15	15	16	16	16	8	10	10	10	16	16	7	7	13	16

*All measurements are in millimeters

TABLE 2
MEASUREMENTS (mm) OF ANTHROPOMETRIC CHARACTERISTICS OF THE CHILDREN*

Characteristics in the standing position	Group	X	SD	Characteristics in the sitting position	Group	X	SD
Stature	1	104.13	9.86	Sitting stature	1	78.15	7.63
	2	103.73	6.35		2	77.46	6.803
Max. vertical reach	1	125.77	9.42	Eye height	1	67.17	4.656
	2	124.92	9.14		2	67.15	5.07
Eye height	1	93.30	8.49	Elbow height	1	34.37	3.32
	2	92.84	6.26		2	34.75	3.83
Elbow height	1	60.16	4.72	Hip breadth	1	22.67	2.05
	2	60.01	4.34		2	22.27	1.82
Forward elbow reach	1	29.31	2.66	One calf thickness	1	7.56	1.18
	2	28.60	2.17		2	7.70	1.24
Forward arm reach	1	51.09	4.68	Two calf thickness	1	16.00	2.32
	2	50.28	4.40		2	15.35	2.12
Shoulder breadth	1	26.22	1.80	Buttock-calf depth	1	27.27	2.20
	2	26.06	1.90		2	28.04	2.24
Elbow to elbow breadth	1	27.68	2.19	Buttock-knee depth	1	33.59	3.32
	2	28.84	21.00		2	33.99	2.83
Waist depth	1	13.62	1.45	Sitting height	1	23.52	2.17
	2	13.37	1.42		2	23.73	2.43

*In groups, 1 – male and 2 – female

Measuring procedure

In this research, various dimensions of furniture and equipment used by children used in crèches were measured. The aim of this is to compare existing furniture dimensions with optimum furniture dimensions based on anthropometric data.

Depth, height and breadth of the furniture that are frequently used in the children’s classrooms and have dominant characteristics were measured. The furniture and equipment that were measured included tables, chairs, beds, bunks, washbasins, toilets, pans, mirrors, TV tables, coat hangers, shoe/toy and equipment cupboards. These measurements were tabulated (see Table 3) along with mean value of each measurement, its standard deviation, and its minimum and maximum values. Thus, measured, empirical values could be compared with calculated optimum values.

Results

Calculations of the depth, height and breadth of the furniture and equipment which are considered to be used rather frequently by children were done. Anthropometric data of children were used when calculating the measurements. Consequently, calculated values and existing furniture measurements were compared in a table and suitability of the optimum measurements with the existing was discussed (Table 4).

TABLE 3
STATISTICS OF EXISTING FURNITURE*

Furniture measurements	X	SD	Min.	Max.
Table depth	614	125	350	850
Table breadth	1,099	437	520	1,990
Table height	512	35	445	570
Chair depth	254	67	300	313
Chair breadth	282	25	245	330
Chair height	281	17	255	310
Bed depth	687	105	535	962
Bed length	1,308	112	1,165	1,550
Bed height	334	138	100	560
Bunk height	1,196	138	920	1,335
W.C. pan depth	331	45	280	440
W.C. pan breadth	288	34	250	375
W.C. pan height	317	35	280	405
Washbasin depth	202	51	130	320
Washbasin height	597	105	440	790
Mirror height	1,018	177	800	1,250
TV height	1,014	286	630	1,370
Coat hanger height	1,036	186	820	1,530
Shoe cupboard height	760	352	160	1,500

*All measurements are in millimeters

TABLE 4
COMPARISON OF MEANS OF CALCULATED OPTIMUM FURNITURE DIMENSIONS WITH THE MEAN VALUES MEASURED*

Furniture	Depth (mm)		Breadth (mm)		Height (mm)	
	X	Calculated	X	Calculated	X	Calculated
Table	614	345	1,099	395	512	490
Chair	254	264	282	267	281	193–285**
Bed	687	626	1,308	1,235+pillow	334	193
Bunk	–	–	–	–	1,196	931
W.C. pan	331	230	288	187	317	193
Washbasin	202	244	–	–	597	515
Mirror	–	–	–	–	1,018	806
TV table	–	–	–	–	1,014	572-TV Height /2
Coat hanger	–	–	–	–	1,036	948
Shoe cupboard	–	–	–	–	760	806

*All measurements are in millimeters

** Minimum and maximum values of chair height

The tables

In sizing tables, two possible sitting positions were considered: sitting facing one another and sitting sideways. Also, the ease of knee and elbow movement must be considered to determine suitable dimensions.

Calculation of table depth (for one person):

The maximum value of forward elbow reach was used in calculating of table depth for one person (Figure 1).

$$\begin{aligned} \text{Table depth (for one person) (max. value)} &= \\ &= \text{Forward elbow reach } (X_{\text{male}}) + \text{SD} \times Z \end{aligned}$$

$$\text{Table depth (for one person)} = 29.31 + 2.66 \times 1.96$$

$$\text{Table depth (for one person)} = 34.52 \text{ cm} = 345 \text{ mm}$$

Calculation of table breadth:

The maximum value of buttock-knee depth was used in calculating of table breadth (Figure 2).

$$\begin{aligned} \text{Table breadth (max. value)} &= \\ &= \text{Buttock-knee depth } (X_{\text{female}}) + \text{SD} \times Z \end{aligned}$$

$$\text{Table breadth} = 33.99 + 2.83 \times 1.96$$

$$\text{Table breadth} = 39.54 \text{ cm} = 395 \text{ mm x (per person)}$$

Calculation of table height:

The minimum value of sitting height and maximum value of two-calf thickness were used in calculating of ta-

ble height. The reason of this is to ensure easy acting of knee on horizontal and vertical ways and the connection between elbow and table (Figure 1).

$$\begin{aligned} \text{Table height (max. value)} &= [\text{Sitting height } (X_{\text{female}}) \\ &+ \text{SD} \times Z] + [\text{Two calf thickness } (X_{\text{male}}) + \text{SD} \times Z] \end{aligned}$$

$$\begin{aligned} \text{Table height (max. value)} &= (23.73 + 2.43 \times 1.96) + \\ &(16.00 + 2.32 \times 1.96) \end{aligned}$$

$$\text{Table height (max. value)} = 49.04 \text{ cm} = 490 \text{ mm}$$

The chairs

In sizing chairs, sitting height, chair depth and breadth are necessary for a comfortable and healthy sitting.

Calculation of chair depth:

The maximum value of buttock- knee depth was used in calculating of chair depth. Chair depth should be 2:3 of buttock-knee depth^{17,21} (Figure 3).

$$\begin{aligned} \text{Chair depth (max. value)} &= [\text{Buttock-knee depth} \\ &(X_{\text{female}}) + \text{SD} \times Z] \times 2:3 \end{aligned}$$

$$\text{Chair depth (max. value)} = (33.99 + 2.83 \times 1.96) \times 2:3$$

$$\text{Chair depth (max. value)} = 26.36 \text{ cm} = 264 \text{ mm}$$

Calculation of chair breadth:

The maximum value of hip breadth was used in calculating of chair breadth (Figure 4).

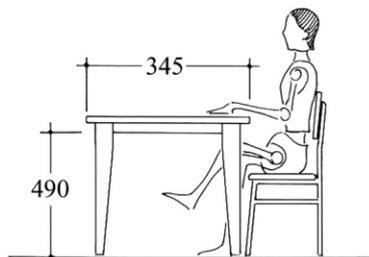


Fig. 1. Table depth and height (mm).

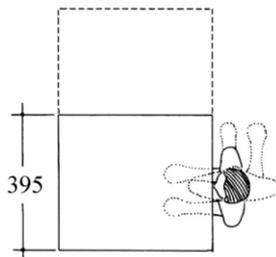


Fig. 2. Table breadth (mm).

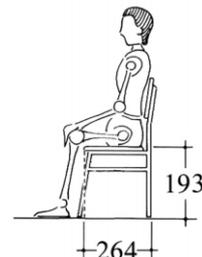


Fig. 3. Chair depth and height (mm).

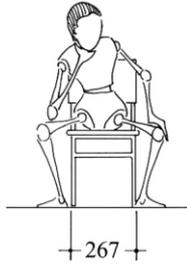


Fig. 4. Chair breadth (mm).

$$\text{Chair breadth (max. value)} = \text{Hip breadth } (X_{\text{male}}) + \text{SD} \times Z$$

$$\text{Chair breadth (max. value)} = 22.67 + 2.05 \times 1.96$$

$$\text{Chair breadth (max. value)} = 26.69 \text{ cm} = 267 \text{ mm}$$

Calculation of Chair height:

The minimum value of sitting height was used in calculating chair height. Because feet must touch to ground and calf must be rest while sitting (Figure 3).

$$\text{Chair Height (min. value)} = \text{Sitting height } (X_{\text{male}}) - \text{SD} \times Z$$

$$\text{Chair height (min. value)} = 23.52 - 2.17 \times 1.96$$

$$\text{Chair height (min. value)} = 19.27 \text{ cm} = 193 \text{ mm}$$

$$\text{Chair height (max. value)} = \text{Sitting height } (X_{\text{female}}) + \text{SD} \times Z$$

$$\text{Chair height (max. value)} = 23.73 + 2.43 \times 1.96$$

$$\text{Chair height (max. value)} = 28.49 \text{ cm} = 285 \text{ mm}$$

Actually, chairs have to be adjusted between minimum and maximum values. If adjustable chairs aren't used, then the minimum height calculation is preferred. For many purposes, the 5th percentile female chair seat height represents the best compromise for a fixed seat height. The seat height should be low enough to avoid excessive pressure on the underside of the thigh^{18,22}. If the seating surface is too high, the underside of the thigh becomes compressed causing discomfort and restriction in blood circulation. To compensate for this, a sitting person usually moves his buttocks forward on the chair seat. This can result in a slumped, kyphotic posture due to lack of back support^{4,19,23}.

The beds and bunks

Calculation of bed depth:

The maximum value of buttock-knee depth was used in calculating of bed depth (Figure 5).

$$\begin{aligned} \text{Bed breadth (max. value)} = & \\ = 2 \times [\text{Buttock-knee depth } (X_{\text{female}}) + \text{SD} \times Z] - & \\ [\text{Waist depth } (X_{\text{male}}) + \text{SD} \times Z] & \end{aligned}$$

$$\begin{aligned} \text{Bed breadth (max. value)} = 2 \times (33.99 + 2.83 \times 1.96) & \\ - (13.62 + 1.45 \times 1.96) & \end{aligned}$$

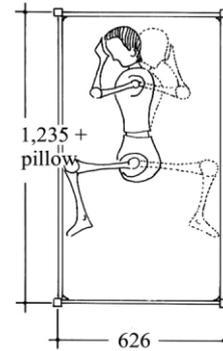


Fig. 5. Bed/bunk breadth and depth (mm).

$$\text{Bed breadth (max. value)} = 62.61 \text{ cm} = 626 \text{ mm}$$

Calculation of bed breadth:

The maximum value of stature was used in calculating of bed length (Figure 5).

$$\text{Bed length (max. value)} = \text{Stature } (X_{\text{male}}) + \text{SD} \times Z + (\text{pillow})$$

$$\text{Bed length (max. value)} = 104.13 + 9.86 \times 1.96 + (\text{pillow})$$

$$\text{Bed length (max. value)} = 123.46 \text{ cm} = 1,235 \text{ mm} + \text{pillow}$$

Calculation of Bed Height:

The minimum value of sitting height was used in calculating of bed height (Figure 6).

$$\text{Bed height (min.value)} = \text{Sitting height } (X_{\text{male}}) - \text{SD} \times Z$$

$$\text{Bed height (min. value)} = 23.52 - 2.17 \times 1.96$$

$$\text{Bed height (min. value)} = 19.27 \text{ cm} = 193 \text{ mm}$$

Calculation of Bunk height:

The maximum value of sitting stature was used in calculating of bunk height (Figure 6).

$$\text{Bunk height (max. value)} = \text{Sitting stature } (X_{\text{male}}) + \text{SD} \times Z$$

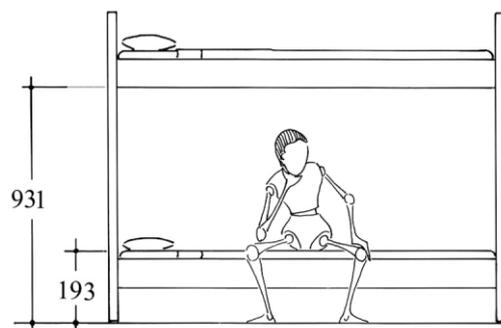


Fig. 6. Bed/bunk height (mm).

$$\text{Bunk height (max. value)} = 78.15 + 7.63 \times 1.96$$

$$\text{Bunk height (max. value)} = 93.11 \text{ cm} = 931 \text{ mm}$$

The W.C. pans

Calculation of W.C. pan depth:

The minimum value of buttock-calf depth was used in calculating of W.C. pan depth (Figure 7).

$$\text{W.C. pan depth (min. value)} = \text{Buttock-calf depth} \\ (X_{\text{male}}) - \text{SD} \times Z$$

$$\text{W.C. pan depth (min. value)} = 27.27 - 2.20 \times 1.96$$

$$\text{W.C. pan depth (min. value)} = 22.96 \text{ cm} = 230 \text{ mm}$$

Calculation of W.C. pan breadth:

The minimum value of hip breadth was used in calculating of W.C. pan breadth (Figure 8).

$$\text{W.C. pan breadth (min. value)} = \text{Hip breadth} \\ (X_{\text{female}}) - \text{SD} \times Z$$

$$\text{W.C. pan breadth (min. value)} = 22.27 - 1.82 \times 1.96$$

$$\text{W.C. pan breadth (min. value)} = 18.70 \text{ cm} = 187 \text{ mm}$$

Calculation of W.C. pan height:

The minimum value of sitting height was used in calculating of W.C. pan height (Figure 7).

$$\text{W.C. pan height (min. value)} = \text{Sitting height} (X_{\text{male}}) \\ - \text{SD} \times Z$$

$$\text{W.C. pan height (min. value)} = 23.52 - 2.17 \times 1.96$$

$$\text{W.C. pan height (min. value)} = 19.27 \text{ cm} = 193 \text{ mm}$$

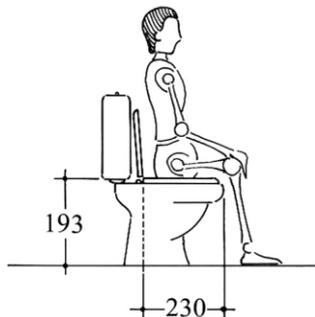


Fig. 7. W.C. pan height, depth (mm).

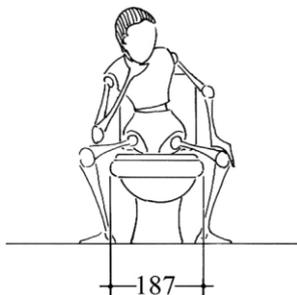


Fig. 8. W.C. pan breadth (mm).

The washbasins

Calculation of distance of tap to person (Depth of the Washbasin):

Tap distance from the person is necessary in tap dimensions. The minimum value of forward elbow reach was used in calculating of distance of tap to the person for reaching out for water easily (Figure 9).

$$\text{Distance of tap to person (min. value)} = \text{Forward} \\ \text{elbow reach} (X_{\text{female}}) - \text{SD} \times Z$$

$$\text{Distance of tap to person (min. value)} = 28.60 - \\ 2.17 \times 1.96$$

$$\text{Distance of tap to person (min. value)} = 24.35 \text{ cm} \\ = 244 \text{ mm}$$

Calculation of washbasin height:

The minimum value of elbow height in standing position was used in calculating of washbasin height (Figure 9).

$$\text{Washbasin height (min. value)} = \text{Elbow height} \\ (X_{\text{female}}) - \text{SD} \times Z$$

$$\text{Washbasin height (min. value)} = 60.01 - 4.34 \times 1.96$$

$$\text{Washbasin height (min. value)} = 51.50 \text{ cm} = 515 \text{ mm}$$

The mirrors

Calculation of mirror height:

Centre point height of mirror must be known for mirror height. The minimum value of eye height in standing position was used in calculating of mirror height (Figure 9).

$$\text{Centre point of mirror (min. value)} = \text{Eye height} \\ (X_{\text{female}}) - \text{SD} \times Z$$

$$\text{Centre point of mirror (min. value)} = 92.84 - \\ 6.26 \times 1.96$$

$$\text{Centre point of mirror (min. value)} = 80.57 \text{ cm} \\ = 806 \text{ mm}$$

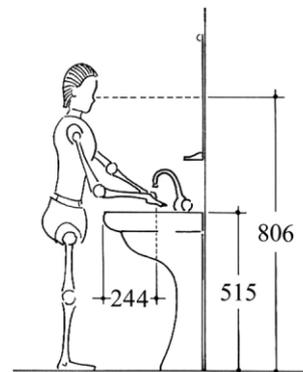


Fig. 9. Washbasin / mirror height and distance of tap to person (mm).

The TV tables

The most important measurement in the design of TV table is the eye height in the sitting position for getting a

perfect view. This height was accepted to be the centre point of the TV height and the table height was calculated according to this situation.

Calculation of centre point of tv height:

The minimum value of eye height in sitting position was used in calculating of centre point height of TV (Figure 10).

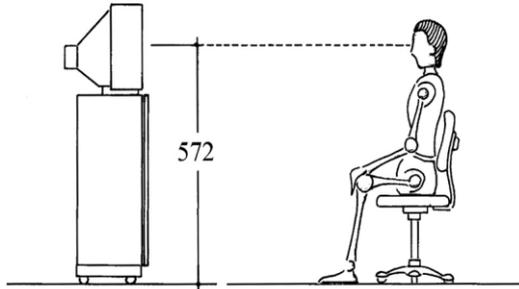


Fig. 10. Centre point of TV height (mm).

$$\text{Centre point of TV height (min. value)} = \text{Sitting eye height } (X_{\text{female}}) - \text{SD} \times Z$$

$$\text{Centre point of TV height (min. value)} = 67.15 - 5.07 \times 1.96$$

$$\text{Centre point of TV height (min. value)} = 57.21 \text{ cm} = 572 \text{ mm}$$

Calculation of TV Table Height:

Centre point height of TV value was used in calculating of TV table height (Figure 10).

$$\text{TV table height (min. value)} = \text{Centre point of TV height} - \text{TV height}/2$$

$$\text{TV table height (min. value)} = 572 \text{ mm} - \text{TV height}/2$$

The coat hangers

Calculation of coat hanger height:

In calculating of coat hanger height, arm is considered to make 45° with the coat hanger while using it. According to this, the formula below was used to calculate the minimum value of coat hanger (Figure 11 and 12).

$$\text{Coat hanger height (min. value)} = \text{Shoulder height (min. value)} + Y (\text{Forward arm reach}/\sqrt{2})$$

First step:

$$\sqrt{2}Y = \text{Forward arm reach}$$

$$Y = \text{Forward arm reach}/\sqrt{2}$$

$$\text{Forward arm reach (min. value)} = \text{Forward arm reach } (X_{\text{female}}) - \text{SD} \times Z$$

$$\text{Forward arm reach (min. value)} = 50.28 - 4.40 \times 1.96$$

$$\text{Forward arm reach (min. value)} = 41.66 \text{ cm} = 417 \text{ mm}$$

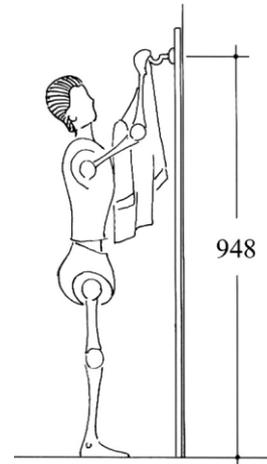


Fig. 11. Calculating coat hanger height (mm).

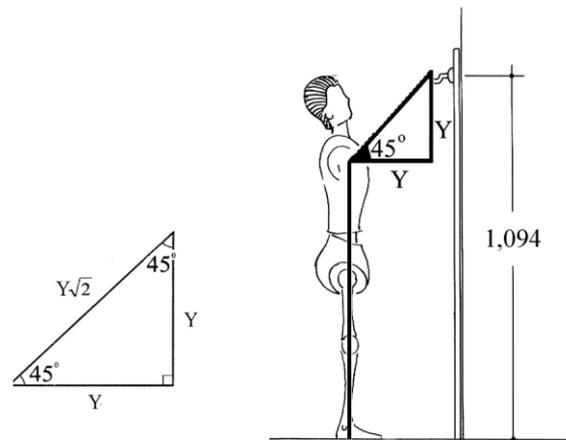


Fig. 12. Coat hanger height (mm).

$$Y = \text{Forward arm reach}/\sqrt{2} = 41.66/\sqrt{2} = 29.46 \text{ cm} = 295 \text{ mm}$$

Second step:

$$\text{Shoulder height (min. value)} = [\text{Maximum vertical reach (min. value)} - \text{SD} \times Z] - [\text{Forward arm reach (min. value)} - \text{SD} \times Z]$$

$$\text{Shoulder height (min. value)} = (124.92 - 9.14 \times 1.96) - (50.28 - 4.40 \times 1.96)$$

$$\text{Shoulder height (min. value)} = 65.35 \text{ cm} = 654 \text{ mm}$$

Third step:

$$\text{Coat hanger height (min. value)} = \text{Shoulder height (min. value)} + Y$$

$$\text{Coat hanger height (min. value)} = 65.35 + 29.46$$

$$\text{Coat hanger height (min. value)} = 94.81 \text{ cm} = 948 \text{ mm}$$

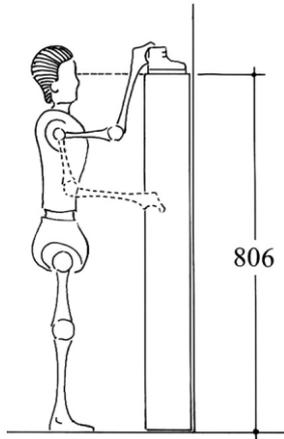


Fig. 13. Shoe, toy and equipment cupboard height (mm).

The toy, shoe and equipment cupboards

Cupboard height is important in designing of shoe and equipment cupboard. The minimum value of eye height in standing position is used in calculating of cupboard height (Figure 13).

Because of the bending forward will take a short time while using toy and equipment cupboard, minimum shelf height wasn't calculated.

Calculation of shoe, toy and equipment cupboard height:

$$\text{Cupboard height (min. value)} = \text{Eye height } (X_{\text{female}}) - \text{SD} \times Z$$

$$\text{Cupboard height (min. value)} = 92.84 - 6.26 \times 1.96$$

$$\text{Cupboard height (min. value)} = 80.57 \text{ cm} = 806 \text{ mm}$$

Findings

Anthropometric measurements are necessary to form the data base which is required for the proper sizing of furniture to match the sizes of its intended users. In this study, we have formed such a data base by collecting the anthropometric data of children (aged 3–5 years) who attend crèches. Using the anthropometric data, the theoretical optimum measurements of furniture frequently

used by the children were calculated. These theoretical optimum dimensions were compared to the measurements of furniture actually in use in crèches (Table 4). It can be seen that the suitability of some types of furniture for use in crèches is questionable. For instance, the mean measured table heights (614 mm) are more than two standard deviations away from the calculated optimal height. Similarly, the mean table breadth (1099 mm) is almost two standard deviations greater than the theoretical optimum breadth (395 mm). However, the mean measured height of the table (512 mm) is easily within one standard deviation of the calculated optimal height (490 mm). Taken together, these data and calculations suggest that manufacturers of children's furniture are getting the height right but that they need to scale down the width and depth of the table dimensions to match the requirements of 3–5 year olds.

The bulleted items shown below summarize the findings of Table 4 in which the mean dimensions of existing crèche furniture are compared to the calculated optimum measurements (Figure 14, 15 and 16):

- Table: The height of the existing table is over the optimum measure (512>490 mm) The depth does not provide adequate distance for two children to eat mutually in comfort. (614<345 × 2 mm). Existing table breadths are too wide for two people and too narrow for three people (1,099>395 × 2 mm).
- Chair: While there is no significant difference between the sitting depth of the available chairs and the calculated value (254<264 mm), the sitting breadth is greater on average than the calculated one (282>267 mm). Additionally, while the mean measured height of chairs is over the calculated minimum value (281>193 mm), it is close to the maximum value (281<285 mm).
- Bed: The mean depth (687>62 mm), height (334>193 mm) and breadth (1,308<1,235 mm) of the existing beds are over the calculated optimum values.
- Bunk: The mean height of bunks (1,196>931 mm) and the mean dimensions of W.C. pans (depth: 331>230 mm, breadth: 288>187 mm, height: 317<193 mm) are greater than the calculated optimum measures. The mean depth of washbasins (202<244 mm) is less than the optimum calculated value, while the mean height (597>515 mm) is greater.

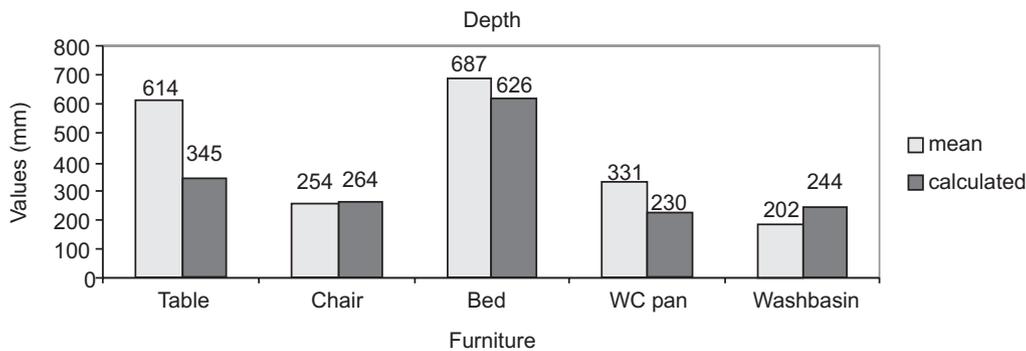


Fig. 14. Comparison of depths of existing furniture measurements and calculated measurements.

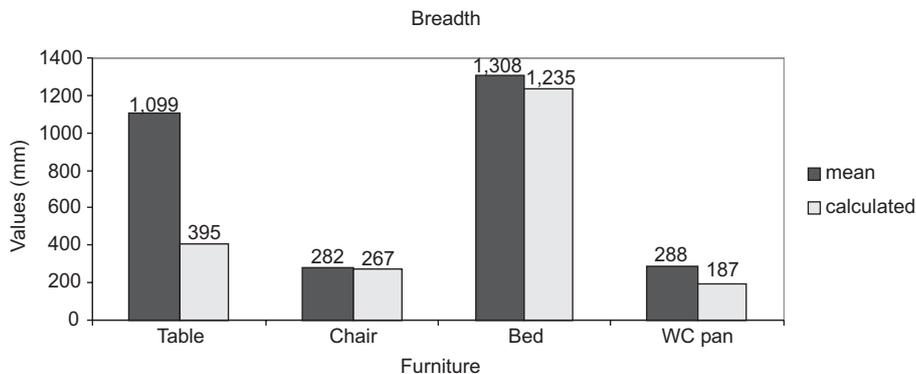


Fig. 15. Comparison of breadths of existing furniture measurements and calculated measurements.

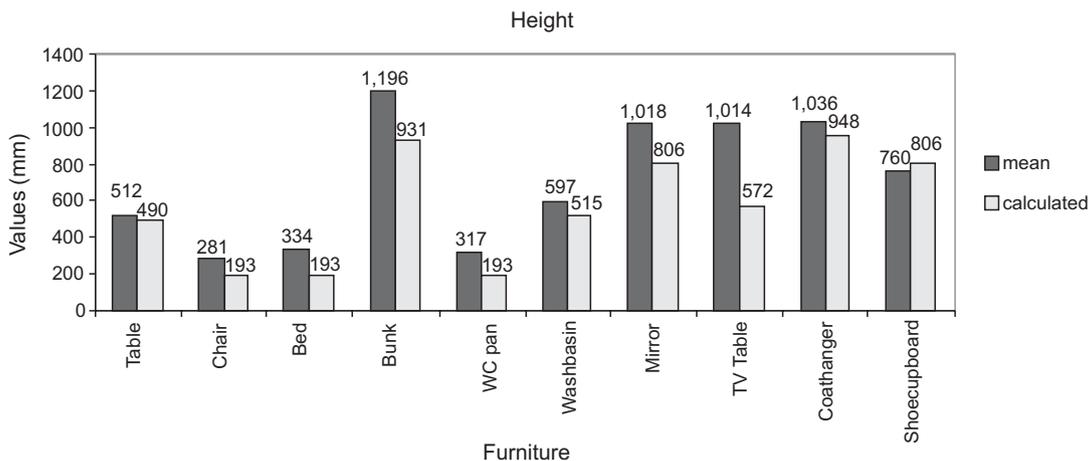


Fig. 16. Comparison of heights of existing furniture measurements and calculated measurements.

- **Mirror:** The mean height of mirrors (1,018 > 806 mm) is over the calculated optimum measure.
- **TV table:** The mean height of TV tables (1,014 > 572 mm) is almost twofold greater than the calculated optimum value.
- **Shoe cupboard:** The mean height of the shoe cupboards (760 < 806 mm) is less than the calculated optimum value.
- **Coat hanger:** The mean height of coat hangers (1,036 > 948 mm) is over the calculated optimum value.

Discussion

The deviations between the existing and calculated optimum furniture measurements were written as percentages (see Table 5). Thus, the relative strength of the differences between the existing furniture dimensions and those of the optimum calculated values can be evaluated.

This study of the dimensions of current accessories used by children attending crèches revealed that the greatest deviation between these measured dimensions and the optimal calculated dimensions are those along the vertical or height coordinate while the smallest deviation occurred along a horizontal coordinate called »breadth«

(Table 5). A one by one comparison of mean measured values against calculated optimal values show that the highest deviation (i) occur in all three dimensions of the W.C. pan, depth, the breadth, the height with deviations of 44%, 54% and 64% respectively; (ii) that deviations in the heights of chairs and beds are large (46% and 73%, respectively); and that (iii) the smallest deviations are found in the depth, the breadth and the maximum height of chair (4%, 6% and 1% respectively), the breadth of beds (6%), the heights of coat hangers and shoe cupboards (9% and 6% respectively).

- **Table:** It is observed that the existing table height is 22 mm less than the ideal measure. This case may cause a child to experience difficulties while moving, sitting at the table and standing up. Additionally, the breadth of table does not make it possible for two people to interact with each other in an activity that requires both people to participate.
- **Chair:** It is observed that the mean measured chair height is 88 mm greater than the desired value, which is the optimal calculated value. This large difference may cause trouble for most children, making it difficult for them to get into chairs and awkward to get out. The current chair depth is almost same with the ideal depth of chair (10 mm) and no ergonomic problems are

TABLE 5
THE DEVIATION RATIOS AND DIRECTIONS OF EXISTING FURNITURE MEASUREMENTS FROM CALCULATED OPTIMUM VALUES

Furniture	Depth (mm)		Breadth (mm)		Height (mm)	
	(+) Deviation	(-) Deviation	(+) Deviation	(-) Deviation	(+) Deviation	(-) Deviation
Table	-	11%	-	-	5%	-
Chair	-	4%	6%	-	46%**	1%***
Bed	10%	-	-	6%	73%	-
Bunk	-	-	-	-	29%	-
W.C. Pan	44%	-	54%	-	64%	-
Washbasin	-	17%	-	-	16%	-
Mirror	-	-	-	-	26%	-
Coat hanger	-	-	-	-	9%	-
Shoe cupboard	-	-	-	-	-	6%
TV Table*	-	-	-	-	-	-

* Because of the height of TV table is changeable according to the dimension of selected TV, the standard deviation of it wasn't calculated.

** The deviation ratio between the existing chair height and ideal min. chair height

*** The deviation ratio between the existing chair height and ideal max. chair height

expected here. It is seen that the mean breadths of chair are about 15 mm wider than the ideal measure. No ergonomic or functional problems are expected as a result of this small difference.

- Bed: It is not expected that a bed will be uncomfortable for its user if the existing mean bed depth (width) is 61 mm larger than the calculated ideal width.. However, that the mean bed length is 77 mm less than the optimal calculated value and that its height is 141 mm greater than the optimal height value suggest the possibility of ergonomic discomfort for the user or, especially in the case of height, the possibility that the user will have difficulty getting in and out of bed.
- Bunk: It is clearly a dangerous situation for children that the mean measured bunk height is 265 mm higher than the ideal. The danger arises from potential for children to fall out of bed while sleeping or falling while trying to climb into bed.
- WC Pan: In all three dimensions, the WC pan is not ergonomically designed to meet the needs of its users. The average WC pan in current use is 124 mm higher, 101 mm larger and 101 mm deeper than the ideal calculated values. It doesn't take much imagination to see the functional problems these differences will cause: inability to use the toilet properly or the possibility that the child will slip into the WC pan.
- Washbasin: The current average washbasin depth is 42 mm less than the ideal. This may cause difficulties for children their washing hands. Clearly children will have trouble using the average washbasin as it is 82 mm higher than the optimal calculated height.
- Mirror: The mean existing mirror height is 212 mm higher than the ideal calculated measure. Therefore, young children cannot use most mirrors currently in

use. It is considered that this case makes discomfort (and corrupts the functionality of furniture).

- TV Table: It was determined that the mean height of existing TV tables is higher than the calculated ideal measure by nearly a factor of 2. This large difference in height between real and ideal suggests that children watching television will be forced to sit in uncomfortable positions possibly causing pain in the neck muscles, eye fatigue, and poor posture.
- Coat hanger: It was determined that the average existing coat hanger height is 88 mm higher than the calculated ideal height. This height difference suggests that most young children will find it difficult or impossible to use the coat hanger.
- Shoe cupboard: It was determined that the mean existing shoe cupboard height is 46 mm less than the calculated ideal height. However, this height, because it is less rather than greater than the ideal value, is still accessible for easy use by young children. Thus, it is expected that the differences (mean measured vs. ideal) will not cause discomfort or lack of use.

The data in this study indicate a substantial degree of mismatch between the furniture measure in crèches and the optimum crèche furniture available to them. Most children are using furniture that are too high, too deep or too breadth (wide-extensive). For instance, according to the calculated ideal measures, some differences considered to cause problems for the comfortable use were detected at the depth and the height of table; at the height of chair; at the length and the height of bunk/bed, at the depth and the height of washbasin; at the depth, the breadth and the height of WC pan; at the heights of mirror, TV table and coat hanger. The positive findings are that chair and bed depth and shoe cupboard were not problem for any student.

While the findings of this study are suggestive, they are based only on data from a convenience sample in a single school district. There may also be systematic variations in body dimensions, based on ethnic/racial characteristics of the students that were not captured in this study. Finally, our definition of mismatch focused on only a few furniture dimensions, such as height, depth and breadth may make to the fit to body dimensions.

If manufacturers are going to continue to produce and sell traditionally designed furniture, schools need to be encouraged to at least provide as much variety in furniture sizes as possible to accommodate the variety of student sizes. In this particular study, crèche furniture simply turned out to be too large for many 3, 4 and 5 year old children. Given the low priority generally assigned to the comfort and functional needs of students, it would not be surprising if school furniture in other school districts show a similar mismatch with students' overall body height. However, it is also important that health professionals working in schools be aware that full accommodation of students' needs would require ergonomically re-designed classroom furniture⁴.

It is known that there are a lot of ergonomic problems in the schools in Turkey and this could increase effectiveness and health problems. Thus, the set of anthropometrical data obtained should be used for the design or adaptation of interior design and furnishing as well as the design of places for variable actions such as sleeping,

studying, playing, eating and etc. In this context, this study is putting forward the optimum-optimal measurements of crèche furniture according to the anthropometrical characteristics of crèche children in Trabzon, Turkey. And it is accepted that the continuity of this kind of studies is necessary for the researches as well as the producers and everyone relating with this concept.

This kind of studies would also put forward the differences between the optimum furniture measurements of children living in different regions in Turkey and the other countries. On the other hand, one of the increasing problems is childhood obesity around the world. The prevalence of overweight and obesity in adults and children is increasing in high-income countries^{20,24}, and is also rapidly emerging as significant health problem in less-developed countries^{21,22,25,26}. It is appears that the increasing problem will affect furniture sizes. Consequently, because of the optimum furniture measurements were based on the data that taken from the children and those will change by the time, this kind of study would be repeated in every decade.

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ANTROPOMETRIJSKA PROCJENA NAMJEŠTAJA U DJEČIJEM VRTIĆU U TURSKOJ

S A Ž E T A K

Dimenzije stambenih prostora u zgradama, kao i odabir materijala različitog namještaja trebao bi biti u skladu sa antropometrijskim mjerama ljudi koji koriste taj prostor. Za dizajn ergonomski povoljnog sistema potrebne su antropometrijske mjere ljudi. Upravo su zbog toga antropometrijske mjere najčešće korišteni ergonomski podaci tijekom dizajniranja. U ovim istraživanjima pokušano je prema antropometrijskim podacima organizirati novu bazu podataka za dizajn namještaja kojeg će koristiti djeca u vrtićima. Početna istraživanja bazirala su se na mjerenju dimenzija namještaja u dječjem vrtiću te ispitivanju kako trenutni namještaj utječe na tjelesne dimenzije i funkcionalne potrebe dječje populacije. U istraživanjima su upotrijebljeni antropometrijski podaci za 3 4 i 5 godišnju djecu. Prema rezultatima mjerenja 18 antropometrijskih karakteristika u djece, napravljena je baza podataka za dizajniranje funkcionalnog prostora i namještaja.

Evaluation of Circle of Willis Aneurysms with Spiral Computed Tomographic Angiography

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ABSTRACT

The aim of this retrospective study is to evaluate the specificity and sensitivity of spiral computed tomographic angiography (SCTA) in the detection of intracranial aneurysms. Patients were included in this study on the ground of the SCTA, digital subtraction angiography, magnetic resonance angiography, neurosurgeons operative findings and autopsy reports. Scanning protocol was slice thickness of 1mm, reconstruction interval of 0.5 mm, pitch 1. Flow rate was 3–4 ml/s, with standard scan delay time of 15–20s. In 18 patients with average age of 49.3 years SCTA results were positive for cerebral aneurysms and confirmed with other methods. On a per aneurysm basis SCTA sensitivity for detection of aneurysms was 89.47% specificity was 86.96%, positive predictive value of 85.00% and negative predictive value of 90.91%. SCTA should be used for the detection of cerebral aneurysms, especially for aneurysms with maximum diameter larger than 5mm.

Key words: computed tomography, angiography, aneurysms

Introduction

Spontaneous subarachnoid haemorrhage (SAH) is a neurological emergency mainly connected with ruptured intracranial aneurysm at the basis of the brain (approximately 85% of cases) with a high mortality rate, results in death in approximately 51% of patients^{1,2}. The incidence is around six cases per 100 000 patients years³. If the patients are untreated within two weeks after initial haemorrhage, rebleeding occurs in approximately 20% with mortality rate of 40%. Most of the intracranial aneurysms are located on the arterial Circle of Willis. Incidence of intracranial aneurysms in the general population varies from 1 to 7%, according to few autopsy series¹. Conventional catheter angiography, usually performed as a selective intra-arterial digital subtraction angiography (DSA) is still the golden standard for detection of intracranial aneurysm before surgery. Complication rate of cerebral DSA in patients with SAH is 1.8% and mortality rate 0.07%⁴.

Spiral (helical) computed tomographic angiography (SCTA) is non-invasive vascular imaging method based

on continuous scanning during the intravascular application of contrast agent. SCTA can be also defined as a minimally invasive imaging method because the patient receives the contrast material through the cannula placed in the peripheral vein. SCTA compared with catheter angiography has advantages in direct visualisation of intraluminal, vascular wall and perivascular changes without need for intraarterial catheterisation^{5,6}. Volumetric data acquisition is a basis for two and three-dimensional reformations of cerebral vasculature. SCTA sensitivity for detection of intracranial aneurysms depends on aneurysm size, location and used scanning protocol. Reported results are better for aneurysms situated in the anterior part of the Circle of Willis^{7–13}. Important role in precise depiction of intracranial aneurysms have twodimensional and threedimensional computer-generated reconstructions (reformations)^{5,6}.

The aim of this retrospective study is to evaluate the specificity and sensitivity of SCTA in the detection of Circle of Willis aneurysms.

Materials and Methods

The records of patients who underwent SCTA of Circle of Willis from May 2001 till May 2006 were analysed. Patients were included in this study on the ground of the SCTA results, DSA findings, MRA findings, neurosurgeons operative findings and autopsy reports. All patients were examined on single-slice spiral CT scanner with tube rotation time 0.8s (High Speed Lxi, GE Medical Systems, Milwaukee, Wisconsin, USA). Standard unenhanced axial head CT scan was performed first with slice thickness of 3mm in the posterior fossa and 7mm above, parallel to orbitomeatal line. SCTA volume of coverage was from the upper contour of C1 arch till the top of the posterior sphenoid clinoids, to cover the region where the intracranial aneurysms are most often situated and to provide valuable anatomy landmarks for neurosurgeons. Scanning protocol parameters were slice thickness of 1mm, image reconstruction interval of 0.5mm, pitch 1 (for faster acquisition and larger volume coverage optional pitch is 1.5), 120kV, 140 to 160mAs. All patients were in supine position during the examination and scan direction was caudocranial. For all patients, non-ionic iodine contrast agents were used, in dose of 100ml and concentration from 300 to 370 mg I / ml, injected by power injector through a needle in peripheral vein (usually cubital vein, and in few patients in central venous catheter). Intravenous line caliber was from 18 to 20 Gauges. Flow rate was 3–4 ml/s, depending on patient cardiac status, with standard scan delay time of 15–20s. Various reformatting techniques has been used for analysis of post-contrast scans, multiplanar reformatting (MPR), maximum intensity projection (MIP), shaded surface display (SSD) and volume rendering (VR). The best reformatting modalities for depiction of intracranial aneurysms were SSD (Figure 1) and VR (Figure 2). Three-dimensional (3D) angiograms were created on accompanying workstation (Advantage Windows 4.0).

For statistical analysis only SCTA results confirmed by other methods (operative finding, DSA or autopsy report) were used. True positives were patients with posi-

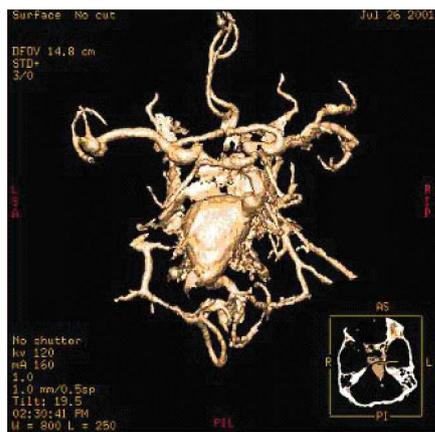


Fig. 1. Shaded Surface Display image. Basilar artery fusiform aneurysm.

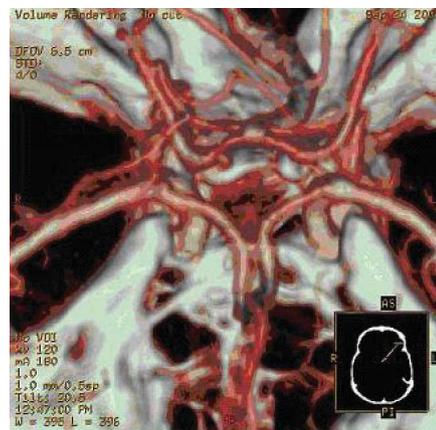


Fig. 2. Volume Rendering image of saccular aneurysm arising on anterior communicating artery.

tive SCTA results confirmed with DSA findings, surgically proven or described in autopsy report. False negatives were negative SCTA cases with intracranial aneurysm diagnosed with other method or found at surgery or autopsy. False positives were defined as positive SCTA results for intracranial aneurysm without confirmation in DSA, surgery or autopsy report. True negatives were patients with negative SCTA results supported by negative DSA or MRA results, or negative surgery or autopsy report. The aneurysm was used as the unit of analysis in calculation of sensitivity, specificity, positive and negative predictive value. Clinical informations were available in all cases before CT scanning procedure.

