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Vindija Cave and The Modern Human Peopling of Europe

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A B S T R A C T

Vindija cave in Croatia has yielded the youngest securely dated Neandertal skeletal remains in Central/Eastern Europe. In addition, these remains have been found in association with archaeological material exhibiting Upper Paleolithic elements. Due to its geographic location and date, the Vindija remains are particularly crucial for the understanding of initial modern human peopling of Europe and the nature of the Neandertal demise. The significance of archaeological and paleontological finds and hominin fossils from this site is discussed in the light of new finds at Vindija and recent developments in the fields of paleoanthropology and prehistoric archaeology. Furthermore, the impact of revised chronology for several crucial specimens and sites throughout Europe, including Vindija, is discussed.

***Key words**: Vindija cave, modern human origins, Neandertals, human evolution, Upper Paleolithic*

Introduction and Brief Site History

The site of Vindija is a large cave, about 50 m in length, 28 m in width, and almost 20 m in height (Figure 1). It is located in the Hrvatsko Zagorje region of Croatia, 9 km northwest of Ivanec and about 20 km west from the center of Varaždin[1] It was first mentioned as a potentially interesting archaeological site by D. Hirc[2]. Initial archaeological excavations were conducted by S. Vuković[3–5] starting in 1928, but it was not until the mid-1970s that large-scale excavations started under the direction of M. Malez[1,6]. It was under his direction that the majority of the paleontological, archaeological, as well as the entire hominin sample was unearthed between 1974 and 1986[7–9]. Since then, several additional hominin fossils have been identified[10–12], and the archaeological and faunal assemblage has been a subject of detailed analyses[12–18].

The stratigraphic sequence of the site is complex, consisting of over 12 m of deposits, divided into 13 basic stratigraphic units (A–M). Complexes F, G and K are further subdivided into Fg, Fs, Fd, Fd/d, G_1 to G_5, and K_1 to K_3 layers[12,19,20]. Units A to D are Holocene, while units D to M yielded material dated to the Pleistocene (Figure 2). Faunal and sedimentological analysis suggests that the climate during the formation of complex G (OIS 3) was variable but at times similar to the recent one, while the younger complex E/F (OIS 2) was deposited under somewhat cooler climatic conditions. Of major interest for the modern human origins debate in Europe is the material from complex G. This stratigraphic unit yielded most of the Neandertal bones from the site. The archaeological assemblage is quite complex. While the tools from G_3 are attributed to the Mousterian with some Upper Paleolithic elements present, the G_1 assemblage provides a more complicated picture[12,15]. It is in this layer that a Neandertal mandible (Vi-207) was found in association with Aurignacian or Aurignacian like split base bone point (Vi-3437) (Figure 3). Additionally, three massive-base bone points (so-called Mladeč type) were found in the same layer. Such bone points are distinctly Upper Paleolithic tools. The stone tool assemblage from G_1 exhibits a mixture of Mousterian and Upper Paleolithic types[15] (Figure 4). One well-made bifacial stone point made from non-local raw material shows similarities to material from Hungary usually attributed to the Szele-

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Fig. 1: Vindija cave (photo: I. Karavanić).

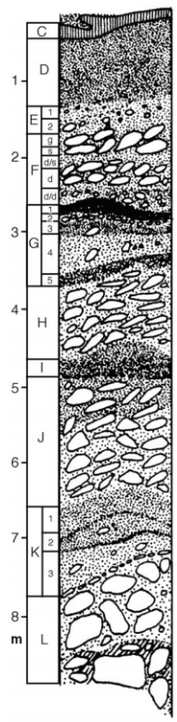

Fig. 2: Stratigraphic profile of the Vindija Cave (modified after Rukavina 1983).

tian industry (Figure 4; 4). Whether the archaeological material from G_1 represents the Aurignacian or some other variant of the initial Upper Paleolithic, as a »transitional« industry (e.g. Szeletian), or the late Mousterian with Upper Paleolithic components remains uncertain[15,21–23]. Complex F has yielded archaeological material attributable to the Aurignacian *sensu lato* (layer Fd/d) and Epigravettian (layers Fd/s, Fs, and Fg), while

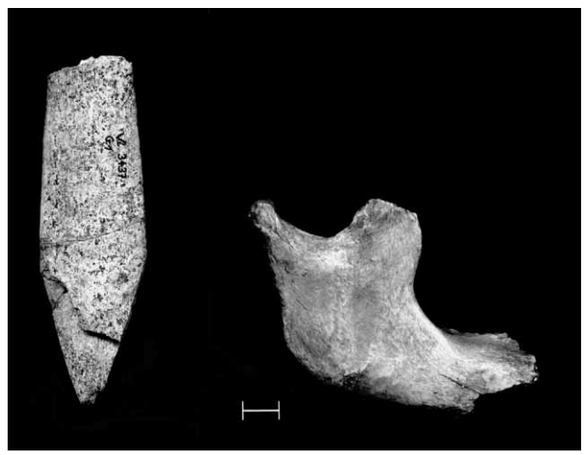

Fig. 3: Split-base bone point Vi 3437 and hominin mandible Vi 207.