Results

38 patients evaluated in the period from May 2001 to May 2006 were included in the statistical analysis. In 18 patients, 10 female and 8 male SCTA has depicted aneurysms and results were confirmed with DSA, during surgery or autopsy. Average age of patients was 49,3 years, in male patients 52.4 years (from 45 to 78 years) and in female patients 48.7 years (from 15 to 78 years). SAH was found in 17 patients with aneurysm. The maximum diameter of the diagnosed aneurysms has ranged from 3.5mm, measured on aneurysm of the ACoA, to 34mm measured on basilar artery aneurysm in 15 year old female patient. 13 patients were treated surgically with clipping, one patient with basilar tip aneurysm was treated with endovascular radiological intervention, with coiling. 2 patients died before treatment after further diagnostic evaluation, one with DSA and one with MRA. 2 patients died before further diagnostic evaluation. Standard scan delay time ranged from 15 to 20s. In twenty patients SCTA did not depict aneurysms on Circle of Willis and SCTA results were confirmed with MRA. Aneurysms were most often positioned on the ACoA in the anterior part of Circle of Willis, and in the posterior part they have mainly arisen on basilar artery, Table 1.

TABLE 1
NUMBER OF ANEURYSMS RELATED TO LOCALIZATION

Aneurysm localization	Total number	Male patients	Female patients
ACoA	6	2	4
ACA	1	/	1
MCA	4	1	3
ACI	1	1	/
PCoA	1	1	/
PCA	/	/	/
BA	5	4	1
<i>Art. pericalosa</i>	1	1	/
	19	10	9

ACoA – anterior communicating artery, ACA – anterior cerebral artery, MCA – middle cerebral artery, ICA – internal carotid artery, PCoA – posterior communicating artery, PCA – posterior cerebral artery, BA – basilar artery

In one case coexistent MCA aneurysm with maximum diameter 2,5mm was overlooked in a patient with ruptured ACoA aneurysm with maximum diameter 9mm. Ruptured ACoA aneurysm was diagnosed with SCTA and confirmed during surgery. SCTA was performed in emergency conditions caused by massive SAH. Overlooked aneurysm was found during the retrospective analysis of volume rendering 3 D angiograms after surgery, it was positioned on superior side of left MCA, cranially oriented. It was false-positive negative SCTA result on a per aneurysm basis but true positive SCTA finding on a per patient basis because symptomatic ruptured aneurysm was diagnosed and correctly described.

There were two cases of false-positive SCTA results on a per aneurysm basis and one false-positive SCTA result on a per patient basis. In one case, which was false-positive on a per aneurysm and a per patient basis, pericalosal artery bending was wrongly described as an aneurysm. DSA indicated by neurosurgeons denied SCTA results. In one patient aneurysm of ICA infraclinoid segment was diagnosed and correctly described but tortuous basilar artery was wrongly reported as a fusiform aneurysm. ICA aneurysm was surgically treated because of SAH. DSA performed after recovery denied false-positive SCTA findings of basilar artery aneurysm. It was a true positive case on a per patient basis but combination of true positive and false positive cases in the same patient on a per aneurysm basis. In one case a pericalosal artery aneurysm was wrongly attributed to anterior cerebral artery, correct diagnosis was established with DSA indicated by neurosurgeons. SCTA sensitivity for detection of aneurysms on a per aneurysm basis in our study was 89.47% specificity was 86.96%, positive predictive value of 85.00% and negative predictive value of 90.91%. On a per patient basis SCTA has achieved sensitivity of 100%, specificity 95.23%, positive predictive value 94.44% and negative predictive value 100%.

Discussion

CTA provides opportunity to evaluate the etiology of SAH immediately after the clinical suspicion of SAH is confirmed with non-contrast brain CT scan. Short scanning time, even on single-slice spiral CT scanner, makes CTA appropriate diagnostic method for emergency patients who can not undergo long-lasting diagnostic procedures (e.g. MR) but DSA is still the golden standard for the diagnosis of cerebral aneurysms in most of the studies¹³.

The role of SCTA in the diagnostic evaluation of patients with cerebral aneurysms of Circle of Willis has been describe in many studies with diferent modalities. In Alberico's prospective study published in1995. SCTA has achieved sensitivity 96% and specificity 100%, aneurysm maximum diameter medium value was 7.9mm¹⁴. In Ogawa's study with scan delay 45s and flow of contrast agent 1.0ml/s, sensitivity was 84%¹⁰. In the study published in 1998. by Velthuis et al. CTA angiography showed 95% of symptomatic aneurysms and 90% of all aneurysms⁸. Korogi et al. reported in their study published in 1999. different sensitivity of three-dimensional CTA for detection of intracranial aneurysms according to size and location of aneurysm, for aneurysms with size less than 4mm was 64–83%, but for aneurysms with size 5–12mm sensitivity was 95%⁹. In the article of Villablanca et al. published in 2002. sensitivity of CTA for very small intracranial aneurysms detection with size less than 5mm was from 98% to 100%, and specificity 100%. Sensitivity and specificity of DSA were 95% and 100%. In 10% of the cases an aneurysm was diagnosed only on the 3D reformatted images and subsequently confirmed and quantitated on 2D images¹¹. Introduction of multi-slice CT scanners (MSCT) has given the new oportunities for further development of CT vascular imaging modalities because MSCT scanners are faster than single-slice CT and can scan longer distance with thinner sections. The sensitivity of MS CTA in Teksam's study from 2004. for detection of aneurysms smaller than 4mm on a per-aneurysm basis was 84%. The sensitivity and specificity of MS CTA for detection of cerebral aneurysms on a per-patient basis were 99% and 98%. Authors stated that CTA, even performed in multi-slice CT technique, is still not sensitive enough to replace DSA as a the criterion standard method in detection of cerebral aneurysms¹³.

Magnetic resonance angiography (MRA) is the non-invasive vascular imaging method based on MR imaging which is not connected with ionizing radiation exposure, and some techniques for depiction of intracranial vasculature like time-of-flight (TOF) does not require intravascular application of contrast material. MR scanning usually requires longer acquisition time than CT scanning. The sensitivity of threedimensional (3D) TOF MRA for detection of aneurysms in patients with SAH was 54 to 79% and slightly better for patients without SAH 65 to 79% in Okahara's study from 2002.¹⁵ Metens et al reported in 2000. 3D contrast-enhanced T1-weighted MRA sensitivity 100% and specificity 94%. Very important characteristic which makes 3D contrast-enhanced T1

weighted sequence appropriate for use in uncooperative patients and emergency situations is short acquisition time¹⁶.

The SCTA study presented in this article has been performed in a general hospital without interventional radiology division and without a neurosurgical ward. We have obtained the informations about further diagnostic procedure and therapeutic measures for only 18 of 34 patients in which SCTA has depicted aneurysms on Circle of Willis, this was the main limitation of this retrospective study. Other limitation is the fact that patients have been evaluated in different neurosurgical institutions, the protocol of MRA and DSA was not the same in all cases. Critically ill patients imaging material and clinical data were transferred to neurosurgeons in university hospitals via telemedicine programe. In all cases SCTA was performed under a general radiologist supervision. Some groups of neuroradiologists, like White et al, insists that non invasive vascular imaging methods should be per-

formed by specialist neuroradiologist¹⁷. The results of The International Cooperative Study on the Timing of Aneurysm Surgery published in 1990 can be strong arguments for those who wish to carry out non-invasive imaging of intracranial vasculature in general hospitals.² Authors agree with opinion exposed by White et al that non-invasive neurovascular imaging can be best performed and interpreted in specialist neuroradiology department but it is doubtful is it always possible to organize patients urgent transfer in emergency conditions.

SCTA limitations are exposure to ionizing radiation, need for intravasculature application of iodine contrast material, operator-dependent two and threedimensional reconstructions and limited vascular territory which can be evaluated.

In conclusion, authors think that spiral CT angiography should be used for the detection of cerebral aneurysms, especially for aneurysms with maximum diameter larger than 5 mm in critically ill patients.

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SPIRALNA CT ANGIOGRAFIJA WILLISOVOG KRUGA

SAŽETAK

Cilj ovog rada bio je ispitati osjetljivost i specifičnost spiralne kompjutorizirane tomografske angiografije (SCTA) u detekciji intrakranijskih aneurizmi. Bolesnici su uključeni u istraživanje na temelju nalaza SCTA, digitalne subtrakcijske angiografije, magnetske rezonancijska angiografije, operativnih nalaza neurokirurga i nalaza obdukcije. Protokol snimanja sadržavao je debljinu sloja 1mm, rekonstrukcijski interval slike 0.5mm, pitch 1. Brzina protoka kontrastnog sredstva bila je 3–4 ml/s s uobičajenim vremenom odgode snimanja od 15 do 20 s. U 18 bolesnika prosječne dobi 49.3 godine SCTA-om su dijagnosticirane aneurizme potvrđene drugim metodama. Gledano po aneurizmama osjetljivost SCTA za detekciju aneurizmi bila je 89.57%, specifičnost 86.96%, pozitivna prediktivna vrijednost 85% i negativna prediktivna vrijednost 90.91%. SCTA treba koristiti za dijagnosticiranje aneurizmi, posebice aneurizmi s najvećim promjerom većim od 5mm.

Association of Methylenetetrahydrofolate (MTHFR) and Apolipoprotein E (Apo E) Genotypes with Homocysteine, Vitamin and Lipid Levels in Carotid Stenosis

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ABSTRACT

The aim of the study was to investigate the association between methylenetetrahydrofolate (MTHFR) genotypes and levels of homocysteine (Hcy), folate, vitamin B12 and lipids as well as the association between apolipoprotein E (apo E) genotypes and levels of lipids in a Croatian healthy control group and a group of patients with >70% carotid stenosis (CS). The study included 98 Croats, 38 patients with >70% carotid stenosis and 60 age- and sex-matched controls. The MTHFR and apo E genotypes were determined by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP), Hcy by enzyme immunoassay, vitamins by immunochemiluminiscence, and lipids by spectrophotometric method. There was no difference between control subjects and CS patients in the distribution of C677T MTHFR genotypes ($p=0.786$) and alleles ($p=0.904$), however, differences in the frequencies of apo E genotypes ($p=0.012$) and alleles ($p=0.029$) were statistically significant. The odds ratio for apo E 3/4 genotype was 3.93 (95% CI 1.23–12.61). Hyperhomocysteinemia ($\geq 15 \mu\text{mol/L}$) was found in 11% of CS patients and 5% of control subjects. Total cholesterol, triglycerides, vitamin B12 and folate were statistically different in »all MTHFR genotypes« ($p<0.001$, $p<0.01$, $p=0.044$ and $p=0.036$, respectively), and in TC/TT ($p<0.001$, $p=0.003$, $p=0.030$ and $p=0.032$, respectively) groups. The levels of total cholesterol, LDL cholesterol and triglycerides in the apo E 3/3, and total cholesterol in the apo E 3/4 group yielded statistical difference. An association was found of apo E 3/4 genotype but not of MTHFR genotypes with the risk of CS. MTHFR and apo E affect blood lipid levels, which was statistically confirmed. An association was also recorded between hyperhomocysteinemia and patients with CS. Vitamin status in CS showed a statistically verified association with TC/TT MTHFR genotype. In the group of patients with TC/TT MTHFR genotype, lower vitamin B12 and higher folate values were recorded. The results of multiple logistic analysis showed that there was no statistical significance of Hcy levels (OR 2.403, $p=0.334$) or conventional vascular risk factors such as smoking habit (OR 0.505, $p=0.149$), age (OR 1.048, $p=0.087$) or sex (OR 2.037, $p=0.112$) in predicting CS.

Key words: MTHFR, apo E, genotype, lipids, vitamin status, homocysteine, carotid stenosis

Introduction

Extracranial atherosclerotic carotid disease is the leading cause of stroke and transient ischemic attacks (TIA) in Western populations¹. In addition to the established risk factors for atherosclerosis (diabetes, tobacco

use, decreased high-density lipoprotein level, hyperlipidemia, hypertension, positive family history, etc.), epidemiologic data indicate that elevated homocysteine (Hcy) concentrations are associated with an increased risk of

cardiovascular disease, including coronary artery disease, cerebrovascular disease and peripheral arterial occlusive disease^{1,2}.

Hcy is a putatively atherothrombotic sulfur amino acid produced during methionine metabolism. It is catabolized to cystathionine and cysteine by cystathionine β -synthase. Significant amounts of methionine may be regenerated by the remethylation pathway, a reaction catalyzed by methionine synthase. Vitamin B12 is a cofactor and methyltetrahydrofolate a substrate in this reaction. Methyltetrahydrofolate, the predominant circulating form of folate found in the blood, is formed in a reaction catalyzed by 5,10-methylene tetrahydrofolate reductase (MTHFR). This enzyme has a strong indirect influence on Hcy remethylation³. The main determinants of mild hyperhomocysteinemia are folate and B12 intake, impaired renal function, and genetic factors³.

A common missense mutation, C677T, has been identified in the MTHFR gene where alanine is substituted by valine, which results in a thermolabile variant of the reductase^{4,5}. The data have shown that the frequency of the mutation varies among different populations^{6,7}, and the association of MTHFR genotype and carotid artery atherosclerosis has been controversial^{7–10}.

High levels of total and low density lipoprotein (LDL) cholesterol and low levels of high density lipoprotein (HDL) cholesterol are known to predispose to the development of atherosclerosis^{11,12}. Also, lipid metabolism in humans is strongly affected by polymorphisms of a number of genes¹¹. The apolipoprotein E (apo E) gene, which also belongs to this group, is located on chromosome 19 and has three common allelic variants known as apo E*2 (ϵ 2), apo E*3 (ϵ 3) and apo E*4 (ϵ 4)¹². Studies of apo E allele distributions in different ethnic groups have shown similar patterns for most Caucasian populations. The ϵ 3 allele is most common, with frequencies between 0.70 and 0.85, ϵ 4 is less frequent, 0.10–0.20, and ϵ 2 is the rarest one with a frequency of 0.05–0.10¹³. Also, it is already known that the prevalence of apo E allele differs among populations¹⁴. Additionally, studies on apo E and carotid atherosclerosis have yielded inconsistent results^{10,15–20}. As this association has not yet been fully clarified, additional research is needed for definite conclusions²¹.

The aim of our study was to investigate the association between MTHFR genotypes and levels of Hcy, folate, vitamin B12 and lipids as well as the association between apo E genotypes and levels of lipids in a Croatian healthy control group and group of patients with >70% carotid stenosis.

Materials and Methods

Subjects

In this study, C677T MTHFR and apo E genotypes as well as blood lipid, homocysteine and vitamin (vitamin B12 and folate) levels were determined in 98 subjects, residents of Zagreb. Patients (38 patients; 9 female and 29 male, mean age 62±9 years) with a severe carotid ste-

nosis (>70%) according to the ECST criteria were included²². All patients had a clinical diagnosis of atherosclerosis based on ultrasonography color Doppler imaging findings. The control group included 60 sex- and age-matched healthy volunteers. EDTA-blood was collected by venipuncture after an overnight fast and important exclusion criteria was using of multivitamins. Patient group was under medication treatment (aspirin, anti-hypertensive or oral antidiabetics) which was administered after blood drawing. An informed consent was received from each subject prior to analysis. The study was approved by the Ethics Committee of the University Hospital »Sestre milosrdnice«, Zagreb.

C677T MTHFR and apo E genotyping

The C677T MTHFR gene mutation and apo E genotypes were detected by a DNA-based method consisting of DNA isolation and polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP)^{4,7,23}. Leukocyte DNA was isolated by the phenol/chloroform/isoamyl alcohol extraction and ethanol precipitation method^{24,25}.

The C677T substitution in the MTHFR was identified as previously described^{4,7}. The apo E genotypes were identified by a slightly modified method described by Dallinga-Thie et al.²⁴. Briefly, target DNA (0.3 μ g) was amplified by PCR using PCR Core Kit (Roche Diagnostics, Mannheim, Germany) and two specific primers (MWG Biotech, Ebersberg, Germany) in a DNA thermal cycler (ProGene thermal cycler, Techne, Duxford, Cambridge, UK). The sequence of primers used for MTHFR gene amplification was: 5'- tgaaggagaaggtgtctgcggga-3' and 5'-aggacgggtgcggtgagagtg-3'; and the sequence of primers for apo E gene amplification was: P1:5'- agaattcgccccgctgtacac-3' and P2: 5'-taagcttggcagcgtctccaagga-3'. The fragments were amplified separately, in reaction volume of 50 μ L using 0.25 μ M primers, 200 μ M dNTP, 1X PCR buffer with 1.5 mM MgCl₂ and 1 U Taq polymerase. Formamide (1 μ L/50 μ L) was added in the reaction mixture of apo E gene amplification as a procedure modification resulting in a higher quantity of specific PCR product with a very sharp and bright band on the gel photography.

Each reaction mixture was subjected to 30 cycles of 30 s at 95 °C, 30 s at 60 °C and 60 s at 72 °C, initial denaturation for 5 min at 95 °C, final extension for 5 min at 72 °C. PCR products (198 bp of MTHFR gene, and 244 bp of apo E gene) were checked electrophoretically in a Clearose BG-ET gel (Elchrom Scientific AG, Basel, Switzerland) using SEA 2000 apparatus (Guest Elchrom Scientific AG, Basel, Switzerland) in 30 mM Tris-acetic-EDTA (TAE) buffer. Negative quality controls generated by inclusion of all reagents except for DNA and positive quality control containing a known genotype were included in every run. Positive quality control containing a known apo E genotype was obtained by the courtesy of Dallinga-Thie GM, Department of Medicine and Endocrinology, Utrecht, The Netherlands.

The specific MTHFR and apo E amplified products were digested using Hinf I (G↓AnTC) and Cfo I (GCG↓C) (Roche Diagnostics, Mannheim, Germany), respectively. Upon digestion, the MTHFR cleaved products were submitted to electrophoresis on 12% PolyNAT gel (Guest Elchrom Scientific AG, Basel, Switzerland), while the apo E cleaved products were analyzed on Sprex EL 400 gel (Guest Elchrom Scientific AG, Basel, Switzerland). Both gels were stained for 30 min by SYBER Green I day (Molecular Probes, Leiden, The Netherlands) and destained overnight by distilled water. Restriction fragments were visualized under UV transillumination and photographs were obtained. MTHFR and apo E genotypes were detected on the basis of restriction fragment length and unique genotype-dependent band combination.

Plasma Hcy determination

Total plasma Hcy was measured by the enzyme immunoassay method (Axis-Shield AS, Oslo, Norway) described by Frantzen *et al.*²⁶. The reference range of Hcy level was 5–15 μmol/L.

Plasma vitamin B12 and folate determinations

Vitamin B12 and folate were determined by the chemiluminescence method using test packages (Bayer Corp., NY, USA) for the Bayer ACS:180 Plus autoanalyzer. The reference values of plasma vitamin B12 and folate were 148–664 pmol/L and 2.5–45.4 nmol/L, respectively.

Plasma lipid determinations

Total cholesterol, HDL cholesterol, and triglycerides were measured by standard methods using available tests (Olympus Diagnostica GmbH, Clare, Ireland) for the Olympus AU 600 autoanalyzer. Total cholesterol and triglycerides were detected spectrophotometrically, whereas HDL cholesterol was analyzed by a combination of spectrophotometric and immunoinhibition method. LDL cholesterol levels were calculated using Friedewald equation, $LDL\text{-cholesterol} = (\text{total cholesterol}/2.18 - \text{HDL cholesterol})$.

Statistical analysis

To analyze Hcy, vitamin B12, folate and lipid differences, comparisons between groups (classified according to genotype and two study groups) were carried out with Mann-Whitney test. The χ^2 -test was used to compare C677T MTHFR and apo E allele and genotype frequencies as well as distribution in value-dependent (Hcy, vitamins and lipids) groups between patients and controls.

The association between homocystein levels (independent variable) and CS (dependent variable) was examined by means of a multiple logistic regression model, with adjustment for age, sex, and smoking as conventional vascular risk factors. Results were expressed as odds ratio (OR) together with their 95% confidence interval (CI). Statistical significance was taken as $p < 0.05$.

The statistical programs used were SigmaStat (version 2.0, Jandel Corporation, Chicago, IL, USA) for Mann-

Whitney, χ^2 -test and multiple logistic regression analysis, and a program found on Internet for OR calculation²⁷.

Results

C677T MTHFR and apo E genotype analysis

The frequencies of C677T MTHFR and apo E genotypes in controls and patients with >70% carotid stenosis (CS) are presented in Figure 1.

The frequencies of MTHFR genotypes (CC/TC/TT) and alleles (C/T) were 45/50/5 and 70/30 in the control group, and 47/45/8 and 70/30 in the patient group, respectively. C677T MTHFR genotypes were in Hardy-Weinberg equilibrium (HWE) for CS patients ($p=0.972$) and controls ($p=0.178$). There was no statistically significant difference between the study groups in the distribution of C677T genotypes ($p=0.786$) and alleles ($p=0.904$).

All three common alleles of apo E were detected, whereas apo E 2/2 and 4/4 genotypes were not found among study subjects. The frequencies of apo E genotypes (3/3, 3/4, 2/3 and 2/4) and alleles (2/3/4) were 80/8/9/3 and 6/88/6 and in the control group, and 50/26/21/3 and 12/74/14 in the patient group, respectively. Apo

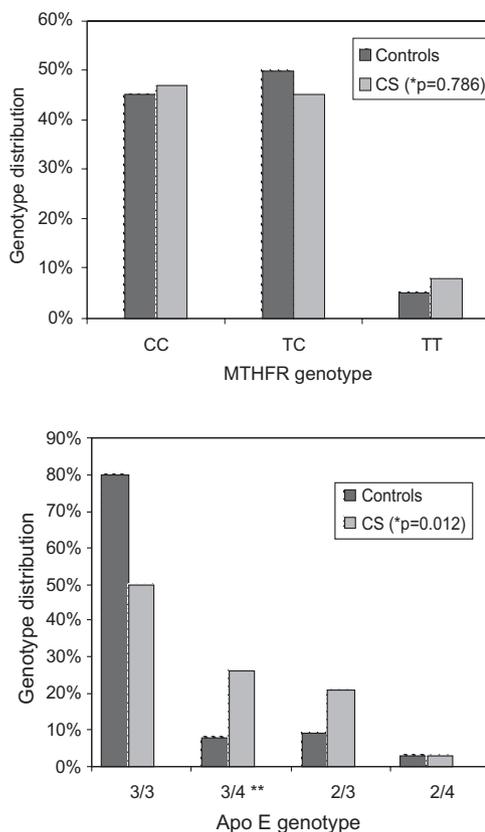


Fig. 1. MTHFR (upper panel) and apo E (down panel) genotype distribution in control subjects (N=60) and carotid stenosis patients (CS, N=38). *p-value (χ^2 -test) for differences among study group genotype frequencies, **OR (odds ratio) of apo E 3/4 genotype was 3.93 (95% CI 1.23–12.61), CI – confidence interval.

TABLE 1
 HOMOCYSTEINE, VITAMIN B12, FOLATE AND LIPIDS IN CONTROL SUBJECTS AND CAROTID STENOSIS PATIENTS WITH DIFFERENT MTHFR GENOTYPES, AND DISTRIBUTION OF STUDY SUBJECTS ACCORDING TO PARAMETER REFERENCE RANGES

	Controls (N=60), CC genotype (N=27), *TC/TT genotype (N=33) median (range)	**CS (N=38), CC genotype (N=18), *TC/TT genotype (N=20) median (range)	p-value
Homocysteine (µmol/L)			
CC MTHFR	8.75 (0.60–15.20)	8.00 (1.30–19.90)	ns
TC/TT MTHFR	8.60 (3.00–18.40)	7.35 (2.90–15.90)	ns
All genotypes	8.70 (0.60–18.40)	7.50 (1.30–19.90)	ns
≥15.00 µmol/L, N (%)	3 (5)	4 (11)	ns
<15.00 µmol/L, N (%)	57 (95)	34 (89)	
Vitamin B12 (pmol/L)			
CC MTHFR	406.53 (75.99–1011.52)	324.63 (57.55–799.04)	ns
TC/TT MTHFR	472.19 (127.64–1274.92)	241.63 (15.49–602.68)	0.030
All genotypes	418.33 (75.99–1274.92)	287.00 (15.49–799.04)	0.044
<148.00 pmol/L, N (%)	3 (5)	9 (23.7)	0.021
148.00–664.00 pmol/L, N (%)	51 (85)	25 (65.8)	
>664.00 pmol/L, N (%)	6 (10)	4 (10.5)	
Folate (nmol/L)			
CC MTHFR	7.37 (0.23–46.08)	11.46 (0.23–46.31)	ns
TC/TT MTHFR	2.50 (0.23–41.09)	19.07 (0.23–43.36)	0.032
All genotypes	3.18 (0.23–46.08)	11.46 (0.23–46.31)	0.036
***<2.5nmol/L, N (%)	26 (43.3)	8 (21)	0.041
≥2.5 nmol/L, N (%)	34 (56.7)	30 (79)	
Total cholesterol (mmol/L)			
CC MTHFR	3.78 (1.98–7.04)	5.92 (3.44–9.94)	<0.001
TC/TT MTHFR	3.36 (2.54–8.82)	5.62 (4.24–8.24)	<0.001
All genotypes	3.70 (1.98–8.82)	5.89 (2.06–12.00)	<0.001
LDL cholesterol (mmol/L)			
CC MTHFR	2.00 (1.00–4.60)	3.60 (0.85–7.20)	0.003
TC/TT MTHFR	2.30 (1.00–6.90)	2.92 (0.75–9.60)	ns
All genotypes	2.20 (1.00–6.90)	3.50 (0.75–9.60)	<0.001
HDL cholesterol (mmol/L)			
CC MTHFR	1.04 (0.49–1.86)	0.99 (0.67–1.71)	ns
TC/TT MTHFR	0.93 (0.62–1.59)	0.92 (0.68–8.24)	ns
All genotypes	0.95 (0.49–1.86)	0.97 (0.67–1.71)	ns
Triglycerides (mmol/L)			
CC MTHFR	1.08 (0.38–6.01)	1.38 (0.65–8.67)	ns
TC/TT MTHFR	1.32 (0.39–3.43)	1.74 (0.85–3.90)	0.003
All genotypes	1.32 (0.38–6.01)	1.70 (0.65–8.67)	<0.01

Statistical analysis: χ^2 -test for differences among value-dependent (homocysteine, vitamin B12 and folate) groups, and Mann-Whitney test for differences among genotype-dependent groups.

* For statistical analysis TC and TT MTHFR genotypes were pooled because of the small number of subjects with TT genotype.

** CS – carotid stenosis.

*** Subjects were divided into two groups because only two subjects in control group and one subject in patient group with folate higher than upper (45.4 nmol/L) level of reference range were found

E genotypes were also in HWE for CS patients ($p=0.447$) and controls ($p=0.920$). There was a statistically significant difference between CS patients and controls in the distribution of apo E genotypes ($p=0.012$) and alleles

($p=0.029$). The odds ratio (OR) was 3.93 (95% CI 1.23–12.61) for apo E 3/4 genotype, 2.93 (95% CI 0.88–9.77) for apo E 2/3 genotype and 0.78 (95% CI 0.07–8.95) for apo E 2/4 genotype.

TABLE 2
MULTIPLE LOGISTIC REGRESSION ANALYSIS OF RISK FACTORS AND HOMOCYSTEIN LEVELS IN ASSOCIATION WITH CAROTIDE STENOSIS

	β^*	SE**	OR (95%CI)***	p-value
Homocystein	0.877	0.907	2.403 (0.406–14.217)	0.334
Smoking	-0.684	0.475	0.505 (0.199–1.279)	0.149
Sex	0.711	0.447	2.037 (0.848–4.896)	0.112
Age	0.046	0.027	1.048 (0.993–1.105)	0.087

* β – estimated coefficient, **SE – standard error; ***OR – adjusted odds ratio with 95% CI, confidence interval

C677T MTHFR genotype and levels of Hcy, vitamin B12, folate and lipids in control subjects and CS patients

Table 1 shows the distribution of Hcy, vitamin B12, folate and lipid values (median and range) among control subjects and CS patients grouped according to MTHFR genotype.

For statistical analysis, heterozygous, TC, and homozygous, TT genotypes were pooled because of the small number of subjects with TT genotype (Figure 1; only 5% of TT genotype in control and 8% in patient group were found, respectively).

There were no statistically significant differences Hcy and HDL cholesterol among study groups. Triglycerides, vitamin B12 and folate were statistically significantly different in the »all genotypes« group ($p < 0.01$, $p = 0.044$ and $p = 0.036$, respectively), and combined (TC/TT) genotype group ($p = 0.003$, $p = 0.030$ and $p = 0.032$, respectively), but not in the group of CC MTHFR genotype. Differences in total cholesterol concentrations reached statistical significance in all three groups ($p < 0.001$), whereas LDL cholesterol did not in the TC/TT group.

Statistical analysis allowed for the results obtained to summarize. The CS patient group with TC/TT genotype showed statistically different vitamin B12, folate, total cholesterol and triglyceride levels in comparison with control subjects with the same genotype. On comparison, only total cholesterol and LDL cholesterol reached statistical difference between CS patients with CC MTHFR genotype and controls with the same genotype. Comparison of Hcy, vitamin B12, folate and lipids irrespective of MTHFR and apo E genotype, between patients and controls yielded statistically significant differences for vitamin B12, folate, total cholesterol, LDL cholesterol and triglycerides, but not for HDL cholesterol. All these values showed higher median in patients than in control subjects, with the exception of Hcy and vitamin B12.

Value-dependent Hcy groups were not statistically different (Table 1), however, hyperhomocysteinemia (≥ 15 $\mu\text{mol/L}$) was found in 11% of patients and 5% of controls. Distribution frequencies of vitamin B12 value-dependent groups showed statistical difference ($p = 0.021$) with 24% of patients and 5% of controls under the lower normal value. Only two-folate value-dependent groups were considered because of the low frequency of patients and controls (two subjects in control and one subject in patient

group) with folate higher than upper (45.5 nmol/L) level of reference range were found. Distributions of these groups showed statistical difference ($p = 0.041$).

The findings from the univariate analysis were further investigated in a multiple logistic model with inclusion of age (in years), sex and smoking habit as a conventional vascular risk factors (Table 2).

The results of multiple logistic analysis showed that there was no statistical significance of homocystein levels (OR 2.403, $p = 0.334$) or conventional vascular risk factors such as smoking habit (OR 0.505, $p = 0.149$), age (OR 1.048, $p = 0.087$) or sex (OR 2.037, $p = 0.112$) in predicting CS.

Apo E genotype and levels of lipids in control subjects and CS patients

Table 3 shows the results of lipid analysis in relation to apo E genotype.

Comparison between the control and CS patient groups classified according to genotype yielded statistical differences for some measured lipids ($p < 0.05$). The levels of total cholesterol, LDL cholesterol and triglycerides in the apo E 3/3 genotype group yielded statistical difference. In the group of the apo E 3/4 genotype statistically significant result was found only for total cholesterol. Levels of all measured lipids were not different in the group of apo E 2/3 genotype. Statistical analysis was not performed in the group of apo E 2/4 genotype group because only one CS patient had this genotype.

Discussion

In the present study, the association of MTHFR genotype with plasma Hcy, vitamin B12, folate and lipids as well as between apo E genotype and lipids was investigated in the groups of Croatian healthy subjects and patients with significant ($> 70\%$) carotid artery stenosis. The frequency of C677T MTHFR genotype in study subjects is presented in Figure 1, showing the genotype distribution to be the same/very similar as previously published^{7,28}, although the present study included a smaller number of subjects. We found no association between CS and MTHFR genotype. Literature data show controversial results concerning the association of MTHFR genotype and carotid artery atherosclerosis, ischemic cerebrovascular disease (ICVD) and stroke^{7–10,29}.

TABLE 3
LIPID LEVELS IN CONTROL SUBJECTS AND CAROTID STENOSIS PATIENTS WITH DIFFERENT APO E GENOTYPES

	Control (N=60); Genotypes: 3/3 (N=48), 3/4 (N=5), 2/3 (N=5) and 2/4 (N=2); (median (range))	*CS (N=38); Genotypes 3/3 (N=19), 3/4 (N=10), 2/3 (N=8) and 2/4 (N=1); (median (range))	**p-value
Total cholesterol (mmol/L)			
3/3	4.04 (1.98–8.82)	6.09 (3.44–9.94)	<0.001
3/4	3.29 (2.96–5.59)	6.50 (3.78–8.36)	0.003
2/3	3.42 (3.10–3.88)	4.08 (2.06–12.00)	ns
2/4	5.14 (5.07–5.20)	5.07	–
LDL cholesterol (mmol/L)			
3/3	2.30 (1.00–6.90)	3.70 (1.22–7.20)	0.007
3/4	1.80 (1.20–3.80)	3.50 (0.75–5.70)	ns
2/3	1.85 (1.00–2.50)	2.40 (0.90–9.60)	ns
2/4	2.53 (2.45–2.60)	2.60	–
HDL cholesterol (mmol/L)			
3/3	1.04 (0.62–1.86)	1.03 (0.67–1.71)	ns
3/4	0.92 (0.49–1.33)	0.94 (0.80–1.51)	ns
2/3	1.04 (0.95–1.11)	0.96 (0.71–1.41)	ns
2/4	1.07 (0.93–1.20)	0.93	–
Triglycerides (mmol/L)			
3/3	1.44 (0.38–3.43)	2.00 (0.77–5.00)	0.035
3/4	1.27 (0.45–6.01)	2.21 (0.82–8.67)	ns
2/3	1.10 (0.70–2.35)	1.44 (0.75–4.00)	ns
2/4	3.40 (3.35–3.45)	3.45	–

*CS – carotid stenosis, **Mann-Whitney test

Comparison of plasma Hcy level between the patients and control group matched for sex, age and place of residence did not show elevated Hcy levels in the CS group (Table 1). The Hcy median was within the normal range in both groups. However, hyperhomocysteinemia (≥ 15.00 $\mu\text{mol/L}$) was detected in 5% of controls and in 11% of patients (Table 1), but did not reach statistical difference. Epidemiologic data show that hyperhomocysteinemia is an independent risk factor for atherosclerosis in general^{2,3} and carotid atherosclerosis in particular^{29,30}, however, there also is a study report that failed to demonstrate the association between Hcy and CS⁹.

Vitamin B12 and folate among controls and CS patients (Table 1) were statistically different in general (group of »all genotypes«) and in the group of TC/TT MTHFR subjects, however, the median of all values was within the normal range. Surprisingly, in the group of patients with TC/TT MTHFR genotype, lower vitamin B12 and higher folate values were recorded in comparison to the controls. Additionally, vitamin B12 concentration below the lowest normal value was found in 24% of patients and only 5% of controls, while there were 79% of patients and 57% of controls with folate over the lowest normal value (Table 1).

The finding of lower vitamin B12 and higher folate concentrations in patients as compared with controls is apparently paradoxical, since neither group of subjects received folate supplementation. It seems that this find-

ing could be explained by the linked metabolism of folic acid and vitamin B12, and by the reaction that transfers methyl group from N⁵-methyltetrahydrofolate to cobalamin³⁰. Namely, in case of cobalamin deficiency, folate is »trapped« as N⁵-methyltetrahydrofolate. It is »metabolically dead« in the absence of vitamin B12 and cannot be recycled as tetrahydrofolate back into the folate pool.

The collected questionnaires of CS patients showed that 80% were regular smokers and alcohol consumers, 70% had elevated blood pressure, and 30% had insulin dependent diabetes mellitus (IDDM). Although a complex disease background of our patient group would predispose to elevated Hcy levels, they were not recorded in our study. In contrast to our results, other authors observed positive Hcy association with alcohol and caffeine intake, smoking, IDDM as well as with hormonal changes, renal failure, cancer, and some drugs^{3,31}. Additionally, Bo *et al.* conclude that a high level of alcohol intake plays a role as an independent risk factor in carotid atherogenesis. Alcohol intake is independent from other risk factors such as arterial hypertension, dyslipidemia, diabetes mellitus, smoking, social status and family history of cardio-cerebrovascular disease³².

Selhub *et al.* showed that Hcy exhibited strong inverse association with folate and weaker association with vitamin B12 in extracranial carotid artery stenosis in the elderly³³. Streifler *et al.* conclude that hyperhomocys-

teinemia and human platelet antigen (HPA)-1 a/b polymorphism are independent risk factors for ischemic events in patients with significant CS ($\geq 50\%$)³⁴.

In contrast, Kostulas *et al.* report that C677T gene polymorphism did not exert any major influence on the risk of developing ICVD or internal carotid artery (ICA) stenosis, and did not cause Hcy level increase as observed in ICA stenosis. Also, it was shown that MTHFR genotype did not correlate with the levels of either Hcy, folic acid or vitamin B12, and did not affect Hcy levels, even in the presence of low blood folate⁹.

The negative results of multiple logistic regression analysis of homocystein levels, smoking habit, age and sex in predicting CS is almost expected because the Hcy median was within the normal range in both groups. There is substantial problem of choosing so called control or healthy group of patients in genetic study. The reason is because there is no warranty that any of age and sex match control subject will get illness for a few years, so in the moment of testing have hyperhomocysteinaemia showing false positive values in control group.

The statistically significant difference in the distribution of apo E genotypes ($p=0.012$) and alleles ($p=0.029$) between CS cases and controls indicated the association between apo E polymorphism and CS. Additionally, OR was 3.93 (95% CI 1.23–12.61) for apo E 3/4 genotype. The association of apo E with CS ($>50\%$) has been previously proposed¹⁰, and was now confirmed and additionally established in severe stenosis ($>70\%$) by the present study. In contrast, Catto *et al.* find no relationship between apo E genotype and cerebrovascular disease³⁵. Also, Frikke-Schmidt *et al.* showed that apo E polymorphism is a risk factor for Alzheimer's disease and other dementia independent of lipid and lipoprotein levels but does not affect the risk of ICVD³⁶. Additionally, no association of apo E polymorphism and carotid atherosclerosis was reported by others^{15,17,18}, however, there also are reports that confirm the existence of such an association^{16,19,20}.

Concerning the results on the association of apo E and MTHFR genetic polymorphism with blood lipids in patients with severe CS, we observed some gene effects on the lipids in the study population. To our knowledge, there are little or no data on such associations in patients with severe SC. Our results demonstrated the association of triglycerides with TC/TT MTHFR ($p=0.003$) but not with wild-type MTHFR genotype. HDL cholesterol was not associated with either wild-type or mutant genotypes, while total cholesterol was associated with both groups of genotypes. LDL cholesterol was statistically different only

for CC MTHFR genotype. It has been reported that fasting and postprandial triglyceride-rich lipoproteins (but not LDLs) are elevated in patients with $\geq 50\%$ CS as compared with controls and particularly identify echolucent, rupture-prone carotid plaques (37). The study of Yatsu *et al.* showed that in Caucasians, carotid artery stenosis was associated with increased plasma total cholesterol and LDL levels and an atherogenic profile but not with Sac I polymorphism for apoprotein AI³⁸.

Comparison between the control and CS patient groups classified according to genotype yielded statistical differences for some measured lipids as it was shown in Table 3. In the group of the apo E 3/4 genotype statistically significant result was found only for total cholesterol, but in the group of the apo E 3/3 genotype total cholesterol, LDL cholesterol and triglycerides were significant. Levels of all measured lipids were not different in the group of apo E 2/3 genotype. Some studies on carotid atherosclerosis showed that total cholesterol and LDL cholesterol were lower in E2 than in E3 and E4 allele carriers^{16,19}, whereas lower HDL cholesterol and higher LDL cholesterol were found in the apo E4 group of subjects³⁹. Similar results, the association of apo E genotypes with lipids, were obtained for the group of coronary heart disease in middle-aged women⁴⁰.

Our study indicated an association of apo E 3/4 genotype but not of MTHFR genotype with the risk of CS. Also, the MTHFR and apo E genotypes were shown to affect blood lipid levels, which was statistically confirmed. This effect was demonstrated through medians of all values (the HDL cholesterol values of controls and CS patients were very close) that were higher in the CS group compared to control subjects with the same genotype. Study results showed an association of hyperhomocysteinemia with heterozygous/mutant MTHFR genotype in the CS group, although not statistically confirmed. Also, the CS group vitamin status was found to be associated with heterozygous/mutant MTHFR genotype, which was confirmed statistically, in comparison to controls with the same genotype. Namely, in the group of patients with TC/TT MTHFR genotype, lower vitamin B12 and higher folate values were recorded in comparison to the controls. Our findings are consistent with the concept according to which neither genes nor the environment but their interactions are responsible for the etiopathogenesis of complex diseases such as vascular disease. This preliminary study of the association between MTHFR genotype and Hcy, vitamin and lipid levels, and between apo E genotype and lipids revealed that there were associations that could be clarified and confirmed in further studies.

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POVEZANOST GENOTIPOVA METILENTETRAHIDROFOLAT REDUKTAZE (MTHFR) I APOLIPOPROTEINA E (APO E) S RAZINOM HOMOCISTEINA, VITAMINA I LIPIDA U ISPITANIKAMA SA STENOZOM KAROTIDA

SAŽETAK

Cilj istraživanja bio je ispitivanje povezanosti genotipova metilentetrahidrofolat reduktaze (MTHFR) s razinom homocisteina (Hcy), folata, vitamina B12 i lipida kao i ispitivanje povezanosti genotipova apolipoproteina E (apo E) s razinom lipida u zdravoj hrvatskoj kontrolnoj skupini i skupini ispitanika s >70% stenozom karotida (CS). U studiju je bilo uključeno 98 Hrvata, 38 ispitanika s >70% stenozom karotida i 60 kontrolnih uzoraka usklađenih po dobi i spolu. Genotipovi MTHFR i apo E određeni su metodom lančane reakcije polimerazom-polimorfizmom duljine restrikcijskih fragmenata (PCR-RFLP), Hcy enzimskim imunotestom, vitamini imunokemiluminiscencijom, i lipidi spektrofotometrijski. Statistički značajna razlika između kontrola i ispitanika s CS nije nađena u distribuciji C677T MTHFR genotipova ($p=0.786$) i alela ($p=0.904$), dok je učestalost apo E genotipova ($p=0.012$) i alela ($p=0.029$) statistički značajna. Faktor rizika (eng. odds ratio, OR) za apo E 3/4 genotip iznosio je 3.93 (95% CI 1.23–12.61). Hiperhomocisteinemija ($\geq 15 \mu\text{mol/L}$) nađena je u 11% CS ispitanika i 5% kontrola. Ukupni kolesterol, trigliceridi, vitamin B12 i folati statistički su značajni u skupinama »svi MTHFR genotipovi« ($p<0.001$, $p<0.01$, $p=0.044$ i $p=0.036$), i TC/TT genotipovi ($p<0.001$, $p=0.003$, $p=0.030$ i $p=0.032$). Statistički značajna razlika dobivena je i za ukupni kolesterol, LDL kolesterol i trigliceride u skupini s apo E 3/3 genotipom, i za ukupni kolesterol u skupini s apo E 3/4 genotipom. Nađena je povezanost apo E 3/4 genotipa, ali ne i MTHFR genotipova s rizikom od CS. Statističkom značajnošću potvrđen je utjecaj MTHFR i apo E na razinu lipida u krvi. Također je zabilježena povezanost hiperhomocisteinemije i ispitanika s CS. Statistički je potvrđena povezanost vitaminskog statusa s TC/TT MTHFR genotipom. U skupini ispitanika s TC/TT genotipom nađena je niža razina vitamina B12 i viša razina folata. Rezultati multiple logističke analize pokazali su da u predskazivanju pojavnosti CS nema statističke značajnosti ni razina Hcy (OR 2.403, $p=0.334$) niti konvencionalni vaskularni faktori rizika, kao što su pušenje (OR 0.505, $p=0.149$), godine (OR 1.048, $p=0.087$) ili spol (OR 2.037, $p=0.112$).

Prevalence of Human Papillomavirus Genotypes in Cervical Cancer and Precursor Lesions

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ABSTRACT

There are no data obtained in biopsy material on the prevalence of human papillomavirus (HPV) and HPV genotypes in Croatian women with cervical carcinoma and precursor lesions. Therefore, the prevalence of HPV and HPV genotypes was investigated in archival material of cervical carcinoma and precursor lesions kept at Department of Pathology, School of Medicine, University of Rijeka. DNA was isolated from formalin fixed, paraffin embedded tissue, histologically classified as cervical intraepithelial neoplasia (CIN) III (n=43), squamous cell carcinoma (SCC) (n=54) and adenocarcinoma (ADC) (n=40). HPV testing was performed by polymerase chain reaction (PCR) using generic and genotype specific primers. The prevalence of HPV DNA was 93.02%, 92.59%, and 92.5% in CIN III, SCC and ADC, respectively. In CIN III and SCC, HPV-16 was the most common high-risk genotype, identified in 65% and 52%, followed by HPV-18 in 22.5% and 28% of cases, respectively. HPV-18 showed a statistically significant prevalence in ADC (67.6%) as compared with SCC ($\chi^2=9.924$; $p \leq 0.01$). Study results revealed a high prevalence of HPV-DNA in examined cervical lesions (>90%). HPV-16 predominated in SCC and HPV-18 in ADC. Single infection was more frequently present than multiple infections in all three histological groups.

Key words: cervical intraepithelial neoplasia, cervical carcinoma, HPV genotype prevalence

Introduction

Approximately 510,000 cases of cervical cancer are reported each year, with nearly 80% in developing countries^{1,2}. Epidemiological studies indicate a strong association of high-risk human papillomavirus (HPV) genotypes with cervical carcinoma and malignant transformation of cervical epithelial cells³. Recent studies have shown that more than 90% of cervical cancers contain HPV DNA, and some are even suggesting that there is no cervical cancer without HPV infection⁴⁻⁷. More than 50 HPV genotypes are known to infect female genital tract, and a subset of ~12 of these are known to have a strong oncogenic potential⁸. Therefore, HPV genotypes have been classified according to their association with cervical cancer and precursor lesions into high-risk (oncogenic) and low-risk HPV genotypes⁹. The most prevalent high-risk HPV genotypes worldwide, which infect uterine cervix, are HPV-16 (~53%), followed by HPV-18 (~15%), HPV-45 (~9%), HPV-31 (~6%) and HPV-33 (~3%)¹⁰.

According to the Croatian National Cancer Registry¹¹, the incidence of cervical cancer in 2002 was 15.9

new cases per 100,000 women, yet there are only a few reports describing HPV genotype distribution in abnormal cervical PAP smears in Croatia¹²⁻¹⁴. There are no data obtained in biopsy material on the prevalence of HPV and HPV genotypes in Croatian women with cervical carcinoma and precursor lesions. Therefore we embarked upon this retrospective analysis to assess the distribution of HPV genotypes in the archival material kept at Department of Pathology, School of Medicine, University of Rijeka. Archival (cervical intraepithelial neoplasia) CIN III and cervical carcinomas were analyzed by polymerase chain reaction (PCR) for the presence of HPV DNA and distribution of HPV genotypes.

Materials and Methods

A total of 137 formalin fixed, paraffin embedded samples were chosen on the basis of availability of tissue in the pathology archive. There were 43 CIN III, 54 squamous cell carcinoma (SCC) and 40 cervical adenocar-

cinoma (ADC) (including 15 adenocarcinoma *in situ* (AIS) specimens obtained from patients treated at Department of Gynecology and Obstetrics, Rijeka University Hospital Center, during the 1995–2005 period. The samples were histopathologically examined by an expert pathologist at Department of Pathology, School of Medicine, University of Rijeka. The mean age of patients with CIN III diagnosis was 31.5, range 24–50 years. In SCC group there were 21 patients with microinvasive carcinoma (MICA). The mean age of patients with MICA and invasive SCC was 40 (range 30–48) and 46 (range 34–67) years, respectively. The mean age of patients with AIS and invasive ADC was 39 (range 26–53) and 45 (range 43–67) years, respectively.

DNA extraction

Total DNA was isolated from formalin fixed, paraffin embedded samples. Great care was taken on sample sectioning to avoid any contamination between the samples. Depending on the amount of biopsy material embedded in paraffin, 4–10 sections (5 µm thick) were placed in a microcentrifuge tube. The sections were deparaffinized by adding 1 mL of xylene and heating at 55 °C for 30 minutes, followed by centrifugation and subsequent removal of the supernatant. Upon dewaxing with three washes of xylene, 1 mL of 100% ethanol was added to remove residual xylene. The tissues were dried at 37 °C for 30 minutes and DNA was isolated using NucleoSpin®Tissue kit (Macherey-Nagel, Duren, Germany) according to the manufacturer's instructions.

PCR analysis

To assess the quality of extracted DNA, β-globin PCRs were performed using four primer combinations spanning 110, 250, 345 and 408 bp (Takara Biomedicals, Japan). Primers targeting highly conserved regions within the L1 and E6/E7 open reading frame (ORF) were used to detect HPV DNA. These included the GP5⁺/GP6⁺¹⁵ and SPF 10 primers (INNO-LiPA Genotyping, Innogenetics N.V., Ghent, Belgium) of the L1 ORF and primers from Human Papillomavirus Typing Set (Takara Biomedicals, Japan), which amplify sequences within E6 and E7 ORF. The HPV types in positive samples were further characterized by using hybridization assay for identification of HPV genotypes (INNO-LiPA Genotyping, Innogenetics N.V., Ghent, Belgium) and type specific PCR amplifying sequences of HPV-16, 18, and 33 within E6 and E7 ORF (Human Papillomavirus Detection Set, Takara Biomedicals, Japan).

Statistical analysis

HPV prevalence was expressed as percentage of all cases tested for HPV in different histological groups (accounted only once). When determining the prevalence of high- and low-risk HPV genotypes, women were counted more than once if they harbored multiple infections with a mixture of both. The prevalence of individual HPV genotypes was determined as they appeared as either single or multiple infections. Multiple high-risk HPV infection

was defined as two or more high-risk HPV genotypes with or without additional low-risk HPV genotypes. The χ^2 test was used to assess statistical significance of differences in the prevalence and distribution of HPV genotypes, and to examine the relationship between multiple and single HPV infection with different histological types of cervical cancer. Statistical significance was established at the $p < 0.05$ level.

Results

Of 137 study specimens, 127 (91.97%) were positive for HPV DNA. HPV prevalence according to histological groups is presented in Table 1 and overall high-risk HPV prevalence in Table 2. The prevalence of HPV infection in CIN III, SCC and ADC was 93.02% (40/43), 92.59% (50/54) and 92.5% (37/40), respectively. In total, 10 differ-

TABLE 1
HPV DISTRIBUTION ACCORDING TO HISTOLOGICAL GROUPS

HPV	Histological group		
	CIN III	SCC	ADC
HPV neg.	3 (6.9)	4 (7.4)	3 (7.5)
6/11	–	–	1 (2.5)
16	17 (39.5)	22 (40.7)	3 (7.5)
18	3 (6.9)	6 (11.1)	20 (50)
31	2 (4.6)	3 (5.5)	1 (2.5)
33	–	–	1 (2.5)
45	–	1 (1.8)	–
52	–	1 (1.8)	–
6/11+16	4 (9.3)	–	–
16+18	4 (9.3)	2 (3.7)	1 (2.5)
16+31	–	–	1 (2.5)
18+31	–	3 (5.5)	–
18+33	2 (4.6)	–	–
31+33	–	4 (7.4)	–
31+52	–	1 (1.8)	–
31+70	1 (2.3)	–	–
45+68	1 (2.3)	–	–
6/11+16+18	–	2 (3.7)	4 (10)
6/11+18+31	–	1 (1.8)	–
16+31+33	–	–	1 (2.5)
16+31+52	1 (2.3)	–	–
31+52+74	–	1 (1.8)	–
x	5 (11.6)	3 (5.5)	4 (10)
Total	43	54	40
		137	
HPV pos. (%)	93.02	92.59	92.5

Values are expressed as number of samples and percentage in parentheses, CIN – cervical intraepithelial neoplasia, SCC – squamous cell carcinoma, ADC – adenocarcinoma, HPV neg. – all samples without positive HPV DNA test result, HPV pos. – all samples with positive HPV DNA test result.

TABLE 2
OVERALL PREVALENCE OF HPV GENOTYPES IN HPV DNA POSITIVE SAMPLES.

Sample type (N)	HPV pos (N)	Overall distribution of HPV types										
		6/11	16	18	31	33	45	52	68	70	74	x
CIN III (43)	40	4 (10)	26 (65)	9 (22.5)	4 (10)	2 (5)	1 (2.5)	1 (2.5)	1 (2.5)	1 (2.5)	0 (0)	5 (12.5)
SCC (54)	50	3 (6)	26 (52)	14 (28)	13 (26)	4 (8)	1 (2)	3 (6)	0 (0)	0 (0)	1 (2)	3 (6)
ADC (40)	37	5 (13.5)	10 (27)	25 (67.6)	3 (8.1)	2 (5.4)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	4 (10.8)
Total (137)	127	12 (9)	62 (49)	48(38)	20 (16)	8 (6)	2 (2)	4 (3)	1 (1)	1 (1)	1 (1)	12 (9)

Because of the existence of multiple HPV infections, women were counted more than once where appropriate, values are number of samples and percentage in parentheses. CIN – cervical intraepithelial neoplasia, SCC – squamous cell carcinoma, ADC –adenocarcinoma, HPV pos. – all samples with positive HPV DNA test result.

ent oncogenic HPV genotypes were identified: 9 in CIN III, 8 in SCC and 5 in ADC. High-risk HPV genotypes that were most prevalent in all three groups accounted for 87.5% (35/40) of all HPV positive cases of CIN III, 90.0% (47/50) of SCC and 86.4% (32/37) of ADC. Low-risk HPVs were detected as part of mixed low/high-risk HPV infections, except for one ADC case. A great diversity of high-risk HPV genotypes was detected in the group of squamous cell lesions, whereas only 4 different HPV genotypes and HPV-X were identified in the ADC group (Table 1). Overall, HPV-16 was the predominant genotype detected in 26/40 (65%) and 26/50 (52.0%) CIN III and SCC HPV positive cases, respectively. The ADC group showed a predominance of HPV-18 genotype, recorded in 25/37 (67.5%) of all HPV ADC positive cases (Table 2). The observed predominance of HPV-16 in CIN III ($\chi^2=13.923$, $p<0.001$) and SCC ($\chi^2=5.717$, $p<0.025$), as well as HPV-18 in ADC ($\chi^2=11.428$, $p<0.001$) was statistically significant (Table 3).

In CIN III group, 77.5% (31/40) of the samples were positive for a single high-risk HPV genotype and 22.5% (9/40) for multiple high-risk HPV genotypes (Figure 1). The overall prevalence of high-risk HPV genotypes found in CIN III in descending order was as follows: HPV-16 (65%), HPV-18 (22.5%), HPV-X (12.5%), HPV-31 (10%), HPV-33 (4.6%), followed by HPV-45, HPV-52, HPV-68 and HPV-70 (Table 2). Single infection was present in 72% and multiple infections in 28% of SCC cases (Figure 1). Considering overall prevalence of high-risk HPV genotypes in the SCC samples, the predominant genotypes

were HPV-16 (52%), HPV-18 (28%), HPV-31 (26%) and HPV-33 (8%), followed by HPV-52, HPV-X, HPV-45 and HPV-74. In the ADC group, 81% (29/36) of the samples were positive for a single high-risk and 19% (7/36) for multiple high-risk HPV genotypes (Figure 1). HPV-18 as the predominant high-risk HPV genotype (67.6%) in the ADC group was followed by HPV-16 (27%), HPV-X (10.8%), HPV-31 (8.1%) and HPV-33 (5.4%) (Table 2). The difference in the predominant HPV-16 genotype distribution in CIN III and SCC *versus* HPV-18 in ADC ($\chi^2=16.121$, $p<0.001$) was statistically significant, and so was the difference yielded by comparison between SCC and ADC ($\chi^2=9.924$, $p<0.01$) samples.

Multiple high-risk HPV infections were found in all three groups without a statistically significant association with the histological diagnosis (Figure 1, Table 1).

TABLE 3
DISTRIBUTION DIFFERENCE OF HIGH-RISK HPV GENOTYPES 16 AND 18 IN CERVICAL CANCER AND CIN III.

Diagnosis	n	Pos.	Neg.	HPV-16 +ve (n)	HPV-18 +ve (n)	χ^2 -test	p
CIN III	43	40	3	26	9	13.923	0.001
SCC	54	50	4	26	14	5.717	0.025
ADC	40	36	3	10	25	11.428	0.001

CIN – cervical intraepithelial neoplasia, SCC – squamous cell carcinoma, ADC – adenocarcinoma

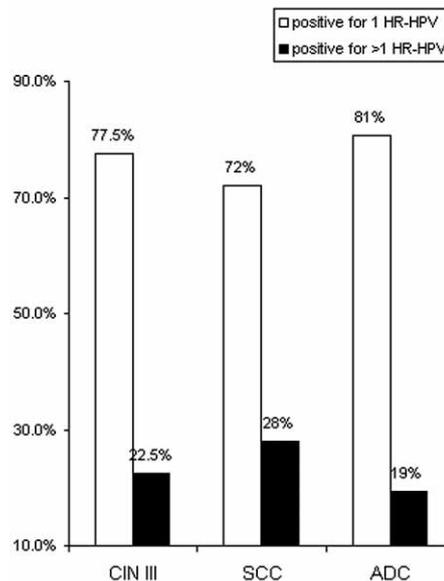


Fig. 1. Prevalence of single and multiple high-risk HPV infections associated with histological groups of cervical carcinoma. HR-HPV – high-risk HPV, CIN – cervical intraepithelial neoplasia, SCC – squamous cell carcinoma, ADC – adenocarcinoma.

Discussion

Epidemiological and molecular biology studies have shown that infection with high-risk HPV is the most important etiologic agent in the pathogenesis of cervical SCC^{10,16}. However, the pathogenic role of HPV in cervical adenocarcinoma remains unclear^{17–19}. Cross-sectional studies indicate that HPV infection is most frequently found among sexually active young women and that its prevalence decreases with age. In most studies HPV-16 was found to be the most prevalent HPV genotype in cytologically normal women, women with CIN, and women with cervical cancer^{7,16}. Yet, recent studies have shown the distribution of HPV genotypes detected in cervical cancer to vary depending on the histological type of the cancer. Whereas HPV-16 is the most frequent genotype in SCC, HPV-18 is a predominant genotype in ADC and AIS^{20–23}. Some studies indicate that both HPV-16 and HPV-18 play a prominent role in the development of ADC of the cervix^{18,19}. The overall frequency of HPV DNA associated with cervical SCC has been reported to be 91%–100%^{7,10}; yet, the HPV DNA detected in adenocarcinomas varies significantly from 32% to 100%, depending on the detection method used¹⁸. The overall prevalence of HPV DNA detected in our study in all three histological groups was high (>90%). We found that HPV-16 was the most prevalent genotype in CIN III and SCC, whereas HPV-18 was most frequent in ADC. Our results are consistent with previous reports, which also indicate a high occurrence of HPV-16 in CIN III and SCC^{22,24}. We demonstrated HPV-18 to be the most common genotype in ADC, which is consistent with several studies conducted in Europe^{20,23,25}. In our study, the HPV genotypes detected in ADC were HPV-18, HPV-16, HPV-X, HPV-31 and HPV-33. The only difference between our study and the meta-analysis reported by Clifford *et al.*²³

was that we did not detect HPV-45 genotype in ADC, which could have been due to some specificity of the HPV genotypes found in this area or a smaller sample size. The difference in the prevalence of high-risk HPV-16 and HPV-18 genotypes recorded in SCC and ADC was statistically significant. In most cancer registries, about 75% of all cervical cancer cases are reported as SCC, whereas ADC and adenosquamous cell carcinoma account for 10%–15% of cervical cancer cases, with increasing incidence rates during the last decade, particularly in younger women²⁶. The influence of multiple HPV infection on the development of cervical carcinoma is still controversial. It has been shown that multiple high-risk HPV infections were less frequent in high grade than in low grade cervical neoplasia²⁷. Multiple infections were significantly less common in ADC, which suggests that the ultimately invasive growth of glandular epithelial cells is triggered by the action of a single HPV genotype rather than by the potentially synergistic action of multiple HPVs^{18,28}. Our study revealed a single high-risk HPV genotype to be more frequent in CIN III, SCC and ADC samples.

Taking into account oncogenic potential of HPV-16 and HPV-18, these data deserve special attention in further development of screening programs for cervical cancer in Croatia, as well as future vaccination programs, to lessen the burden of this neoplasm.

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PREVALENCIJA GENOTIPOVA HUMANOG PAPILOMA VIRUSA U KARCINOMU CERVIKSA I PREKURSORSKIM LEZIJAMA

S A Ž E T A K

Ispitali smo prevalenciju humanog papilomavirusa (HPV) i distribuciju HPV genotipova u arhivskom materijalu karcinoma cerviksa i prekanceroznih lezija, prikupljenom u periodu 1995–2005 godina u Zavodu za patologiju, Medicinskog fakulteta Sveučilišta u Rijeci. Iz tkiva uklopljenog u parafin, histološki klasificiranog kao cervikalna intraepitelna neoplazija (CIN) III (n=43), pločasti karcinom cerviksa (SCC) (n=54) i adenokarcinoma cerviksa (ADC) (n=40) izolirana je DNA, a HPV analiza je izvršena pomoću polimeraza lančane reakcije (PCR) koristeći generičke i genotip specifične PCR primere. Prevalencija HPV-DNA utvrđena je u 93.02% slučajeva CIN-a III, 92.59% SCC-a i 92.5% ADC-a. Od visoko rizičnih genotipova HPV-16 je bio najčešći u 65% CIN-ova III i 52% SCC-a, dok je HPV-18 utvrđen u 22.5% CIN-ova III i 28% SCC-a. Pronađena je statistički značajna prevalencija HPV-18 genotipa u ADC-u, 67.6%, u usporedbi sa 28% u SCC-u, ($\chi^2=9.924$; $p \leq 0.01$). U manje od 15 % slučajeva utvrđene su miješane infekcije s HPV genotipovima 31, 33, 45, 52, 68, 70 i neutvrđenim genotipom X. U sva tri histološka tipa infekcija s jednim HPV-genotipom bila je dominantna u odnosu na miješanu infekciju.

The Immunomodulation Effect of Allogenic Blood Transfusion in Colorectal Cancer – A New Approach

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ABSTRACT

The total number of 542 patients with colorectal cancer surgery have been analyzed in order to estimate the effect of re-ceiving transfusion local recurrences, and the disease free – survival. It should be examined whether there are changes in general immunity indicators which would be connected with perioperative transfusion. A significant connection has been found between local recurrences and blood transfusion ($p < 0.0001$), the most noticeable being in Dukes A ($p = 0.045$), localization on rectum ($p = 0.036$). The receiving of blood transfusion is linked significantly with disease free – survival reduction ($p = 0.0068$; log rank), the most significant being in Dukes A stage ($p = 0.0123$; log rank) and with localization on rectum ($p = 0.0231$). The analysis of general immunity indicators has shown significant immunocompromitiation of patients just before the surgery and this could have effect on immunomodulation caused by transfusion and just as on the treatment prognosis of colorectal carcinoma.

Key words: colorectal cancer, immunomodulation, allogenic blood transfusion

Introduction

Intravenous blood transfusion introduces large quantities of foreign antigens, i.e. peripheral blood cells, proteins, lipids, preservatives and anticoagulants. Tests on animal models showed that the intravenous infusion of allogenic tissue leads to immunosuppression. The mechanism of development of natural immunological tolerance was explained in classic experiments by Medwar, which showed that exposure to foreign bodies during the fetal life leads to lifelong acceptance of skin transplants taken from organism to which the animal was exposed during its fetal life¹.

Those and similar studies have given hope that the tolerance may also be stimulated in patients who were intended for transplantation, and that this would help to reduce the rejection of the transplant. Clinical studies in the later period confirmed the assumption that the use of blood preparations prior to kidney transplantation was a very successful method for improved survival of renal allograft².

Previous research on the animal model proved that the infusion of extracts of different tissues from the allogenic donor before the tumor implantation accelerated growth of the tumor³.

Gantt's hypothesis⁴ that the immunosuppressive influence of transfusion, which was noticed in kidney transplantations, can suppress immunological functions in patients with malignant diseases and in that way contribute to the appearance of local relapse and distant metastases, had been investigated for more than 20 years in over 130 studies, most of them related to colorectal cancer, is still controversial and without final conclusion.

Nor random studies^{5–8}, nor meta-analyses^{9–12} have provided the answer of whether the allogenic transfusion influences the outcome of treatment of colorectal cancers or not. The transfusion of allogenic blood causes in the recipient the change in immunological response^{13,14}, such as the reduction of NK activities and T lymphocytic blastogenesis, and the increase of suppres-

sive T lymphocytic activity, which can, it is assumed, reduce the resistance to the infection or lead to dissemination of malignant cells.

The studies of the influence of allogeneic blood transfusion on specific changes in immunological functions were carried out. The following was observed: reduced secretion of interleukin 2 (IL-2)¹⁵, reduced activity of natural killer cells (NK cells)¹⁶, reduced proportion of T lymphocyte helper cells (CD 4+ cells) / cytotoxic T lymphocytes (CD 8+ cells) – CD 4/CD 8 proportion¹⁴, reduced macrophagic functions¹⁷, and reduction of postponed type of oversensitivity¹⁸.

Clinical syndromes, whose mechanism is still to be defined, connected with transfusions of allogeneic blood in medical literature are called allogeneic blood transfusion – associated immunomodulation (TRIM)¹⁹.

The goal is to investigate whether there is connection in patients operated on account of colorectal cancer, between the appearance of local relapse and periods of disease free-survival with the transfusion and the quantity of perioperative blood compensation, and to investigate whether there are changes in indicators of general immunity which are related to perioperative blood compensation.

Review of the databases showed that thus far not one analysis of the influence of perioperative allogeneic blood compensation in patients operated on account of colorectal cancer has analyzed the influence of preoperative immunological stage on the possible influence of allogeneic transfusion on the outcome of the treatment.

Patients and Methods

We analyzed the patients who were operated on account of colorectal cancer in the »Sisters of Mercy« Clinical Hospital in Zagreb in the period from 1995 – 1999, who in the moment of operation did not have distant metastases, were not operated on if they were in an inoperable stage or if the palliative operation had been performed. The patients who died during the postoperative period of 30 days, and those who had primary relapsing colorectal cancer were not included in the study. The research also excluded patients who in the period of one month before they were admitted received some kind of blood preparation, who had malignant tumors of other localization, autoimmune diseases, who had corticosteroid therapy, and those whose patohistological analysis determined that their resection margine is not tumor-free.

The data on the acceptance of blood derivatives in perioperative period were recorded – immediately before the surgical intervention, during the operation of colorectal cancer and in the first ten days of the postoperative period. The mentioned period is defined as perioperative period. The transfusion has been defined as reception of any other blood preparation: erythrocyte concentrate, full blood or fresh frozen plasma. The quantity of transfusion was also measured: non-reception or

reception of less than 500 ml, more than 500 and less than a thousand milliliters, and more than a thousand milliliters of transfusion. Since most of the patients who received the transfusion also received a combination of several preparations, the analysis of the influence of separate blood preparations was not possible because of mixing of accompanying effects.

The operated patients were postoperatively monitored in days after the surgical intervention. During control examinations, the data about the manifestation of cancer relapse were registered local relapses and distant metastases and/or death, up to 1825 days after the operation. In some randomly chosen patients we measured the indicators of general immunity one day before the operation and on the tenth day after the operation. The scope of subjects was from 42 to 75. Stage of colorectal cancer according to Dukes, as well as the localization in those subjects, were equally represented in patients in all performed analyses.

Also, the same immunological parameters were measured in healthy subjects. The scope of healthy examinees was from 45 to 239. Immunoassays were performed at the Immunology Department at the Clinic for the Oncology and Nuclear Medicine at the »Sisters of Mercy« Clinical Hospital. Blood for the analysis of immunological parameters was taken one day before the operation, and on the tenth day after the operation. In order for the subjects to be accepted for this study, they needed to fulfill the following criteria: not to have received blood preparation transfusion one month before the beginning of the study, not to have had an intestinal obstruction, perforation, intraabdominal abscess, nor the signs of acute inflammatory disease which would influence their immunological status. All patients who were included in this study were operated by surgeons who specialized in colorectal surgery. None of the patients whose immunological indicators were measured on the tenth postoperative day showed signs of sepsis, anastomosis dehiscence, pneumonia, urological infection, or anything that would affect the immunological parameters. All patients whose general immunity indicators were measured on the tenth postoperative day received some transfusion preparation – erythrocyte concentrate, full blood or fresh frozen plasma. Prior to the operation, the values of immunological parameters between the healthy subjects and those who had colorectal cancer were compared. Since all patients received some kind of blood preparation, in the analysis of impact of transfusion on immunological status the patients were divided in respect to the median of received blood preparations. Out of the indicators of general immunity, functional and morphological changes were measured:

- the number of leukocytes, percentage and total number of granulocytes, lymphocytes and monocytes (from standard smears of peripheral blood);
- percentage and total number of T lymphocytes and their subpopulations (CD 4 and CD 8), B lymphocytes (surface immunoglobulins) and natural killer

cells (NK cells, CD 56) by monoclonal antibodies using the method of immunofluorescence;

- functional tests for determining NK activities (cytotoxicity with ^{51}Cr -K562) and phagocytic functions of granulocytes and monocytes (fluorescence with acridine orange and live saccharomyces as target cells).

The instruments and methods used for determining immunological parameters are:

- the light microscope OPTON Axioskop (peripheral blood smears analyzed)
- surface immunocompetent cells (CD 3, CD 4, CD 8, CD 56) were determined with fluorescent microscope with the help of OPTON photomicroscope equipped with epifluorescent condenser III RS;
- the fluorescent method was used to determine phagocytic functions of granulocytes and monocytes, by coloring with acridine orange and with saccharomyces as target cells. The results were examined with OPTON photomicroscope with the use of epifluorescent condenser III RS;
- the radioactive method was used to determine NK – activity with the target cells K-562 marked with chrome ^{51}CR . Radioactivity released in the supernatant of incubation mixture was measured with LKB gamma-counter, model 1272 Clinigamma.

The process of determining fagocytic functions of granulocytes and monocytes. After the incubation of phagocytes with microorganisms, the preparations were colored with acridine orange and microscoped under the ultraviolet light. Dead microorganisms fluoresce red, and the live ones green. The level of microbicidity was determined by comparing the number of ingested dead microorganisms in relation to the total amount of ingested particles.

The process of determining the ingestion and intracellular microbicidity by fluorescence with the aid of saccharomyces as target cells. With the addition of heparin, peripheral blood sediments, which results with the separation of plasma rich in white blood cells which are then rinsed, counted and their concentration set to a determined value. At the same time, a culture of saccharomyces is prepared, which are then washed and their concentration set to a determined value. On the slide, with the help of a small plastic ring and vaseline, a chamber is prepared in which a prepared cell suspension is pipetted and incubated in an incubator for cell culture for 30 minutes, because of the sedimentation of cells and adherence to the base. Non-adhered cells are then rinsed, and fungus suspension is added into the chamber, then it is incubated in the incubator for cell culture, free fungi are rinsed, the cells which adhered to the glass are colored with acridine orange and then microscoped under ultraviolet light at the increase of 80x. In that way we have obtained the data on:

- a) type of phagocytes (monocyte, granulocyte)
- b) number of ingested fungi (ingestion index)

c) percentage of cells which fagocyted (phagocytic activity)

d) number of dead, killed fungi (percentage of microbicidity).

Determining of NK-activity. Mononuclear cells from peripheral blood were separated in Ficoll-Hypaque gradient (Pharmacia, Sweden) and incubated with ^{51}Cr -K562 target cells in RPMI cell culture (Institute for Immunology, Croatia) which contains 10 % of calf serum (Ruđer Bošković Institute, Zagreb) by a proportion of 50:1 over the period of 18 hours. Radioactivity measured with gamma counter was expressed as percentage of cytotoxicity, as explained earlier.

The impact of transfusion and the quantity of received transfusion in particular factors upon the appearance of local relapses were compared with the standard χ^2 test, with measurement of proportion of prospects and limits – the confidence interval at the level of 95%. Kaplan-Meier method was used to present the curve of periods of disease free – survival, and differences in survival were compared by using the log-rank test and Brestow test. In statistical processing the tests of rank sum were used – Wilcoxon test and Mann-Whitney U test.

Results

In the period from 1995 – 1999 on the Surgery Clinic at the Sisters of Mercy Clinical Hospital in Zagreb, 711 patients were operated on account of colorectal cancer. There were 542 (76%) patients who fulfilled the criteria, stated above, for admission into the study, while 169 (24%) of patients did not fulfill the criteria. Dukes A was found in 29.3 % (n=159), Dukes B in 37.8 % (n=205), and Dukes C in 32.8 % (n=178) of patients.

The average reception of erythrocyte concentrate per patient was 699.5 ml (median 580 ml), the reception of full blood was 214 ml (median 0 ml), and fresh frozen plasma 325 ml (median 190 ml). Statistically important connection between the manifestation of local relapse and transfusion was discovered ($\chi^2=11.774$, $df=1$, $p<0.0001$, Odds ratio – 5.139, CI 95% 1.833–14.412), and also that the quantity of received transfusion is statistically significantly connected with the manifestation of local relapses ($\chi^2=14.09$, $df=2$, $p=0.001$). As for transfusion, there was significant manifestation of local relapses for rectum and rectosigmoid cancer ($\chi^2=4.836$, $df=1$, $p=0.036$, Odds ratio 3.581, CI 95 % 1.072–11.967), and even more significant with regard to the quantity of received transfusions ($\chi^2=9.766$, $df=2$, $p=0.008$). There is important connection between the manifestation of local relapses in the transfusion for Dukes A in the data processing with χ^2 test and obtained p value ($\chi^2=4.282$, $df=1$, $p=0.045$), but that is not confirmed by proportion of prospects and the reliability interval of 95% (Odds ratio 6.66, CI 95 % 0.856–51.666). Also, transfusions are statistically marginally related to the manifestation of local relapses in Dukes B ($\chi^2=4.301$, $df=1$, $p=0.53$, Odds

ratio 6.586, CI 95% 0.862–50.38). In Dukes C there was no statistical connection found between the manifestation of relapse and transfusion ($\chi^2=2.477$, $df=1$, $p=0.171$, Odds ratio 3.153, CI 95% 0.704–14.114). In evaluation of statistical significance of local relapses in respect to the quantity of transfusions, there was significance in Dukes A ($\chi^2=9.551$, $df=2$, $p=0.008$), while in Dukes B ($\chi^2=4.156$, $df=2$, $p=0.125$) and Dukes C ($\chi^2=4.016$, $df=2$, $p=0.134$) no significant connection was found. There was significant shortening of disease free – survival period in patients who received transfusions ($p=0.0069$; log rank, $p=0.0139$; Brestow signif) (Figure 1).

There was significant shortening of disease free – survival period in the increase of the quantity of received transfusion ($p=0.008$; log rank, $p=0.0139$; Brestow signif) (Figure 2).

There was considerably shorter of disease free – survival period in case of transfusion for rectum cancer

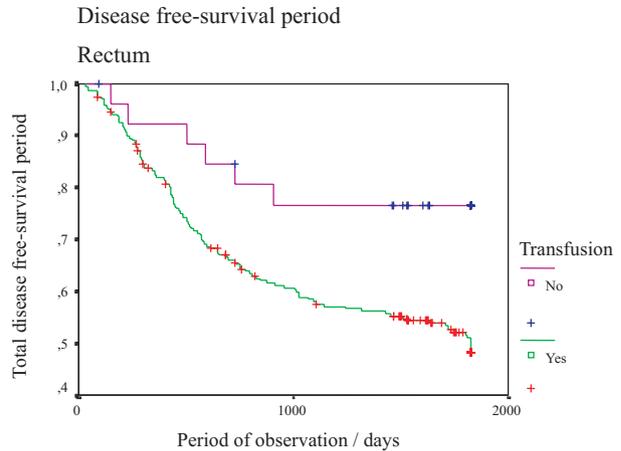


Fig. 3. Curve – disease free – survival period with regard to transfusion for rectum cancer.

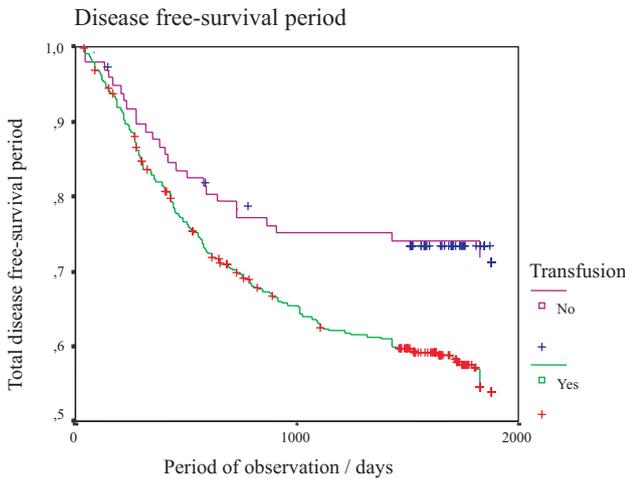


Fig. 1. Curve – disease free – survival period with regard to transfusion.

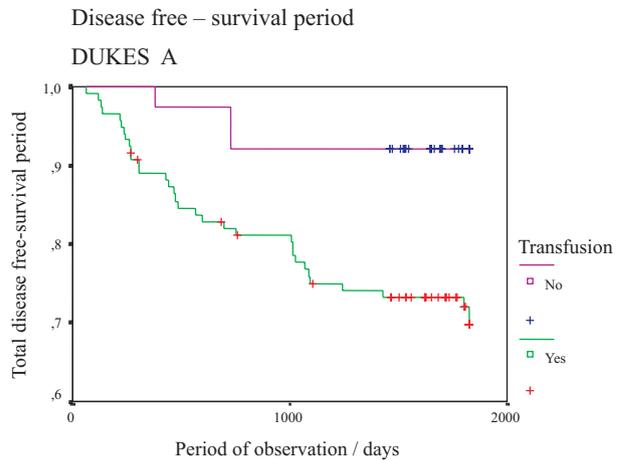


Fig. 4. Curve – disease free – survival period with regard to transfusion for Dukes A.

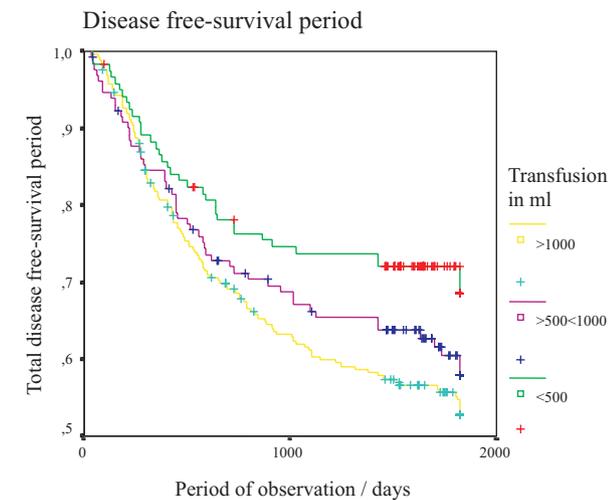


Fig. 2. Curve – disease free – survival period with regard to quantity of received transfusion.

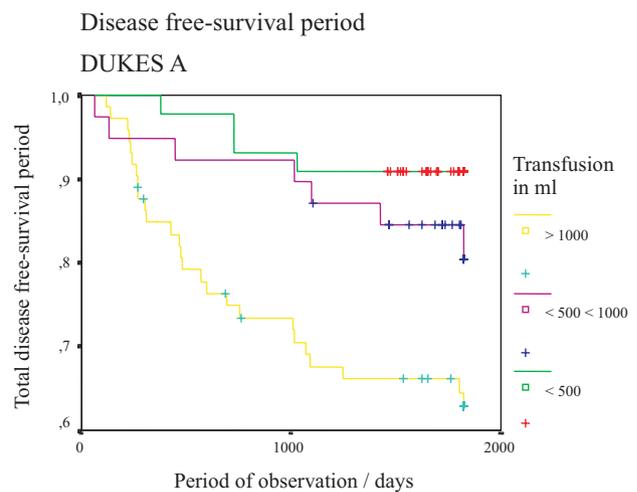


Fig. 5. Curve – period of recurrence free – survival with regard to quantity of received transfusion for Dukes A.

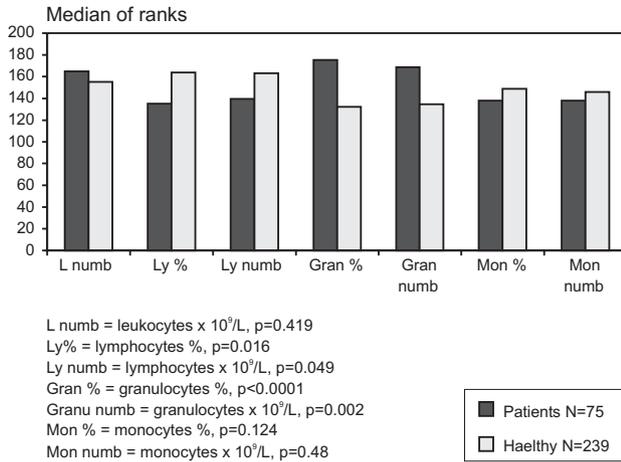


Fig. 6. Number of leukocytes, percentage and number of lymphocytes, granulocytes and monocytes (standard smear of peripheral blood) in healthy subjects and in patients before the operation, i.e. before the transfusion.

($p=0.0231$; log rank, $p=0.0316$; Brestow signif) (Figure 3), but not in respect to the quantity of received transfusion ($p=0.0654$; log rank, $p=0.0867$; Brestow signif).

There was no important influence to the disease free – survival period in cases of colon cancer with transfusion ($p=0.3312$; log rank, $p=0.3643$; Brestow signif), as well as with regard to the quantity of received transfusion ($p=0.7853$; log rank, $p=0.6309$; Brestow signif). It was found that transfusion significantly shortens the disease free – survival period in Dukes A ($p=0.0123$; log rank, $p=0.0131$; Brestow signif) (Figure 4), as well as the quantity of received transfusion ($p=0.0026$; log rank, $p=0.0021$; Brestow signif) (Figure 5).

There was no influence of transfusion to the disease free – survival period for Dukes B ($p=0.2098$; log rank, $p=0.3442$; Brestow signif), as well as in respect to the quantity of received transfusion ($p=0.6273$; log rank, $p=0.7151$; Brestow signif). There was no influence of transfusion on the disease free – survival period for Dukes C ($p=0.8413$; log rank, $p=0.8501$; Brestow signif), as well as in respect to the quantity of received transfusion ($p=0.8699$; log rank, $p=0.8338$; Brestow signif).

There was significant decrease of lymphocyte percentage in patients ($p=0.016$), as well as of the total number of lymphocytes ($p=0.049$), and the increase of percentage of granulocytes ($p<0.0001$), as well as of the total number of granulocytes ($p=0.002$) (Figure 6).

By comparison of patients with healthy subjects, it was noticed that there was significant decrease of percentage of T lymphocytes – CD 3⁺ ($p<0.0001$), of total number of CD 3⁺ ($p=0.002$), percentage of CD 4⁺ ($p=0.001$), and percentage of CD 8⁺ ($p=0.015$) (Figure 7).

There was significantly greater percentage of large granulated lymphocytes in patients ($p=0.024$).

It was noticed that in patients, as compared to healthy subjects, there was significant decrease of percentage of granulocyte function ($p=0.006$), granulocytic in-

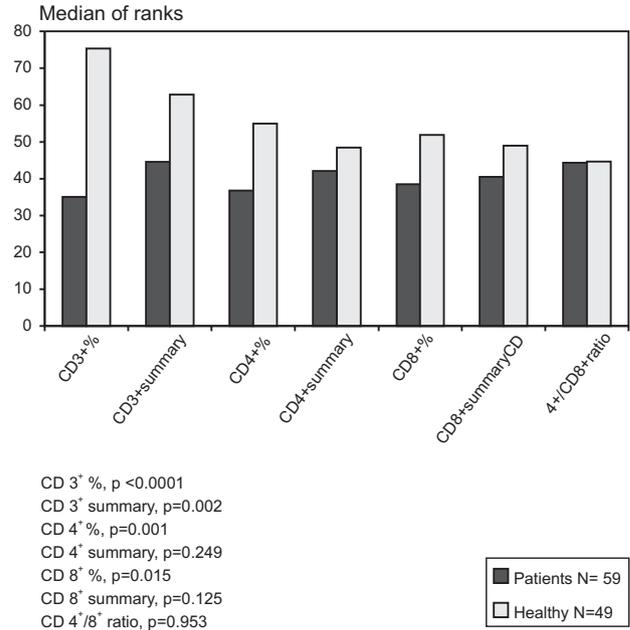


Fig. 7. T lymphocytes (CD 3⁺) and their subpopulations (CD 4⁺, CD 8⁺) in healthy subjects and in patients before the operation, i.e. transfusion.

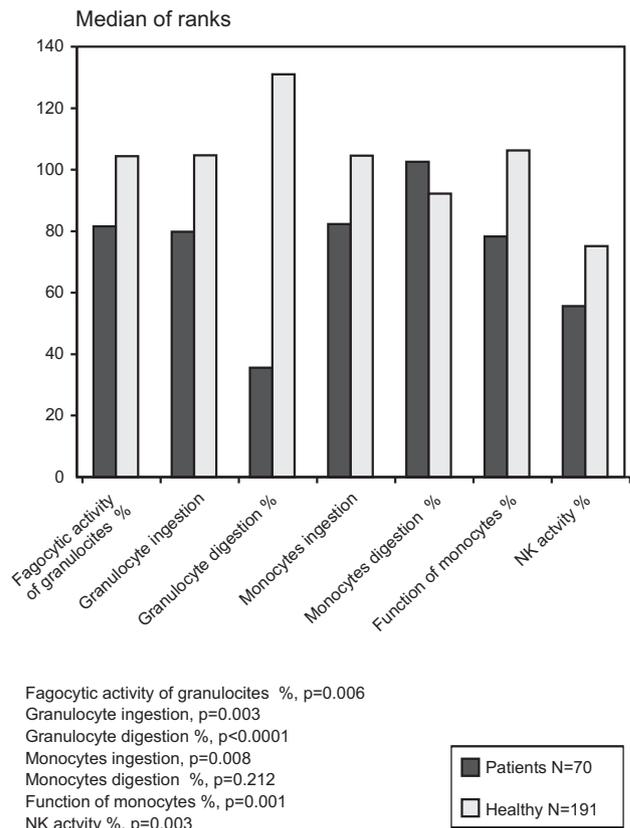


Fig. 8. Tests of phagocytic functions of granulocytes, monocytes and NK activities in healthy subjects and patients before the operation, i.e. transfusion.