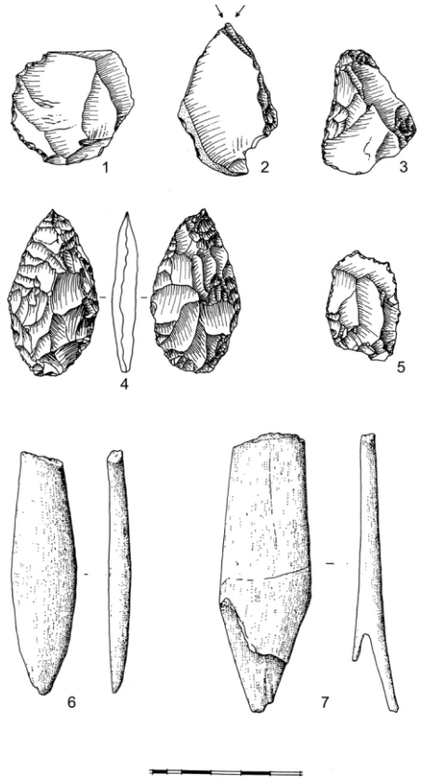

Fig. 4: Selected artefacts from Vindija level G1: 1. probable a pseudo- tool (previously published as denticulated piece), 2. burin. 3. sidescraper. 4. leaf-shaped bifacial piece, 5. flake with marginal retouch on distal end (previously published as an endscraper on flake), 6. massive base bone point, split base bone points. (Modified after Karavanić 1995: Fig. 3; Drawing by Marta Perkić.

the E layer is Epigravettian[12–14]. In layer D, modern human (*Homo sapiens sapiens*) skeletal material has been found alongside material attributed to the Epigravettian. The majority of the anatomically modern human sample comes from this layer, although the inscriptions on several fragments suggests that they were found near the border with the E sequence, and a few fragments might belong to the Holocene layer B. In this paper, we will concentrate on the finds from complex G, as those are crucial to the »Neandertal question« and the modern human peopling of Europe.

Vindija Faunal Sample

During the Upper Pleistocene, Vindija cave was situated on the southern edge of the Alpine ice sheet, which at the times of the glacial maximum covered the Alps. However, Vindija also lies near the edge of the Pannonian Plain, which explains the steppe elements in the classical forest faunal community during the OIS 2 and 3. As majority of the Vindija finds are faunal, the zooarchaeological sample from this site has been studied at numerous

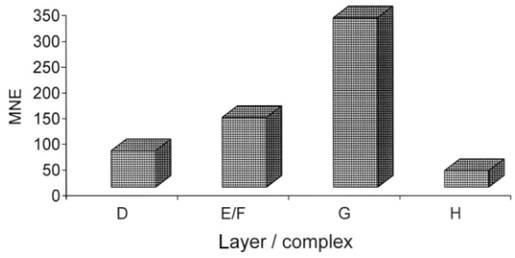

Fig. 5: Accumulated fossil remains of ungulata in taphodermes of layers/complexes D, E/F, G, and H.

times[19,20,24–26]. With a better understanding of taphonomy of the site and more detailed studies of specific taxa, new patterns emerged.

A recent revision of the ungulate fauna removes *Coelodonta antiquitatis* (woolly rhino), *Saiga tatarica* (saiga antelope), and *Equus* cf. *germanicus* from D, E/F, and G complexes at Vindija[18]. The presence of the first two taxa was considered to be evidence of extremely cold paleoclimatic conditions, while the equid was considered indicative of open, steppe environments during the period in which these complexes were deposited[19]. *Rangifer tarandus* (reindeer) is representative of the tundra zones and more open parts of the taiga. Fossil remains of reindeer were reported to be present in complexes D, E, F, and G[19], but now we know that only a few skeletal remains (MNE 5) were accumulated in complexes E/F and G. On the other hand, our recent revision has added *Capreolus caprolus* (roe deer) to the faunal lists of the E/F and G complexes. Results have shown that the abundance of ungulate remains is highest in complex G (Figure 5). The revised faunal associations better accord with the palaeoclimatic reconstructions based on sedimentological characteristics[27] and paleovegetation[28]. Results of this new revision of the Vindija faunal assemblage call into question the previous reconstruction of alternating »cold« and »warm« faunal communities during the deposition of the E, F and G complexes.

The Vindija ungulate assemblage has undergone a complex taphonomic history. Traces of animal modification (e.g. gnaw marks) point to the activities of small-sized carnivores (e.g. fox and marten) and rodents. Of the larger-sized carnivores, *Ursus spelaeus* (cave bear) is ubiquitous throughout the Vindija sequence, and it is the only large carnivore present in the lowest strata of complex G[19]. Cave bears probably occupied the cave for hibernation[17]. The other larger-sized carnivores are *Panthera spelaea* (cave lion) and *Canis lupus* (wolf); both are present in the assemblages of complexes D, E, F and G. There are only rare gnaw marks, most probably from wolf, on the ungulate remains, and these appear to have been made on bone refuse left by the hominins. In contrast, our new taphonomic analyses have produced widespread evidence of hominin selection and modification (e.g. body part selection, breakage patterns, butchery marks). This shows that hominins were the most important accumulators of the ungulate assemblage.