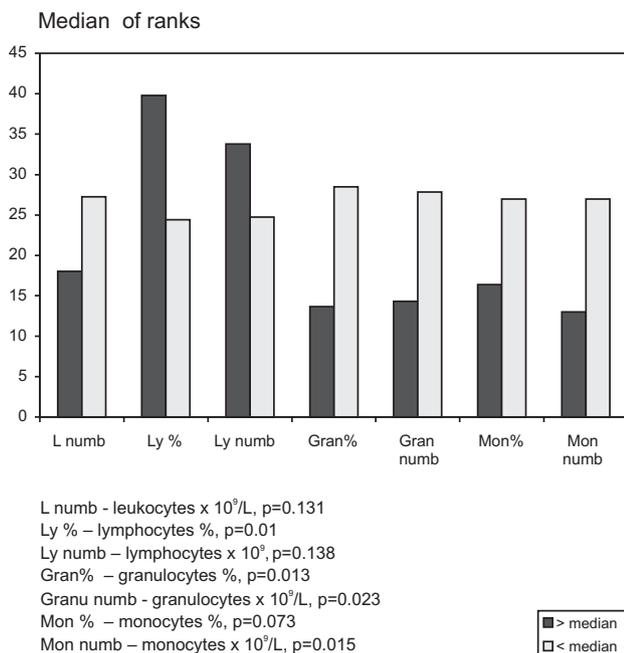


Fig. 9. Number of leukocytes, percentage and number of granulocytes, lymphocytes and monocytes (standard smear of peripheral blood) in patients after the operation, i.e. transfusion, and division in respect to the median of received transfusion in perioperative period.

gestion ($p=0.003$), percentage of granulocytic digestion ($p<0.0001$), monocytic ingestion ($p=0.008$), percentage of monocyte function ($p=0.003$), and percentage of NK activity ($p=0.003$) (Figure 8).

In patients who received more than a median of transfusion, there was significant decrease of percentage of lymphocytes ($p=0.01$), significant increase of percentage of granulocytes ($p=0.013$), and significant increase of total number of granulocytes ($p=0.015$), as well as of total number of monocytes ($p=0.015$) (Figure 9).

There were no changes in values of T lymphocytes and their subpopulations (CD 4⁺, CD 8⁺) in patients after the operation, in respect to the median of received transfusion in perioperative period.

There was decrease of value of total number of B lymphocytes in those who received more than a median of transfusion preparations ($p=0.022$).

There were significantly higher values of percentage of granulocytic digestion in patients after the operation, in patients who received more than a median of blood preparations ($p=0.021$).

Discussion

The mechanism of immunological changes, which emerge under the influence of transfusion, has not been fully explained. Many authors believe that transfusion is connected with cellular immunosuppression^{22–24}.

Since the first report on the influence of transfusion on the prognosis for colorectal cancer operation²⁵, until today, studies often dealt only with the survival in respect to the reception or non-reception of transfusion without including into the studies the other factors which influence both the loss of blood during the operation and the manifestation of relapse of the disease. The later studies started to include the other accompanying factors in the analysis of transfusion influence. But it was the work on randomized studies that provided the clearer image of the possible influence of transfusion to the relapse of colorectal cancer. Three randomized studies compared the frequency of relapse of cancer between buffy-coat reduced allogeneic concentrate of erythrocytes and receiver of autologous full blood⁷ or concentrate of erythrocytes⁵ or leukocytes reduced, buffy-coat reduced, concentrate of erythrocytes⁶, in patients who were operated on account of colorectal cancer. Generally, only one⁶ of those three studies reported on relation between allogeneic transfusion and relapse of cancer, and where the control group comprised the patients who received leukocyte reduced concentrates of erythrocytes. Only a few studies had more than one hundred patients with Dukes A²⁶, and the same was reported by Beynon et al.²⁷ in his analysis in which he found out that there were not enough patients with Dukes A to make the final conclusion – in his analysis of 519 patients only 77 were those with Dukes A. In that work, the patients who received transfusion had the frequency of relapse 25.5 %, while in the group who did not receive transfusion this frequency was considerably lower and amounted to 6.7 %. Modin et al.²⁸ in 57 patients with Dukes A who had colon cancer, found the unfavorable influence of transfusion on the period of disease free – survival. According to Modin et al.²⁸ period of disease free – survival for a six-year period for patients who did not receive transfusion amounted to 69 %, while for patients who received transfusion it was 48 % ($p < 0.05$). Weiden et al.²⁹ in his work even found the favorable influence of transfusion in C2 stage on the relapse of disease.

Our study, which included 156 patients in Dukes A, is significant in that respect. Our results suggest that there is connection between transfusion, and particularly the quantity of received transfusion for rectum and rectosigmoid cancer with the manifestation of local relapses, more than the influence on the disease free – survival period. Localization of rectum and rectosigmoid cancer on account of the nature of its localization, and frequent forming of anastomosis deep in pelvis, opens up the question of influence of transfusion on the prognosis of disease. Data on greater connection between transfusion, and particularly the quantity of received transfusion to the manifestation of local relapses, more than to the final prognosis for disease, suggests that there is greater influence of the surgical operation and more aggressive manipulation of tumor in the narrow pelvis area, by which the transfusion is just an indicator of such work, than TRIM influence. The same conclusion was reached by Busch et al.³⁰ and Brand et al.²⁶

The immunological parameters in patients with colorectal cancer and in healthy subjects we compared. It was found out that there was significant deviation from normal distributions in differential blood count in patients with colorectal cancer, in comparison with healthy subjects. It can be seen that the number of leukocytes is not significantly different between patients with colorectal cancer and healthy subjects, while there is significant decrease of percentage of lymphocytes ($p=0.016$), the total number of lymphocytes ($p=0.049$), and increased percentage of granulocytes ($AP < 0.0001$), as well as the total number of granulocytes ($p=0.001$) (Figure 6). The inconsiderable difference in total number of leukocytes, lymphopenia and relative granulocytosis was found by Elsasser-Biele et al.³¹ and Ordemann et al.³². Goto et al.³³ also found the inconsiderable difference in the total number of leukocytes, with significant decrease in the number of lymphocytes, and even more significant decrease of percentage of lymphocytes in patients with several different malignant tumors, as compared to healthy subjects. Also, as well as in our sample, there was no significant change of the total number and percentage of monocytes³³, Ordemann et al.³² discovered significant increase of percentage of monocytes in patients with colorectal cancer in relation to healthy subjects.

It was found out that the percentage ($p < 0.0001$), as well as the total number ($p=0.002$) of T lymphocytes (CD 3⁺) were significantly reduced in patients with colorectal cancer, as compared to healthy subjects. The percentage of helper lymphocytes (CD 4⁺) is significantly reduced in patients ($p=0.001$), but not the total number CD 4⁺ ($p=0.249$). Also, the percentage of cytotoxic lymphocytes (CD 8⁺) is significantly reduced in patients ($p=0.015$), but not the total number ($p=0.125$). The proportion of CD 4/CD 8 did not change ($p=0.953$, Figure 7).

Ordemann et al.³² discovered significant decrease of CD 4/CD 8 ratio in patients with colorectal cancer in comparison with healthy subjects ($p=0.045$). They also noticed that with the progress of the disease, stronger immunocompromise would appear. Goto et al.³³ did not find any significant difference between CD 4⁺ cells, CD 8⁺ cells and the proportion of CD 4/CD 8 between patients with cancer and healthy subjects.

It was observed that there was no significant difference in the percentage and the total amount of B-lymphocytes, between healthy subjects and patients. The percentage of large granulated lymphocytes is significantly increased in patients with colorectal cancer in comparison to healthy subjects ($p=0.024$), although a significant difference in the total amount ($p=0.864$) was not found. The percentage of NK cells is higher, but not significantly ($p=0.064$) in patients with colorectal cancer, while the total amount was not significantly altered ($p=0.259$).

Mentioned data suggest that there is a significant decrease of cell immunity in patients with colorectal cancer in comparison to healthy subjects.

The most observable changes were found by tests measuring phagocyte activity of granulocytes, monocytes and NK activity in patients in relation to healthy subjects.

The percentage of phagocyte activity in granulocytes is significantly reduced in patients ($p=0.006$). Likewise, the granulocyte ingestion is significantly reduced in patients ($p=0.003$) and the percentage of granulocytes digestion even more ($AP < 0.0001$). It has been observed that the function of monocyte ingestion is significantly reduced in patients ($p=0.008$). The percentage of monocyte digestion is not significantly changed in patients in comparison to healthy subjects ($p=0.212$). The percentage of monocyte function is significantly reduced in patients with colorectal cancer in comparison to healthy subjects ($p=0.001$).

The percentage of NK activity is significantly reduced in patients with colorectal cancer ($p=0.003$, Figure 8).

Many authors find that the influence of the amount of received transfusions is an even more important factor which influences the relapse of the illness in case of colorectal cancer^{28,34–36}. In patients who received less than a median of blood preparations during the perioperative period no changes were observed in the total number of leukocytes in comparison to those who received more than a median of blood preparations ($p=0.131$). It has been observed that the percentage of lymphocytes was significantly reduced in patients who received more than a median of blood preparations ($p=0.01$), but the total amount of lymphocytes did not significantly change ($p=0.138$), while the percentage of granulocyte significantly increased ($p=0.013$), as well as the total amount of granulocyte in patients who received more than a median of blood preparations ($p=0.023$). The number of monocytes was significantly increased in patients who received more than a median of blood preparations ($p=0.015$, Figure 9).

No important difference was observed in subpopulations of T lymphocytes (CD 4⁺, CD 8⁺, ratio CD 4 / 8) in patients who received less, compared to those who received more than a median of blood preparations. A significant decrease was observed in the total amount of B lymphocytes in patients who received more than a median of blood preparations ($p=0.022$), while the percentage of B lymphocytes was marginally reduced ($p=0.059$). The total number of large granulated lymphocytes, percentage of NK cells (CD 56) and total amount NK cells (CD 56) were not significantly changed. Ziv and al.³⁷ also found out a significant decrease in the number of B lymphocytes in patients who received transfusion, and were operated on because of colorectal cancer in Dukes C. Also they did not find any important differences in percentage and total amount of leukocytes, lymphocytes, T lymphocytes, helper T lymphocytes, cytotoxic T lymphocytes and NK cells, in the first and the fifth week after the operation.

In our patients, who were divided in a different way, a lymphopenia with reactive granulocytosis and monocytosis was observed and, likewise, a significant decrease in the total number of B lymphocytes which appeared with the increase of the amount of transfusion.

Intake of antigens changes the flow of lymphatic cells in lymph nodes and other lymphatic organs. During 24 hours after the intake of antigens, the lymphatic reactive

clone completely disappears from the blood circulation and can be found in the area of lymph nodes. In fact, clone members accidentally enter the lymph node, but then the reaction to the antigen retains them there. Only after a couple of days, lymphocytes come out from the lymph node, mostly in the shape of lymphoblasts, and spread through the body. Mentioned facts are a possible cause for the decrease in the number of B lymphocytes, which this study observed in patients who received more than a median of blood preparations.

In the test analysis of phagocyte functions of granulocytes, monocytes and NK activity, a significant rise in the percentage of granulocyte digestion was observed in patients who have taken more than a median amount of blood preparations ($p=0.021$), while no significant results were noticed in other tests. Kaplan et al.³⁸ found a decrease in the proportion of CD 4/8 in patients who received an allogeneic transfusion, whereas Waymackas et al.³⁹ found a decrease in macrophages functions. On the whole, neither of the previous analyses has taken into consideration that patients were already immunocompromised before receiving transfusions, i.e. prior to the operation, which should be taken into account in the future studies.

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It is also possible that a different level of immunocompromise before the operation, which is linked to the cancer stage, could be connected to the influence of transfusion on the outcome of the treatment. In patients with Dukes A, which are less immunocompromised before the very operation, immunocompromisation caused by allogeneic transfusion could be strongly manifested, while in patients with dissemination malignant illnesses (Dukes C) this influence would not be manifested.

In the further research an analysis of the immunological status should be carried out prior to the operation, which would be connected with the stage of disease, then patients should be divided according to the type of received transfusion – autologous / leucoreduced vs. allogeneic, and a postoperational analysis of the immunological status needs to be carried out. Furthermore, the development of the local relapses and distant metastases should be monitored, taking into account all prognostic factors which influence the course of colorectal cancer treatment. All of this would give a clearer picture of the possible immunomodulation activity of allogeneic transfusion.

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IMUNOMODULACIJSKI UČINAK PRIMANJA TRANSFUZIJE KOD KOLOREKTALNOG KARCINOMA

S A Ž E T A K

Analizirano je 542 bolesnika operiranih zbog kolorektalnog karcinoma radi procijene utjecaja primanja transfuzije na pojavu lokalnih recidiva i na period bez bolesti – preživljavanje. Istražene su promjene u pokazateljima opće imunosti i njihovu moguću povezanost sa perioperacijskom transfuzijom. Nađena je značajna povezanost pojave lokalnih recidiva i primanja transfuzije ($p < 0,0001$), najizraženije kod Dukesa A ($p = 0,045$), lokalizacije na rektumu i rekto-sigmoidu ($p = 0,036$). Količina transfuzije najviše je povezana sa pojavom lokalnih recidiva kod Dukesa A ($p = 0,008$), lokalizacije na rektumu ($p = 0,008$). Primanje transfuzije ($p = 0,0123$; log rank) značajno je povezano sa skraćenjem perioda bez bolesti – preživljavanje, najznačajnije kod Dukesa A stadija ($p = 0,0123$; log rank), te pri lokalizaciji na rektumu ($p = 0,0231$; long rank). Analizom pokazatelja opće imunosti uočena je značajna imunokompromitacija bolesnika prije same operacije, što bi moglo djelovati na stupanj imunomodulacije uzrokovane transfuzijom, a tim i na prognozu liječenja kolorektalnog karcinoma.

Variations in Mugwort (*Artemisia* Spp.) Airborne Pollen Concentrations at Three Sites in Central Croatia, in Period from 2002 to 2003

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ABSTRACT

In spite of the low atmospheric pollen levels, *Artemisia* sensitisation and allergy has been reported widely. The aim of the study was to determine the length of pollen season, intradiurnal, daily and monthly pollen variation, and the effect of some meteorological parameters on atmospheric pollen concentrations in Central Croatia. Seven-day Hirst volumetric pollen and spore traps were used for pollen sampling. The *Artemisia* pollen season lasted from the end of July until the end of September with the highest concentrations in August. The percentage of the total pollen count ranged from 0.52% to 0.92%. The intradiurnal peak occurred between 10 a.m. and 12 a.m. Statistical analysis showed a significant correlations between higher air temperature and high pollen concentration as well as high precipitation and low pollen concentration. Results of this study are expected to help in preventing the symptoms of allergic reaction in individuals with *Artemisia* pollen hypersensitivity.

Key words: aeropalynology, meteorological parameters, *Artemisia*, pollen season

Introduction

The genus *Artemisia* belongs to a very extensive plant family *Asteraceae* (*Compositae*), which includes a number of allergenic representatives¹. *Artemisia* is a typical and widespread weed. The most common species in Europe are *Artemisia vulgaris* (mugwort), abundantly present all over the continent, and *A. verlotorum* and *A. annua*, predominantly in the southern half of Europe^{2,3}. Many species of *Artemisia* preferably grow on ruderal and disturbed soils, in urban and suburban areas, at industrial and building sites, in rural areas and in the countryside along roads. Although this weed is widely spread, its highly allergenic pollen is rarely found at high concentrations in the air, accounting for only 0.5% to 5% of the total annual pollen concentration at particular monitoring sites in Europe^{2,4,5}. In spite of the low atmospheric pollen count, the incidence of pollen allergies (asthma, allergic rhinitis and allergic conjunctivitis) caused by *Artemisia* pollen ranges between 3% and 10% of the overall prevalence of pollinosis in some European countries. *Artemisia* is an important cause of sensitisation and allergy in Germany⁶, in Italy and France with sensitisation prevalence increasing^{7–9}, Poland^{10,11}, south Hungary¹² and in Switzerland^{13,14}. *Artemisia* pollen sen-

sitisation has also been reported from Sweden and Finland, but during 12-year period, showed a decrease, as compared to other pollen allergens¹⁵.

The first research into allergenic pollen distribution in Croatia was launched in 1959 at four locations: Zagreb, Hvar, Crikvenica and Dubrovnik¹⁶. Using gravimetric method, data were collected weekly throughout the year. Since 1973, allergenic pollen has been continuously studied in Zagreb¹⁷. Modern aerobiologic investigations using daily volumetric monitoring of atmospheric pollen began in Croatia in 2002¹⁸.

The aim of the study was to determine the length of pollen season, intradiurnal, daily and monthly pollen variation, effect of some meteorological parameters on atmospheric pollen concentration, and possible variation in the occurrence and concentration of *Artemisia* pollen at three monitoring sites in Central Croatia over 2002–2003. Results of this aeropalynological study are expected to contribute to the prevention of allergic symptoms in individuals with *Artemisia* pollen hypersensitivity, thus improving their quality of life.

Material and Methods

Monitoring sites

This study was conducted in 3 localities of Central Croatia from January 5, 2002 to December 20, 2003. The samplers were placed in Zagreb (45° 49' N and 15° 59' E, 157 m above the sea level and 19.7 m above the ground level), in Ivanić Grad (45° 43' N and 16° 24' E, 101 m above the sea level and 18.5 m above the ground level), and in Samobor (45° 48' N and 15° 43' E, 168 m above the sea level and 17.3 m above the ground level). The distance between recording sites is about 30 km (Figure 1). All monitoring sites have a continental climate (Table 1). The meteorological data for each location were provided by different stations of the Croatian Weather Bureau (Zagreb-Grič, Čazma and Samobor).



Fig. 1. Monitoring sites in central Croatia.

Pollen sampling and counts

A 7-day Hirst volumetric pollen and spore traps were used for pollen sampling¹⁹. The sampler absorbs 10 L air per minute, allowing determination of pollen concentration at 2-hour intervals (for practical sampling details see^{20,21}). It is supplied with a clock-work driven drum which moves adhesive tape (2 mm/h) for pollen grains to stick to. The tape was cut to a length corresponding to 24-hour pollen sampling, applied onto a glass slide and embedded in the following medium: 70 g polyvinyl alcohol (Gelvatol) and 4 g phenol C₆H₆O, and dissolved in 200

mL of distilled water. After overnight rest, 100 mL glycerol C₃H₈O₃ were added and warmed up in water bath until the solution was clarified. Then, 4 drops of alcohol solution of basic fuchsin C₂₀H₂₀CIN₃ per 100 mL were added. Recognition and counting of pollen grains was performed under light microscope (400x). Pollen grain counts were expressed as pollen grains per cubic meter of air^{22,23}. Pollen data set in relation to the meteorological parameters (mean air temperature and precipitations) was tested with Spearman's rank test.

Results

In 2002, *Artemisia* pollen grains initially occurred in the second half of July at all monitoring sites (Zagreb: July 20, Samobor: July 19, and Ivanić Grad: July 21). Peak daily pollen concentrations were recorded from August 9 to August 16 at different monitoring sites. A high concentration of *Artemisia* pollen (72 pollen grains/m³ air per day) was only recorded on a single day at the Zagreb monitoring site (August 9), whereas low to moderate concentrations were measured on all other days of observation. At other monitoring sites, air concentration of *Artemisia* pollen grains did not exceed moderate levels during the 2002 pollen season. The percentage of *Artemisia* pollen in the total annual atmospheric pollen concentrations was low at all monitoring sites, ranging from 0.52% in Ivanić Grad to 0.92% in Zagreb. In 2003, the pollen season started earlier than the previous year in Zagreb, whereas at the other two sites it was delayed by ten days (Zagreb: July 14, Samobor: July 27, and Ivanić Grad: August 2). However, total *Artemisia* pollen count exceeded that of the previous year's, despite the shorter pollen season. This did not apply for the Zagreb, where an inverse pattern was observed. The peak daily concentration did not exceed moderate levels at any of the monitoring sites (Table 2).

At all monitoring sites, *Artemisia* pollen concentrations were significantly influenced by temperature and precipitation. Concerning weather conditions, the *Artemisia* pollination months in 2002 were warm and wet, with average temperature and total precipitation (July 21.7–23 °C, 60.9–148.2 mm; August 21–23 °C, 128.5–151.0 mm), whereas a temperature decline and increased precipitation were recorded in the last ten days of September (15.6–17 °C, 67.5–94.9 mm). The number of pre-

TABLE 1
MEAN YEARLY AIR TEMPERATURES AND PRECIPITATIONS COMPARED TO THE LONG-TERM MEANS

Meteorological station	Temperature (°C)			Precipitation (mm)		
	Long-term average (1961–1990)	2003	2004	Long-term average (1961–1990)	2003	2004
Zagreb	11.2	12.9	11.2	882.8	624.4	918.4
Samobor*	10.4	11.7	11.2	1181.9	728.4	1044.6
Čazma	10.5	11.4	10.9	880.3	692.0	972.0

* long-term average for Samobor (1972–1990)

TABLE 2
DATA CONCERNING THE PRESENCE OF *ARTEMISIA* POLLEN ON THREE MONITORING SITES IN CROATIA, 2002–2003.

Site	Year	Period of occurrence	Peak day	Peak day concentration	Total pollen	<i>Artemisia</i> pollen	% of <i>Artemisia</i> pollen in total pollen
Zagreb	2002	July 20 th – September 28 th	August 9 th	72	71,286	667	0.92
	2003	July 14 th – September 29 th	August 10 th	50	72,556	506	0.69
Samobor	2002	July 19 th – September 30 th	August 11 th	28	50,112	294	0.58
	2003	July 27 th – September 30 th	August 10 th	30	52,996	345	0.65
Ivanić Grad	2002	July 21 th – September 28 th	August 16 th	39	70,698	374	0.52
	2003	August 2 nd – September 28 th	August 11 th	48	76,254	474	0.62

precipitation days during these three months ranged from 33 to 45 at the three monitoring sites. Temperature decline was recorded on rainy days and was associated with a decrease in daily pollen concentration at all three monitoring sites (Figure 2). In 2003, the *Artemisia* pollination months were very warm and dry (July: 22.2–23.8 °C, 53.5–78.6 mm; August: 22.2–26.2 °C, 17.4–57.4 mm), followed by temperature decline and precipitation increase

toring sites (Figure 2). In 2003, the *Artemisia* pollination months were very warm and dry (July: 22.2–23.8 °C, 53.5–78.6 mm; August: 22.2–26.2 °C, 17.4–57.4 mm), followed by temperature decline and precipitation increase

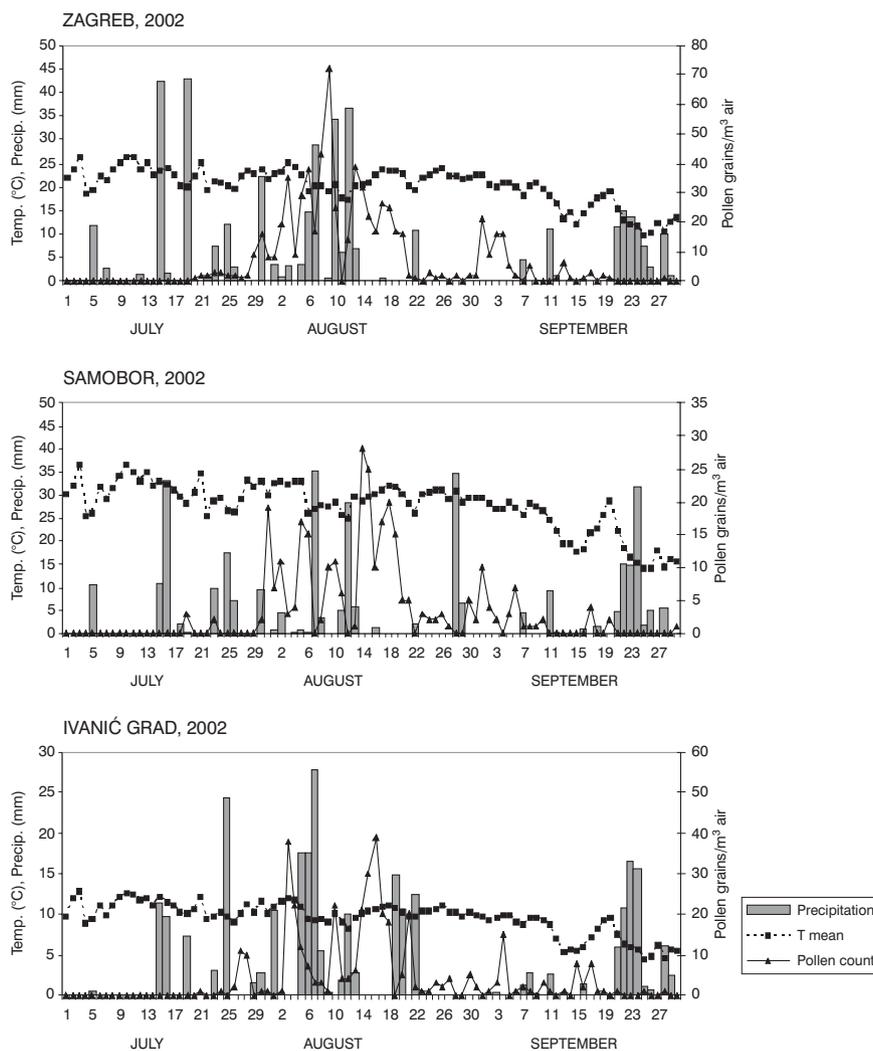


Fig. 2. Daily variations in *Artemisia* pollen concentrations, temperature (Temp.) and precipitations (Precip.) in three different sites in Central Croatia over 2002.

in September (16.1–17.2 °C, 60.7–106.6 mm). The number of rainy days was lower than the year before, ranging

from 29 to 33 (Figure 3). Finally, the *Artemisia* pollen concentrations showed a statistically significant correla-

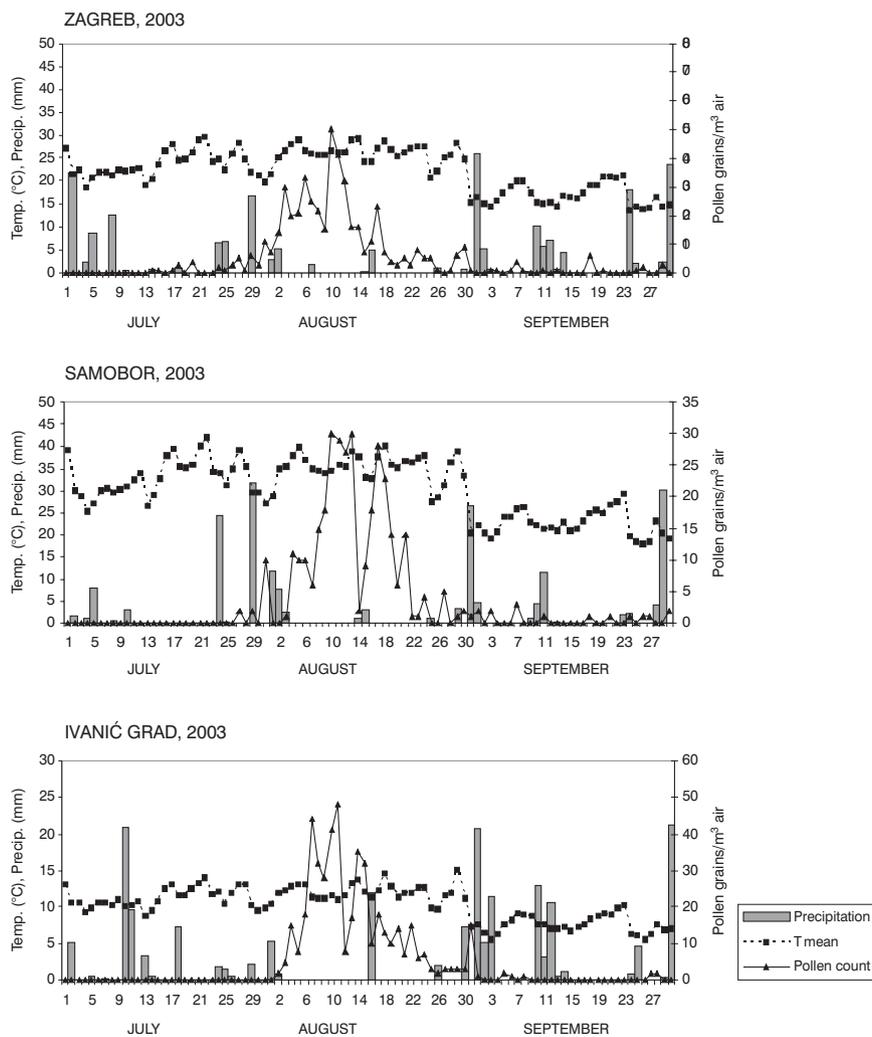


Fig. 3. Daily variations in *Artemisia* pollen concentrations, temperature (Temp.) and precipitations (Precip.) in three different sites in Central Croatia over 2003.

TABLE 3.
SUMMARIZED RESULTS OF THE STATISTICAL ANALYSIS POLLEN DATA IN RELATION TO THE METEOROLOGICAL PARAMETERS

Pair of variables	Spearman rank order correlations			
	Valid N	Spearman rank	t(N-2)	p-level
Temperature and pollen				
Zagreb	62	0.471838	4.11060	0.000124*
Samobor	62	0.099544	0.76843	0.445298
Ivanić Grad	62	0.414697	3.50054	0.000891*
Precipitation and pollen				
Zagreb	62	-0.429622	-3.65443	0.000550*
Samobor	62	-0.462305	-4.00468	0.000176*
Ivanić Grad	62	-0.282265	-2.26002	0.027526*

Marked values (*) are statistically significant ($p < 0.05$), pair of variables – variables in correlation, Valid N – valid number of variables, t(N-2) – t distribution on N-2 degrees of freedom, p-level – probability level

tion with temperature, however, no such correlation was found in Samobor. In both seasons at all monitoring sites there was a statistically significant negative correlation with precipitation (Table 3.)

Intradiurnal pollen distribution was almost identical in 2002 and 2003 at all three monitoring sites. A slight decrease in pollen concentration was recorded between 2 and 4 a.m. relative to 0–2 a.m., and an abrupt increase after 8 a.m. Peak concentrations were recorded at 10–12 a.m., followed by a decline by the end of the day. A major difference in the peak pollen concentration was only recorded at the Zagreb monitoring site, where a total of 181 pollen grains were found between 10 and 12 a.m. throughout the 2002 pollen season (July, August and September), whereas a nearly half this concentration (95 pollen grains) was observed in 2003 (Figure 4).

Discussion

In spite of the widespread and common occurrence of *Artemisia* plants, their airborne pollen concentrations have hardly ever been reported to reach high levels. In Central Croatia, a high concentration of *Artemisia* pollen was recorded on a single day at one of the three sampling sites in 2002. At the other two sampling sites, the concentration did not exceed 50 pollen grains per m³ in either 2002 or 2003. The percentage of *Artemisia* pollen in the total annual pollen count was low, ranging from 0.52% to 0.92%, which is consistent with the lower Euro-

pean sample limit of 0.5% to 5%^{24–26}. In Central Croatia, the *Artemisia* pollen season starts in the end of July and terminates towards the end of September. Major concentrations of *Artemisia* pollen grains were recorded in August. A similar pattern has also been reported from other European countries^{2,3,25–27}. In Croatia, the pollen season was somewhat prolonged in the two study years. Intradiurnal pollen distribution at the three monitoring sites showed peak concentrations between 10 and 12 a.m. in both 2002 and 2003. The timing of peak *Artemisia* pollen concentration depends on the sampling height, because of different wind speed²⁸. Generally, *Artemisia* releases its pollen in the early morning hours, before the air turbulence convection preventing pollen grains from being lifted up to atmospheric layers higher than 3–10 m, which is why this production peak is not recorded by pollen traps located on the building roof tops, their usual positions. When later in the day insolation at the Earth surface causes air turbulence, the *Artemisia* pollen has already deposited on the available substrates, while hardly any further pollen release occurs. As pollen trap located close to the ground level will measure the early morning peak, leading to the observation of higher daily airborne concentrations and a distinct diurnal pattern²⁹. However this phenomenon is sometimes much more complex: influence of topography and regional vegetation. The pollen released to the atmosphere is mostly influenced by temperature and precipitation^{30–32}. Statistical analysis showed that mean temperature and precipi-

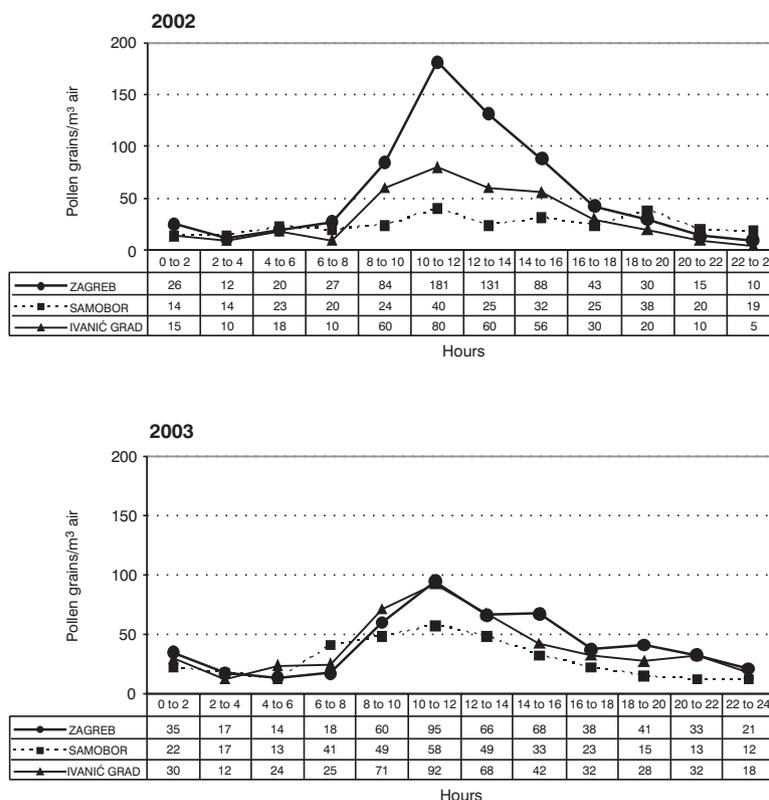


Fig. 4. Intradiurnal variations of *Artemisia* pollen concentrations in Central Croatia, July-September 2002 and 2003.

pitation are parameters that yealds highest correlation with *Artemisia* pollen in the atmosphere. On rainy days, the pollen concentration decreased to the minimum, as indicated by our results.

Daily concentration variation is usually useful for published pollen reports prophylaxis of allergenic individuals, because whenever possible they could plan their activities and by choosing the time and place of holidays they can avoid exposure to large doses of the allergen.

Data of intradiurnal concentration variation are the most important for establishing relations between pollen counts and local meteorology. These conclusions have been made on the basis of two years investigation; future studies over a longer study period will be necessary to gain a clearer insight into the relationship between weather parameters and airborne pollen concentrations in the air.

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VARIJACIJE KONCENTRACIJA PELUDA PELINA (*ARTEMISIA* Spp.) U ZRAKU SREDIŠNJE HRVATSKE, 2002–2003

SAŽETAK

Usprkos niskim koncentracijama peluda *Artemisia* u zraku, alergije na tu vrstu peluda široko su rasprostranjene. Cilj rada bio je odrediti duljinu peludne sezone, intradiurnalne, dnevne i mjesečne varijacije koncentracije peluda *Artemisia* u zraku središnje Hrvatske. Za uzorkovanje se koristio sedmodnevni volumetrijski uzorkivač za pelud i spore, Hirst-ovog tipa. Sezona pojavljivanja peluda *Artemisia* u zraku traje od kraja srpnja, do kraja rujna, sa najvišim koncentracijama u mjesecu kolovozu. Postotni udio peluda *Artemisia* ima raspon od 0.52% do 0.92% od ukupnog godišnjeg broja peluda svih ostalih taksona. Najviše intradiurnalne koncentracije zabilježene su između 10:00 i 12:00 sati. Statističkom analizom dobivene su pozitivne signifikantne korelacije peluda s temperaturama zraka te negativne s količinama padalina.

Analysis of the Feeding Sites for some Horse Flies (*Diptera, Tabanidae*) on a Human in Croatia

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ABSTRACT

*The landing patterns of horse flies on the human body were observed in Croatia. A total of 386 horse flies belonging to 22 species were sampled. The five most commonly collected species were used in the analysis. The stochastic linear connection is tight among the landings of the species *Tabanus bromius*, *Tabanus maculicornis*, *Tabanus tergustinus*, and *Philipomyia graeca* on the human body regions (matrix *R*). The preferred feeding area for these four species was the lower leg, whereas for the species *Haematopota pluvialis* it was the head and neck. Of the total number of horse flies that landed 44.81% were on the lower leg. Only 0.26% landed on the forearm. Chi-square analysis indicated non random landing patterns on human by these horse flies.*

Key words: Diptera, Tabanidae, human, feeding sites, Croatia

Introduction

Many different species of Diptera are implicated in mechanical transmission of diseases agents, but haematophagous species are the most important^{1,2}. The Tabanidae are considered to be among the major Dipteran pests of human and animals worldwide³. Tabanids are known as important mechanical vectors of viruses, bacteria, protozoans and helminths, which cause diseases of wild and domestic animals, and also of humans^{3,4-8}. These flies cause a painful bite owing to their blood feeding habit, and in many areas of the world they are very abundant during the summer season, when people visit outdoor recreation areas. To the host, a feeding fly results in annoyance and loss of blood at best, and significant disease risk and, ultimately, death at worst⁹. To minimize these risks people have developed many mechanisms to prevent or mitigate attack by horse flies. Many faunistic and ecologic studies of horse flies were performed in the world during the last 50 years. However, there is no information on the feeding sites on humans for this important group of nuisance insects. The present paper provides new data, which expand the knowledge about the feeding sites of horse flies on the human body in Croatia.

Material and Methods

Horse flies (*Tabanidae*) were collected between 1995 and 2005 at 29 localities in Croatia. No field trips were made particularly for the collecting of horse flies landing on humans. Specimens of horse flies were hand picked from volunteers during the various fieldworks. Collected horse flies were stored in the field in 70% alcohol, separately with respect to the landing places on the human, collection locality, and date. We have divided the surface of the human body into 8 areas (Figure 1). Absolute and relative frequencies of horse flies arriving on different parts of the human body, specified as: back (B=E₁ variable), chest (C=E₂ variable), forearm (F=E₃ variable), head (H=E₄ variable), lower leg (L=E₅ variable), neck (N=E₆ variable), upper leg (R=E₇ variable), upper arm (U=E₈ variable), were recorded. Species identification was made according to Chvála et al. (1972)¹⁰. In the analysis of the acquired data, the probability statistical method and Chi-square test was applied¹¹. All the collected horse flies are deposited in the insect collections of the JJ Strossmayer University Department of Biology, Osijek.

TABLE 2
FREQUENCIES OF HORSE FLIES (TABANIDAE) THE HUMAN BODY

Ordinal number	Body part (E _i)	Absolute frequencies f _i	Cumulative frequency below	Cumulative frequency above	Relative frequency p _i
1	E ₁	17	17	386	0.0440
2	E ₂	4	21	369	0.0103
3	E ₃	1	22	365	0.0026
4	E ₄	100	122	364	0.2590
5	E ₅	173	295	264	0.4481
6	E ₆	63	358	91	0.1632
7	E ₇	22	380	28	0.0569
8	E ₈	6	386	6	0.0155
		386			0.9996

E₁ – back, E₂ – chest, E₃ – forearm, E₄ – head, E₅ – lower leg, E₆ – neck, E₇ – upper leg, E₈ – upper arm

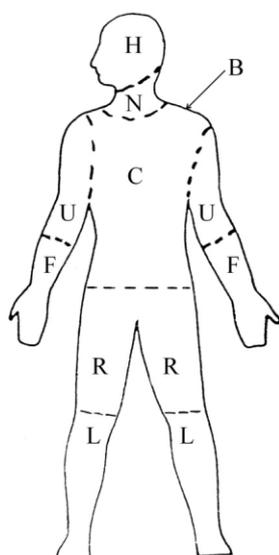


Fig. 1. Parts of the human body on which horse flies take their blood meal. B – back, C – chest, F – forearm, H – head, L – lower leg, N – neck, R – upper leg, U – upper arm.

With regard to the value of elements r_{ij} ($i \neq j$) of the correlation matrix, we can observe: that gradual landings of pairs (T₁, T₂), (T₁, T₃), (T₁, T₅), (T₂, T₃), (T₂, T₅) and (T₃, T₅) species of horse flies are manifested unidirectionally, whereas other pairs do not manifest themselves unidirectionally. The stochastic linear connection between landings of horse flies in pairs (T₁, T₄), (T₂, T₄), (T₃, T₄), (T₄, T₅) is entirely insignificant. Just the opposite, between landing pairs (T₁, T₂), (T₁, T₃), (T₁, T₅), (T₂, T₅), (T₃, T₅) is very tight and between landings (T₂, T₃) it is identical, because the species *T. bromius*, *T. maculicornis*, *T. tergestinus* and *P. graeca* mostly landed on the lower leg whereas the species *H. pluvialis* mostly landed on the head and neck body regions. The χ^2 analysis of landings by these horse flies on respective parts of the human bodies showed that horse flies landing patterns significantly differ among parts of the human body: *T. bromius* ($\chi^2=199.91$ p<0.05), *T. maculicornis* ($\chi^2=464.79$

TABLE 3
THE MORE NUMEROUS SPECIES OF HORSE FLIES
TAKING BLOOD MEAL

Ordinal Number	Body part (E _i)	T ₁	T ₂	T ₃	T ₄	T ₅
1	E ₁	–	–	–	–	–
2	E ₂	–	–	–	3	–
3	E ₃	–	–	–	1	–
4	E ₄	–	–	–	88	–
5	E ₅	32	66	30	–	37
6	E ₆	–	–	–	46	–
7	E ₇	3	–	–	4	1
8	E ₈	–	–	–	2	–
	Σ	35	66	30	144	38

E₁ – back, E₂ – chest, E₃ – forearm, E₄ – head, E₅ – lower leg, E₆ – neck, E₇ – upper leg, E₈ – upper arm, T₁ – *T. bromius*, T₂ – *T. maculicornis*, T₃ – *T. tergestinus*, T₄ – *H. pluvialis*, T₅ – *P. graeca*

p<0.05), *T. tergestinus* ($\chi^2=212.80$ p<0.05), *P. graeca* ($\chi^2=253.11$ p<0.05), *H. pluvialis* ($\chi^2=405.30$ p<0.05). Horse flies were distributed unequally among the 8 body regions. In general, lower leg was preferred compared with the others body regions.

Discussion

The presence of 22 species of horse flies on the human body has been documented. Five species comprised 81.08% of horse flies sampled on human bodies. It has been determined by testing, although it is immediately visible, that the differences in landing of the same species according to parts of the bodies of human are very significant. Proportions of landings of horse flies according to species on different body parts are not equal. This character is not accidental; horse flies significantly distinguish between parts of the bodies of human. During the research period, the largest number of horse flies landed on the lower leg. Unfortunately, we cannot comparing our results obtained from human bodies with others, be-

cause there are no data from similar studies. However, some authors have reported that horse flies also significantly distinguish between particular parts of the bodies of domestic animals^{12,13-16}. Comparative studies on the landing site preferences of mosquito species showed that olfactory cues emanating from particular areas of the human body enhance landing on those areas for the specialised species, whereas generalists feeders land at random¹⁷. De Jong and Knols¹⁷ also learned that out of the eight species of mosquitoes they studied, five of them prefer receiving blood meals at the head and upper torso¹⁷. Of these five, three preferred biting the face directly, as did *H. pluvialis* in our research. Their study suggests that carbon dioxide is very powerful attractant and it tends to attract mosquitoes to the area of the body that emits the largest amount of compound. Removal of the carbon dioxide from human breath decreases attraction to the host, but the catch of tabanids increased 100 fold when traps were baited with carbon dioxide¹⁸⁻²⁰. Furthermore, studies on mosquitoes showed that lactic

acid and sweat enhanced the number of landings in the presence of carbon dioxide on the human hand and legs²¹. Our study showed that horse flies are highly selective for landing sites on humans. Data on landing sites of tabanids on the human bodies are very scarce. Because of this direct comparisons between our data and data of other investigators are not possible. Despite the fact that studies of vector ecology are essential to understand predict and control insect-borne diseases, relatively few studies have been conducted about blood feeding by tabanids on humans. This points out the necessity of further research.