Chronometric Dating and the Early Modern Human Sequence in Europe

The significance of establishing a reliable chronological framework in human evolutionary studies cannot be overemphasized, and improvement in dating techniques and redating of a number of finds has led to major reevaluations of both data and interpretations concerning modern human origins[29]. Recently, several key fossils have been redated by more precise methods. This led to exclusion of several specimens previously held to be among the earliest modern humans in Europe from the debate (e.g., Vogelherd and Velika Pećina, now dated to the Neolithic[30,31]). As the Vindija Neandertal remains are crucial to the debate, dating of various stratigraphic layers of this site has been attempted several times, but not without problems[32]. Neandertal remains from later G_1 were directly dated by AMS and yielded a date of 28–29 kya, thus making them the youngest Neandertals in the region[30]. Recently, the new technique of ultrafiltration of collagen samples has been applied and the same G_1 fossils have been redated to 32–33 000 ^{14}C years ago[33]. Until the same methods are applied to other crucial specimens of approximately same time period (both late Neandertals and early anatomically modern humans in the region) it is impossible to create the much needed timeframe of overlap of these two populations in Europe. The main problem with radiocarbon dating is a high error margin for material older than about 30 kya. Newer techniques, such as AMS, ultrafiltration, etc., add to the accuracy of dating and make these methods less destructive[34]. However when the time of overlap is expected to be several thousand years at best, the error margin is still unacceptably high. Further, many specimens from this crucial time period (e.g. Mladeč, Kostenki, etc.) are likely to be older than reported[35,36]. Therefore, the redating of the Vindija specimens does not necessarily widen the temporal gap between indigenous European Neandertals and anatomically modern newcomers.

At present, and based on the radiocarbon dates of the finds, candidates for the oldest anatomically modern human remains from Europe are those from Kent's Cavern, England, Brassempouy and La Quina in France, Kostenki in Russia, Oase, Cioclovina and Baia de Fier in Romania, and Mladeč in Czech Republic. However, there are problems with all of these sites. Kent's Cavern 4 is a human maxillary fragment found in 1927 in a large cave system near Torquay, England[37]. It was found below the layer containing what was described as »Aurignacoid» industry[22,38–40], making the association of archeological industry and human fossil questionable. The fossil was directly dated to around 31 kya[40,41], but it may be as old as 35–37 kya[42]. Although this specimen was described as modern in morphology, the fragmentary state makes this assesment uncertain[36] and new analyses are still in progress. The exact nature of the »Aurignacoid» industry also needs to be subjected to careful re-analysis. Several isolated teeth and phalanges found at Brassempouy in France yielded dates between 30000 and 33500 years ago[43]. As is the case with Kent's Cavern finds, the archaeological industry of this site needs serious reexamination before it can be confirmed as Aurignacian *sensu stricto*. An additional problem is that the metric values of the human fossils fall both within modern human and Neandertal ranges[43 contra 44]. La Quina 25 is stratigraphically associated with the radiocarbon date of around 32 kya[45], and not directly dated. Further, the specimen is juvenile which always presents an additional problem in taxonomic assesments. The Kostenki 1 specimen has recently been directly dated to around 32 kya[46], but a detailed morphological analysis is still unpublished.

Recently, human fossils from several Romanian sites have been directly dated[47]. A skull, tibia and scapula from the Woman's cave (Baia de Fier) were found in 1952 and the postcranial remains have been dated to around 30 kya[48]. The archaeological finds from the site have been described as Mousterian, while the upper layers contain some type of Upper Paleolithic industry. As the layers in the cave are mixed, the association of archaeological industries, as well as various human fossil elements are unclear. The skull from Cioclovina cave, most likely male[49] is now dated to around 29 kya[50] has been described by Rainer and Simionescu[51] as »*Homo sapiens fosillis*…with Neanderthalian characters«, and although it is morphologically modern in overall gestalt, its supraorbital region is very robust and there is bunning on the occipital bone[22,36,49]. Cranial and postcranial remains from Pestera Muierii[48] are approximately 30 000 years old[50], but not associated with archaeological industry. The most recent finds come from Pestera cu Oase in Romania and are dated to arround 35 kya[52,53]. These were also not found in association with archaeological material. Trinkaus and colleagues[52,53] note the presence of several archaic features on these otherwise anatomically modern specimens (e.g. pronounced juxtamastoid eminence on Oase 3, robust and laterally oriented zygomatic bones and large molars in Oase 2). At least one feature (lingual bridging of the mandibular foramen present on the left ramus of Oase 1 mandible) is unknown in modern humans predating Oase remains but is common in Neandertals and some of the later modern humans in Europe[52–53]. No archaeological industry was found at this important site, limiting our knowledge of these earliest anatomically modern humans in Europe to their anatomical features.

New direct dating of the human remains from Mladeč (Lautsch) in Moravia, Czech Republic[54] suggests an age of around 31 kya for these anatomically modern humans[55]. Although the association with Aurignacian lithics was previously suggested, the exact nature of the deposition at the site is uncertain[22] and while Mladeč type bone points were found, the lithic material is scarce, and the split base bone points that are common in other Aurignacian-like industries of the earliest Central/Eastern European Upper Paleolithic are absent[22]. Therefore, the question of whether these tools represent an early Aurignacian-like (transitional) industry, or later Aurignacian *sensu stricto*, remains open. In addition, as in Oase sample, several archaic features are seen in some of

the Mladeč specimens. These include occipital bunning in Mladeč 3, 5 and 6, and robust supraorbital regions in Mladeč 5 and 6, as well as large palatal and dental dimensions and some other anatomical details in the sample[22,49,55–64], all features that are common in earlier Neandertal populations.