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ANALIZA MJESTA ISHRANE NEKIH VRSTA OBADA (DIPTERA, TABANIDAE) NA LJUDIMA U HRVATSKOJ

SAŽETAK

Obavljena su istraživanja mjesta ishrane obada (*Tabanidae*) na ljudima u Hrvatskoj. Uzorkovano je ukupno 386 obada svrstanih u 22 vrste. Analizirano je pet najbrojnijih vrsta obada. Stohastička linearna veza između nadolazaka vrsta *Tabanus bromius* L., 1758, *Tabanus maculicornis* Zetterstedt, 1842, *Tabanus tergstinus* Egger, 1859 and *Philiopomyia graeca* (Fabricius, 1794) vrlo je tijesna jer slijeću na iste djelove tijela čovjeka (matrica R). Izbor mjesta hranjenja odnosno uzimanja krvnog obroka ovih četiriju vrsta je potkoljenica, dok je za vrstu *Haematopota pluvialis* (L., 1758) glava i vrat. Od ukupnog broja pristiglih obada 44.81% skupljeno je na potkoljenici. Jedino 0.26% od ukupnog broja obada slijeće na podlakticu. *Hi*-kvadrat test pokazuje da ove vrste obada ne slijeću nasumce na tijelo čovjeka, već signifikantno razlikuju pojedine dijelove tijela čovjeka.

Hirschsprung's Disease and Rehbein's Procedure – Our Results in the Last 30 Years

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ABSTRACT

Hirschsprung's disease is congenital anomaly of the intestine and Harald Hirschsprung gave the first description of this disease¹. The aim of this follow-up study was to evaluate the results of Rehbein's procedure in the treatment of Hirschsprung's disease in the last 30 years in Children's Hospital Zagreb. Hirschsprung's disease is congenital intestinal aganglionosis as the results of arrested fetal development of the myenteric nervous system. Hirschsprung's disease is affecting between 1:5000 to 1:8000 live births. A total of 124 children underwent Rehbein's lower anterior resection at Children's Hospital Zagreb. The principle of Rehbein's procedure is to remove aganglionic narrow segment and dilated sigmoid colon and anastomosis between normal intestine with rectal stump. The postoperative outcome was analysed for early and late complications like wound infections, abscesses, anastomotic insufficiency, postoperative enterocolitis, constipation, fecal incontinence, need for reoperation, ileus and mortality. On the basis of our results and data from literature we concluded that Rehbein's procedure is an excellent method for treatment Hirschsprung's disease.

Key words: Hirschsprung's disease, Rehbein's procedure, children

Introduction

Normal intestinal motility depends on a coordinated segmental contraction wave immediately preceded by smooth muscle relaxation as it propagates caudally. Patients with Hirschsprung's disease lack functional myenteric nervous system in the affected distal intestine and have ineffective distal peristalsis^{2,3}. The clinical outcome are failure to pass meconium shortly after birth, failure to pass the first stool within 24 to 48 hours after birth, constipation, abdominal distension, palpable loops of bowel, vomiting, watery diarrhoea in the newborn, poor weight gain, slow growth and malabsorption. The aganglionic distal segment of the bowel is reason for dilatation of the proximal part of the colon or opening debility of the anal sphincter system^{4,5}.

The Rehbein's procedure was developed in 1953 by Fritz Rehbein, Germany⁶. This operative treatment offers few advantages: 1. No risk of damages autonomic nerve plexus of the small pelvis which supplies bladder, urethra, genital organs and the rectum. 2. Avoidance of incontinence. 3. Technical easy operation 4. Extraperitoneal location of the anastomosis⁷.

The purpose of the present study was to present our results of treatment of Hirschsprung's disease with Rehbein's procedure.

Materials and Methods

The diagnosis of the Hirschsprung's disease was established on clinical signs, roentgen examination, anorectal manometry and rectal biopsy. In normal individuals, transient rectal distension causes relaxation of the internal anal sphincter. Anorectal manometry detects the absence of the relaxation reflex of the internal sphincter after the distension of the rectal lumen. In Hirschsprung's disease contraction occurs^{8,9}. RTG examination with gastrografin enema shows us a spasmotic distal intestinal segment with dilation of the proximal bowel. This spastic transitional segment may be best seen on the lateral view. Findings in neonates are difficult to interpret because it often fails this transition zone, which takes time to develop. A suggestive finding is the failure to evacuate barium from the colon within 24

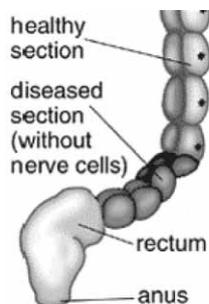


Fig. 1. Hirschsprung's disease.

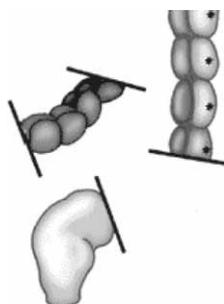


Fig. 2. - Removal of aganglionic intestine.

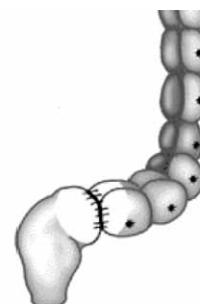


Fig. 3. End-to-end anastomosis (normal colon+rectal stump).

hours of the performance of the study. The definitive method for obtaining tissue for pathologic examination is by a full-thickness rectal biopsy. Also simple suction rectal biopsy has been used to obtain tissue for histologic examination. The histologic and histochemical examinations suggest the absence of intramural ganglionic cells (submucosa Meissner's plexus and myenteric Auerbach's plexus) and the presence of excess nonmyelinated nerves in the distal intestine in an adequate rectal biopsy establishes the diagnosis^{10,11}. The diagnosis of Hirschsprung's disease was confirmed during or after the operation. Rehbein's procedure removes aganglionic intestine (spasmodic part of intestine) with sigmoid colon and the dilated part of the intestine end-to-end anastomosis with rectal stump can be performed (Figures 1-3). The size of the rectal stump measures 3-4 cm in infants and small children and 5-7 cm in older children. A plastic tube was inserted through the anastomosis^{11,12}. In patients with large colonic enlargement of the proximal part of intestine a right transverse colostomy was the procedure of choice for decompression. When we make definitive procedure we close the colostomy^{13,14}.

From 1974 to 2004 a total of 124 children underwent Rehbein's procedure and were followed up. The follow up period was 1 month to 12 years after operative treatment.

Results and Discussion

In the last 30 years 124 patients underwent Rehbein's procedure for treatment of Hirschsprung's disease. 97 (78.2%) patients were male and 27 (21.8%) patients were female. The sex ratio male to female was 3.6 to 1¹⁵. The follow up period was 1 month to 12 years after operative treatment and the average period was 4.5 years. The mean age of the patients at the time of operation was 4 years and 7 months, the range was 1 year to 17.8 year. 118 (95.1%) of patients were younger of 5 years at the time of operation.

Incidence of early complications was 8.1% (10 patients) wound infections, 10.5% (13 patients) were anastomotic stenosis, and 2.4% (4 patients) suffered anastomotic insufficiency. Only one patient (0.8%) died from toxic enterocolitic (Table 1).

In 44.4% (55 patients) preoperative colostomy was performed. One year after colostomy Rehbein's procedure was done. During the Rehbein's procedure closure of the colostomy was done in 72.7% (40 patients). 15 patients had one more operative treatment for closure colostomy. The most common complications of colostomy are prolapse or strictures. We have had no colostomy complications.

71.7% (89 patients) had stool every day, 13.7% (17 patients) had stool every second or third day and 14.5% (18 patients) showed rare bowel movement. The late postoperative complications were constipation in 12.9% (16 patients), intermittent diarrhea in 15.4% (19 patients) and fecal incontinence in 5.7% (7 patients). In total of 124 Rehbein's procedures 46% (57 patients) had recurrent achalasia of internal sphincter muscle and these patients required sphincter and anastomosis dilatation. In 95% of patients dilatations were successful and only 5% of these patients needed postoperative sphincterotomy. Stenosis of the anastomosis had 6.5% (8 patients) and these patients needs reoperation and resection of these anastomosis. Only 1.6% (2 patients) had adhesive ileus.

In total, excellent results means bowel evacuation every day, without any complications. Excellent results oc-

TABLE 1
COMPLICATIONS AFTER REHBEIN'S PROCEDURE

	Patients	Percentage
Wound infection	10	8.1%
Anastomotic stenosis	13	10.5%
Anastomotic insufficiency	4	2.4%
Toxic enterocolitis	1	0.8%

TABLE 2
LATE RESULTS AFTER REHBEIN'S PROCEDURE

	Patients	Percentage
Excelent	75	60.5%
Good	38	30.6%
Poor	11	8.9%

curred in 60.5% (75 patients). Good results had 30.6% (38 patients) and good results mean occasional laxative or enema for daily stool. Poor results had 8.9% (11 patients) and it means fecal incontinence or laxatives and enema were given regularly for stool (Table 2).

Conclusion

The world surgical literature describes different surgical procedures for the definitive surgical treatment of aganglionosis or Hirschsprung's disease. Every method involves bringing normal bowel as low in the rectum as is technically possible by resecting or bypassing the aganglionic bowel¹⁶⁻¹⁸. Rehbein's procedure removes aganglionic intestine (spasmodic part of intestine) and the dilated part of the intestine end-to-end anastomosis with rectal stump can be performed. Rehbein's procedure does

not disturb the sphincter mechanism. Sigmoid colon was always resected because in this part of colon the water was absorbed. Without sigmoid colon stool was fluent and passed easily by through rectal stump. The size of the rectal stump measures 3-4 cm in infants and small children and 5-7 cm in older children. The technique is simple and can be used for treatment even in early infancy. We avoid preoperative colostomy when possible and make primary definitive operation. In our hospital we perform only Rehbein's procedure for treatment of Hirschsprung's disease. Due to the excellent and good results achieved in 91.1 % of the patients, we regard Rehbein's method excellent for the treatment of Hirschsprung's disease^{19,20}.

In the last year we performed a laparoscopic Duhamel pull-through method for Hirschsprung's disease. If it is not possible to perform a laparoscopic method we perform Rehbein's procedure²¹⁻²⁵.

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HIRSCHSPRUNGOVA BOLEST I REHBEINOVA METODA LIJEČENJA – NAŠI REZULTATI U ZADNJIH 30 GODINA

S A Ž E T A K

Hirschsprungova bolest je urođena anomalija crijeva, a Harald Hirschsprung je prvi opisao tu bolest¹. Svrha ovog rada je evaluacija rezultata operacije Hirschsprungove bolesti po metodi Rehbein u zadnjih 30 godina u Klinici za dječje bolesti Zagreb. Hirschsprungova bolest je urođena bolest crijeva koja nastaje radi zastoja u fetalnom razvoju myenteričkog nervnog sustava. Sama bolest zahvaća jedno od 5,000 do 8,000 živorođene djece. Ukupno je operirano 124 djece po metodi Rehbein u našoj bolnici. Osnovna zamisao ovog operativnog zahvata je odstranjenje uskog dijela crijeva bez ganglijskih stanica zajedno sa sigmoidom te spajanje zdravog crijeva sa ostatkom rektuma. Postoperativni rezultati su evaluirani kao rane i kasne komplikacije i to kao upala rane, gnojni procesi u trbuhu, slabost ili suženje učinjene anastomoze, postoperativni učestali proljev ili zatvor, nemogućnost kontrole stolice, potreba za ponovnom operacijom, ileus te smrtni ishod liječenja. Na osnovu naših rezultata i podataka iz literature možemo zaključiti da je metoda po Rehbeinu odlična za liječenje Hirschsprungove bolesti.

Eighteen Years Of Heart Transplantation – A Single Center Experience

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ABSTRACT

The best option for the treatment of a failing heart is heart transplantation. The transplantation program at the University Hospital Center Rebro Zagreb started in 1988. To the best of our knowledge this is the first retrospective study on cardiac transplantation in Croatia looking into survival following heart transplantation. Between 1988 and 2006, we performed 81 heart transplantations at the University Hospital Center Rebro Zagreb. Our study focused on the last ten years after establishment of the Department of cardiac surgery as a separate institution. There were thirteen different hospitals throughout Croatia, which contributed to the donor network. Average age of the heart recipient was 48 ± 11.8 years (range 14–72), and average age of the heart donor was 34 ± 10.7 years (range 14–56). There were more women among the heart donors (34%) than among the heart recipients (18%). During the first ten years, from 1988–1998, the average number of cardiac transplantations was 3 per year. In the period from 1998–2006, average number of cardiac transplantations increased to 6 per year. The average thirty-day mortality for the last nine years was 27%. It declined from 30% and 40% in 1998 and 1999, respectively down to 0% in the last two years. Average age of the patients who died was 50 ± 6.5 years (range 44–62) and did not significantly differ from those who survived. The donor network has grown up to fourteen different hospitals throughout Croatia. The limiting factor in cardiac transplant surgery is the number of available donors. Therefore in attempt to form a good transplant program it is crucial to form an efficient donor network. The number of performed cardiac transplantations is expected to rise until it reaches the number of available donors. With advances in operative technique and postoperative management – immunosuppressive therapy we have observed a remarkable drop in the early operative mortality in the studied period.

Key words: heart transplantation, living donor, immunosuppression, mortality

Introduction

The treatment of heart failure utilizes a substantial proportion of funds in health care systems of developed countries. According to Cowie, 1999, a crude prevalence is 3 to 20 per 1000 in the general population¹ with constantly increasing incidence. Heart failure has a substantial risk of death, which is around 66% for men and 50% for women in the period of five years^{2,3}. Heart failure is one of the most common reasons for hospital admissions in the elderly. Readmission is quite common even within 6 months. EuroHeart failure survey showed that suspected or confirmed heart failure caused 20% of readmissions⁴.

The heart transplant surgery begun with French surgeon Alexis Carrel, who performed the first heterotopic canine heart transplant at the very beginning of the 20th century^{5,6}. The era of human heart transplantation started with Christian Barnard who performed the first successful human to human heart transplantation in South Africa in 1967⁷. Shumway and his colleagues at the Stanford University revitalized cardiac transplantation in the late 1970s. Josip Sokolic performed the first successful human to human heart transplantation in Croatia in 1988 at the University Hospital Center Rebro Zagreb. Introduction of transvenous endomyocardial biopsy as effi-

cient means of monitoring allograft rejection and recent progress in immunosuppression and infection control has transformed cardiac transplantation and marked the beginning of the modern era of successful cardiac transplantation. Cardiac transplantation is widely accepted as a therapeutic procedure of choice for patients with end-stage heart failure. Ventricular assisting devices are now additional efficient means of treatment of heart failure used as a destination therapy or as a bridge to transplantation.

Patients with ischemic heart disease or idiopathic dilated cardiomyopathy that is not amenable to medical or surgical therapy comprise the group of candidates for heart transplantation. Those are patients with left ventricular ejection fraction lower than 20%, with high pulmonary capillary wedge pressure classified in New York Heart Association class III or IV with^{8–10}. Oxygen consumption MVO₂ should be equal or less than 14 L/min/kg.

Expected 1-year survival in the group of patients that are considered to meet the above mentioned criteria for heart transplantation is less than 50% without transplantation^{11–17}.

Heart transplantation has its contraindications. Major concern in the early postoperative period is the function of the right ventricle. There are several reasons for such concern. First, depending on the method of delivery, cardioplegia reaches right heart chambers less efficiently than the left ones. This is often times a cause of myocardial stunning and reperfusion injury. On top of that, given usual long duration of heart failure before patients meet criteria for transplantation, recipients often times have some degree of pulmonary vascular hypertension and increased pulmonary vascular resistance, which both put the right heart under a higher strain and can both precipitate right heart failure. Fixed pulmonary vascular resistance greater than six Wood units is an absolute contraindication for heart transplantation and those patients should undergo heart-lung transplantation if possible^{18–25}. General contraindications for cardiac transplantation are active infection, renal or hepatic dysfunction, chronic lung disease, severe peripheral atherosclerotic vascular disease, severe systemic connective tissue disorder (SLE), sarcoidosis and malignancy to mention some of the most common ones. End-organ damage such as nephropathy or retinopathy in diabetes is contraindication for heart transplantation^{26,27}. The upper age limit, as a cut off for heart transplant, is currently defined as age > 70 years. Older patients tend to have more advanced systemic diseases and somewhat higher rate of complications after surgery. However the quality of life after transplantation and survival is comparable to the younger ones.

Patients and Methods

We performed first retrospective study on cardiac transplantation in Croatia since it first began in 1988. There were total of 81 human-to-human cardiac transplantations performed at the University Hospital Center Rebro Zagreb, but our database covered only the period

of last 9 years. During that period of time we performed fifty-one heart transplantations. Technique that we used was orthotopic heart transplantation with no heterotopic transplantations. Average age of the heart recipient was 48 ± 11.8 years (range 14–72), and average age of the heart donor was 34 ± 10.7 years (range 14–56). There were more women among the heart donors (34%) compared to the heart recipients group (18%). There were no heart re-transplantations and none of heart recipients has undergone major cardiac surgery such as valve replacement or aortocoronary bypass after successful cardiac transplantation.

Immunosuppressive protocol at our institution included combination of corticosteroids, cyclosporine, and azathioprine. Azathioprine was later on excluded from the protocol and replaced with mycophenolate mofetil due to its potential toxicity. We titrated cyclosporine to achieve a serum level between 100 and 300 ng/mL. Corticosteroids were first line therapy for acute rejection. Recently we included polyclonal anti-T-cell preparations (ATG) in the early postoperative period. In our current immunosuppressive protocol ATG is replaced with cyclosporine after 3 days. Corticosteroid regimen started with solumedrol at 1.5 mg/kg four times per day. By the postoperative day seven solumedrol was replaced with prednisone starting at 50mg twice a day and further titrated depending on the level of rejection. The bicaval heart transplantation technique as we perform it is shown in Figure 1a–d.

Results

We observed an average number of 3 heart transplantations per year till 1998. After the Department of the cardiac surgery was formed as a separate institution in 1998 average number of cardiac operations increased to 6 per year (Figure 2).

There were 51 cardiac transplantations in the last nine years compared to 30 during the first ten years.

First nineteen explantations were all performed at the University Hospital Center Rebro Zagreb. Ever since the first donor heart came from Rijeka in the year 1994, donor network has expanded to a total of fourteen Hospitals throughout Croatia (Table 1).

Average thirty-day mortality for the last nine years was 27%. It dropped from 30 and 40% in 1998 and 1999, respectively down to 10% in the last four years (table 2). Average age of the patients who died was 50 ± 6.5 years (44–62) and did not significantly differ from patients who survived ($p=0.066$ according to student T test).

Discussion

Long-term survival of the transplanted heart recipient is limited firstly by allograft rejection and side effects of immunosuppression. It was the advent of cyclosporine that significantly changed survival, marking the beginning of modern era of cardiac transplantation. Immu-

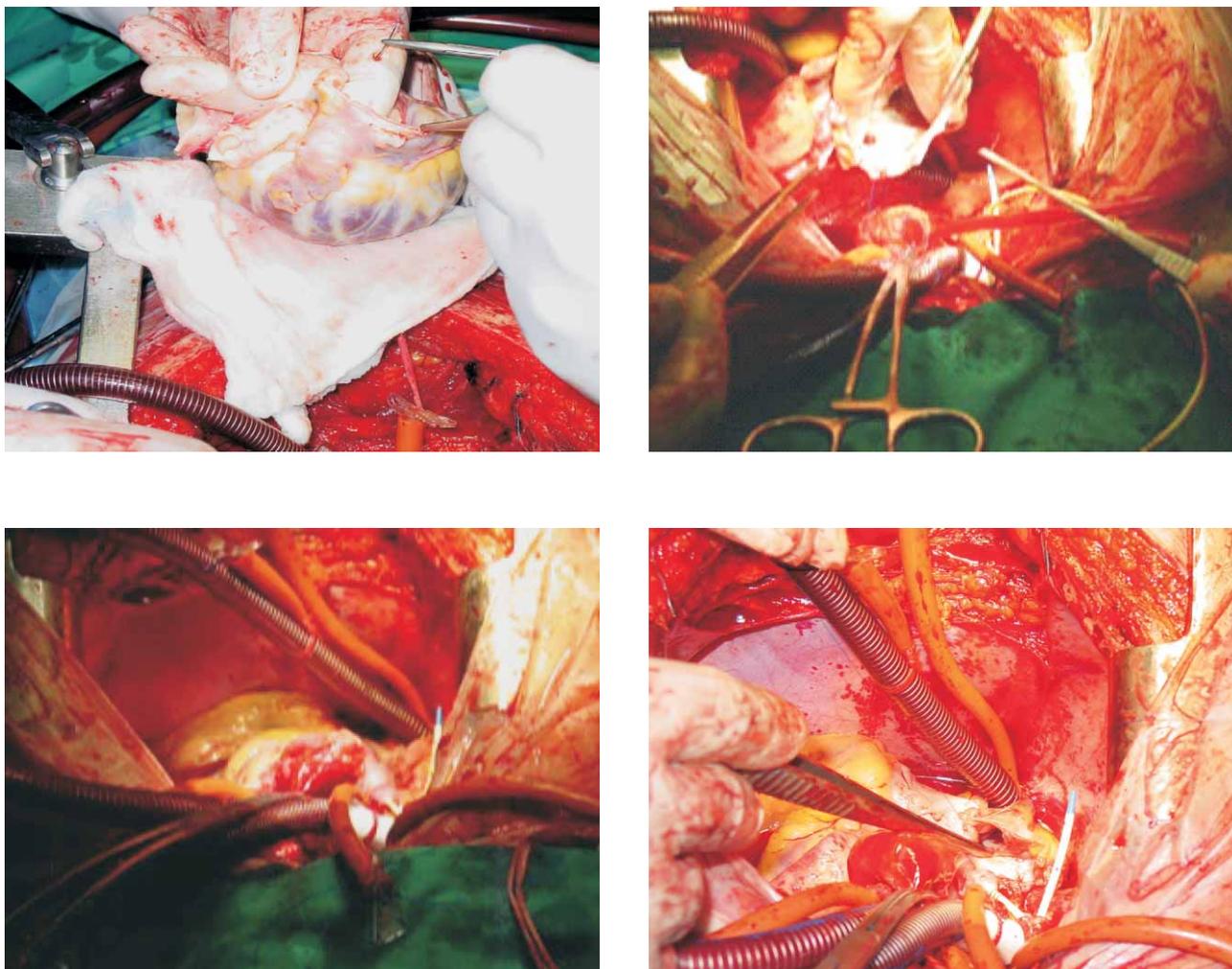


Fig. 1. Bicaval heart transplantation technique: a) first, left atrial anastomosis is created, b) followed by inferior vena cava and aorta (as shown on the figure). c) Transplantation is completed once pulmonary artery and finally d) superior vena cava have been created.

nosuppression is crucial to the success of cardiac transplantation, especially in the early induction phase. Most centers use triple immunosuppressive regimen with var-

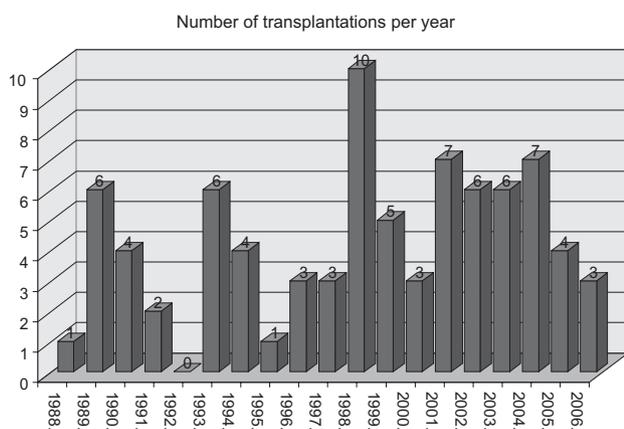


Fig. 2. Number of cardiac transplantations per year.

TABLE 1
THE DONOR NETWORK – CONTRIBUTION OF DIFFERENT HOSPITALS TO THE HEART TRANSPLANT PROGRAM

City	Number of donors	%
Zagreb	42	52.5
Split	6	7.5
Varazdin	6	7.5
Rijeka	5	6.3
Osijek	4	4.4
Dubrovnik	3	3.7
Pula	3	3.7
SI. Brod	2	2.5
Zadar	1	1.3
Sibenik	1	1.3
Karlovac	1	1.3
Cakovec	1	1.3
Koprivnica	1	1.3

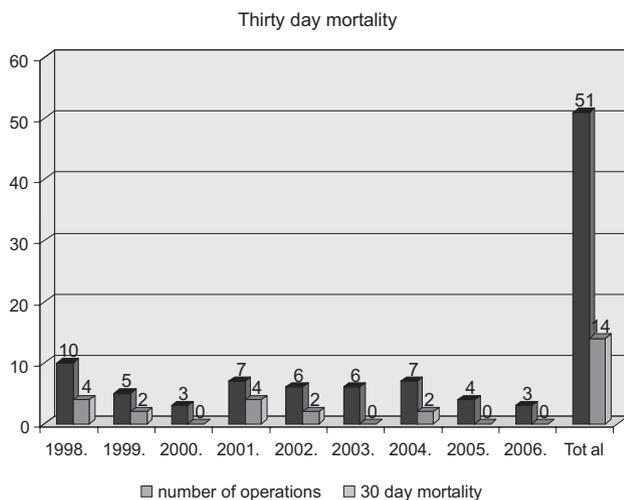


Fig 3. Thirty-day mortality following the heart transplantation.

ious dosages. In patients that develop complications associated with immunosuppressive agents some nonpharmacological immunosuppression may play a significant role. Radiation to lymphatic tissues provides several weeks of nonspecific immunosuppression. Peripheral mononuclear antibodies obtained via leukopheresis have a suppressor effect on T lymphocytes and they may reverse acute rejection. Plasma exchange may permit removal of circulating antibodies.

Operative (i.e., 30-day) mortality for cardiac transplantation ranges from 5 to 10%²⁸. We reported a higher 30-day mortality (average 27%), but it has been significantly reduced in the last four years to 10% and it is expected to further decline with larger series of patients. Average age of the heart recipient (48 years) in our group is still well below 65 years of age which is generally considered upper limit for cardiac transplantation eligibility. We expect that the average age of our patients rises. Some of our patients will need retransplantation and some will undergo valve replacement or aortocoronary bypass procedures given the natural course of coronary artery disease and valve degeneration.

The increased success of the cardiac transplantation resulted in more operations per year with the same number of available organs. Number of cardiac transplanta-

tions in Croatia is still below number of available donors. In most western countries number of donors is the limiting factor for cardiac transplantations. Xenograft transplantation may become an additional source of donor organs once xenograft rejection is resolved.

Critically ill patients are admitted to intensive care unit prior to the transplantation. Some may require aggressive pharmacologic support or even placement of an intra-aortic balloon pump is necessary^{29–35} to maintain adequate cardiac output and tissue perfusion prior to transplantation. Mechanical devices such as Ventricular assist devices (VAD) or total artificial hearts (TAH) may provide a bridge to transplantation³⁶. Patients with mechanical devices have improved survival and quality of life (REMATCH trial). Approximately 70% of patients are successfully bridged to transplantation³⁷.

Clinical outcome of heart transplantation has dramatically improved with potent immunosuppressive regimen resulting in lesser degree of rejection and improved survival. The success of cardiac transplantation should not be measured only by the survival of the patients, but also by their quality of life.

Conclusion

The number of cardiac transplantations per year is expected to rise until it reaches the number of available donors. With advancement in cardioplegic solutions, operative technique and immunosuppressive protocol we have observed a dramatic drop in the early operative mortality in the studied period. We have reached the point of learning curve where we cannot expect further dramatic decrease in mortality. Additional survival benefit may be seen after introduction of ventricular assisting devices as a mean of therapy at our institution. Development of a ventricular assisting device program will not eradicate the need for heart transplantations but has a well-established potential to postpone heart transplantation. The cut off time for heart ischemia for a transplanted heart is 4–6 hours; therefore we are emphasizing the importance of logistic support (hospital network, staff education, efficient transportation) to assure adequate graft preservation. Heart transplantation can be safely performed at our institution with acceptable mortality.

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OSAMNAEST GODINA ISKUSTVA U TRANSPLANTACIJI SRCA – ISKUSTVA JEDNOG CENTRA

S A Ž E T A K

Najbolji rezultati u liječenju srčanog zatajenja postižu se transplantacijom srca. Transplantacijski program na Kliničkom Bolničkom Centru Rebro Zagreb započeo je 1988. Prema našim spoznajama ovo je prva retrospektivna studija o transplantaciji srca u Hrvatskoj koja analizira preživljenje nakon transplantacije srca. Između 1988. i 2006. izvedena je 81 transplantacija srca na KBC Zagreb. Naša studija se fokusirala na posljednjih 10 godina nakon formiranja Klinike za kardijalnu kirurgiju kao zasebnog odjela. Trinaest različitih bolnica u Hrvatskoj sačinjava mrežu za donaciju organa. Prosječna dob primaoca bila je 48 ± 11.8 godina (raspon 14–72), a prosječna dob donora bila je 34 ± 10.7 godina (raspon 14–56). Žene su češće bile donori srca (34%), nego primaoci (18%). Tijekom prvih deset godina od 1988–1998 prosječno je bilo tri transplantacije srca godišnje. U razdoblju od 1998–2006 prosječan broj transplantacija srca porastao je na šest transplantacija godišnje. Prosječan 30 dnevni mortalitet za posljednjih devet godina iznosi 27%. Mortalitet je pao s 30% i 40% u 1998 i 1999 godini na 0% u posljednje dvije godine. Prosječna dob umrlih bolesnika iznosila je 50 ± 6.5 godina (raspon 44–62) i nije se statistički razlikovala od dobi preživjelih bolesnika. Mreža bolnica donatora povećala se na četrnaest bolnica diljem zemlje. Transplantacijski programi su limitirani brojem dostupnih donora. Stoga je za formiranje dobrog transplantacijskog programa ključno oformiti učinkovitu donorsku mrežu. Broj transplantacija očekivano raste dok ne dosegne broj dostupnih donora. Napredak u operacijskoj tehnici i poslijeoperacijskom tretmanu – imunosupresivnoj terapiji rezultirao je zamjetnim padom mortaliteta u ranom poslijeoperacijskom razdoblju.

Acute Coronary Syndrome Frequency in Western Herzegovina over the Fifteen Year Period (1987–2001)

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ABSTRACT

All patients who suffered from the acute coronary syndrome in western Herzegovina over the fifteen year period (1987–2001) are included in this retrospective epidemiological study. The population that was undertaken by the study is relative stable and did not emigrate during the war period. The study compared the time before the war (1987–1991), during the war (1992–1996) and after the war (1997–2001). The data were acquired from the archives of the patients of the Mostar hospital and Clinical hospital Split during the war period. A total of 2022 acute coronary syndrome patients were found, 1305 men and 717 women. More patients were treated during the war compared to the time before the war for both male and female patients ($p < 0.0005$). During the after-war period the number of treated patients was greater ($p < 0.0005$) compared to the war-time for both sexes. The comparison of the after-war period and the pre-war period reveals a statistically significant difference as the number of treated patients (male and female) is larger in the after-war period. The number of patient who are 65 years old and older than that is greater, and that is statistically significant ($p = 0.0005$). We can conclude that the stress caused by the war and other factors have influenced a larger number of treated patients of acute coronary syndrome. Therefore, further epidemiological researches of acute coronary syndrome with the accent on prevention and treatment are needed.

Key words: war, acute coronary syndrome, western Herzegovina

Introduction

During the war and after-war period the population of Bosnia and Herzegovina was exposed to different risk factors that could affect the health condition of the population especially with respect to non-infective (coronary) diseases. It is well known that the stress is the risk factor for the development of the coronary disease. Since the influence of stress on the frequency of patients being treated for acute coronary syndrome was not previously investigated, we decided to conduct this research in the

population of western Herzegovina. Our goal is to present the frequency of patients being treated for acute coronary syndrome during pre-war, war and post war period (1987–2001).

Methods

In this retrospective study the period of fifteen years (from 1987 to 2001) was taken as the referent period. We

have compared frequency of patients treated for acute coronary syndrome in pre-war period (1987–1991), war (1992–1996) and post war period (1997–2001). The relatively stabile population of Mostar and neighboring municipalities – Čapljina, Stolac, Čitluk, Neum, Prozor, Široki Brijeg, Posušje, Grude and Ljubuški was included in the study. The number of inhabitants according to the Census from 1991 was 182.000 inhabitants.

We collected the information about the patients hospitalized due to acute coronary syndrome (category I 20, 21, 22 – X revision ICD) or treated in hospital during the pre-war period in Mostar, during the war period in Mostar and in the Republic of Croatia, as well as in post-war period in the Clinical Hospital in Mostar, where that population belongs according to their health insurance. The data about the treated patients due to the acute coronary syndrome were taken from the hospital archive.

The acute coronary syndrome encompassed acute myocardial infarction, with or without ST segment elevation, and unstable angina pectoris.

Myocardial infarction (first sad recurrent) was based on at least two criteria of the following three: (1) typical acute symptoms (chest pain for >30 minutes), (2) enzyme level elevations (creatine kinase or creatine kinase isoenzyme MB levels twice the normal upper limit within 72 hours after the onset of acute symptoms), and (3) electrocardiographic (ECG) changes, with or without ST segment elevation. Myocardial infarction with ST segment elevation was defined if ST segment elevation of ≥ 1 mm was present for two or more consecutive ECG leads for the inferior region (ECG leads II, III, and/or a VF: inferior myocardial infarction) or ST segment elevation of > 2 mm was present for ECG leads for the anteroseptal or lateral region (ECG leads V1–V6, a VL and/or I: myocardial infarction with anteroseptal or lateral localization). Dorsal localization of myocardial infarction was defined with new R wave development for ECG leads V1 and V2. Myocardial infarction without ST segment elevation was defined when > 1 mm depression of the ST segment at two consecutive ECG leads or T wave inversion at two or more consecutive leads was present.

We have collected the following data for every patient: age, gender, residency, as well as their medical record for acute coronary syndrome in the period from 1987 to 2001. Although there were changes in the number of population that came to live in Herzegovina, this research applies to the population that lived in that area during the period under research and did not emigrate. Their number is relatively stabile. The frequency of acute coronary syndrome is shown with the absolute numbers and percentages. The differences were tested with t-test, and $p < 0.05$ was considered as statistically significant.

Results

Total number of patients that were treated from acute coronary syndrome is 2022 (1343 treated from acute myocardial infarction, 105 from repeated myocar-

dial infarction, and 574 from instable angina pectoris), out of that 1305 men (865 acute myocardial infarction, 64 repeated myocardial infarction and 376 from instable angina pectoris) and 717 women (478 treated from acute myocardial infarction, 41 repeated myocardial infarction and 198 from instable angina pectoris). In the pre-war period 514 patients were treated from acute coronary syndrome (365 from acute myocardial infarction, 24 from repeated myocardial infarction and 125 from instable angina pectoris), out of that 344 men (246 from acute myocardial infarction, 14 repeated myocardial infarction and 84 from instable angina pectoris), and 170 women (119 from acute myocardial infarction, 10 from repeated myocardial infarction and 41 from instable angina pectoris). In the war period 665 patients were treated from acute coronary syndrome (428 from acute myocardial infarction, 52 from repeated myocardial infarction and 185 from instable angina pectoris), out of that 409 men (267 from acute myocardial infarction, 33 repeated myocardial infarction and 109 from instable angina pectoris), and 256 women (161 from acute myocardial infarction, 19 from repeated myocardial infarction and 76 from instable angina pectoris). In the post war period 843 patients were treated from acute coronary syndrome (550 from acute myocardial infarction, 29 from repeated myocardial infarction and 264 from instable angina pectoris), out of that 552 men (352 from acute myocardial infarction, 17 repeated myocardial infarction and 183 from instable angina pectoris), and 291 women (198 from acute myocardial infarction, 12 from repeated myocardial infarction and 81 from instable angina pectoris). The largest percentage of treated from acute coronary syndrome with both gender (48.4%), out of that women (62.9%) is in the oldest age groups in post war period and men (51.7%) in the pre-war period, and the least (12.8%) with both genders together and women (3.5%) in the pre-war period, and with men (17.4%) in the post war period. When the number of the treated patients is compared, it is obvious that largest number of treated patients is in 1998 – 187 patients, and the least number of patients was in 1992 – 81 patients hospitalized from acute coronary syndrome. The number of patients treated from acute coronary syndrome in the war period is much bigger comparing to the pre-war period with both genders together ($p < 0.001$), with men ($p < 0.018$) and with women ($p < 0.001$). Statistically, significantly larger number of treated patients in the post-war period comparing to the war period with both genders together ($p = 0.0000$), with men ($p = 0.0000$). Comparing the post war and pre-war period, we get the larger number of treated men and women in post war period ($p = 0.0000$), and larger number of treated from acute coronary syndrome ($p < 0.05$) in the age of 65 and more in the post war period, which is statistically significant. There are no statistical significances according to age and gender in three research period (Table 1). Statistically, number of treated patients from instable angina pectoris with men in post war period comparing to war period is more important ($p < 0.05$), and pre-war period ($p < 0.05$), as well as larger

number of treated from instable angina pectoris in the war period comparing to pre-war period ($p=0.019$).

The number of treated from acute myocardial infarction with men in post war period comparing to war period ($p=0.0006$), and pre-war ($p<0.0001$), period was bigger, and with women, number of treated was bigger in the war period comparing to per-war period ($p=0.01$), and in post war comparing to war period, which has proven as the statistically significant difference. The incidence of the repeated myocardial infarction is more significant in the war period comparing to pre-war period ($p=0.0005$), and post war period ($p=0.02$) with men.

Discussion

Many researches that were conducted during the previous years correlate war stress and its influence on the development of specific diseases, as well as on the coronary diseases¹⁻⁴. The psycho-social factors, psycho and physical stress, eating disorders are listed as examples of war stress factors^{5,6}. The significance of the war stress for getting sick and increased mortality from coronary disease⁷⁻¹⁰ was the subject of many researches in Lebanon, Israel, Korea and Croatia¹¹⁻¹⁵. While certain authors found connection between the influence of the war stress on increased number of sick patients from acute coronary syndrome^{7,15} others were not able to find that connection¹³. Besides the war stress, earthquakes can also cause increased number of sick patients from acute coronary syndrome¹⁶ and that was obvious after the earthquake in Athens in 1981 and in Los Angeles in 1994^{17,18}.

During the war in Bosnia and Herzegovina, citizens of Bosnia and Herzegovina were subject to many difficulties due to various factors, and many of those could influence their health condition. Citizens were spending lots of time in shelters, due to general danger from bombing. Everyday life was completely changed and was filled with stressful situations. Besides physical and mental stress, the eating habits were changed, physical activity was reduced, and communication among people, smoking and alcohol habits, and etc, and all that could influence general health of population. This study showed that significantly larger number of patients treated from acute coronary syndrome was in the war and post war period comparing to the number of patients before the war. Twenty years after the war in Vietnam, the veterans still suffer and die from acute coronary syndrome, which is the same in our country after the war¹⁹. The number of patient who is suffering of acute coronary syndrome is bigger in countries in transit, as ex countries of SSSR and Yugoslavia because of unemployment, stress, unhealthy way of living, health prevention which is late for few years, and reconstruction of health care is still on^{20,21}.

The least number of patients treated from acute coronary syndrome was in 1992 (unexpectedly), and the biggest was in 1998 (Table 2).

The largest number of treated patients 66.4% is for the patients from acute myocardial infarction, while 28% are for the instable angina, although in the medical literature angina pectoris is the most frequent diagnosis of acute coronary syndrome, and here it is about the patients treated in hospital, and it is most possible that some of the patients were treated in primary care. In this study it is obvious that significantly number of patients treated from acute coronary syndrome in the oldest age groups, and that can be explained by long-term chronic stress as the risk factor for atherosclerosis. Also, the number of treated women in the war and post war period in comparison to the pre war period is bigger. This refers to the women older than 50, since in the age group under 50, number of the women with acute coronary syndrome was little and this can be explained by the protective influence of estrogens in generative age of women. Significantly larger number ($p<0.05$) of treated women from acute myocardial infarction in the age from 60–69 in the war period is already described (1.3%). The number of women that died in the hospital from acute myocardial infarction was the least during the war (18.6%) in comparison to the per-war period (32.8%), most probably due to the fact that women had difficulties in coming to the hospital, and probably more have died prior to coming to the hospital, while in the pre-war period accessibility to the hospital was much better¹.

It was determined that those differences were not noticed with men, that in the huge number were obliged to work and military service, and access to the hospital was much easier to them comparing to women¹.

Greater mortality of women in hospitals was described in other studies^{19,20,22,23}. That can be explained by the fact that women were older in the time of myocardial infarction, have more often hypertension, diabetes mellitus, chronic heart failing, and other chronic diseases.

Greater morbidity and mortality from coronary disease is visible during the war in Israel and war in Croatia. That is how in Zagreb, during the war number of patients from acute myocardial infarction and sudden death¹⁵ was increased, while authors in Split during the time of general danger periods did not determine bigger number of sick or dead from acute myocardial infarction¹³. Regional differences in mortality of AIM during the war can be explained by bigger accessibility of urgent medical service and hospital care in bigger cities (Zagreb), while that was the case in geographically indented region (Split). Some authors have found larger number of treated from acute myocardial infarction in the age of 45 in Split¹⁴ and age 49 in Bosnia and Herzegovina.

In the conclusion we can say that our research has showed statistically significant larger hospital frequency of acute coronary syndrome in the war and post war period with the stress as one of the risk factors, although in other transition countries mortality and morbidity from coronary diseases is larger, due to the fact that primary prevention is late for couple of decades after western countries. Definitely, we need to conclude that war trau-

mas have short-term and long-term negative impact on mental and physical health of population whose consequences could also be in the development of the coronary diseases. The reason for this are most probably the con-

sequences caused by PTSP that was caused by the war, as well as some negative living habits acquired during the war, and difficult economical situation that continued after the war.