Industries of the Earliest Upper Paleolithic of Europe

If we use the traditional approach based primarily on typology and technology in order to define Middle (Mousterian and its variants) vs. Upper Paleolithic industries in Europe, we face the problem of several so-called 'transitional' industries. These include the Châtelperronian of France and northern Spain, Szeletian and Jankovichian of central and parts of eastern Europe, Uluzzian of Italy (Tuscany, Calabria, southern Adriatic part, Uluzzo Bay, etc.), Streletskian of eastern Europe, Jerzmanowician of eastern Germany and Poland, Althmülian of southern Germany, Bohunician of Czech Republic, Brynzeny and Kostenki Szeletian of Russia and several other unnamed or site-specific assemblages from Poland, Slovakia, Czech Republic, Romania, etc. in which various elements of Mousterian appear alongside the Upper Paleolithic types or types produced using technology commonly associated with the Upper Paleolithic. All these industries seem to have their origin in local Mousterian variants and no abrupt change can be seen[22,65–97]. Except for documented associations of Neandertal remains and Châtelperronian artifacts from La Roche à Pierrot at St. Cesaire and Grotte du Renne at Arcy-sur-Cure[98–101] there are no diagnostic hominin fossils associated with any of these earliest Upper Paleolithic finds[22,102–103]. Thus, even if we accept the earliest Aurignacian as a single industrial complex that has its origins outside this area[104] (both of these premises being far from proven) and attribute it to anatomically modern newcomers (for which there are no known hominin/industrial associations) we are left with the problem of who is responsible for these pre-Aurignacian assemblages.

Typological thinking is responsible for the acceptance of the Aurignacian as a single widespread complex commonly associated with the spread of morphologically modern humans into Europe[21,22]. We believe that, in light of the currently available evidence (or the lack thereof) this view should be carefully reexamined. Simplification of this model can be summarized as follows:

As more and more studies[66–70,73,75,76,78,81,82,105] show that the earliest Upper Paleolithic (»transitional«) industries in Europe develop within the local framework from (and including various elements of) the Mousterian complex, the earliest distinctly Upper Paleolithic industry associated with anatomically modern humans should be Aurignacian, brought here as they move into the region[106]. Here authors vary in opinion on whether and how much influence modern newcomers and their culture had on the technological/behavioral change of late Neandertals. Thus, in this model, the Aurignacian is regarded as a single imported complex that can be recognized in the archaeological record by the appearance of certain tool types and automatically assigned to anatomically modern populations.

While this sounds simple enough, it is not. First, detailed archaeological studies show that several tool types (especially bone tools) used as indicative of Aurignacian are in fact commonly found in various aforementioned »transitional« industries[21,67,89,95,107,108]. Further, the Early Aurignacian differs from the Late Aurignacian[21]. Finally, there are great differences between assemblages of typical Aurignacian from Western Europe, and that of Central/Eastern Europe[15,21,90,108].

All this makes it clear that there may be a different pattern of behavioral, and most likely, populational change in Western vs. Central/Eastern Europe. This is in agreement with several anatomical studies[49,57,109]. While this transition (whatever the mode of it) was more abrupt in Western Europe, evidence suggests a more gradual pattern for Central and Eastern areas of this region. Therefore, we believe it is quite likely that some Neandertal populations had a significant role in the formation of early modern European gene pool (via assimilation into anatomically more modern populations), while other Neandertal groups had none.

As in the case of the initial Upper Paleolithic (aka »transitional«) industries, except for the Châtelperronian, makers of the earliest Aurignacian *sensu lato* are unknown as there is no clear association of diagnostic hominin and archaeological material. Although a new study and dating of an interstratified sequence of Châtelperronian and supposed Aurignacian suggests coexistence of these industries at least in some sites[110], determination of this industry as Aurignacian should be reexamined. As mentioned, tool types indicative of Aurignacian commonly appear in other transitional industries of Central Europe. Again, no association of human bones was found in these layers, therefore all we can say is that there are two contemporaneous yet somewhat different cultural traditions present at the site. One of these is known to be associated with late Neandertals.

One more point concerning the appearance of the Aurignacian should be mentioned. Although its origins were commonly seen in the Middle Eastern assemblages of anatomically modern humans, some authors trace its initial rise in several independent centers in Europe[111]. This explanation makes more sense if the Aurignacian is not a single widespread complex but actually represents different Early Upper Paleolithic assemblages that share several tool types (previously considered to be indicative of a single industrial complex). In this light there is no need to see these industries as a product of a single population. This also raises possibilities of different explanations for shared similarities (trade, influence, population mixing, etc). However, we should bear in mind that population contacts differ in their pattern. Interbreeding and peaceful coexistence, trade, etc., might dominate some of these interactions, while in others patterns of contact might differ. Therefore, models based on data from Western Europe should not be used for Central/Eastern Europe.

The Middle to Upper Paleolithic Transition at Vindija and its Significance for the Modern Human-Peopling of Europe

Vindija Cave has an important place in the understanding of the initial anatomically modern human peopling of Europe. The significance of the association of Neandertal remains with an Upper Paleolithic industry has been a subject of a considerable debate[15,80,112–114], as it has been argued that the association is in fact artificial and the result of the cryoturbation that has been noted in some parts of the cave. A partial Neandertal mandible (Vi-207) found in direct association with the characteristic Upper Paleolithic tool type (a split-based bone point, Vi-3437) adds to the complexity of the picture. We contend that the arguments presented in favor of artificial mixing of these are weakened by careful consideration of data.