TABLE 1
THE FREQUENCY OF ACUTE CORONARY SYNDROME ACCORDING TO THE AGE IN THE TIME PERIOD OF 1987 TO 2001 IN WESTERN HERZEGOVINA

Male	≤49		50–64		≥65		Total
	N	%	N	%	N	%	
Before war	60	17.4	178	51.7	106	30.9	344
War time	72	17.6	205	50.1	132	32.3	409
After war	107	19.4	220	39.9	225	40.7	552
Total	239	18.3	603	46.3	463	35.4	1305

Female	≤49		50–64		≥65		Total
	N	%	N	%	N	%	
Before war	6	3.5	70	41.2	94	55.3	170
War time	13	5.1	104	40.6	139	54.3	256
After war	21	7.2	87	29.9	183	62.9	291
Total	40	5.6	261	36.4	416	58.0	717

Male and Females	≤49		50–64		≥65		Total
	N	%	N	%	N	%	
Before war	66	12.8	248	48.2	200	39.0	514
War time	85	12.9	309	46.5	271	40.7	665
After war	128	15.2	307	36.4	408	48.4	843
Total	279	13.8	864	42.7	879	43.5	2022

TABLE 2
THE INCIDENCY OF ACUTE CORONARY SYNDROME ACCORDING TO THE AGE IN THE TIME PERIOD OF 1987 TO 2001 IN WESTERN HERZEGOVINA

Year	Acute infarct of myocardium			Repeated infarct of myocardium			Angina pectoris		
	Number of treated patients from acute coronary syndrome	Database 1987	Chain index	Number of treated patients from acute coronary syndrome	Database 1987	Chain index	Number of treated patients from acute coronary syndrome	Database 1987	Chain index
1987	77	100.00	100.00	2	100.00	100.00	11	100.00	100.00
1988	75	97.40	97.40	7	350.00	350.00	25	227.27	227.27
1989	65	84.42	86.67	5	250.00	71.43	29	263.64	116.00
1990	78	101.30	120.00	6	300.00	120.00	38	345.45	131.03
1991	70	90.91	89.74	4	200.00	66.67	22	200.00	57.89
1992	58	75.32	82.86	3	150.00	75.00	20	181.82	90.91
1993	87	112.99	150.00	14	700.00	466.67	35	318.18	175.00
1994	103	133.77	118.39	12	600.00	85.71	40	363.64	114.29
1995	93	120.78	90.29	15	750.00	125.00	51	463.64	127.50
1996	87	112.99	93.55	8	400.00	53.33	39	354.55	76.47
1997	97	125.974	111.4943	10	500	125	40	363.6364	102.5641
1998	114	148.05	117.53	6	300.00	60.00	67	609.09	167.50
1999	119	154.55	104.39	5	250.00	83.33	48	436.36	71.64
2000	111	144.16	93.28	3	150.00	60.00	46	418.18	95.83
2001	109	141.56	98.20	5	250.00	166.67	62	563.64	134.78

In retrospective epidemiological research of patients treated in hospital from acute coronary syndrome in fifteen years period (1987–2001), pre-war period (1987–1991) war period (1992–1996), and post war period (1997–2001) were included. Statistically, number of treated from acute coronary syndrome in the war period comparing to the pre-war period with both genders together, men and

women is more important, post war period comparing to the pre-war period with both genders. Number of treated men and women is larger in post war period comparing to the pre war period and that is statistically significant. The number of treated in the age of 65 and more in post war period is statistically significant.

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UČESTALOST AKUTNOG KORONARNOG SINDROMA U PETNAESTOGODIŠNJEM RAZDOBLJU (1987–2001) U ZAPADNOJ HERCEGOVINI

S A Ž E T A K

Radi se o retrospektivnoj epidemiološkoj studiji liječenih od akutnog koronarnog sindroma u petnaestogodišnjem razdoblju (1987–2001) u zapadnoj Hercegovini. Obradena je relativno stabilna populacija koja se za vrijeme rata nije iseljavala. Uspoređeno je referentno prijeratno (1987–1991), ratno (1992–1996) i poslijeratno (1997–2001) razdoblje. Podaci su dobiveni iz arhive o liječenim bolesnicima u bolnici Mostar u petnaestogodišnjem razdoblju i KB Split u ratnom razdoblju. Obradena su 2022 bolnička slučaja akutnih koronarnih sindroma, a od toga 1305 muškaraca i 717 žena. Značajnije je, da je veći broj liječenih od akutnog koronarnog sindroma u ratnom u odnosu na prijeratno razdoblje kod oba spola zajedno ($p < 0.0005$). Veći je broj liječenih u poslijeratnom razdoblju kod oba spola zajedno ($p < 0.0005$) u odnosu na ratno, a kada usporedimo poslijeratno i prijeratno razdoblje vidimo statistički značajno veći broj liječenih i muškaraca i žena u poslijeratnom razdoblju ($p < 0.0005$). Statistički je značajnije veći broj oboljelih u dobi 65 i više ($p = 0.0005$). Zaključno se može reći da je ratni stres uz ostale čimbenike rizika imao utjecaja na veći broj liječenih od akutnog koronarnog sindroma, što otvara mogućnost epidemioloških istraživanja akutnog koronarnog sindroma te mogućnosti prevencije i liječenja.

Of Mice and Men: Teratomas and Teratocarcinomas

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ABSTRACT

Teratomas and teratocarcinomas are tumors containing tissue derivatives of all three germ-layers. They can be induced by transplantation of animal embryos to ectopic microenvironment. Development of malignant teratocarcinomas depends on embryonic stage, species-specificity and immunological competence of the host. In the man, teratomas and teratocarcinomas usually represent a subtype of germ-cell tumors but sacrococcygeal teratomas arise from the remnants of the pluripotent primitive streak. Undifferentiated embryonal carcinoma (EC) cells are responsible for the malignancy of experimental mouse teratocarcinomas. Mouse EC cells injected to the adult give rise to tumors and upon injection to early embryos to differentiated tissues – thus resembling normal mouse embryonic stem cells (mESC). Epigenetic changes rather than mutations are associated with transformation of mESC to EC cells. Human EC and ES cell-lines (hESC) contain chromosomal abnormalities and can form teratocarcinoma after transplantation. ES cells are among those proposed for cell replacement therapy in the man. Suicide gene introduction should be recommended prior to their use in vivo to ablate them in case of malignant transformation.

Key words: teratoma, teratocarcinoma, embryo, EC cell, ES cell, cell therapy

Experimental Animal Teratoma and Teratocarcinoma

Experimental teratomas, tumors containing a disorganized mixture of various tissues, were induced in laboratory animals such as rat by subcapsular kidney transplantation of postimplantation embryos or their parts. In such a favorable *in vivo* microenvironment well differentiated derivatives of the ectoderm, mesoderm and endoderm (the three germ-layers) such as brain tissue, epidermis, bone, muscle and gut epithelium regularly differentiated. Tissues were sometimes even forming organotypic structures resembling e.g. the tooth, fingers with phalange etc. Švajger, Levak-Švajger and Škreb have discovered that, depending upon the stage of embryonic development, single germ-layers differ in their potential to give rise to differentiated tissues. Embryonic epiblast (pre-gastrulation primary ectoderm) formed a teratoma containing differentiated tissues derivatives of all three definite germ-layers. Primary endoderm (hypo- blast

isolated at this stage was resorbed. After the formation of the primitive streak, embryonic ectoderm was able to give rise to ectodermal and mesodermal derivatives but not of the endodermal so that a restriction of its developmental potential occurred in older embryos. The general conclusion, later corroborated by experiments of others, was that all three definitive germ-layers in mammals are originating from the primitive ectoderm (epiblast)^{1,2}.

Postimplantation rat embryos (E9, 5) cultivated for two weeks *in vitro* also gave rise to experimental teratomas. Surprisingly, their developmental potential was partly executed even in the protein-free chemically defined medium³. In this medium, targeted changes of differentiation were achieved by addition of defined growth, differentiation or morphogenetic factors (e.g. transferrin promoted differentiation of the ocular lens cells and RA

changed differentiation from epidermis to columnar epithelium)⁴⁻⁶. It was also noticed that the restriction of developmental potential for neural tissue differentiation found after serumless *in vitro* culture itself was retained in transplants in spite of the fact that this microenvironment is very favorable for differentiation of directly transplanted embryos^{7,8}.

Solter, Škreb and Damjanov have found that by transplantation of the mouse egg-cylinder under the kidney capsule, not only teratomas, but also retransplantable teratocarcinomas could develop. Among differentiated tissues, teratocarcinomas contained undifferentiated embryonal carcinoma cells (EC)⁹. Older embryos going through the process of neurulation were not able to give rise to teratocarcinoma, probably because ectoderm was already developmentally committed and has lost its pluripotentiality^{10,11}. Development of tumors occurred also in testicular transplants, but their weight was significantly lower. Embryo-derived tumors caused splenomegaly in hosts which was greater in animals bearing teratocarcinoma¹². Interestingly, experimental teratocarcinoma was not found in rat transplants, but sometimes yolk-sac carcinoma developed. Yolk-sac carcinoma developed also in the mouse but after a longer period of time¹⁰. Teratocarcinoma development was dependent on the mouse strain. However, strains that did not permit teratocarcinoma development did so in F1 hybrid hosts¹¹. In immunologically compromised mice, teratocarcinoma development was rare¹³. From the above described experiments it can be concluded that development of malignant teratocarcinomas depends on embryonic stage, species-specificity and immunological competence of the host. Although genetic factors seem to be important for the rise of embryo-derived teratocarcinoma, main culprits for the development of this malignant tumor are seemingly epigenetic factors from the microenvironment at the ectopic site which change gene expression and potential for differentiation of transplanted embryos¹⁴.

Human Teratoma and Teratocarcinoma

Human germ cell tumors (GCT) can be either testicular or ovarian. They may share important etiological factors but incidence of female GCT is much lower^{15,16}. The research of their cause has been guided by the hypothesis that the disease process starts in fetal life with the abnormal differentiation of fetal primordial germ cells. Testicular germ cell tumors are divided into two groups: seminomas and nonseminomatous germ cell tumors (NSGCT). Intratubular germ cell neoplasia of unclassified type (IGCNU) seem to be a precursor for those lesions¹⁵. NSGCT¹⁶ are thought to have a clonal origin and to recapitulate embryogenesis, their pattern of differentiation being directed toward the formation of one or more of the components of the embryo and related structures. The specific direction this differentiation takes will determine the morphologic appearance of given tumor and hence its name^{15,17}. There are four basic patterns of NSGCT: embryonal carcinoma (primitive carcinoma like cells with minimal or without signs of differentiation), mature and immature teratoma

(differentiation toward structures of the embryo proper), choriocarcinoma (presence of well-developed trophoblastic elements in an organoid fashion), yolk sac tumor (formation of extraembryonic endoderm and mesoderm). Tumors exhibiting two or more of these patterns are designated as mixed NSGCT. The combination of embryonal carcinoma and teratoma is also known as teratocarcinoma. Current morphologic, cytogenetic and DNA ploidy data are showing that seminoma probably serves as precursor in the formation of NSGCT^{17,18}.

A cryptorchid testis is 30–50 times more likely to develop a malignant neoplasm than a normally placed organ. The incidence of testicular cancer is also increased in men with hypospadias and with inguinal hernia. There are some tumors that have occurred in a family setting, suggesting a genetic background but also environmental exposures to pesticides, textile dust, organ solvents seem to be important^{15,19}.

The majority of testicular germ cell tumors manifest aneuploid DNA contents with minimal intratumoral heterogeneity. Seminoma and IGCNU cells are hypertriploid and NSGCT are hypotriploid. They have at least one X and one Y chromosome. Mature teratoma of the prepubertal testis is the only TGCT lacking gross chromosomal aberrations. TGCT of all other types are characterized with two abnormalities of chromosome 12²⁰, |i(12p)| in about 80% and |del(12q)| in 20%. It has been postulated that these deletions cause the loss of one or more tumor suppressor genes whose products regulate the normal proliferation of spermatogonial germ cells^{20,21}. |i(12p)| is also detected in these types of tumors in ovary, mediastinum and midline of the brain. Persons with 46, XY or 45, X/46, XY are at very high risk of gonadal germ cell tumor. Telomerase activity is present in all types of TGCT which can be explained with biallelic expression of multiple imprinted genes. Cyclin E has a higher expression in embryonal carcinoma than in other NGCT and Fas gene mutations are also common in that tumor¹⁵. Teratocarcinoma were found to be hypermethylated while seminomas were hypomethylated which epigenetic difference might reflect the normal developmental switch in primordial germ cells from an undermethylated genome to a normally methylated genome^{22,23}.

Sacrocoxygeal teratoma (SCT) is developing at the caudal end of the primitive streak (a transient formation of the gastrulating embryo containing pluripotent cells), probably from its remnants which did not disappear on time²⁴. It is predominantly benign and can contain all kinds of differentiated tissues among which even cells of the ocular lens²⁵. Sacrocoxygeal teratoma expresses several oncoproteins and tumor suppressor proteins such as ras, fos and jun, nm23 and p53 but no correlation was found between intensity of their expression and tumor size, age and survival of patients neither between mature and immature type of tumor²⁶.

EC and ES Cells

EC cells were isolated from experimental animal teratocarcinomas and human teratocarcinomas and subjec-

ted to extensive investigation from 1954 until today. In the mouse, introduction of a single EC cell to the blastocyst was able to produce normal chimeric mice, while in adults subcutaneous or intraperitoneal introduction of EC cells produced teratocarcinoma^{14,27–29}. Adult chimeras, or later stages of postimplantation embryos especially after completion of organogenesis³⁰, also develop tumors and it seems that the microenvironment of the early embryos is more favorable for the suppression of malignant phenotype. This shows that the development of the teratocarcinoma is epigenetically regulated by the microenvironment and not caused by mutations which are found in majority of other types of tumors¹⁴.

Because pluripotentiality of EC cells resembles pluripotentiality of ES cells – cells of the inner cell mass from the blastocyst which gives rise to the embryo proper, EC cell lines were established *in vitro* to investigate biologically active molecules in mouse development. Later on, establishment of pluripotent mouse embryonic stem cells (mESC) in cultures *in vitro* made possible the production of transgenic gene knock-outs in mice. Pluripotent ES rat cells could not be cultivated²⁸. So in the rat, two species-specific differences in comparison to the mouse were found, namely no teratocarcinoma in embryonic transplants and no pluripotent ES cell-lines *in vitro*. Mouse embryonic stem cells (mESC) could also induce subcutaneous tumors, but they were growing at a much slower rate in direct comparison to teratocarcinoma derived from transplantation of an euploid EC cell-line. Gene expression profiling on microarrays was done to investigate differences in gene expression between teratocarcinoma and ES control in both cell cultures and in nude mice tumors. Results have shown the involvement of several pathways, and especially the cell cycle pathway in induction of teratocarcinoma³¹.

Human EC cell-lines were established from human germ cell primary or metastatic tumors both *in vivo* and *in vitro*. Human EC cell-lines are virtually always aneuploid and only few can differentiate into well recognizable cell types. In both males and females extra-gonadal germ-cell tumors are usually diploid and very few cell-lines have been developed from them²⁷. Specific biological differences between animal and human EC cells include a distinct pattern of surface antigen expression³².

Human embryonic stem cells (hESC) were recently cultivated *in vitro* and cell lines were established. They seem ready to develop chromosomal abnormalities in long-term *in vitro* cultures among which i(12p), strongly implicated in human germ cell cancer³³. It was also reported that long-term cultivated hESC can induce teratocarcinoma after transplantation to immunodeficient SCID mice³⁴.

Cell Replacement Therapy

The latest rise of hopes in regenerative medicine based on cell replacement therapy, tissue or organ en-

gineering^{35,36} are in fact funded upon developmental biology research aimed to investigate potential for growth and differentiation of various immature cells in an embryo, teratocarcinoma and in an adult organism²⁹. Cells that are today in focus for therapeutic purposes are pluripotent embryonic stem cells (ES) from the blastocyst obtained either from the surplus of embryos after *in vitro* fertilization or possibly after therapeutic cloning. Of wide developmental potential are also PGE (primordial germ cells), cord blood stem cells or stem cells isolated from the adult organism which are not derived from sources that are subjected to wide ethical discussions as the ones previously mentioned^{35,36}.

Cell replacement therapy can be exerted through direct approach by transplanting cells directly from one organism to the other, or even to an embryo³⁷. Fetal human mesenchymal stem cells from the liver of an unrelated donor were shown to alleviate a case of osteogenesis imperfecta (a disease characterized with multiple prenatal and postnatal bone fractures) in a three-year-old child subjected to therapy *in utero*³⁸. Recently a therapeutic success was reported with the human bladder in several patients. Muscle and urothelial cells were taken from the miniature bladders of the patients themselves and propagated *in vitro* upon a degradable scaffold. Thus a whole organ was constructed and successfully transplanted back to the patient³⁵.

Although a lot is known about the biology of various kinds of undifferentiated cells, in order not to compromise the therapeutic effect with an aberrant development resulting with tumors, basic research is still necessary. In fact, one case of the tumor development was reported in a cell therapy experiment in the mouse. Mouse ES cells were differentiated into neural cells *in vitro* and subsequently transplanted subretinally. After two months, a teratoma appeared making the whole eye nonfunctional. Probably the *in vitro* differentiation process was not completed in every ES cell and some remained undifferentiated producing a tumor after transplantation³⁹. In the latest functional engraftment of human ES cell-derived dopaminergic neurons to the parkinsonian rats, potential for phenotypic instability and undifferentiated expansion was reported⁴⁰. Another danger lies in usage of immunocompromized mice for testing the developmental potential in transplants of various undifferentiated human cells because it was shown that in immunologically compromised mice, teratocarcinoma development from transplanted embryos was rare¹⁰. If immunologically compromised mice are also not able to readily produce tumors from undifferentiated human cells, then these tests are not totally reliable. Therefore, research involving the transduction of suicide genes to mouse or human stem cells seems to be especially important because such genes could render stem cells prone to ablation on demand and make a »fail-safe protection against cellular misbehavior«^{41,42}.

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O MIŠEVIMA I LJUDIMA – TERATOM I TERATOKARCINOM

SAŽETAK

Teratomi i teratokarcinomi su tumori koji se sastoje od tkivnih derivata svih triju zametnih listića. Moguće ih je inducirati transplantacijom animalnih zametaka u ektopični mikrookoliš. Razvoj malignog teratokarcinoma ovisi o stadiju razvoja zametka, species-specifičnosti te imunološkoj kompetenciji domaćina. U čovjeka, teratomi i teratokarcinomi obično predstavljaju podtipove tumora spolnih stanica, ali sacrococcygealni teratom nastaje iz zaostataka pluripotentne primitivne pruge. Nedefinirane stanice embrionalnog karcinoma (EC) odgovorne su za malignost eksperimentalnog mišjeg teratokarcinoma. Mišje EC stanice injicirane odraslom stvaraju tumore, a injicirane u rane zametke diferencirana tkiva te stoga nalikuju normalnim mišjim matičnim stanicama zametka (mESC). Epigenetske promjene, prije nego li mutacije, povezane su s transformacijom mESC u EC stanice. Ljudske EC i ES stanične linije (hESC) sadrže kromosomske aberacije i mogu formirati teratokarcinom nakon transplantacije. ES stanice su među stanicama predloženim za staničnu nadomjesnu terapiju čovjeka. Trebalo bi preporučiti da se u njih unesu samoubilački geni prije upotrebe *in vivo*, kako bi se mogle odstraniti u slučaju maligne transformacije.

Primary Sjögren's Syndrome Associated with Non-Hodgkin's Lymphoma of Salivary Gland and Cystic Lung Disease

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ABSTRACT

A rare case of a young nonsmoker woman with Sjögren's syndrome and salivary gland non-Hodgkin's lymphoma, diagnosed one year later, is presented. Three years after treatment of the lymphoma, asymptomatic progression of the Sjögren's syndrome was observed with pulmonary involvement – predominantly bullous or cystic lung disease.

To our knowledge, this is the only report of Sjögren's syndrome associated with non-Hodgkin's lymphoma in salivary gland, and complicated with multiple lung cysts.

Key words: *Sjögren's syndrome, non-Hodgkin's lymphoma, salivary gland, lymphocytic interstitial pneumonia, cystic lung disease*

Introduction

Sjögren's syndrome is a chronic inflammatory autoimmune disorder characterized by lymphocytic infiltration of the exocrine glands. The diagnostic criteria include keratoconjunctivitis sicca and xerostomia. Additionally, different types of lung involvement have been described in Sjögren's syndrome of which the bullous/cystic lung disease is a very rare manifestation^{1,2}.

Patients with Sjögren's syndrome are at increased risk of lymphoma development. Most of these lymphomas involve the neck organs³. Salivary gland mucosa-associated lymphoid tissue (MALT) lymphomas are very rare, but their close association with autoimmune diseases confirms their possible role in pathogenesis of these lymphomas⁴.

We describe a case of Sjögren's syndrome associated with non-Hodgkin's lymphoma in salivary gland, and complicated with multiple lung cysts.

Case report

In nonsmoker female patient, born in 1973, the diagnoses of Sjögren's syndrome and sialolithiasis of the left parotid gland were confirmed in 1999. The diagnosis of Sjögren's syndrome was based on classification criteria by Vittali et al.⁵ with patient's positive ocular and oral

symptoms, minor salivary gland biopsy, diagnostic test for salivary gland involvement and raised levels of auto antibodies. The patient reported enlarged neck lymph nodes in October 2000 followed by extirpation of an enlarged lymph node and left submandibular gland. Histology confirmed marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue (MALT) type in salivary gland and lymph node follicular hyperplasia. She had been received chemotherapy till June 2001 and radiotherapy of salivary glands region afterwards.

The initial chest radiogram was normal as well as follow-ups every 6 months until April 2004 when rare discrete parenchymal opacities measured 1 – 2 cm have been depicted in both lower lung zones. High-resolution computed tomography (HRCT) scans showed multiple cystic formations predominantly in the middle and lower lung zones (Figure 1), measured from 1 to 4 cm in diameter. Four parenchymal consolidations were found in the same areas, measured from 0.5 to 2 cm (Figure 2). The findings were interpreted as pulmonary cysts of unknown origin (not resembling those in lymphangioleiomyomatosis, Langerhans cell histiocytosis or irregular emphysema) and parenchymal consolidations suggesting infectious origin with *Pneumocystis carinii* as a possible agent.

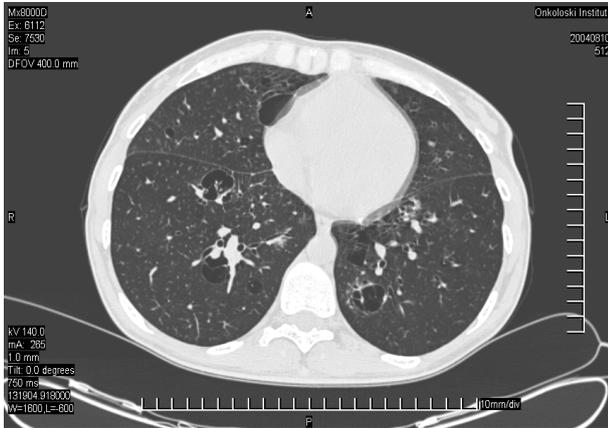


Fig. 1. High-resolution computed tomography in August 2004: Multiple lung cystic lesions.

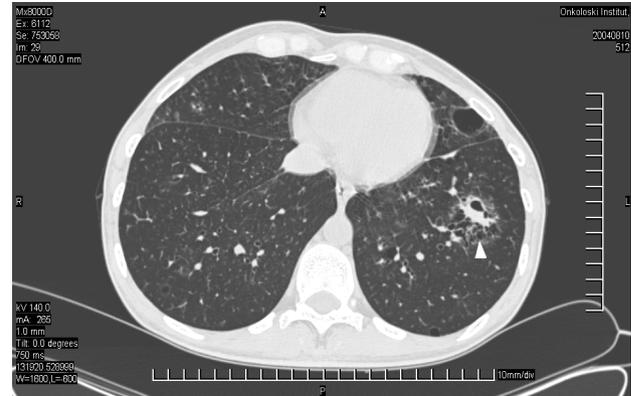


Fig. 3. High-resolution computed tomography in August 2004 at the same level as Figure 2. The most of parenchymal consolidation was replaced by small thin walled cysts.



Fig. 2. High-resolution computed tomography in May 2004: Parenchymal consolidation in the left lower lobe.

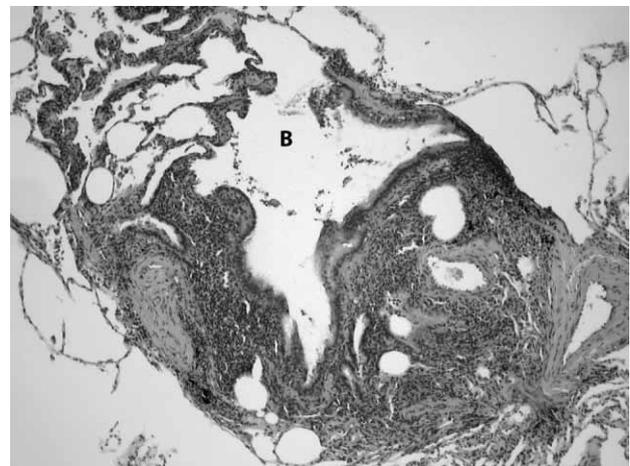


Fig. 4. Transbronchial biopsy: cystic-like bronchioloectasia (B) with lymphocytic infiltration in bronchiolar walls.

The patient had no respiratory symptoms although pulmonary function tests revealed mild restrictive ventilatory dysfunction ($VC=3.7$ l, $\%VC=80\%$, $FEV_1=3.4$ l, $FEV_1\% = 92\%$, $\%DL_{CO}=64\%$). Transbronchial biopsy specimen was interpreted as chronic non-specific inflammation with no signs of lymphoma infiltration. *Streptococcus pneumoniae* and *Candida glabrata* were isolated from the alveolar lavage and the patient was consecutively treated by antimycotic drugs for four weeks without regression of parenchymal consolidations on chest radiographs.

The follow-up HRCT showed progression of pulmonary cystic lesions in number and size (Figure 1). Some of the parenchymal consolidations were transformed into small cysts (Figure 3), suggesting a rare cystic/bullous form of Sjögren's syndrome in the lung.

The second transbronchial biopsy taken from left lower lobe consolidation showed irregular and intense lymphocytic interstitial infiltration with widened small

airways (Figure 4). Alveolar lavage taken from this area was interpreted as CD8 lymphocytic alveolitis with CD4/CD8 index of 0.5, thus confirming autoimmune involvement of pulmonary interstitium.

Discussion

Sjögren's syndrome is an autoimmune disease with lymphocytic infiltration of glandular and extraglandular tissues⁶. In most patients, it is confined to the salivary and lacrimal glands, however, extraglandular infiltration has been identified in 5 to 10% of the affected patients⁷.

The lung with abundant mucosal glands is a primary target. Several pulmonary complications have been described, including interstitial lung disease, airway disease, and nodular infiltrates⁸. Cystic or bullous disease is a rare complication of Sjögren's syndrome with only 15 cases in the literature^{1,2}. Compared to the other cases, our patient is the youngest of all with cystic lung disease.

Meyer et al⁹ suggested that stenosis with obstruction of bronchioles by peribronchiolar lymphocyte infiltration is the cause of air trapping with check-valve mechanism leading to formation of the multiple lung cysts. On the contrary, Johkoh et al¹⁰ proposed lymphocytic interstitial pneumonia to be the common feature in the cases of thin-walled cystic formations. Nevertheless, the radiological differential diagnosis of lung cysts in Sjögren's syndrome includes cystic abnormalities associated with Langerhans cell histiocytosis, lymphangiomyomato-

sis, different types of emphysema, honeycomb cysts in idiopathic pulmonary fibrosis, and thin walled cysts in *Pneumocystis carinii* pneumonia.

We are presenting the first report of Sjögren's syndrome associated with non-Hodgkin's lymphoma in salivary gland and complicated with multiple lung cysts and some parenchymal consolidations. Therefore one should know that such lung manifestations of Sjögren's syndrome could be also associated with salivary gland MALT lymphoma.

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PRIMARNI SJÖGREN OV SINDROM UDRUŽEN S NE-HODKINOVIM LIMFOMOM PAROTIDNIH ŽLIJEZDA I CISTIČKOM BOLEŠĆU PLUĆA

S A Ž E T A K

Prikazan je vrlo rijedak slučaj mlade žene, nepušačice, s Sjögrenovim sindromom, koja je godinu dana nakon postavljanja diagnoze oboljela također i za ne-Hodkinovim limfomom parotidnih žlijezda. Nakon tri godine liječenja limfoma pojavljuje se asimptomatsko napredovanje Sjögrenove bolesti u plućima sa buloznim ili cističkim promjenama pluća. Prema našim podacima radi se o prvom prikazu Sjögrenovog sindroma s ne-Hodkinovim limfomom parotidnih žlijezda, koji se zakomplicirao cističkom bolešću pluća.

Hemophagocytic Syndrome – Should We Consider it More Often?

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ABSTRACT

Hemophagocytic syndrome (HPS) is a rare condition characterized by overactive histiocytes, hepatosplenomegaly, fever and cytopenia, with two major types: familial, autosomal recessive genetic disease and acquired that can occur during systemic infections, immunodeficiency or malignancy. Inappropriate activation of macrophages by cytokines is the major mechanism of the disease. We report a case of an adult patient with HPS. After thorough clinical investigation, we have not been able to establish the underlying disease, and corticosteroids therapy was initiated empirically. After 8 months follow-up the patient is well with normal laboratory findings.

Key words: hemophagocytic syndrome, hemophagocytic lymphohistiocytosis, secondary

Introduction

Hemophagocytic syndrome (HPS), also known as hemophagocytic lymphohistiocytosis (HLH) or erythrophagocytic lymphohistiocytosis (EL) is a rare condition characterized by overactive (otherwise normal) histiocytes, hepatosplenomegaly, fever and cytopenia¹.

There are two major types of HPS². Primary (i.e. familial – FHL) is the inherited form, autosomal recessive genetic disease with onset in the first year of childhood. Secondary (i.e. acquired HPS) occurs after strong immunologic stimulation that can be a part of systemic infections, autoimmune diseases, immunodeficiency or malignancy.

Accepted theory for pathophysiology involves inappropriate activation of macrophages by activated T cells³. Large quantities of cytokines, primarily tumor necrosis factor- α (TNF- α), interferon gamma (INF- γ) and granulocyte-macrophage colony-stimulating factor (GM-CSF) cause macrophage proliferation and activation with further release of interleukin-1 (IL-1) and IL-6⁴. Interleukin-18 seems to have an important role in the activation also⁵. Activated macrophages phagocytose blood cells throughout reticuloendothelial system which is the main site of involvement.

The triggering mechanisms that lead to inappropriate immune activation are different in familial and acquired HPS. Perforin, a membrane protein of cytotoxic cells such as NK cells has an important role in the genesis of

at least one type of FLH^{6,7}. It is a part of cytoplasm granules of NK cells and cytotoxic T-cells that are released after stimulation to form pores in target cells thus damaging and ultimately destroying it by osmotic lysis. Patients with perforin deficiency have impaired NK activity that could lead to activation of T-cells in an inappropriate manner. Other NK cell disorders have been associated with FLH⁸. Secondary HPS can be initiated by a number of pathogens, of which the best understood is the pathogenesis of Epstein-Barr virus (EBV) associated HPS^{9,10}. Any kind of tumor, but more frequently lymphomas can lead to production of cytokines whether by direct production or immune system stimulation. Overproduction of TNF- α , INF- γ and other cytokines can lead to the cascade of reaction leading to macrophage over-stimulation.

Clinical presentation always includes fever, cytopenia and splenomegaly. Skin rash, hepatomegaly, lymphadenopathy, CNS disorders, jaundice and coagulopathy may also be present. Laboratory findings in addition to cytopenia include hyperfibrinogenemia, hypertriglyceridemia, high ferritine and low haptoglobine concentrations, liver damage and hyponatremia. Characteristic finding is cytological or histological confirmation of hemophagocytosis in an aspirate or biopsy of bone marrow, liver, spleen, lymph node or skin. Diagnostic criteria for HPS have been established^{1,2}. NK cell activity can be determined as an aid in determining between HPS types, since

reactive disease has normal NK activity, unlike familial FLH. NK cell is also impaired in secondary HPS especially in systemic onset as juvenile rheumatoid arthritis. Perforin expression can also be determined, as well as PRF-1 gene mutations and other gene mutations.

Familial types of HPS are treated with different approaches that include corticosteroids, immunosuppressive and antineoplastic drugs¹¹. The main goal is to achieve clinical stability. In cases of disease refractory to such treatment, bone marrow transplantation (BMT) should be considered¹². In the case of reactive HPS, underlying disease must be sought and treated appropriately if present. The same treatment as for FHL can be applied and for non-reactive patients a BMT must be considered.

Case Report

A 59-year-old male was admitted because of a fever that lasted for 6 weeks, anemia, leucopenia and weight loss of 8 kg over several months. Fever occurred usually in the afternoons, without chills and there were no other symptoms.

Patient's history was scarce: he had suffered a brainstem infarction 4 years before, and had his gallbladder removed due to gallstones. Initial examination revealed fever (37.8 °C), pale skin, hepatomegaly and splenomegaly (4 cm and 5 cm below costal margin respectively). Blood count showed neutropenia and anemia, whereas thrombocytes were only slightly reduced. Hyponatremia, hypertriglyceridemia, elevated liver enzymes and lactate dehydrogenase activity, elevated ferritine and low haptoglobine concentrations were present. Other electrolytes were normal, no alterations in coagulation tests were present and electrophoresis of serum proteins was normal as well as the values for available tumor markers (PSA, CEA, AFP, Ca-19-9, Ca-125).

Imaging methods (chest radiogram, abdominal ultrasound and CT) revealed no pathology in the thorax, enlarged spleen and liver with homogenous structure and several enlarged retroperitoneal lymph nodes.

Clinical presentation and laboratory findings were suggestive for hematological or infectious disease, but numerous microbiological tests performed during the course of hospitalization were negative. Several blood and urine cultures, also pharyngeal and nasal swabs were taken and all were negative. Serology for B and C hepatitis viruses and HIV virus was negative. IgG antibodies for Cytomegalo and Epstein-Barr virus were positive, but IgM were negative. Available tests for leishmaniasis, shistosomiasis and malaria were negative.

Cytology of sternal aspirate was done early, and showed normal hematopoiesis, but histiocytes that phagocyte mostly erythrocytes and rarely granulocytes were present, as well as some multinuclear cells. Following that finding, spleen biopsy and bone marrow biopsy were done and phagocytosis of erythrocytes was found in both. The diagnosis of hemophagocytic syndrome was set.

In the following patient evaluation possible causes for secondary HPS were considered. No proof for malignant disease was found after examination of gastrointestinal, respiratory and urinary systems. No criteria for systemic autoimmune disease were met. Besides the positive anti-EBV and anti-CMV IgG, no infection could have been connected with the patient's condition.

Without any known condition that could have been treated, corticosteroid treatment was chosen. A daily dose of 40 mg (0.5 mg per kg) methylprednisolone was administered orally and clinical condition improved rapidly. Fever disappeared three days after initiating treatment and blood count normalized on 7th day of treatment, patient was discharged three days after that. The dose of methylprednisolone was reduced gradually over eight weeks to 12 mg per day, but that led to a fall in blood count, primarily thrombocytes (110,000/ml) and leucocytes (3,000/mL). The values normalized with increase of steroid dose. During the eight-month follow up, our patient developed steroid diabetes, but other laboratory findings remained normal even after another reduction of drug dose to 16 mg daily, with well general condition of the patient. Repeated abdominal ultrasounds showed gradual reduction of spleen size which normalized six months after initiation of treatment.

Discussion

In the presented case of an adult male patient with HPS, no underlying condition that could be »accused« for the condition could be proven. Malignant disease, bacterial and parasitic infection, as well as autoimmune or other immunology disorder has been ruled out. The patient was serologically positive for a past EBV and CMV infection (positive IgG and negative IgM). Clinical course of the disease is not in concordance with known course of EBV associated HPS which is mainly much more severe. Any infection in theory could trigger the disease, and since there has been at least six weeks from the onset of symptoms to first examination, it is possible that an unknown infectious agent other than CMV or EBV was involved. No actual infection however was present during the hospitalization.

HPS is usually described as severe even in the case of secondary type. We have managed to achieve complete remission after several weeks of low dose corticosteroids treatment, but maintenance therapy is still required for our patient.

Although clinical presentation of the condition is anything but characteristic, and laboratory findings are also not specific, histology or cytology finding of hemophagocytosis is very characteristic, and necessary for the diagnosis. In cases of unexplained fever with hepato and splenomegaly it should be considered, especially if the laboratory findings are suggestive. There are reports that up to 60% of initial bone marrow aspirates can be negative for hemophagocytosis, so the examination should be repeated at least once if there is clinical suspicion of HPS.

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HEMOFAGOCITARNI SINDROM – TREBAMO LI ČEŠĆE MISLITI NA NJEGA?

SAŽETAK

Hemofagocitarni sindrom (HFS) je rijetko stanje karakterizirano hiperaktivnošću histiocita, hepatosplenomegalijom, vrućicom i citopenijom. Postoje dva osnovna oblika sindroma: obiteljni, autosomno recesivna nasljedna bolest te stečeni oblik koji može nastati za vrijeme sistemskih infekcija, imunodeficijencije ili uz maligne bolesti. Neodgovarajuća aktivacija makrofaga citokinima osnovna je u mehanizmu nastanka ove bolesti. Opisujemo odraslog bolesnika s HFS kod kojega nakon temeljite kliničke obrade nije otkriven primarni uzrok bolesti, a sistemska terapija kortikosteroidima empirijski uključena. Nakon osam mjeseci kontrole bolesnik je klinički dobro s urednim laboratorijskim nalazima.

Pulmonary Embolism Due to the Right Atrial Myxoma

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ABSTRACT

A 47-year-old man was admitted to the hospital with a pleuritic pain, dyspnea, nonproductive cough and low-grade fever. An ECG documented a sinus tachycardia with S₁Q₃T₃ pattern and incomplete right bundle branch block, and lung scintigraphy showed multiple perfusion defects. The initial diagnosis was pulmonary embolism. Echocardiography, undertaken before application of the anticoagulant therapy because of hematological disturbances reflecting possible coagulopathy (elevated erythrocyte sedimentation rate, increased leukocyte count, decreased platelet count), revealed a large mobile tumor in the right atrium. Tumor was surgically removed, and histological findings was supported a diagnosis of the cardiac myxoma. The right cardiac myxoma should be considered in the differential diagnosis of pulmonary embolism, particularly in cases presented in conjunction with constitutional symptoms and/or hematological disturbances. In these patients echocardiography should be undertaken early to exclude the rare but treatable diseases of the right heart.

Key words: echocardiography, myxoma, pulmonary embolism

Introduction

Myxoma is the most common cardiac tumor^{1,2}. Despite its benign pathologic nature, catastrophic results can occur because of systemic or pulmonary embolism and/or intracardiac obstruction^{1–3}. In some cases myxoma can be accompanied with nonspecific extracardiac symptoms and hematological disturbances that fail to suggest the proper etiology^{4,5}. We report a case of pulmonary embolism and hematological disorder secondary to a large right atrial myxoma.

Case Report

A 47-year-old man was admitted to our hospital with a right-sided pleuritic chest pain, dyspnea, nonproductive cough and low-grade fever. Dyspnea and nonproductive cough had appeared 8 months previously but the severity and frequency of symptoms worsened in the previous 2 weeks, especially in exertion and supine position. In addition to these symptoms he has become weak and easily fatigued. He has no conventional cardiovascular risk factors.

On physical examination patient was feverish (37.6 °C) and tachypnoic (a respiratory rate of 28/min). There was no jugular venous distention. Peripheral pulse examination revealed normal carotid and extremity arterial pulsation. His arterial pressure was 140/85 mmHg, and pulse 110 beats per minute. Cardiac auscultation revealed regular rhythm, and no murmurs or extra heart sounds. Lungs were clear to auscultation. There was no lower extremity edema or evidence of peripheral venous thrombosis.

An ECG documented a sinus tachycardia (110/min) with S₁Q₃T₃ pattern, and incomplete right bundle branch block. Chest radiography demonstrated mild cardiomegaly. Radionuclide lung perfusion scan showed multiple moderately sized segmental and subsegmental defects in both lungs. The patients erythrocyte sedimentation rate (47 mm/h), total leukocyte count (16.700/mm³), C-reactive protein (32.5 mg/L), D-dimers (1375 µg/L), and serum lactate dehydrogenase level (1478 U/L) were increased, while platelet count (83.000/mm³) was decreased. Other hematological and biochemical parameters were in the normal limits. In blood gas analysis there were only

slightly decreased pO_2 (8.4 kPa) and O_2 saturation (93%). Antinuclear and anticardiolipin antibodies were negative, and no lupus anticoagulant was detected.

The initial diagnosis was pulmonary embolism. Because of the decreased platelet count and possibility of an underlying coagulopathy, patient was not treated with anticoagulant therapy. Also, patient's medical history and laboratory findings suggested possibility of cardiac myxoma. Therefore, patient was referred to echocardiography.

Transthoracic echocardiography (TTE) using Vivid 3, General Electric, Milwaukee, WI, USA, machine demonstrated normal left ventricular size and function, normal left atrial size, and normal function of the aortic and mitral valves. The right atrium (RA=56 mm) and right ventricle (RV=45 mm) were enlarged. A large mobile mass was identified in the right atrium. There is no pericardial effusion. Transesophageal echocardiography (TEE) using a 5-MHz multiplane imaging transducer revealed a tumors mass in the right atrium, attached with a stalk close to the entry of the inferior vena cava. The tumor extended during diastole through the tricuspid valve into the right ventricle almost to the right ventricular apex causing severe relative tricuspid valve stenosis (Figure 1). Also, a moderate tricuspid regurgitation (angiographic grade +2/+3) was registered by color Doppler. Right ventricular systolic pressure estimated from tricuspid regurgitant jet (RVSP=45 mmHg), as well as shorter pulmonary valve acceleration time (PVaccT = 89 ms), suggested moderate pulmonary hypertension.

Immediate surgical treatment was indicated because of the high risk of recurrent, potentially fatal, embolism. The surgeon completely removed a tumor with the surrounding endocardium. Macroscopically, the resected tumor was a well-defined encapsulated mass with a smooth contour weighted 129 g and measured 98 x 56 x 44 mm in size (Figure 2). Hystologically, the tumor consisted of a

hypocellular mass of a myxoid matrix, rich in acid mucopolysaccharides, with a supporting structure of spindle-like, elongated or stellate cells scattered in an abundant stroma. These findings supported diagnosis of myxoma.

Excision of the tumor resulted in marked symptomatic and hematological improvement. The patient was discharged 10 days after the operation. Six months later the patient was asymptomatic. Follow-up echocardiography showed normal cavities dimensions and ventricular function. However, mild to moderate tricuspid regurgitation (angiographic grade +1/+2) was still evident. Also, there was a slightly elevated pulmonary artery pressure (RVSP=33 mmHg, PVaccT 106 ms). One year later, the patient remains well, with normal echocardiographic finding and pulmonary artery pressure.

Discussion

Primary cardiac tumors are uncommon with the incidence between 0.0017 % and 0.33%^{1,2}. About two-thirds of these tumors are myxoma that typically arises in the left atrium (80%) along the interatrial septum near the fossa ovalis. Occasionally, myxomas arise in the right atrium (15%), the ventricles (3–4%), or valves. Rarely, the tumor is present in more than one cavity^{1,2}. Cardiac myxomas arise more frequently in women and usually present between the ages of 50 and 70 years^{1,2}. Sporadic cases of myxoma are almost always single. However, approximately 7% of cardiac myxomas are a component of a complex hereditary syndrome that affects multiple organs¹.

Clinical manifestations of cardiac myxomas are most often determined by tumor size, location and mobility, and can be separated into three sessions: (1) symptoms of mechanical valvular obstruction, (2) embolization to the pulmonary or systemic circulation and (3) nonspecific constitutional symptoms and/or hematological findings^{1–5}.