It is true that the excavations at Vindija, in many ways, followed techniques that had already been abandoned in Paleolithic archaeology at that time in most of Europe (especially in France where the past mistakes of numerous excavations during the early part of the 20th century led to deeper understanding of the importance of careful and detailed collecting and documenting of finds and features). However, such arguments can only go so far. Practice of selective collecting of »more important« or bigger and diagnostic finds of recognizable importance does not automatically cast a shadow on all of the data. While important data was lost, resulting from non-collecting or selective collecting of items (such as debitage or smaller non-diagnostic fragmentary bones etc), the majority of recognizable tools, bones, bigger pieces of debitage etc. were collected and recorded according to stratigraphic units.

Cryoturbation, while present at the site[115], has not been noted for the part of the cave where the associated mandible and bone point have been found[8,12,15,112]. Further, G_1 consists of characteristic reddish clay, easily recognizable and distinct from both upper and lower parts of the sequence. This reddish clay was embedded in both Vi-207 mandible and the Vi-3437 bone point and can still be observed on another massive bone point from this stratigraphic layer. In a recent paper, Ahern and colleagues[12] reported additional Neandertal remains, one of which (a proximal radial shaft Vi 13.8) has embedded reddish clay sediment that is characteristic of layer G_1. Neandertal attribution of this specimen[12] is suggested by the strong curvature of the shaft and the medial orientation of radial tuberosity[116,117]. The presence of further Neandertal specimens from layer G_1 additionally disproves the claim for artificial mixing of layers and arguments against the Neandertal association with the G_1 Upper Paleolithic industry.

There is an interesting pattern when we compare archaeological assemblages of various Vindija layers. In older layers (unit K) typical Mousterian tools predominate and there is a clear evidence of the use of Levallois technology that is common in most European Mousterian assemblages. The most abundant raw material in unit K is local quartz[16,118], and flake technology predominates in tool production. Level G_3 presents a mixture of typical Mousterian tools, such as sidescrapers, but there are also Upper Paleolithic types of stone tools (such as endscrapers), and alongside flake technology, bifacial and blade technology was used in production of tools from this layer. It is important to note that no evidence of Levallois technology is seen in layer G_3 of Vindija[15]. There is also evidence of more selective use of raw material, as there are more tools on chert in this layer[12,16,118].

The level G_1 assemblage shows an even more pronounced shift towards the use of higher quality raw material (i.e., chert) compared to the older layers of the site, and there are no tools made on quartz[12,16,118]. Upper Paleolithic elements in stone tools are more abundant than in layer G_3, and bone points from G_1 layer represent a new distinctly Upper Paleolithic element that is not seen in any of the older layers[13–15].

At several Slovenian sites, such as Divje Babe I and Mokriška Jama, bone tools similar to those of Vindija have also been found[119–120]. Similarly »Aurignacian« as-

Fig. 6: Comparison of Vindija 202 (left) and Krapina 4 (right) frontal bones (photo: J.C.M. Ahern).

semblage of Potočka Zijalka also differs in pattern from the »classical Aurignacian« assemblages[120]. In fact, this assemblage was previously referred to as Olschewian[121].

All hominins from the Vindija G complex can be recognized as a part of Neandertal populations on the basis of their overall gestalt. However, most of the commonly noted »Neandertal features« (for a detailed list see[64,122–129] and references therein) do not represent autapomorphies, but are instead either plesiomorphic characters inherited from preceding archaic hominins or shared with contemporary and/or post-Neandertal populations[60]. It is clear that there are many temporal and geographic differences. Several studies have shown that later Neandertals differ in morphological details from earlier »classic« members of this population, for instance in the reduction of facial dimensions and projection[8,9,12,49,56,130–131] as well as in other details of their anatomy. This is true for the Vindija G_1 Neandertals, as shown by several studies, especially on the supraorbital and mandibular material[56,130,132]. Analyses reveal the intermediate position of the Vindija supraorbitals, both in projection and shape compared to the older Krapina sample (Figure 6). The Vindija supraorbital tori have relatively greater degrees of pinching above the orbits compared to the earlier Neandertals[49,130,133,134]. Recent study of a newly reconstructed partial cranial vault from G_3 level comprised of supraorbital and frontal fragments (Vi 284, Vi 230, Vi 255, Vi 256) again suggests anatomical change in the direction of anatomically more modern morphology[12]. Change in the direction toward a more modern human pattern is also seen in the Vindija mandibular sample, suggesting facial reduction, and the Vindija mandibles have more vertical symphyses than earlier Neandertals and exhibit incipient eminences, though not a true modern human chin[133,135–136]. Observed gracility and change in shape is not due to body size[109] or age and/or sex bias in the sample[12,131,132,136] and could suggest gene exchange with anatomically modern populations. »Neandertal« traits are not present in earlier anatomically modern humans (samples predating 40 kya from Africa and Asia) that are the likely ancestors of Upper Paleolithic populations that came to Europe. Thus, the appearance of several »Neandertal» traits in the youngest modern groups in Europe (such as Mladeč or Predmostí)[49,56,60,63,137,138] and the later Gravettian child from Lagar Velho[139] is easily explained by interbreeding and would best fit within the framework of the Assimilation model of modern human origins[36,57–58].

The Impact of Molecular Data on the Modern Human Origins Debate

After the field of genetics entered the modern human origins debate with the initial claims for exclusively African origins[140], several authors emphasized that the results could be explained in different ways[141–145]. Moreover, mtDNA results do not seem to be in agreement with results obtained from other parts of genome[146,147]. Newer analyses of mtDNA isolated directly from Neandertal bones added another dimension to the debate[148–152]. Although these sequences are different from those of living humans, various processes (e.g. bottlenecks, selection, drift, populational expansions etc.) could cloud our insight into the past events. Among these specimens, several Vindija fossils were included[151–152] and were reported to fall outside both contemporary modern human, as well as Upper Paleolithic hominid ranges. However, ancient DNA was extracted from Vindija fossils that are both undiagnostic and of uncertain context (Vi 77, Vi 80, Vi 75). While a more meaningful insight into the question of whether or not Neandertals and anatomically modern humans interbred could be provided by extraction of DNA from the earliest modern humans in Europe[152], alas, problems with extraction and contamination of ancient DNA, as well as with the small size of the available fossil sample of these crucial specimens makes it impossible to answer this question solely based on genetic evidence. In sum, some amount of interbreeding between these two late Pleistocene populations cannot be excluded and distinction of Neandertals at the species level is refuted by the current evidence[58,141–143,147,148,151,152,154]. Any molecular analysis dealing with the question of Neandertal and anatomically modern human interaction must take into account the complex pattern of population movements, population size, bottlenecks, etc. Even then, known problems such as small sample size and difficulties with extraction and contamination of DNA would make such analyses questionable. Until these questions are answered, the genetic picture drawn from both ancient DNA studies, as well as of models based on contemporary modern human genetic research allows for different explanations and should not be taken as a proof that no interbreeding between these populations took place.

Conclusion

Vindija cave in Croatia has yielded the youngest securely dated Neandertal skeletal remains in Central/Eastern Europe. In addition, these remains have been found in association with archaeological material exhibiting Upper Paleolithic elements. Due to its geographic location and date, the Vindija remains are particularly crucial for the understanding of the initial modern human peopling of Europe and the nature of the Neandertal demise. We argue that the association of an early Upper Paleolithic industry with late Neandertals at Vindija is not likely to be a result of artificial mixing of specimens from different strata, but rather that these artifacts are reasonably considered to be products of the Vindija Neandertals. Although similar archaeological samples in Europe have traditionally been regarded as Aurignacian and automatically assigned to anatomically modern humans, we believe that many of earliest Upper Paleolithic assemblages are in fact derived from the local Mousterian, and the question of which population is responsible for the production of these assemblages remains open.

The so-called transitional industries such as Uluzzian of Italy and Szeletian of Hungary and adjacent areas were quite likely a product of local Neandertal groups, as they have their origin in preceeding local Mousterian. In Europe at least, only Neandertals have been associated with Mousterian assemblages. Likewise, the only clear association of hominin remains and the Initial Upper Paleolithic thus far has been Neandertals with the Châtelperronian (at Arcy-sur-Cure and St. Cesaire[98,100]). Although it can be argued that the anatomically modern newcomers are the likely producers of the earlier distinctly Upper Paleolithic industry of Europe (later Aurignacian, or Aurignacian *sensu stricto*), this still remains to be proven. However if, as we argue, Aurignacian should no longer be considered a single Pan-European industrial complex, but rather represents a number of local early Upper Paleolithic assemblages, the association of Neandertals and Early Upper Paleolithic is not so surprising.

The Upper Paleolithic industry at Vindija is not Aurignacian *sensu stricto*, but one of many »transitional» industry assemblages. This suggestion is supported by the presence of significant Mousterian types, one bifacial stone point typical of Szeletian, as well by significant differences in the assemblage compared to Western European sites[21,90,112,155]. While we cannot equal industry with biological populations, the simplest explanation would be that late Neandertals developed at least some of these »transitional» industries. Further, we should reexamine the Aurignacian sequence at various sites, especially in Central and Eastern Europe, and try to detect whether these are in fact Aurignacian *sensu stricto*, or another »transitional» industry. If the later proves to be the case, the association of the split-base bone point (and therefore the Upper Paleolithic sequence) and late Neandertals at Vindija should not come as a surprise at all.

The first modern people to come to Europe might have been small groups and it is unclear how much they contributed to the later modern human groups (e.g. Gravettians etc.). Therefore we must bear in mind that it is not only the issue of Neandertal genetic contribution to the initial anatomically modern newcomers, but also the relation of these first groups to the later modern humans that needs to be taken into account. Unfortunately the relatively short time frame of the populational overlap between late Neandertals and early moderns, possible differential site use, and numerous factors, including sedimentation rates, preservation of the sediment which is erroding more quickly than forming differences in site use, etc., will result in rare preservation of such evidence.

Therefore, the Vindija G_1 layer is a rare and important find. Anthropological analyses demonstrate that the late Neandertals at Vindija exhibit a more modern pattern of morphology compared to most other European Neandertals. We believe that both the anatomical and archaeological characteristics of Vindija are best explained by the Assimilation model of modern human origins.

The studies on the Vindija cave anthropological, archaeological and paleontological material is by no means over. New dating, DNA and various other skeletal analyses, as well as the recently published newly recognized hominids allow for a better insight into the human evolutionary past. There are many questions still to be answered and still more to be created by these answers. No doubt the material from the Vindija Cave will have a crucial part in answering some of them.