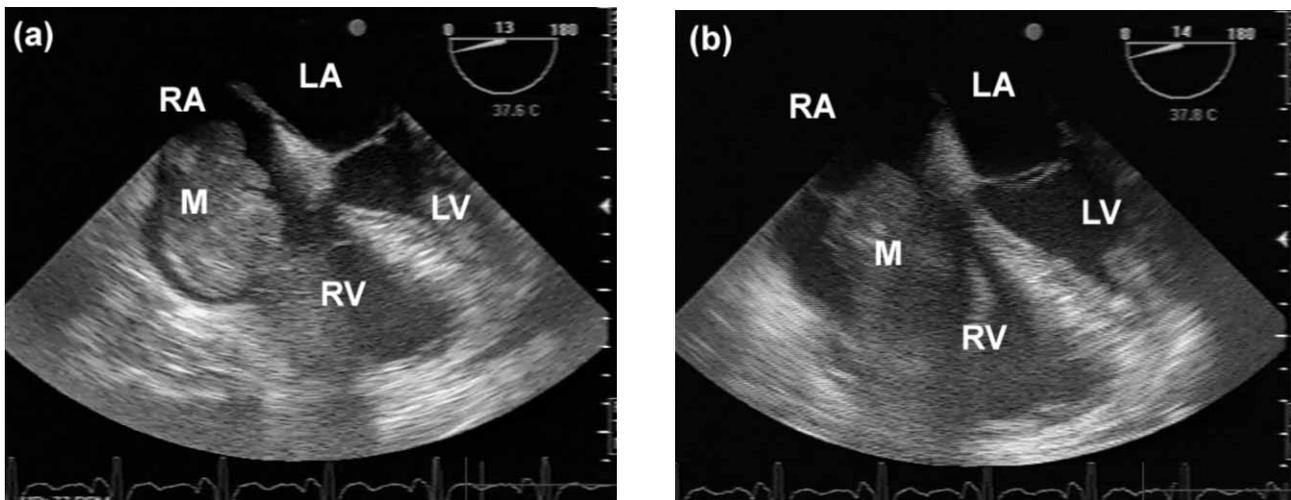


Fig. 1. Midesophageal four-chamber echocardiographic study in (a) systole and (b) diastole, revealing a right atrial (RA) mass (M) with a stalk (arrow) extended through the tricuspid valve into the right ventricle (RV). LA denotes left atrium, LV – left ventricle.

Obstruction to blood flow may occur at the orifice of any valve mimicking the clinical picture of valve stenosis. If the tumor is large enough, soft and easily deformable, and if it has a long stalk, temporary complete valve obstruction may occur, resulting in syncope or sudden death. However, the most frequently obstructive symptom is dyspnea that occurs in approximately 80% of patients with atrial myxoma^{1,2}. Left atrial myxoma produces symptoms when it reaches approximately 7 cm in size. Those in the right atrium that produce symptoms are usually approximately twice as large and sometimes several-fold larger⁶.



Fig. 2. The tumor weighed 129 g and measured 98 x 56 x 44 mm in size.

Embolic phenomenon in cardiac myxoma is common, with the incidence ranging from 30% to 40%^{1,2}. In left-sided cardiac myxomas the emboli generally display a predilection for the central nervous system, but also can affect other organs such as the liver, spleen, kidney, retina, coronary vessels, abdominal aorta, and distal arterial tree^{1,2,5,7}. In right-sided myxomas, clinically evident embolism is uncommon. Nevertheless, in these cases, there have been reports not only of repeated microembolization into the pulmonary vessels with subsequent pulmonary hypertension, but also of lethal pulmonary embolism³. The myxoma size is a significant determinant of valvular obstruction and constitutional symptoms, whereas the villous or papillary type and irregular surface is a risk factor for embolism^{4,5}. Also, males are statistically at greater risk than females of developing embolic complications^{4,5}. Our case is unusual because a large solid myxoma with a smooth surface cause pulmonary embolization.

Constitutional symptoms (fever, weight loss, weakness, fatigue, Raynaud's phenomenon, erythematous rash, digital clubbing, arthralgias) and laboratory findings (anemia, elevated erythrocyte sedimentation rate, elevated leukocyte count, decreased platelet count, positive seroreactive protein, and abnormal serum proteins)

are reported in 10–45% of patients^{1,2,5,6}. These symptoms may mimic infective endocarditis, collagen vascular disease, or occult malignancy, and may be due to the release of the acute phase reactant interleukin-6 from the tumor leading to inflammatory and autoimmune manifestations⁵. Plasma interleukin-6 concentration correlated positively with both the myxoma size and constitutional symptoms^{4,5}. Although nonspecific constitutional symptoms and laboratory findings are most often in patients with left atrial myxoma⁴, our case serves to remind the clinician that these symptoms can also be found in patients with a right-sided myxoma.

Cardiac myxomas range from small (< 1 cm or < 10 g) to large (> 10 cm or > 100 g)^{1,5}. Lazarides was reported a right atrial myxoma that weighed 450 g⁶! However, how fast atrial myxomas grow has never been clarified. It was estimated that recurrent atrial myxomas grow an average of 0.24–1.6 cm per year¹. Therefore, the large tumors size in our patient assumed that this intracardiac pathology existed for some years prior to diagnosis.

Before the introduction of echocardiography, the time interval between the onset of symptoms and diagnosis was 5.5–12 months and there has been a trend toward shortening of that interval to approximately 3 months⁸. Although the incidence of myxoma did not change between the early decade and the latest 10 years, small-sized and asymptomatic myxomas were more frequently found during the later decade⁸. It can be explained by the development and widespread introduction of echocardiography. However, as in our patient, many patients with myxoma experience a significant delay in diagnosis that can be attributed to the absence or misleading of cardiac symptoms, or to the presence of extracardiac symptoms that fail to suggest the proper etiology.

Although it is usually possible to detect intracardiac tumors with TTE the TEE examination produces spectacular images of a small tumors (1 to 3 mm in diameter), especially in patients with poor TTE images, and makes diagnosis, particularly of atrial masses, relatively easy⁹. The TEE also permits a clearer picture of the attachment or stalk of the tumor and more precise characterization of the size, shape, and location of the mass¹⁰. However, although TEE is a semi-invasive diagnostic technique with a very low incidence of significant complications, catastrophic pulmonary embolism during TEE examination it has been reported¹¹.

In cases in which the echocardiography characterization of intracardiac mass is incomplete, cardiac magnetic resonance imaging (CMR) and multislice spiral computed tomography (MSCT) are particularly helpful in determining the relationship to normal intracardiac structures and tumor extension to adjacent vascular and mediastinal structures, infiltration into the pericardium, influence on cardiac function and surgical planning^{12,13}. Additionally, superior to echocardiography, CMR and MSCT could strengthen the diagnostic accuracy by additional information on tissue characterization using different imaging sequences^{12,13}. These two imaging techniques can differentiate tissue composition, making it

possible to identify solid, liquid, hemorrhagic, and fatty, space-occupying tumors. Typically for cardiac myxomas, contrast enhancement is moderate and delayed enhancement can be found in the outer circumferential tumor margins only¹².

The accepted treatment of cardiac myxoma is operative excision¹⁴. Surgical removal of a tumor is important for preventing valvular obstruction, eliminating systemic or pulmonary emboli, maintaining systolic function, and restoring biventricular diastolic function. Surgical treatment leads to complete resolution with low rates of recurrence and good long-term survival¹⁴. The overall risk of recurrence is about 1–3% for sporadic myxoma of-

ten because of inadequate resection^{1,14}. Annual TTE or TEE review is suggested for a period of 3 to 4 years when the risk of recurrence is greatest. No chemotherapeutic or radiotherapeutic approach has been shown to be effective in preventing the recurrence of myxoma.

In conclusion, the right cardiac myxoma should be considered in the differential diagnosis of pulmonary embolism, particularly in cases presented in conjunction with constitutional symptoms and/or hematological disturbances. In these patients echocardiography should be undertaken early to exclude the rare but treatable diseases of the right heart.

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PLUĆNA EMBOLIJA UZROKOVANA MIKSOMOM DESNE PREDKLIJETKE

SAŽETAK

47-godišnji muškarac je hospitaliziran zbog desnostrane pleuritične boli, zaduhe, neproduktivnog kašlja i blago povišene tjelesne temperature. EKG-om je potvrđena sinusna tahikardija sa S₁Q₃T₃ obrascem i nepotpuni blok desne grane, a scintigrafijom pluća su pokazani višestruki ispadi perfuzije. Početna dijagnoza je bila plućna embolija. Ehokardiografskim pregledom učinjenim prije primjene antikoagulantne terapije, zbog hematoloških otklona koji su ukazivali na moguću koagulopatiju (ubrzana sedimentacija eritrocita, leukocitoza, trombocitopenija), potvrđen je veliki, pomični tumor u desnoj predklijetki. Tumor je kirurški odstranjen, a histološki nalaz je potvrdio dijagnozu miksoma. U diferencijalnoj dijagnozi plućne embolije, osobito ukoliko je praćena i konstitucionalnim simptomima i/ili hematološkim otklonima, potrebno je razmatrati i miksom desne predklijetke ili klijetke. U tih je bolesnika potrebno učiniti rani ehokardiografski pregled kako bi se isključile rijetke ali izlječive bolesti desnih šupljina srca.

Partial Cecal Necrosis Treated by Laparoscopic Partial Cecal Resection

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ABSTRACT

Acute colonic ischemia is the common cause of colitis in elderly population. However, isolated ischemic necrosis of cecum is rare entity, often associated with variety of conditions. Here we present a case of a 73-year old woman with a past history of hypertension presented with clinical symptoms of right lower quadrant abdominal pain and tenderness localized to the right lower quadrant, guarding and rebound tenderness. With diagnosis of acute appendicitis, the patient underwent laparoscopy where the cecal partial necrosis was discovered. Necrotic area of cecum was excised using two endoscopic cutters and laparoscopic appendectomy was performed. Pathologist report showed thrombosis of vessels and necrosis of entire cecal wall. The patient completely recovered without any surgical complications. This is the first case of partial cecum necrosis laparoscopically managed and with a partial cecal resection only.

Key words: cecal necrosis, laparoscopy, partial cecal resection

Introduction

Acute colonic ischaemia is a common cause of colitis in the elderly population however isolated ischemic necrosis of the cecum is rare and is often associated with conditions such as chronic heart disease, systemic sepsis, opportunistic fungal infections, hypovolemic shock and rheumatic fever¹⁻³.

Several cases of isolated cecal necrosis have been described in surgical literature^{1,4,5}. The patients in these earlier reports underwent laparotomy to establish diagnosis of the acute appendicitis with eventual cecectomy, ileocolic resection or right colectomy were performed^{1,4,5}.

Older patients are liable to have various pathologies such as tumors of the cecum, tubo – ovarian abscess, twisted or ruptured ovarian cyst etc. So in such patients differential diagnosis is very essential. Acute colonic ischaemia is a common cause of colitis not only in the elders but can also occur in the young population. This depends mainly on the factors that can cause ischaemia of the colon⁶. The clinical approach should not be limited to physical and laboratory examinations, but must be extended to pan colonoscopy, ultra sound of lower abdominal region. In this case operation was based on physical and laboratory examinations because the patient was op-

erated in emergency, when the diagnosis of acute appendicitis was established and the emergency does not allow any additional diagnostic elaboration.

Case Report

Here we describe the case of a 73-year old woman with a past history of hypertension who presented following two days of right lower quadrant abdominal pain associated with nausea and diarrhea. Physical examination revealed tenderness localized to the right lower quadrant with both guarding and rebound tenderness. Blood tests revealed a white cell count of 17,500/mm³.

Diagnosis of the acute appendicitis was made and the patient underwent laparoscopy at which time partial cecal necrosis was discovered. The necrotic area was localized to the antimesenteric side of cecum, measured approximately 4 cm in diameter and was adherent to the anterior abdominal wall (Figure 1). After thorough laparoscopic examination no other pathology was identified within the abdomen. The appendix was situated 3 cm from the necrotic area and was without any sign of inflammation. The mesoappendix was divided using har-



Fig. 1. Partial cecal necrosis.

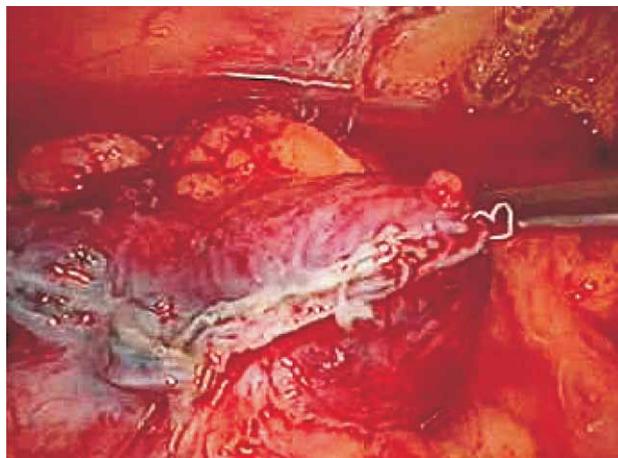


Fig. 3. Operation field after the resection.



Fig. 2. Resection using endoscopic cutter.

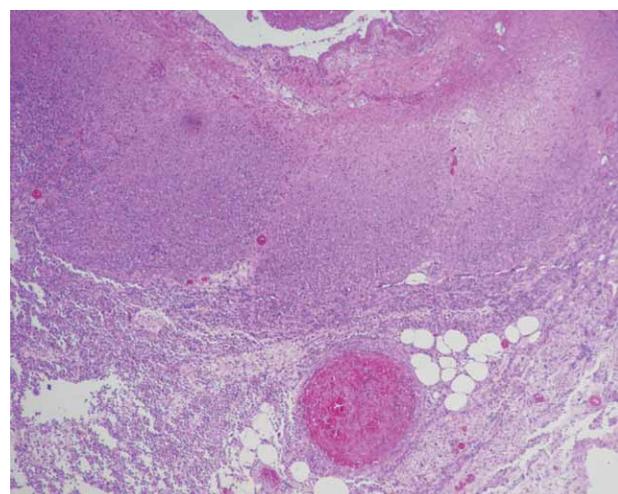


Fig. 4. Pathological finding - necrosis of the cecal wall and thrombosis of the vessels.

monic scalpel and an endo-loop was placed around the base of the appendix. The necrotic area of the cecum was excised using two endoscopic cutters (Figure 2) and several reabsorbable interrupted sutures were placed to cover the resection line (Figure 3). The use of two cutters is quite sufficient, but the additional sutures were placed because the resection line appeared low irrigated. Thorough peritoneal lavage was performed and an abdominal drain placed in the ileocecal region. The subsequent

pathological report showed thrombosis of the vessels supplying the excised tissue with necrosis of the entire cecal wall (Figure 4).

The patient recovered well without any surgical complications. This experience showed that partial cecal necrosis can be managed laparoscopically with partial cecal resection only.

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PARCIJALNA NEKROZA CEKUMA TRETIRANA LAPAROSKOPSKOM PARCIJALNOM RESEKCIJOM CEKUMA

S A Ž E T A K

Akutna ishemija kolona je uobičajeni uzrok kolitisa kod starije populacije. Izolirana ishemijska nekroza cekuma rijedak je slučaj, uglavnom udružen s različitim drugim stanjima. Ovdje predstavljamo slučaj 73. godišnje žene s hipertenzijom koja se prezentirala kliničkim simptomima boli u desnom donjem dijelu trbuha. S dijagnozom akutnog apendicitisa pacijentica je podvrgnuta laparoskopskom zahvatu, kada je ustanovljena parcijalna nekroza cekuma. Nekrotično područje cekuma ekscidirano je pomoću dva endoskopska staplera, a učinjena je i laparoskopska apendektomija. Nalaz patologa pokazao je trombozu krvnih žila i nekrozu cekuma. Bolesnica se potpuno oporavila bez ikakvih kirurških komplikacija. Ovo je prvi slučaj parcijalne nekroze cekuma dijagnosticiran i tretiran laparoskopski samo parcijalnom resekcijom cekuma.

Different Therapeutic Modalities in a Patient with Multiple Spontaneously Developed Keloids – A Case Report

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ABSTRACT

Keloids are benign tumors that usually develop as an excessive healing response to injury. They remain a challenging therapeutic problem to this day. Numerous treatment approaches are available, yet therapeutic results are often not satisfactory. A female patient with multiple spontaneously developed keloids on her trunk is presented. In this patient, four different therapeutic options were employed at different sites, with variable responses. The first option included cryotherapy, with poor effect. Slight flattening was observed after intralesional corticosteroid therapy. Treatment with excision followed by radiotherapy resulted in recurrence after 3 months. The best effect was noticed when excision and injection of corticosteroids into surgical margins were followed by radiotherapy. To the authors' knowledge, this is the first report of three-modal therapy in the management of keloids, which resulted in no recurrences over a 3-year follow-up.

Key words: keloids, excision, intralesional corticosteroids, radiotherapy

Introduction

Keloid is a benign, well-demarcated area of fibrous tissue overgrowth, which may occur after injury or other skin lesions. In contrast to hypertrophic scars, keloids extend beyond the border of the initial defect¹. Keloid formation is influenced by both local and inherited factors. The earlobes, chin, neck, shoulders, upper trunk, especially presternal region, and proximal parts of extremities are the most commonly affected sites. These anatomic areas are susceptible to increased skin tension^{1,2}. Afro-Caribbeans and individuals of Mediterranean descent are more prone to develop keloids than other populations. The occurrence of keloids may also run in families³.

Most keloids appear within a year after local trauma, for example after surgery, burns, vaccination and acne vulgaris. However, the patients sometimes have no recollection of prior injury^{1,4}. Such »spontaneous« keloids may be the result of an unnoticed microtrauma and are usually refractory to various treatments⁵.

The pathogenetic mechanisms that cause keloids are still unclear. A number of abnormalities in cellular function were observed, such as proliferation abnormalities,

apoptosis, expression of growth factors and extracellular matrix proteins. Recent studies suggest that single genes may act as major regulators of keloid development⁶. Initially, keloidal scars are raised, firm, pink or red plaques that may grow for months or years. The surface becomes smooth and shiny with the color of alabaster. It is often indolent and painful on pressure¹.

The major histologic changes usually occur in the lower dermis. Large, unencapsulated nodules of densely packed, irregularly arranged, coarse, and homogeneous collagen bundles are observed. In keloids of recent onset, the number of fibroblasts is increased. Elastic fibers are largely absent⁷.

To date no method of treatment for keloids has proved fully satisfactory due to the high rate of recurrence. Treatment options include intralesional steroids, compression therapy, fractionated soft x-ray radiotherapy, cryotherapy, silicone gel sheeting, interferon, laser therapy, and surgical excision. Combined therapies involving various agents have also been tried, such as intralesional steroid injection or radiotherapy following surgical excision⁸⁻¹¹.

Case Report

A healthy 41-year-old woman presented to our Department with multiple irregularly shaped, firm, hyperpigmented keloids on her trunk (Figure 1). Their size varied from 1.5 to 6 cm in diameter. The first keloid occurred 23 years before, after variola vaccination, at the site of inoculation, i.e. on her left shoulder. In the same year, excision of the keloid was performed, soon followed by a recurrence. Three years later, new keloids appeared spontaneously on her back. Later, every few years a number of new keloids developed on the patient's trunk without previous injury. Her family history revealed no occurrence of keloids in her relatives.

Before she was examined at our Department, she had received 15 treatments with cryotherapy using cotton-tipped method of liquid nitrogen application at one-month intervals, without therapeutic effect.

On admission, laboratory testing produced normal complete blood count and chemistry findings. We used three therapeutic options for the lesions on her trunk. On the back, two excisions were done. After both excisions, the diagnosis of keloidal scars was confirmed by histology. The first excision on the patient's back was followed by fractionated soft x-ray radiotherapy initiated on the day after excision and then every other day, with a daily dose of 200 cGy, total dose of 1000 cGy. Radiotherapy was accompanied by compression bandage. After three months, a slight recurrence was observed. In this lesion we continued intralesional application of triamcinolone acetonide crystalline suspension 10 mg, diluted 1:3 with xylocaine, every 3 weeks. After three months, one half underwent atrophy, while the other half partially regressed (Figure 2, marked with asterisk).



Fig. 1. The patient's back before treatment.



Fig. 2. The patient's back three years after treatment. The asterisk denotes keloid recurrence after surgical excision followed by superficial radiotherapy. The arrow shows normal scar after keloid excision, followed by corticosteroid application into surgical margins and radiotherapy.

The second keloid was located in her lumbar region. After excision, triamcinolone acetonide crystalline suspension 10 mg, diluted 1:3 with xylocaine, was injected intradermally into the wound margins. After one day, fractionated soft x-ray radiotherapy started, with a daily dose of 200 cGy, administered every other day to a total dose of 1000 cGy. The wound showed normal healing. The sutures were left in place for 10-14 days since the use of intralesional corticosteroids would interfere with the normal rate of scar development. There were no signs of recurrence during the 3-year follow-up (Figure 2, marked with arrow).

Smaller keloids were treated by intralesional corticosteroids as monotherapy. Triamcinolone acetonide suspension 10 mg, diluted 1:3 with xylocaine, was used at 3-week intervals over 3 months. Some of the keloids showed partial, and none complete flattening.

Discussion

Therapeutic management of keloids is still a challenge, since the results of treatment are usually unsatisfactory. There is no universally efficacious treatment, and numerous treatment modalities have been attempted, often with disappointing results. Traditionally, these included the use of cryotherapy, intralesional corticosteroids, or pressure therapy. The best results were often achieved by combining different invasive methods such as cryotherapy, surgery, intralesional corticosteroids, laser or radiotherapy^{8,9}.

Cryosurgery has been used alone or in conjunction with injections of corticosteroids. Rusciani et al. report flattening of 70% of keloidal scars after cryosurgery monotherapy, with pigmentary disturbances in all cases¹⁰. Hirshowitz et al. examined the use of cryotherapy with concomitant intralesional application of triamcinolone acetonide. Although this therapeutic combination led to complete regression in 70% of study patients, the true success of this therapeutic approach was difficult to evaluate because they were not able to determine the rate of recurrence¹¹. Monotherapy with intralesional corticosteroids can also produce partial or full flattening of keloidal scars, especially of smaller lesions. As surgical excision of keloids shows a high rate of recurrence, it is usually used with adjunctive therapies such as intralesional corticosteroids or radiotherapy⁸. A study of Tepmongkol involving the use of radiation with or without surgery suggested there would be no benefit of preoperative irradiation of keloidal scars¹². Sclafani et al. carried out a prospective study to compare the effects of postoperative radiotherapy and corticosteroid injections, and found no statistically significant difference¹³. In the available literature, we found no data on combining intralesional corticosteroids and radiotherapy after surgical excision in the same lesion.

Our patient spontaneously developed multiple keloids, although the first keloid occurred at the site of variola vaccination. This event may be considered as a

precipitating factor for the first lesion, but the causes of subsequent multiple keloids remained unclear. According to some authors, spontaneously developed keloids are more likely to be refractory to various treatments⁵.

When our patient presented to our Department, she had a history of 15 cryotherapy sessions, without any effect. We decided to perform different therapeutic options to different sites. In addition to monotherapy with intralesional corticosteroid injection, we decided to employ two combinations of different methods. In one lesion, we performed surgical excision followed by superficial radiotherapy, and after 3 months we noticed the first sign of recurrence. Although partial regression occurred after intralesional application of corticosteroids to recurrence lesions, the result was not satisfactory.

Therefore, we decided to treat the other keloidal scar by a combination of surgical excision, intradermal injection of corticosteroids into the wound margins prior to final wound closure, and subsequent fractionated soft x-ray radiotherapy beginning from the second postoperative day. It was our own therapeutic protocol modification, which included a three-modal treatment, to our knowledge not yet described. This therapeutic option proved fully successful and in this case most efficacious as the patient remained free from any sign of recurrence at 3-year follow-up. However, the efficacy of this method has to be proved through the prospective controlled study in future.

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RAZLIČITI TERAPIJSKI PRISTUPI U BOLESNICE S VIŠESTRUKIM SPONTANO NASTALIM KELOIDIMA – PRIKAZ SLUČAJA

SAŽETAK

Keloidi su dobroćudni tumori koji obično nastaju nakon ozljede, kao posljedica neprimjerenog tkivnog odgovora pri cijeljenju. Radi se o promjenama koje u današnje vrijeme još uvijek predstavljaju terapijski problem. Iako su na raspolaganju brojne terapijske mogućnosti, rezultati liječenja obično nisu zadovoljavajući. Opisuje se bolesnica s višestrukim, spontano nastalim keloidima na trupu. Četiri terapijska pristupa su primijenjena na različitim keloidima u iste bolesnice, s različitim terapijskim odgovorom. Krioterapija kao prvi terapijski pristup nije dovela do zadovoljavajućeg

učinka. Intralezijskom primjenom kortikosteroida postignuta je djelomična regresija promjene. Keloid koji je liječen ekscizijom i potom radioterapijom ponovno se je pojavio nakon tri mjeseca. Najbolji rezultat je postignut kombinacijom ekscizije, intralezijske primjene kortikosteroida u rubove operacijskog reza i zatim radioterapije. Prema saznanjima autora, ovo je prvi opis trimodalnog terapijskog pristupa u liječenju keloida, nakon kojeg nije nastao recidiv tijekom trogodišnjeg praćenja bolesnice.

Progressive Chronic Inflammatory Demyelinating Polyneuropathy in a Child with Central Nervous System Involvement and Myopathy

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ABSTRACT

Chronic inflammatory demyelinating polyneuropathy (CIDP) is a chronic disorder, manifesting with monophasic or relapsing course. Progressive course is rare in children. The article presents a boy with progressive generalized muscle weakness and areflexia since the age of two, developed after viral infection. Electromyoneurography showed severe neurogenic lesion, with myopathic pattern in proximal muscles. Increased serum ganglioside antibody titers (anti-GM1 and anti-GD1b) were registered. Sural nerve biopsy revealed demyelination and onion bulbs. Inflammatory perivascular CD3 positive infiltrates were present in muscle and nerve biopsies. Brain magnetic resonance imaging showed cortical atrophy, hyperintensities of the white matter and gray matter hypointensities. Improvement occurred on intravenous immune globulins and methylprednisolone treatment. Demyelination might develop in central and peripheral nervous system associated with inflammatory myopathy in patients with progressive course of CIDP.

Key words: chronic inflammatory demyelinating polyneuropathy, inflammatory myopathy, central nervous system, child, antiganglioside antibody

Introduction

Chronic inflammatory demyelinating polyneuropathy (CIDP) manifests itself with monophasic, relapsing or progressive course. Children with CIDP present with more severe clinical features than adults¹. It is characterized by multifocal demyelination involving nerve roots, intermediate nerve segments and nerve terminals. Demyelinating changes of the brain have been reported in some adult patients with CIDP², and scarcely among pediatric patients, mainly in the white matter³. The pathogenesis of CIDP is still unknown, but involvement of the immune system has been firmly established⁴. Different ganglioside autoantibodies have been detected in adults with CIDP⁵.

We present a case of a boy with early onset progressive muscle weakness since the age of 2, associated with focal central nervous system (CNS) involvement of white and gray matter, inflammatory myopathy and positive anti-GM1 ganglioside antibodies.

Case Report

A boy of normal psychomotor development, now at the age of 10, was born after normal pregnancy and labor. At the age of two he manifested progressive generalized muscle weakness, hypotonia and small muscles hypotrophy, developed three weeks after viral infection. Electromyoneurography performed after six months showed axonal lesion and mild loss of motoneurons and myopathic pattern in proximal muscles. Muscle biopsy showed muscle fiber regeneration and necrosis, perivascular inflammatory infiltrates, and subsarcolemal accumulation of mitochondria without visible structural abnormalities. Brain magnetic resonance imaging (MRI) showed mild cortical atrophy, hyperintensities of the white matter and the gray matter hypointensities, whereas angiography was normal. He was treated with physiotherapy, without improvement.

At the age of 4.5, examination revealed muscle weakness (grade 4), generalized hypotonia and small muscles

hypotrophy, peroneal gait, positive Gowers' sign, areflexia, and right abducens palsy. Functional score of motor deficit was estimated according to Hughes grading scale (grade 0 – healthy, grade 1 – minor signs or symptoms not interfering with normal social life, grade 2 – able to walk without support of a stick but incapable for manual work, grade 3 – able to walk 5 meters with assistance, grade 4 – confined to bed or chairbound, grade 5 – requiring assisted ventilation, grade 6 – dead)⁶. Electromyoneurography showed severe neurogenic lesion, spontaneous activity, low compound muscle action potentials (1 mV), decreased motor nerve conduction velocity (7.9–11.3 m/s; normal values 56.14 ± 4.96 m/s)^{7,8}, prolonged distal latency, and myopathic pattern in proximal muscles. Cerebrospinal fluid examination revealed increased protein content (0.40 g/L). Proton magnetic resonance spectroscopy indicated decreased levels of N-acetyl-aspartat in cerebral cortex and increased lactate. Positive serum anti-GM1 (IgM, 2000 Bühlmann Titer Units-BTU) and anti-GD1b (IgG, 2300 BTU) antibodies were detected. CK was 97–127 U/L (normal values 75–230 U/L at 37 °C). Follow-up brain MRI showed multiple white matter lesions, and repeated muscle biopsy showed neurogenic atrophy. Sural nerve biopsy revealed demyelination and onion bulbs. Inflammatory perivascular CD3-positive infiltrates were present in both biopsies. Visual evoked potentials showed bilaterally prolonged latencies. Metabolic and immunological tests were normal, same as aryl-sulphatase A and galactocerebrosidase activities. Mutations for Charcot-Marie-Tooth disease 1A and hereditary neuropathy with pressure palsies, as well as connexin and spinal muscle atrophy mutations were excluded. Mitochondrial genome analysis showed gene polymorphism in sequence T13933A in ND5 gene, confirmed by mitochondrial genome sequencing. Methylprednisolone treatment (1 mg/kg/day) induced significant improvement after four weeks. Tapering off the steroids, a few months later, caused serious neurological impairment, so steroids were reintroduced at a lower dose.

On examination at the age of 6, peroneal gait, positive Gowers' sign, muscle weakness (grade 2), small muscles hypotrophy, areflexia, distal hypesthesia, left talocrural and interphalangeal 5th finger contractures were present. Follow-up electromyoneurography showed progression of the neural loss, inelicitable compound muscle action potentials on the left peroneal nerve and very low (250 μ V) on the right, with prolonged distal latency and completely absent sensory neural potentials. Further clinical improvement occurred after intravenous immune globulins (IVIg) treatment (2 g/kg/5days). The following electromyoneurography showed very low, but elicitable left peroneal (250 μ V), and increase of the amplitude of the right peroneal compound muscle action potentials. On examination at the age of 7.5, he manifested slight improvement of muscle strength (grade 1), without additional changes in neurological examination. The recent electromyoneurography, at the age of 9.5, showed inelicitable left peroneal nerve compound muscle action potentials (Table 1 and Figure 1). Azathioprine was in-

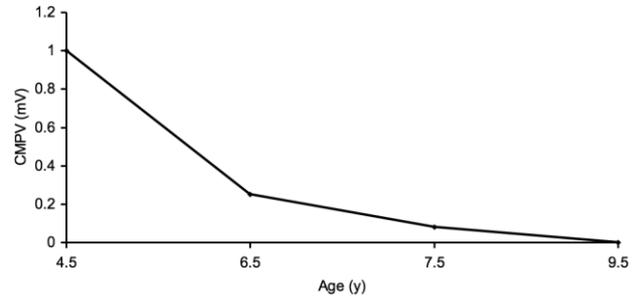


Fig. 1. Decreasing of compound muscle action potential amplitude during long term follow-up recorded in extensor brevis mus-

troduced (1 mg/kg/day). He was treated with maintenance steroid dose (0.5 mg/kg) on alternate day, azathioprine (1mg/kg/day) and periodic intravenous immune globulins treatment (1 g/kg/3days) every 8–10 weeks. Follow-up brain MRI and proton magnetic resonance spectroscopy at the age of 8 showed improvement with hyperintensities of the white matter mostly in frontal region (Figure 2).

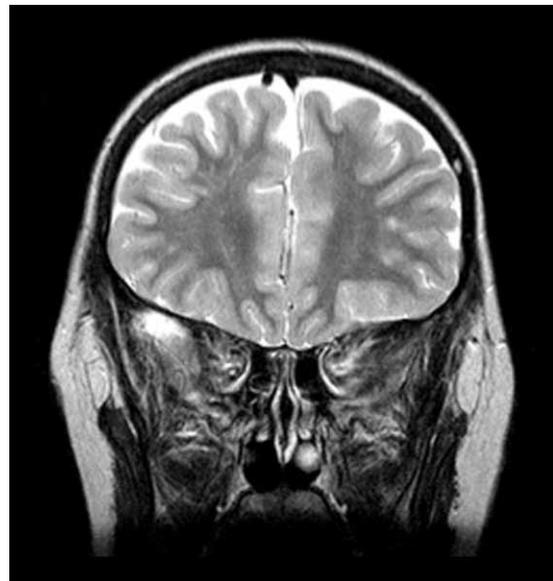


Fig. 2. T2 – weighted brain magnetic resonance imaging (MRI) coronal section (repetition time (TR), 5800.0 / echo time (TE), 90.0 ms). Hyperintensity of the white matter in left frontal region.

Discussion

Our patient manifested limb girdle muscle weakness and progressive small muscles atrophy associated with CNS involvement. Electromyoneurographical findings fulfilled diagnostic criteria for CIDP^{9,10}, which was confirmed by nerve biopsy, whereas muscle biopsy showed myopathy with perivascular inflammatory infiltrates. CIDP manifests itself with broad heterogeneous clinical

TABLE 1
FOLLOW UP OF NEUROGRAPHIC ABNORMALITIES

Age (yrs.)	MNCV (m/s)		CMAP (mV)		DL (ms)	
	M	P	M	P	M	P
4.5	11.3 56.4±4.22*	7.9 56.14±4.96*	nd	1 7.1±4.76*	0.71 2.27+7/-0.45*	2 3.01±0.43*
6.5#	15.5 59.5±5.1*	8.33 57.05±4.54*	S1 – 1 S2 – 0.5 12.37±4.79*	S1 – 0.25 S2 – 0.5 8.15±4.19*	1.17 2.73±0.44*	0.9 3.25±0.51*
6.5##	nd 59.5±5.1*	12 57.05±4.54*	nd	S1 – 1 S2 – 1 8.15±4.19*	nd	1.11 3.25±0.51*
7.5	14.2 59.5±5.1*	13 57.05±4.54*	0.6 12.37±4.79*	0.08 8.15±4.19*	1.11 2.73±0.44*	0.5 3.25±0.51*
9.5	13.3 59.5±5.1*	0 57.05±4.54*	0.8 12.37±4.79*	0 8.15±4.19*	0.93 2.73±0.44*	0 3.25±0.51*

MNCV – motor nerve conduction velocity, CMAP – compound muscle action potential, DL – distal latency, M – median nerve, P – peroneal nerve, S1- distal stimulation point, S2 – intermediate stimulation point, y – years, nd – not done, *normal values, #2 years after steroid treatment introduction, ##4 weeks after intravenous immunoglobulin (IVIG) treatment

and electrophysiological symptoms². Axonal features are observed rarely in children¹⁰. Children with CIDP manifest more proximal muscle weakness¹¹. The diagnosis of CIDP in our patient was established according to proposed electrophysiologic criteria by American Academy of Neurology in 1991⁹. His electromyoneurography showed the conduction abnormalities in both distal and intermediate segments with prolonged distal latencies more than 125% of the upper limit, and pronounced decreasing of motor nerve conduction velocity. Multifocal demyelination is a diagnostic hallmark of CIDP, but the degree and distribution of demyelination is variable¹². Recently, conduction block/temporal dispersion have been proposed as more sensitive and reliable electromyoneurographical criteria for the diagnosis of CIDP¹³. Other than pronounced demyelinating polyneuropathy, electromyoneurography in our patient showed absent or very low compound muscle action potentials, especially on distal stimulation, suggesting severe and afterwards complete axonal degeneration. Primary demyelination is the main pathological feature in CIDP. However, axonal degeneration in distal parts of the nerves was seen on autopsy in patients with CIDP¹⁴. Axonal degeneration is often responsible for neurological impairment in autoimmune and inherited disorders of central and peripheral nervous system¹⁵. Distribution pattern of electrodiagnostic abnormalities in inflammatory demyelinating polyneuropathies correlates with clinical features, response to treatment and outcome¹². In accordance with observation reported, our patient showed diffuse pattern with slowly progressive and relapsing course.

Onion bulbs and inflammatory perivascular CD3 positive infiltrates were registered in his nerve biopsy. Inflammatory infiltrates are registered often around epineurial blood vessels, consisting of CD4 and CD8 T lymphocytes¹⁰, thus suggesting the important role of the

cellular immunity in the pathogenesis of CIDP. Macrophages are involved in striping of the myelin from axons with variable axonal loss.

Proximal muscle weakness, myopathic pattern in electromyoneurography and CD3 positive infiltrates in muscle biopsy of our patient are compatible with the diagnosis of inflammatory myopathy. Normal CK values do not exclude the muscle fibers involvement in our patient. Polymyositis associated with CIDP has never been reported. However, Kimura et al. described a boy with myasthenia gravis and CIDP, suggesting the spreading of immune response from neuromuscular junction to peripheral nerve¹⁶.

Cerebrospinal fluid analysis of our patient revealed slightly increased protein content (0.40 g/L, normal values up to 0.37 g/L) and suspected IgG intrathecal synthesis, suggesting dysfunction of the blood – cerebrospinal fluid barrier surrounding the nerve roots.

IgG and IgM anti-GM₁ antibodies were also present in our patient as reported previously in adult patients with CIDP⁵. The role of these antibodies in the pathogenesis of the disease is unknown. Gangliosides may act as target antigens in the immunopathogenesis of neuronal/myelin damage. Our patient manifests both central and peripheral nervous system involvement. None of the reported patients with CIDP and elevated IgM anti-GM1 antibodies manifested definite CNS involvement, but showed spontaneous activity and similar electrophysiological findings as our patient¹⁷. In patients with amyotrophic lateral sclerosis expression of antiganglioside antibodies varies between 5.5–78%^{18,19} also suggesting that increased anti-GM1 IgM titers are closely associated with purely motor or resembled motor neuron disease mostly reversible by treatment.

CNS involvement in CIDP presenting initially with spinal symptoms and MRI intramedullary lesion has been observed in the child. Later on, demyelination developed in the brain and peripheral nerves³. Differential diagnosis of CIDP in children includes hereditary sensory and motor neuropathies, leukodystrophies (metachromatic, Krabbe) and other demyelinating disorders of CNS, as well as aryl-sulphatase A pseudodeficiency²⁰, which were excluded in our patient. Some patients with Charcot-Marie-Tooth disease show worsening and inflammatory infiltrates in their biopsies²¹ clinically responding to corticosteroids. It might also be possible that patients with CIDP and mutations found in Charcot-Marie-Tooth disease manifest more pronounced symptoms²².

Some of the clinical symptoms in our patient resemble mitochondrial encephalomyoneuropathies. CNS involvement included radiological signs of both white and gray matter. Proton magnetic resonance spectroscopy revealed high lactate and decreased levels of N-acetyl-aspartate while the first biopsy performed at the age of 3 showed subsarcolemmal accumulation of mitochondria without visible structural abnormalities. Both of these findings are not specific and might be present secondary to different inflammatory conditions^{23,24,25}. Visual evoked potentials analysis showed signs of demyelinating optic neuropathy. Optic neuropathy is not an uncommon finding in CIDP. The association of a multiple sclerosis (MS)-like disease and pathogenic Leber's hereditary optic neuropathy (LHON) mutations was reported previously in several publications^{26,24,27}. Very recently the pathology of the MS-like lesions on autopsy was investigated in a patient carrying the mitochondrial DNA 14484 pathogenic LHON mutation²⁸. Nuclear and mtDNA analysis in our patient revealed only gene polymorphism, confirmed by mitochondrial genome sequencing, thus excluding pathogenetic role of mitochondrial DNA mutation.

Immunomodulating treatment has been shown to be effective in children with CIDP^{10,1}. IVIG is effective as the first line therapy in CIDP according to the controlled clinical trials. IVIG action in CIDP includes inhibition of complement and membranolytic attack complex activation beside modulation of autoantibodies as well as of inhibition or activation of receptors²⁹. Remissions are usu-

ally achieved after IVIG treatment but the vast majority of patients still need additional intermittent IVIG treatment at the dose 0.15–0.4 g/L every 6–8 weeks. The tapering off the steroids in our patient resulted in clinical deterioration and progression of small muscles atrophy. Some authors reported steroid dependant course of relapsing CIDP, which did not respond well to IVIG³⁰. Children with rapid progression of CIDP are more responsive to steroids^{30,10}. Azathioprine has been shown to be effective in the treatment of CIDP in children after initial remission induction with steroids. None of the serious side effects occurred during 12 years of follow-up¹⁰. The efficacy of interferon β 1 (a or b) still waits for proper investigation of its place in treatment of CIDP.

Follow-up electromyoneurography showed slight improvement of distal compound muscle action potentials in our patient after IVIG treatment, as previously reported in adults³¹. Clinical improvement thus correlated with resolution of conduction block in distal nerve segments. However, inelicitable peroneal nerve and severe axonal loss were observed most recently in our patient. Resistance to the treatment might be induced by the axonal involvement during the long course of the illness¹².

Clinical outcome and response to treatment are excellent in children with CIDP, although electrodiagnostic parameters of pronounced demyelination or axonal damage might remain unchanged during long-term follow-up²⁰.

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KRONIČNA UPALNA DEMIJELINIZIRAJUĆA POLINEUROPATIJA PROGRESIVNOG TIJEKA U DJEČAKA UDRUŽENA SA ZAHVAĆANJEM SREDIŠNJEG ŽIVČANOG SUSTAVA I MIOPATIJOM

SAŽETAK

Kronična upalna demijelinizirajuća polineuropatija se očituje monofazičnim tijekom ili ponavljajućim pogoršanjima bolesti. Progresivan tijek bolesti u djece je rijedak. U članku prikazujemo dječaka sa progresivnom generaliziranom mišićnom slabošću i arefleksijom, koja se razvila u dobi od 2 godine, nakon preboljele virusne infekcije. Elektromiografijom je nađeno teško neurogeno oštećenje s miopatskim uzorkom u proksimalnim mišićnim skupinama. U tijeku obrade dokazan je povišeni titar serumskih protutijela na GM1 i GD1b gangliozide a pregledom biopta suralnog živca potvrđena je hipertrofična demijelinizirajuća neuropatija i formacije poput lukovica. Upalni CD3 pozitivni infiltrati nađeni su u bioptatima mišića i živca. Magnetska rezonancija mozga pokazala je kortikalnu atrofiju, hiperintenzitete bijele tvari i hipointenzitete sive tvari. Poboljšanje je uslijedilo nakon intravenske primjene imunoglobulina i terapije metilprednisolonom. Demijelinizacija se može razviti u središnjem i perifernom živčanom sustavu udružena s upalnom miopatijom u bolesnika s progresivnim tijekomom kronične upalne demijelinizirajuće polineuropatije

Use of DNA Probes in the Diagnosis and Treatment of Periodontitis – A Case Series

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ABSTRACT

Aggressive periodontitis is characterized by rapid attachment and bone loss with no underlying systemic disease and is associated with specific bacteria like Actinobacillus actinomycetemcomitans (Aa) and Porphyromonas gingivalis (Pg). In this case series 25 patients were diagnosed with aggressive periodontitis by the aid of DNA probes for Aa and Pg and other periodontal pathogens. The use of DNA probes for the detection of periodontal pathogens may aid in the diagnosis and treatment of aggressive periodontitis. Clinical experience suggests that lowering periodontal pathogens to undetectable levels could improve the long-term stability of periodontal health.

Key words: periodontitis, periodontal diseases, diagnosis, treatment

Introduction

Aggressive periodontitis as defined by the 1999 International Workshop for a Classification of Periodontal Diseases and Conditions is characterized by the following:

1. Patients that have no underlying systemic disease.
2. Have rapid attachment and bone loss.
3. Have a family history of the disease.

Secondary features may include elevated proportions of *Actinobacillus actinomycetemcomitans* (Aa) and in some cases a high level of *Porphyromonas gingivalis* (Pg)¹. A correct diagnosis is essential in the treatment of aggressive periodontitis. The elimination or significant reduction of periodontal pathogens is one of the major treatment goals. The use of the DNA probes for diagnosis of aggressive periodontitis is well documented²⁻⁶. It appears to be a good alternative to culturing, with faster results^{2,7}. One of the drawbacks of this technique is that it is limited to the known periodontal pathogens that the specific DNA probes is designed for.