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ŠPILJA VINDIJA I DOLAZAK ANATOMSKI MODERNIH LJUDI NA PROSTORE EUROPE

S A Ž E T A K

U gornjopleistocenskim sedimentima špilje Vindije u SZ Hrvatskoj, u vertikalnoj sukcesiji s ostacima modernih ljudi, nađeni su ostaci najmlađih neandertalaca središnje i istočne Europe. Sedimentološki, paleontološki i arheološki sadržaji u autohtonim su startigrafskim relacijama s ostacima hominida, što Vindiju određuje kao ključno nalazište, koje se već nekoliko desetljeća interdisciplinarno istražuje. U potrazi za rasvjetljavanjem interakcije i sukcesije neandertalskih i modernih hominidnih populacija, dosadašnje spoznaje prezentirane su u svjetlu suvremenih saznanja i teorijskih pomaka. Izneseni su novi podaci o faunskim asocijacijama pojedinih stratigrafskih članova koji mijenjaju ranije spoznaje o prehrambenim resursima paleolitskih lovaca Vindije. Naglašen je značaj novih kronoloških podataka u repozicioniranju nekih ključnih gornjopleistocenskih nalaza i nalazišta Europe.

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Original scientific paper

The Prehistoric Hillfort at Grad (Pelješac, Dalmatia) – Preliminary Results of Intensive Surface Survey

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A B S T R A C T

An intensive surface survey, covering an area of approximately 10.000 m^2, was carried out at Grad, a hillfort in southern Dalmatia. Its aims were to obtain information relevant for determining the spatial extent of the site, its function(s), periods of occupation, degree of preservation, and potential for further investigation. Research strategy included mapping of the visible structural remains and systematic recovery of all surface finds. Majority of the collected finds are coarse Hellenistic ceramics followed by Bronze Age and Iron Age pottery. The central area of the hillfort was intensively used during the last few centuries B.C., while its origins can be traced back to the Bronze Age. The recorded structures and the recovered finds hint at a residential and defensive function of the site, while its central, elevated area may have been a focus of special activities.

Key words: *hillfort, Pelješac, Adriatic, Bronze Age/Iron Age pottery, Hellenistic ceramics, surface survey*

Introduction

Hillforts are among the most prominent man-made features of the late prehistoric landscape of the eastern Adriatic and its hinterland. Locally known as *gradine*, the majority of those monumental structures date from the last two millennia B.C. (Bronze Age and Iron Age). Strategically located on hilltops or other elevated locations, typically they consist of an oval area enclosed by a drystone wall or multiple concentric drystone ramparts, except in places where the steep terrain by itself provides ample protection.

Traditionally, the eastern Adriatic hillforts were considered as remains of fortified settlements[1,2]. A few decades ago, however, several authors[3–5] noted the great variability in their size and shape, the kind and extent of their enclosing structures, as well as the kind and quantity of domestic and other debris that they contained. They interpreted this as evidence for functional variability and proposed that, aside from fortified settlements, hillforts may have served as refugia, cattle corrals, elite residences, or ritual foci. More recently, the simplistic »fortified settlement« concept was further challenged by Gaffney *et al*.[6,7] who propose that many of these enclosures may be better interpreted as public monuments, associated with control of land through common rituals, and reflecting the power of the local potentates.

It is clear that many different kinds of hillforts were constructed and maintained during the periods in question. The main reason why we still know so little about them, in spite of their great number and conspicuous presence in the landscape, is that only a very few, such as Monkodonja[8], Varvara[9] or Ošanići[10] have been extensively excavated. The main reason for this is the high cost of large-scale excavations, which require long-time commitment, as well as conservation of the recovered structural remains. On the other hand, small-scale test excavations, carried out on numerous hillforts, sometimes produce valuable results, but they can yield only limited information about a few selected spots within those large and often complex sites.

Intensive surface survey, consisting of systematic recovery of removable finds, as well as mapping of structural remains and other features visible at the surface, is an alternative, low-budget approach that can easily cover

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the entire site. Regardless of it many shortcomings, such survey can be a serious analytical tool when used judiciously[11–13]. In the eastern Adriatic region, this kind of survey was attempted only on a few hillforts that were investigated in the course of the »Adriatic Islands Project«[14]. We present here the preliminary results of another such investigation, carried out recently on a hillfort in southern Dalmatia.

For our case-study we chose Grad, a major hillfort located near the western tip of Pelješac peninsula. Our choice was guided by the fact that the micro-region around Grad is well covered archaeologically. The area has been a focus of a long-term field project that included general surface survey and mapping, as well as excavation of another major prehistoric site, Nakovana Cave[15]. Data from that survey offer comprehensive information about over a hundred prehistoric sites in Grad's immediate neighborhood[16], while the deep excavation trench at Nakovana Cave provides reliable temporal controls[17,18].

Centrally located within this microregion and dominating the relatively fertile Nakovana plateau, the hillfort clearly represents its most prominent prehistoric site. Grad is a natural fortress, located on an almost impregnable rocky hilltop that is surrounded on all sides by vertical or overhanging cliffs some 20 meters high. The only approach to its high plateau is from the southwest, where scant traces remain of a drystone enclosure wall. From there, one can easily ascend to its barren, rocky summit (Figure 1). Grad has been mentioned in scientific reports since the late 19th century[19], and was described as an important prehistoric site by Nikša Petrić[20].

Methodology and Techniques Applied

Our intensive survey of Grad, carried out in May 2005, had multiple aims. The immediate ones were to roughly determine the spatial extent of the site and the periods during which it had been occupied. We also hoped to gain an initial insight into its function, estimate the degree of its preservation, and identify potential dangers posed to it by natural elements and human agency. Our further intention was to gather information that would allow a reliable estimate of the site's potential for future research, and could serve as a secure base for planning a more extensive field investigation.