DNA probes could also be used to monitor sites before and following therapy, in order to validate the desired outcome of reduction of the subgingival pathogens to undetectable levels⁸⁻¹⁰. Several studies linked periodontally affected sites with elevated levels of periodontal patho-

gens¹¹⁻¹⁸. Further studies have shown that sites left populated with periodontal pathogens like Pg and *Tannerella forsythensis* (Tf) (formerly *Bacteroides forsythus*) lost more clinical attachment and had more bleeding on probing^{9,19}. In addition, it was demonstrated that sites that improve clinically have lower levels of Pg²⁰.

In this report 25 cases of aggressive periodontitis that were treated in the practice of one of the authors (GZ) are documented. Diagnosis of aggressive periodontitis was based on clinical and radiographic evaluation of the periodontal condition, the absence of underlying systemic disease, and by utilizing DNA probes to analyze their subgingival microbial flora. DNA probes were also used to monitor those profiles throughout treatment. Three case reports will be presented to demonstrate the use of DNA probes in conjunction with periodontal therapy, to assure reduction of periodontal pathogens to undetectable levels, and thus stabilizing the periodontal condition of the patients.

Materials and Methods

Twenty-five patients were treated in a Center for Periodontology. They were referred for evaluation and

treatment of their periodontal condition by their general dentists. In addition to a regular clinical and radiographic evaluation subgingival plaque samples were obtained and their microbial profile was analyzed in the following way:

Immediately after sampling, paper points were dipped into 50 μ l of guanidinium thiocyanate buffer²¹⁻²³ in tight-sealing 100 μ l screw-cap tubes. The samples were heated to 70 °C for 10 minutes, thoroughly vortexed and stored frozen until analysis (no longer than 2 weeks). A microbiologist, blinded to treatment allocation, performed the analysis of the samples.

For analysis, the samples were diluted and aliquots of each were applied on 5 nylon membranes mounted in dot-blot manifolds (Inotech AG). The membranes were processed by standard procedures²⁴. Each one was then hybridized to one of five²², P-labelled, specific probes for the small subunit ribosomal RNA's (ssRNAs) of Aa, Tf, Pg, *Treponema denticola* (Td) and to a universal bacterial probe (UP). Probes Aa, Bf, Pg and UP were obtained

from Microprobe (Microprobe Corporation, Bothell, WA, USA), probe Td was designed by IAI (Institute of Applied Immunology). Hybridization and wash conditions were set as recommended by the manufacturer of the probes (Microprobe)²⁵. A processing control was attained by sampling a known amount of lysed *Escherichia coli* on each membrane. Blots were quantified by direct counting in a Trace-96 system (Inotech AG). No calibration curves were necessary because the cpm readings turned out to be linear from 2×10^4 to 600×10^6 bacteria.

Denatured reference standards for each of the 4 bacteria were applied along with the samples to each membrane. Counts for each bacterial species were determined by comparison to the homologous standard. To determine total bacteria, a pool of the 4 standards, consisting of quantified dilutions of plasmids containing a cloned full DNA copy of the ssrRNA of each of the 4 bacterial species were used. Countswere transferred in to 'millions of bacteria' by using an arbitrarily set standard of each bacterium being equivalent to 10^4 copies of ssrRNA.

TABLE 1
PERIODONTAL PATHOGENS AND ANTIBIOTIC REGIMEN (A-R) IN THE TREATED CASES

Cases	Periodontal pathogens (at baseline)	A-R 1	Periodontal pathogens (after SRP)	A-R 2	Periodontal pathogens (at maintenance)	A-R 3
1	A.a, Pg	X 1	A.a	X 2	∅	∅
2	A.a	X 1	∅	∅	∅	∅
3	A.a	X 1	∅	∅	∅	∅
4	A.a, Pg, Pi, Tf	X 1	A.a, Pg	X 2	∅	∅
5	Pg, Pi, Tf	X 4	Pg	X 4	∅	∅
6	A.a, Pg	X 1	∅	∅	∅	∅
7	Pg, Pi, Tf	X 4	Pg, Tf	X 4	∅	∅
8	A.a, Pg, Pi, Tf	X 1	A.a, Pi, Tf	X 2	∅	∅
9	A.a, Pg	X 1	A.a	X 3	∅	∅
10	Pg, Tf, Td	X 4	∅	∅	∅	∅
11	A.a, Pg	X 1	A.a, Pg	X 2	∅	∅
12	A.a	X 3	A.a	X 3	∅	∅
13	A.a, Pg, Tf	X 1	Pg	X 4	∅	∅
14	A.a, Pg	X 1	A.a	X 3	∅	∅
15	A.a	X 3	A.a	X 3	∅	∅
16	Pg, Pi, Tf	X 4	∅	∅	∅	∅
17	A.a, Pg, Pi, Tf	X 1	A.a, Pg	X 2	A.a	X 3
18	A.a	X 3	∅	∅	∅	∅
19	Pg, Pi, Tf	X 4	∅	∅	∅	∅
20	Pg, Pi, Tf	X 4	Pg	X 4	∅	∅
21	A.a, Pg	X 1	∅	∅	∅	∅
22	A.a, Pg, Pi, Tf	X 1	A.a, Pg	X 2	A.a	X 3
23	A.a, Pg, Pi, Tf	X 1	Pg, Pi	X 4	∅	∅
24	A.a, Pg, Tf	X 1	A.a, Tf	X 2	A.a	X 3
25	Pg, Pi, Tf	X 4	Pg, Pi	X 4	∅	∅

A.a – *Actinobacillus actinomycetemcomitans*, Pg – *Porphyromonas gingivalis*, Pi – *Prevotella intermedia*, Tf – *Tannerella forsythensis*,

X 1 – Amoxicillin 1000 mg daily plus Metronidazole 800 mg daily, 1 week

X 2 – Augmentin 1000 mg daily plus Metronidazole 800 mg daily, 1 week

X 3 – Doxycyclin 200 mg daily, 1 week

X 4 – Metronidazole 800 mg daily, 1 week

Case 1

The patient (43 year-old female) presented in the office for an initial periodontal examination in February 1993. Her chief complaint was constant gingival pain, swelling and bleeding on probing. She denied any systemic disease, allergies, or taking any medication on a regular basis.

Intraoral examination

The intraoral examination revealed unsatisfactory restorative treatment. The gingiva in maxilla and mandible was edematous and bulbous (Figure 1). The gingival margins were generalized rolled, and bled spontaneously or upon light probing. Gingival recession was evident throughout (Figure 1). The probing depth (PD) in the areas of # 3, 8, 9, 12–14, 19–21, 28–30 ranged from 8–10 mm. Purulent exudate was present in localized sites. The oral hygiene index (OHI)²⁶ was 50%, bleeding on probing (BOP) was generalized (100%).



Fig. 1. Facial view baseline, case #1.

Radiographic analysis

Generalized horizontal bone loss ($\geq 50\%$) with localized vertical defects in the area of the mandibular incisors and 1st molars (both jaws) was observed in the radiographs.

Microbiology

The microbiological analysis of the subgingival plaque samples collected from the deepest sites revealed elevated levels of Aa (2×10^3) as well as Pg (1×10^3 ; Table 1).

Diagnosis – aggressive periodontitis

Treatment phase

The patient was informed about the etiology of periodontal disease and was instructed and motivated in self-performed plaque control. During the initial treatment phase supragingival plaque was removed, and the teeth were polished. Scaling and root planing (SRP) was performed under local anesthesia with lidocaine hydrochloride and epinephrine (Xylestesin-S, 3M ESPE). Following SRP the patient was put on a systemic antibiotic regimen

of Amoxicillin (Ratiopharm GmbH) 1000 mg daily and Metronidazole (Aventis Pharma) 800 mg daily for one week.

Ten weeks post SRP subgingival plaque samples were collected. The result of the DNA analysis showed elevated levels of Aa (1×10^3 ; Table 1).

Subgingival deep scaling and root planing (SRP) under local anesthesia was repeated and the patient was put again on a systemic antibiotic regimen (Augmentin 1000 mg daily and Metronidazole 800 mg daily for one week) were repeated.

In order to reduce probing pocket depths, and to ensure thorough removal of subgingival deposits, flap surgery was performed in the areas of the localized defects. Surgical treatment was covered by systemic use of Doxycyclin 200 mg once a day for a period of 10 days. The patient refused to have any type of regenerative treatment. Ten weeks after medication subgingival plaque samples were collected and analyzed for detection of periodontal pathogens. The results of the analysis were negative.

Maintenance phase

The patient was enrolled in a 3-month interval maintenance program. Subgingival plaque samples were collected and analyzed microbiologically. The analysis of the plaque samples was performed once a year, starting six months after completion of the treatment. The results were negative for periodontal pathogens (Table 1). PDs had decreased to 2–3 mm. The OHI improved (8%), and BOP was reduced (5%).



Fig. 2. Facial view ten year recall, case #1.

Due to the patient's excellent plaque control, the patient recall schedule was adjusted to every 6 months. The PDs (3 mm), OHI (6–10%) and BOP (0–5%) remained stable throughout the maintenance phase to date during the 10 years of follow-up (Figure 2).

Case 2

The patient (31 year-old female) was referred in December 1993 for periodontal treatment by her general dentist. The medical history was negative for any chronic



Fig. 3. Facial view at baseline, case #2.

disease and no medications were taken on a regular basis. She was not aware of any drug allergies. Previous attempts by her general dentist to reduce PPDs by performing gingivectomies had failed. Therefore the patient was referred to the periodontist.

Intraoral examination

The intraoral examination revealed localized discolored gingiva. The gingival margins were rolled. Generalized gingival recession was evident (Figure 3). The PDs of the incisors, 1st molars and canines of both jaws ranged between 8–10 mm. The oral hygiene index (OHI) was 10%, BOP was (5%).

Radiographic analysis

Horizontal bone loss with localized vertical defects that extended up to 50% of the root length could be observed on several teeth (Figure 4).

Microbiology

The microbiological analysis of the subgingival plaque samples collected from the deepest sites revealed elevated levels of Aa (1×10^4 ; Table1).

Diagnosis – aggressive periodontitis.

Treatment phase

The patient was informed about the etiology of periodontal disease and was instructed in plaque control methods.

Scaling and root planing (SRP) was performed with local anesthesia which was followed by a systemic antibiotic regimen (Amoxicillin 1000mg daily and Metronidazole 800mg daily for one week).

Ten weeks later subgingival plaque samples were collected again. The result of the DNA analysis was negative for periodontal pathogens (Table 1).

After microbiological examination, in the area of the maxillary and mandibular molars. No augmentive procedures were performed. Doxycyclin 200 mg was given for 10 days post-operatively.

Maintenance phase

The patient was enrolled in a supportive periodontal therapy (SPT) program. Subgingival plaque samples were collected and analyzed microbiologically. The analysis of the plaque samples was performed once a year, starting six months after completion of the treatment. The results were negative for periodontal pathogens (Table 1). PDs had decreased to 2–3mm. The OHI improved to 4%, and BOP was reduced 0%. The gingiva appeared firm and pink (Figure 5).

The patient was placed on a 6 months recall schedule. The PDs (2–3 mm), OHI (4–6%) and BOP (0–2%) re-



Fig. 4. Full mouth radiographs at baseline, case #2.



Fig. 5. Facial view 8-year recall, case #2.

remained stable throughout the maintenance phase. Additional gingival recessions did not occur during a follow-up period of 9 years.

Case 3

The patient (36 year-old female) presented for an initial periodontal examination in July 1995. The chief complaint was constant gingival pain, swelling and bleeding. She denied any systemic disease, allergies, or taking any medication on a regular basis.

Intraoral examination

The intraoral examination revealed unsatisfactory restorative treatment. The gingiva in maxilla and mandible was edematous and bulbous. The gingival margins appeared »rolled«. Localized gingival recession was present. The papillae were edematous and bled upon light probing (Figure 6). Probing depths up to 10mm were detected in the areas of the canines and first molars of both jaws with out purulent exudate. The oral hygiene index (OHI) was 20% and BOP was 30%.



Fig. 6. Facial view at baseline, case #3.

Radiographic analysis

Radiographic analysis (Figure 7) revealed generalized horizontal bone loss with localized vertical defects that

extended to the apical third of the roots ($\geq 50\%$). A panoramic radiograph of this patient is presented in Figure 7.



Fig. 7. Panoramic radiograph at baseline, case #3.

Microbiology

The microbiological analysis of the subgingival plaque samples collected from the deepest sites revealed elevated levels of Aa (2×10^4 ; Table 1).

Diagnosis – aggressive periodontitis.

Treatment phase

The patient was educated about the etiology of periodontal disease and was instructed and motivated in self-performed plaque control. Scaling and root planing was performed under local anesthesia and followed by a systemic antibiotic regimen (Amoxicillin 1000 mg daily and Metronidazole 800 mg daily for one week).

Ten weeks post SRP subgingival plaque samples were collected. The result of the DNA analysis was negative for periodontal pathogens (Table 1).

After this microbiological examination, flap surgery was performed in the areas of the localized defects. Doxycyclin 200 mg once a day for a period of 10 days was administered post-operative.

Maintenance phase

The patient was enrolled in a periodontal maintenance program. Subgingival plaque samples were collected and analyzed microbiologically once a year, start-



Fig. 8. Facial view 8-year recall, case #3.

ing six months after completion of the treatment. The results were negative for periodontal pathogens (Table 1). PDs had decreased to 2–3mm. The OHI improved to 10%, and BOP was reduced to 5%.

The patient was on a 6 months recall schedule. The PDs (2–3 mm), OHI (10%) and BOP (0–5%) remained stable throughout the maintenance phase. No further gingival recession was detected during the 8-year follow-up period (Figure 8).

Discussion

This case series presents various treatment modalities for aggressive periodontitis patients with the common aspect that the patients were monitored for periodontal pathogens levels by DNA probes. It was observed in these 25 additional cases of aggressive periodontitis treated in the authors practice that ensuring undetectable levels of periodontal pathogens by using a combination of non-surgical, antimicrobial and surgical treatment modalities could prevent future periodontal destruction could be prevented and long-term stability achieved.

Our clinical experience confirms the findings of Shiloah et al. (1998), who observed that sites infected with

Pg or Tf showed more attachment loss at the one year follow-up irrespective of the treatment modality used¹⁹. The follow-up periods of all 25 cases ranged from 3–10 years. All patients showed stability during this time period. In the treatment of periodontitis, especially when dealing with aggressive forms of the disease that fact that periodontitis is a bacterial infection needs to be addressed. Reducing a amount of microbial periodontal pathogens to undetectable levels should be a goal of treatment, thus allowing the hosts immune system to better cope with the infection²⁷. DNA probes are a fast way to determine periodontal pathogen levels and help to indicate necessary treatment adjustments.

Conclusion

This case series suggests that the use of DNA probes to detect periodontal pathogens, may provides the clinician with the opportunity to decide at immediately if supplemental antibiotic therapy is necessary, in addition to non-surgical or surgical therapy and to monitor periodontal pathogen level during maintenance to assure periodontal stability.

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UPOTREBA DNA-SONDI U DIJAGNOSTICI I TRETMANU PARODONTITISA – SERIJA SLUČAJEVA

S A Ž E T A K

Agresivni parodontis karakterizira brzi gubitak pričvrstka i kosti bez prisustva sistemnih bolesti, a povezan je s specifičnim bakterijama kao što su *Actinobacillus actinomycetemcomitans* (Aa) i *Porphyromonas gingivalis* (Pg). U ovoj seriji od 25 slučajeva kod pacijenata je dijagnosticiran agresivni parodontitis pomoću DNA sonda za Aa i Pg i ostale parodontopatogene. Rezultati pokazuju da upotreba DNA sonda za detekciju parodontopatogenih bakterije može pomoći u dijagnozi i terapiji agresivnog parodontitisa. Pored toga, prema našim kliničkim iskustvima smanjenje parodontnih patogena rezultira dugoročno stabilno parodontno zdravlje.

Subacute Sclerosing Panencephalitis – The Continuing Threat

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ABSTRACT

Clinical, epidemiological and laboratory findings of four patients with subacute sclerosing panencephalitis (SSPE), diagnosed in Croatia in 2002, were examined. Patient age at disease onset ranged from 5–11 years. All patients were vaccinated regularly with MMR-vaccine. Two patients had a history of measles infection at the age of six and seven months, respectively. In the other two patients, the disease started immediately after the varicella infection. Complement fixing antibody titre to the measles virus (MV) ranged from 1:1024 to 1:65536 in serum, and from 1:16 to 1:128 in cerebrospinal fluid (CSF). In CSF, no antibodies to varicella-zoster virus were found. Brain tissue samples were obtained at autopsy from two patients. In one patient, electron microscopy demonstrated intranuclear viral inclusions (MV nucleocapsids). MV antigen was detected in brain imprints using IFA in both of them. Viral RNA was found in brain tissue samples only, while plasma, serum and CSF were negative. Nucleotide sequence analysis showed that the viruses detected in brain tissue belong to the wild-type MV D6 genotype¹⁴.

Key words: subacute sclerosing panencephalitis, measles, MMR

Introduction

Subacute sclerosing panencephalitis (SSPE) is a rare, fatal, and late complication of measles. Although SSPE rates reported in the literature vary, it is estimated that approximately one case of SSPE occurs per 10,000 to 1,000,000 reported measles cases^{1–3}. The disease occurs 5–10 years after the initial attack of measles. Mental deterioration and myoclonus usually characterize the disease onset, leading to a vegetative, decorticated state, coma and death within 1–3 years. In approximately 10% of cases, there is a fulminant course with death few months after the onset⁴. The exact pathogenic mechanism of SSPE remains unclear. While large amounts of measles antigens are present within inclusion bodies in infected brain cells, no virus particles mature. Viral replication is defective owing to the lack of production of one or more viral gene products, often the matrix protein⁵. It is unknown whether there is a specific predisposing im-

mune defect. A possible risk factor for the development of SSPE is measles infection at an early age, especially before the age of two years⁶. Demonstration of intrathecal measles virus (MV)-specific antibody synthesis or detection of MV-RNA from brain tissue confirms diagnosis⁷.

We describe the four SSPE cases diagnosed in Croatia in 2002.

Case Reports

Patient 1

Patient 1 was born in December 1997 in a very high-risk twin pregnancy. She was vaccinated regularly with the measles-mumps-rubella (MMR)-vaccine according to the official immunization schedule⁸. At the age of six months, she was hospitalized for morbilliform rash. In

August 2002, she developed dysarthria, ataxia and myoclonal jerks. The electroencephalogram (EEG) showed diffuse paroxysmal discharges accompanied by sharp-wave complexes and valproate therapy was started. Computed tomography (CT) of the brain was normal. Because of persistent seizures, the girl was admitted to the hospital. CSF analysis revealed the IgG synthesis within the central nervous system (CNS) with oligoclonal bands of immunoglobulins. Repeated CT of the brain revealed diffuse atrophic changes. Despite being on valproate therapy she experienced no improvement. The girl's condition continued deteriorating. She became soporiferous and tetraparetic. The disease had a fulminant course and the girl died in December 2002. Her identical twin sister also had a morbilliform rash at the age of six months but she didn't exhibit any symptoms of SSPE until the end of 2004.

Patient 2

Patient 2 was born in November 1994 in a normal pregnancy. He was vaccinated regularly with the MMR-vaccine⁸. At the age of seven months, the boy had a febrile disease with morbilliform rash. During 1995, he was hospitalized on three occasions for febrile convulsions for which he was placed on prophylactic therapy with phenobarbitone. In October 1996, the boy was readmitted to the hospital for relapsing convulsions. Because of multifocal EEG discharges, he was placed on valproate therapy. In September 2001, he entered the primary school. Eight months later, mental deterioration started accompanied by memory dysfunction, dysgraphia and dyslexia. Two months later, he developed ataxia and was admitted to the hospital. EEG revealed hysarrhythmia. CSF analysis revealed the IgG synthesis within the CNS with oligoclonal bands of immunoglobulins. CT of the brain was normal. A topiramate therapy was started. During the hospitalization, he still occasionally had partial seizures in the extremities accompanied by short losses of consciousness and absences. His condition continued deteriorating. The patient was unable to sit voluntarily, stopped talking and lost control of the sphincters. As the disease was progressive, the boy died in January 2003.

Patient 3

Patient 3 was born in May 1990 in a normal pregnancy. He was vaccinated regularly with the MMR-vaccine⁸. At the age of six years, he had the first partial seizure. The EEG revealed dysrhythmic discharges and a therapy with primidone was started. In March 2002, he was hospitalized for varicella when the first generalized seizure occurred. On an EEG, irritative bilateral changes were then detected. CT of the brain was normal. Five months later, his mother noted changes in his behavior, ataxia and occasionally indistinct speech. The boy was readmitted to the hospital. At the end of August 2002, he developed the signs of milder respiratory infection. The following day he became febrile and markedly somnolent, waking up only at a stronger stimulus. Repeated

EEG showed generalized dysrhythmic discharges with paroxysms of high-voltage slow waves and occasional sharp-pointed waves. CSF analysis revealed the IgG synthesis within the CNS with oligoclonal bands of immunoglobulins. MRI of the brain was normal. On several occasions during the hospitalization, he suffered from generalized convulsions. A therapy with phenobarbitone and carbamazepine produced no improvement. The disease had a progressive course and the boy died in November 2003.

Patient 4

Patient 4 was born in November 1997 in a normal pregnancy. He was vaccinated regularly with the MMR-vaccine⁸. The patient developed varicella in October 2002. On the third day of the disease, his mother noted his head dropping down on several occasions. The patient was hospitalized and treated with acyclovir intravenously. Then ataxia and myoclonic seizures of the arm and head developed. The treatment with carbamazepine resulted in temporary improvement. Next diplopia arose accompanied by dysarthria, swallowing difficulties and inability to control the sphincters. EEG showed continuous slowness with paroxysmal discharges. CSF analysis revealed the IgG synthesis within the CNS with oligoclonal bands of immunoglobulins. On brain MRI, discrete hyperintensive lacunar regions subcortically in white matter were found. During hospitalization he was treated with phenobarbitone and carbamazepine but no improvement was observed. Due to progressive disease, the boy died in December 2003.

Methods

Serologic testing of serum and CSF samples for MV and for varicella zoster virus (VZV)-specific antibodies was performed at WHO National Measles Laboratory, Croatian National Institute of Public Health (CNIPH). Antibody titer was determined by using the complement fixation (CF) test (micromethod)⁹ and enzyme-immunosorbent assay (Measles Virus-EIA, Institute Virion; Anti-VZV-ELISA, Euroimmun). Antigen used in CF test was obtained from Edmonston strain of MV at Virology Department, CNIPH⁹. Guinea-pig complement and haemolysin were prepared commercially at the Institute of Immunology, Zagreb. Brain tissue samples of two patients obtained at autopsy were examined by light microscopy, electron microscopy (EM; Neuropathology Department, Clinical Hospital Centre, Zagreb) and indirect immunofluorescence (IFA – Measles IFA Kit, Light Diagnostics; Virology Department, CNIPH).

Results

In 2002, SSPE was diagnosed in four patients (three boys and one girl) in Croatia. Their age at disease onset ranged from 5–11 years. They all had been vaccinated regularly against measles according to the official immunization schedule⁸. Two had a history of measles infec-

TABLE 1
CLINICAL AND EPIDEMIOLOGICAL DATA OF FOUR SSPE PATIENTS

Patients	Sex	Year of birth	Place of birth	MMR-vaccination /revaccination (age)	Measles infection (age)	Date of SSPE onset	Age at diagnosis	Duration of disease
1	F	1997	Požega	13 mo / –	6 mo (morbilliform rash)	August 2002	4 yr 8 mo	4 mo
2	M	1994	Koprivnica	20 mo (only measles) / 6 yr	7 mo (morbilliform rash + fever)	May 2002	7 yr 6 mo	8 mo
3	M	1990	Rovišće	12 mo / 11 yr	–	August 2002	11 yr 3 mo	15 mo
3	M	1997	Garčin	12 mo / –	–	October 2002	4 yr 11 mo	14 mo

mo – months

TABLE 2
LABORATORY AND HISTOPATHOLOGIC FINDINGS OF FOUR SSPE PATIENTS

Patients	Complement-fixing test CSF / serum	EIA – IgG CSF / serum	Electron microscopy (brain tissue)	IFA (brain imprint)		
1	I 1:128	1:32768	positive	positive	autolysis, without MV nucleocapsids	positive
	II 1:128	1:65536	positive	positive		
2	I 1:16	1:4096	positive	positive	intranuclear inclusions (MV nucleocapsids) in neurons and astrocytes	positive
	II 1:32	1:4096	positive	positive		
3	I 1:16	1:1024	positive	positive	–	–
4	I 1:32	1:2048	positive	positive	–	–

tion (diagnosed clinically) at the age of six and seven months, respectively (Table 1). In the other two patients, the disease began immediately after varicella infection. We detected antibodies to MV in the CSF and serum of all patients. The CF-antibody titers ranged from 1:1024 to 1:65536 in serum and from 1:16 to 1:128 in CSF (Table 2). In CSF, no antibodies to VZV were found. For two patients brain tissue was obtained post mortally. Using IFA, the MV antigen was detected in the brain imprints of both patients (Table 2). The histopathologic examination of the brain tissue from the second patient revealed neurofibrillar degeneration with acidophil intranuclear inclusions in the neurons (Figure 1a) and glial cells (Figure 1b). Using EM, intranuclear viral inclusions composed of MV nucleocapsids were demonstrated (Figure 1c and 1d). Strong autolysis prevented finding the characteristic changes in the brain tissue from the first patient (Table 2).

Discussion

The incidence of acute measles infection and SSPE has been dramatically reduced because of the widespread administration of measles vaccine. Despite vaccination, mainland Croatia had two measles outbreaks in the past ten years with 697 and 648 cases notified in 1995 and 1998, respectively (data from Reference Epidemiology Centre, CNIPH). The last registered SSPE case before those reported in the present study dates from 1994.

Studies involving the genetic characterization of viral material from brain tissue of SSPE patients have all reported the wild type MV^{10–13}. In plasma, serum and CSF samples of our patients, the RT-PCR revealed no viral RNA. Only brain tissue samples from both of them were positive. The nucleotide sequence analysis showed that viruses detected from the brain tissue belong to the wild-type MV D6 genotype¹⁴.

In spite of some reports of SSPE occurring after the administration of the MMR vaccine^{3, 15}, there is no evidence that the measles vaccine could cause SSPE (no vaccine strain has ever been discovered). Similarly, no increased risk associated with the administration of a measles vaccine to the children who had already had measles was observed¹⁶. It is likely that SSPE cases with no history of natural measles infection may have resulted from a subclinical or unrecognized infection that occurred before the administration of vaccine¹⁷. Before the routine use of measles vaccine, Krugman et al. reported finding that 15–20% of the children whose parents had reported no history of measles were immune to this disease. These children presumably were exposed at a time when maternal antibodies modified the disease but did not prevent asymptomatic or non-specific infection¹⁸. The other possibility of SSPE in vaccinated individuals is due to poor seroconversion or vaccine failure. This could happen either due to poor quality of vaccine or to its administration at an earlier than the recommended age at 12–15 months¹⁹.

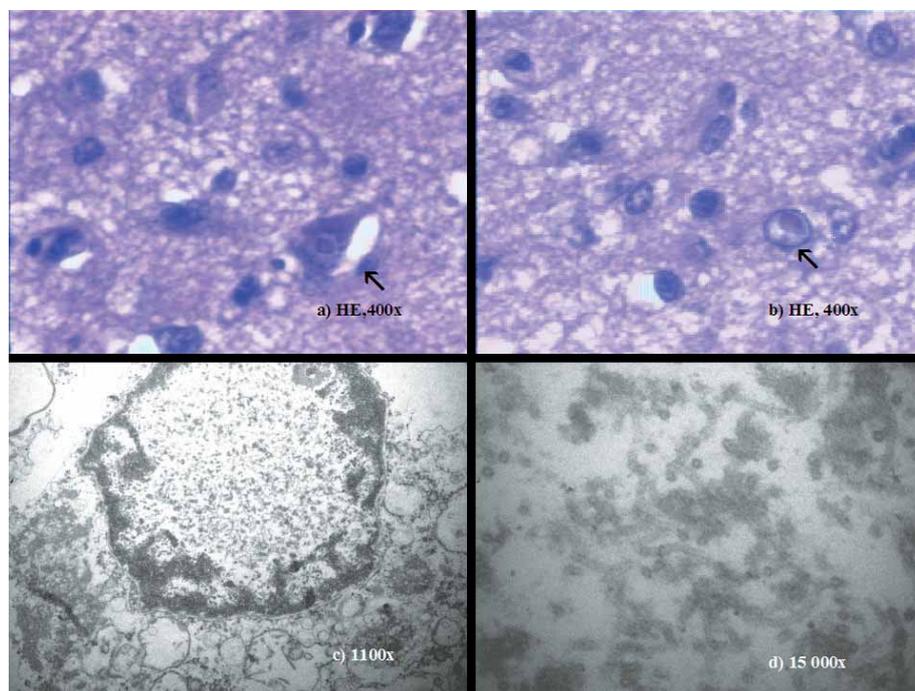


Fig. 1. Neuron contains eosinophilic nuclear inclusion (a), nuclear inclusion is also in glial cell (b). On electron microscope analysis, intranuclear viral inclusions are composed of measles virus (MV) nucleocapsids (c and d).

In 2002 in northwest Croatia, an exceptional cluster of four SSPE patients was diagnosed in a 6-month period. They all were vaccinated regularly against measles at an age of 12–20 months⁹. Two patients had measles as infants at the age of six and seven months, respectively, during the 1998 and 1995 measles outbreaks. In both patients, the disease was diagnosed retrospectively after taking detailed medical histories, but was no serologic confirmation. No vaccine strain was detected in the brain tissue although both these patients were immunized after a natural measles infection. According to the reports of other authors^{10–13}, they have been infected with the wild-type MV before vaccination¹⁴. In the other two cases, the disease began immediately after a varicella infection. No antibodies to VZV were found in the CSF of these patients. Anlar et al. have investigated the hypothesis of other viruses contributing to the pathogenesis of SSPE. They tested the CSF of 43 SSPE patients for DNA, RNA and antibodies against several viruses, VZV included, comparing them with those from 39 patients with other non-infectious neurological disorders. Forty-one point eight percent of SSPE patients had antibodies to more than one virus synthesized intrathecally. VZV DNA was found in 11.6% patients with SSPE compared to 15.3% in controls. Likewise, antibodies to VZV were present in 10.2% of SSPE patients and in 10.0% of controls. Their data do not support a specific role for this agent in SSPE²⁰.

Although the pathogenic mechanisms that result in SSPE following measles are not understood, several theories are proposed. One theory holds that affected pa-

tients have a hyperimmune response²¹ resulting in antibodies masking the infected cell surface antigens and making them unrecognizable to cytotoxic T-cells². Another theory suggests that neurons and glia fail to envelope and transport the antigen to the membrane, leading to an inability by the immune system to recognize infected cells²².

There have been several reports in the literature about SSPE in twins. Vieker et al.⁶ reported the occurrence of SSPE in two brothers two years after measles infection. The occurrence of two cases in one family is suggestive of a genetic predisposing factor. In contrast, Dhib-Jalbut and Haddad²³ described SSPE in one member of identical twins that emphasizes the importance of other factors in the pathogenesis of SSPE. One our patient was born in a twin-pregnancy. Her identical twin sister was also vaccinated against measles after a natural measles infection at the age of six months but she didn't exhibit any symptoms of SSPE.

Because SSPE reporting to the Epidemiological Service in Croatia is not obligatory, its incidence is unknown. This study describes a cluster of four SSPE patients in 2002 in Croatia, after an eight-year interval with none reported. The last notified diagnosed case of SSPE was a 10-year old girl in 1994. She had a short period of disease progression, but remained stable long thereafter. Still alive, she suffers from severe neurological defects (spastic tetraparesis, blindness and aphasia). The rate of SSPE in Croatia during observing period is higher than reported worldwide. We suppose that many

cases of measles were missed or not reported to the Epidemiological Service during the epidemics.

In conclusion, we feel that all SSPE case reporting should be mandatory and supported by laboratory confirmation and molecular characterization of the MV strains. In addition, all cases of febrile disease accompanied by morbilliform rash in early childhood in the differential diagnosis should consider measles.

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SUBAKUTNI SKLEROZIRAJUĆI PANENCEFALITIS: STALNA PRIJETNJA

SAŽETAK

Analizirani su klinički, epidemiološki i laboratorijski nalazi četvero bolesnika sa subakutnim sklerozirajućim panencefalitisom (SSPE) u Hrvatskoj tijekom 2002 godine. Dob bolesnika iznosila je od 5–11 godina. Svi su bolesnici redovno cijepljeni protiv morbila (Morbili-Parotitis-Rubela cjepivo). Dvoje bolesnika je imalo anamnestički podatak o preboljelim morbilima u dobi od šest odnosno sedam mjeseci. U druga je dva bolesnika bolest počela neposredno nakon preboljelih vodenih kozica. Titar komplement-fiksirajućih protutijela na virus morbila (MV) iznosio je od 1:1024 do 1:65536 u serumu te od 1:16 do 1:128 u cerebrospinalnom likvoru (CSL). Protutijela na virus varicella-zoster u CSL nisu nađena. Postmortalno su dobiveni uzorci moždanog tkiva od dvoje bolesnika. U jednog je bolesnika elektronska mikroskopija pokazala intranuklearne virusne inkluzije (MV nukleokapside). Metodom IFA, MV antigen dokazan je u moždanom otisku u oba bolesnika. Virusna RNA nađena je samo u uzorcima moždanog tkiva, dok su plazma, serum i CSL bili negativni. Analizom sekvenci nukleotida dokazan je divlji tip MV D6 genotipa¹⁴.

Multiple Medical Realities – Patients and Healers in Biomedical, Alternative and Traditional Medicine

(Editors: Helle Johannessen and Imre Lázár, Berghan Books, New York, Oxford, 2006)

Hrvoje Čargonja

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»Multiple Medical Realities – Patients and Healers in Biomedical, Alternative and Traditional Medicine« is a title to a reader comprised of 10 articles selected by Helle Johannessen and Imre Lázár with the intent to bring together two major theoretical trends in medical anthropology: one based on its fundamental concept of medical pluralism and the other based on phenomenological studies of body and self. The work of authors of these studies, all prominent European social anthropologists with very diverse research focuses situated worldwide (Mexico, Ghana, India, Norway, etc...) uphold the optics on plurality of provision, use and understanding of medical practices as cultural universal. Multiple views on multiple medical realities, as given by this well rounded book, will be inspiring and invaluable reading for any contemporary interest or research in this field.

The initiative to assemble this 4th volume of European Association of Social Anthropologists (EASA) Series was conceived at the workshop of 7th biennial conference of EASA held in Copenhagen, Denmark, 2002. Apart from the research reports, 197 pages of this volume contain two major points of synthesis, one introductory and the other one in epilogue written by editors. There is also a preface to the book written by Thomas Csordas. The book is organized in two parts: the first one with focus on social and the other on individual or phenomenological aspects of body and self in medical pluralism.

Bringing together two major theoretical trends in medical anthropology, as the title of the Introduction suggests: »Body and Self in Medical Pluralism«, the editor Helle Johannessen is proposing major theoretical framework which she will deploy to review each article of this volume. She is expounding on the work of N. Schepher-Hughes and M. Lock who suggest three perspectives from which a body can be viewed and thus analyzed: 1) phenomenally experienced individual body self; 2) social body and 3) body politics – an artifact of social and political control. The author goes on to establish a plurality of phenomena on each of those three levels of analysis mutually connected by multiple organizing principles. Rela-

tionships between those three levels of analysis are regarded as relations of similarity and opposition between organizing principles of each level. At this point Johannessen complements her theoretical framework by another concept: the relationships between organizing principles are seen as »elective affinity«. This originally chemical notion independently used by sociologists B. Turner and S. Lang as a central concept for understanding connections between power, knowledge and the body, is defined as »relation between two social factors which are able to coexist in a stable relation with no opposition and tension between them«.

Thus constructed theoretical tool, as Johannessen writes, allows us to uncover order in local medical pluralism which is not regarded as the sum of separate and distinct medical systems, but as fluid and flexible networks which connect across objects, knowledge, social institutions and persons by affinal relationships. Along with that, phenomenological experience of the body and self is also seen as flexible. It may be linked up to »different networks of praxis, knowledge and power as patients and practitioners move in and out between networks«. This consideration culminates in a model of »complex bodies and flexible selves« where complex bodies have »eyes that see the body and discourse which explains it«, and flexible selves which are »in constant struggle for social recognition where complex body plays a significant role«. Local specifics are thus differences in configurations and individual hierarchies of the elements and patterns of this network.

Pat I starts off with two articles on medical pluralism in Hungary. However, first contribution differs from all the rest from this book in that it is a demographic survey of status and uses of alternative medicine. In statistical terms the study is pointing out some specificities of a transitional conditions in the Hungary which are contributing to the growing popularity of alternative medicine especially between people of higher education and income status.

The core of the second paper is a case study of miraculous outcome of a therapy administered by Taltos healers, a postmodern syncretic medical practice consisted of puzzles with esoteric, spiritualist, neoshamanic and Hungarian ethno-cultural features. In his analyses the author is trying out phenomenological approach leading to an interesting stand point where cultural phenomenology of the body is seen as the »last station before the Transcendent«. To bridge the »gulf between knowledge from the experiment and knowledge from experience« and to enlighten »transspatial and transtemporal medical reality of Taltos healers« the author is concluding with application of Turnerian structure /»communitas« model.

Next story about a mentally ill patient in Ghana tells us how pills and exorcisms in another syncretic practice can successfully complement each other. Author is using the concept of »elective affinity« to describe the relationships between three main acting value systems in this case: biomedical, Christian Charismatic religious and Ghanaian traditional religious system. Although with even conflicting mutual relationships, all three come in play when patient tries to come to terms with her condition. A model of »subjunctive acts of appropriation« is discussed to shed some light on this example of how people »try to act and give meaning to conditions through their entanglements and not against them.«

Following two studies demonstrate how flexible the formation of network in a medical pluralism can be. First study is exploring the motives and attitudes of heterodox German medical doctors, practicing homeopathy, acupuncture or ayurveda and the other one is ethnography on varieties of homeopathic provision in the area of South London, England. In other words, first one is about plurality between biomedical practitioners and the other one is about plurality between users of homeopathy. Interestingly enough, both have reached similar conclusions in grouping their informants in two categories: pragmatic or those who will try out or administer certain mode of therapy mainly out of practical reasons; and committed or those who are more engaged and influenced by philosophical background of particular mode of therapy.

Last study of the part one is about plurality of social interpretations of biomedical concepts. On the example of a couple from a rural Greek village that went through a great lengths to conceive children author is demonstrating how aspects of health seeking activity are expressed as a function of individual's relation to the community. The couple is navigating through the network of many different explanations depending on the social contexts, unwilling to talk openly about health problems out of fear that others may not hold the same »medicalisation of ideas« which could possibly impact on their privacy and reputation.

With a following study on childbirth and medical pluralism in South Asia begins a second part of this reader. The article is shortly reviewing a notion of »modal states«, a theoretical tool which allows for analysis of human experience as a part of mind-body-society-environment

complex, as opposed to biomedical explanations whom the author sees as focused only on the level of the body. Thus the birthing rituals in South Asia, although maybe not appropriate from the biomedical perspective, are presented as more informative and supportive to the woman in labor than the available biomedical alternative. To use the author's words: »If the traditional (medical) procedures can have a positive žplacebo effect', biomedical practices may well have a negative one.«

The author of the next study describes his cooperation as physician with traditional healers and shamans of Naporuna in Ecuador. By asking the question what people did and thought of his work, he gains a better understanding of the *samay*, a local concept in the line of »life force« which could well correspond to mind-body-society-environment point of view from the previous study in this book. This insight not only improves the practice of the author but also leads him to question analytic categories like »medical system« and »explanatory model« as unspecific.

The story of the next chapter is about Tamil refugees in Northern Norway who are experiencing diffuse and vague aches and pains that are difficult to diagnose and cure for Norwegian biomedical personal. The reasons for that are best left to be said by one of the Tamil informants in the study: »Norwegian doctors only see my body and organs. They don't see my person.« Through additional elucidations on massive social and cultural rearrangements this new community is facing, the author illustrates how process of healing is connected with construction of self and identity.

The concluding case study of this book is a disturbing story about a mentally ill woman from Chiapas, Mexico, who is, in quest for healing, being pushed in the »spider's web« of medical realities by her relatives and friends. The study is a warning how proliferation of medical realities can only worsen the condition when the process of handling them is not with the control of the individual. Author sees a life history of his informant as a »constant battle between different explanatory models embedded in the social experience of multiple agents, who have endeavored to heal her soul.«

The epilogue begins with Helle Johannessen and Imre Lázár asserting of the multifaceted or holistic conception of sickness and healing, (i.e. medical plurality) as inherent and universal for complex societies. The editors are giving us another punch line where they see medical plurality as reflection of the complexity of the body. Such statement will lead them to reconsider its epistemological and ontological implications. Firstly, they will throw a suspicion on naturalistic ontology of western science arguing for cultural influences on its main axiom: nature and truth as universals, independent of time and space and of human consciousness, morality and culture. As an alternative to naturalistic ontology they will take into consideration a metaphysical one which complements model of body-psycho-social medicine by an additional attribute – spiritual. Confronting notions like social psychophysiology and psychoneuroimmunology on the one side with the reference to several, experimental stud-

ies, seriously challenging naturalistic ontology, on the other, the editors will offer a bypass to this polarizing discussion. Successful navigation through contradictory character of our own internal and external everyday life, especially when seeking healing, is a »common sense« clearly demonstrated through the chapters of this reader. Thus, driven by this »postmodern pragmatic eclecticism« the authors will take shelter under the framework of »ecosystem of healing practices embedded in a wider social, technological, natural or even supernatural reality«. This will finally open doors for the last, culminating concept of the book hinted in its title – the concept of multiple medical realities. Based on the works of A. Schutz who defines reality as »the meaning of the experiences and not (as) ontological structure of objects« the authors will reach a following statement: »medical realities do not exist as coexistence of separate and independent sociocultural system of medicine but are embedded in networks based on affinal organizing principles linking medical narratives and forms of praxis to issues of power and social relations«. The epilogue is concluded with applicable suggestions of such understandings.

All articles in this reader focus on the plural use of health care. However, the complementing thematic selection of the articles is evident from the following facts: all studies are result of fieldwork but one which gives us an impersonal, statistical viewpoint; all are focused on the relationship between alternative and biomedicine except one which is committed to the plurality within biomedicine; the majority of papers are pointing out affir-

mative features of medical plurality except the last one which is showing us the negative ones, etc. Within and in between articles of this reader the matter is examined from the viewpoints of multiple classes of actors included in the process of participating and observing multiple medical realities: pragmatic or committed practitioner or patient, anthropologist and even anthropologist practitioner. Lastly, the author of each article deploys one or more different theoretical models not necessarily in line with the one used by editors in their synthetic texts.

By this impressive and well balanced composition of updated articles of this reader the editors have successfully achieved a plurality of the perspectives on this complex matter of ecological network of healing practices. It is interesting to notice how the multiplicity of selected themes, viewpoints and theories, presented through chapters, give rise to the concept of multiple medical realities. This is strikingly analogous to the idea of the editors that the medical pluralism reflects the inherent need of the self to deal with the complexity of the body. The universal traces of our thought patterns are thus interwoven in the structure and the conclusion of this volume. This book is motivating and exciting reading and could easily serve as a textbook on the core topics of medical anthropology which are, by the word of Thomas Csordas:

»the misery of those who are ill, the pity of those who become healers and the unwillingness by either to tolerate such pitiful misery.«

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