We covered only the central area of the hillfort, its high plateau bounded by cliffs, which extends over an area of approximately 10.000 m^2. Our research strategy required mapping of all visible structural remains and systematic collection of all surface finds. In order to ac-

Fig. 1. View of the Grad hillfort, rising above the Nakovana plateau.

complish this, a 10 x 10 meters square grid was laid out over the area to be surveyed, using compass and measuring tapes, and taking into account the ground slope wherever necessary.

Each 10 x 10 meter square was documented individually on a standardized recording sheet prepared for the purpose. We estimated and recorded relative proportions of soil and bedrock exposed at the surface, the relative area covered by vegetation, and kinds of vegetation present. Ground visibility, which critically influences survey data, was estimated and recorded on ordinal scale, ranging from 0 (no visibility) to 5 (excellent visibility). A rough plan of each square, drawn to a scale of 1:100, included all structural remains and other features, together with their descriptions. This served as the base for the composite plan of the site, which was produced cumulatively in the field (Figure 2).

All removable artifacts visible at the surface were collected, bagged by class (pottery, lithics, other), and tagged for later laboratory treatment, and their presence/absence was noted on the recording sheet. Majority of the finds were small, heavily weathered pottery fragments, damaged by long exposure at the surface.

A total of 5461 potsherds, weighing almost 60 kilograms, made up by far the largest of all the recovered artifact assemblages. Since most of them were non-diagnostic due to fragmentation and weathering, they were classified into four rough technological categories: (1) sherds of Bronze Age/Iron Age hand-made vessels; (2) sherds of thick-walled hand-made vessels with a characteristic wall section (red-gray-red); (3) sherds of Hellenistic wheel-made vessels; and (4) glazed sherds. Each category was counted and weighed by the square, and the data were used as the base for further analysis of their spatial distribution across the site.

As expected, distribution diagrams plotted from raw data revealed that ground visibility had a major impact on the apparent distribution of finds. A linear and an exponential correction for visibility were therefore applied, the latter apparently producing more realistic plots. Three of those plots are reproduced in Figures 3–5, which are further discussed below.

Results of the Survey

The highest, eastern part of the hillfort, and the western end of the high plateau, are eroded to bedrock in most places, while its central part is covered by soil. This area was under cultivation in recent historic times, but the fields are now abandoned, and there seems to be little danger that modern agricultural activities might damage the site. Concentration of finds and structural remains is highest in the central area, and there is a distinct possibility that intact archaeological deposits are preserved underneath the plowzone. The site is being actively eroded away, as indicated by denuded areas along all cliff edges, which are strewn by numerous potsherds washed out form the sediment.

Earlier researchers[20] noted that the site was not confined to the high plateau. In the course of our fieldwork, it became abundantly clear that the artifact scatters extended well beyond it and encompassed large areas on

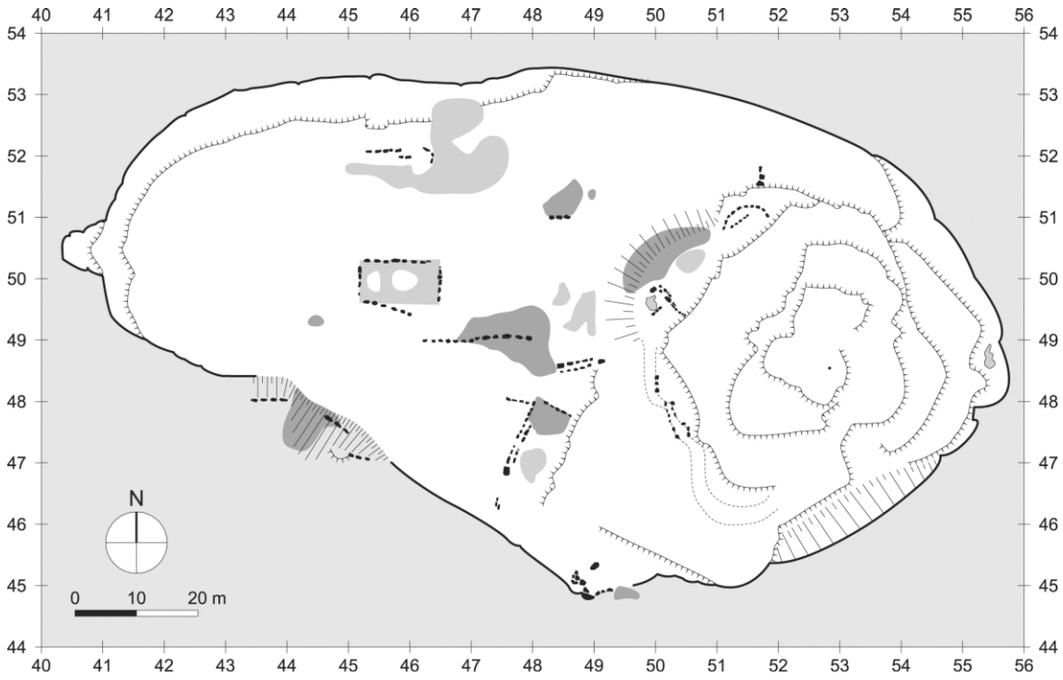

Fig. 2. Plan of the central area of the Grad hillfort.

Grad's slopes. Since our systematic survey was not extended over the high plateau's edges, we could not identify the full spatial extent of the site.

Periods of Occupation

The nine flaked stone artifacts and a couple of ground stone fragments (probably, an axe and a grindstone) are

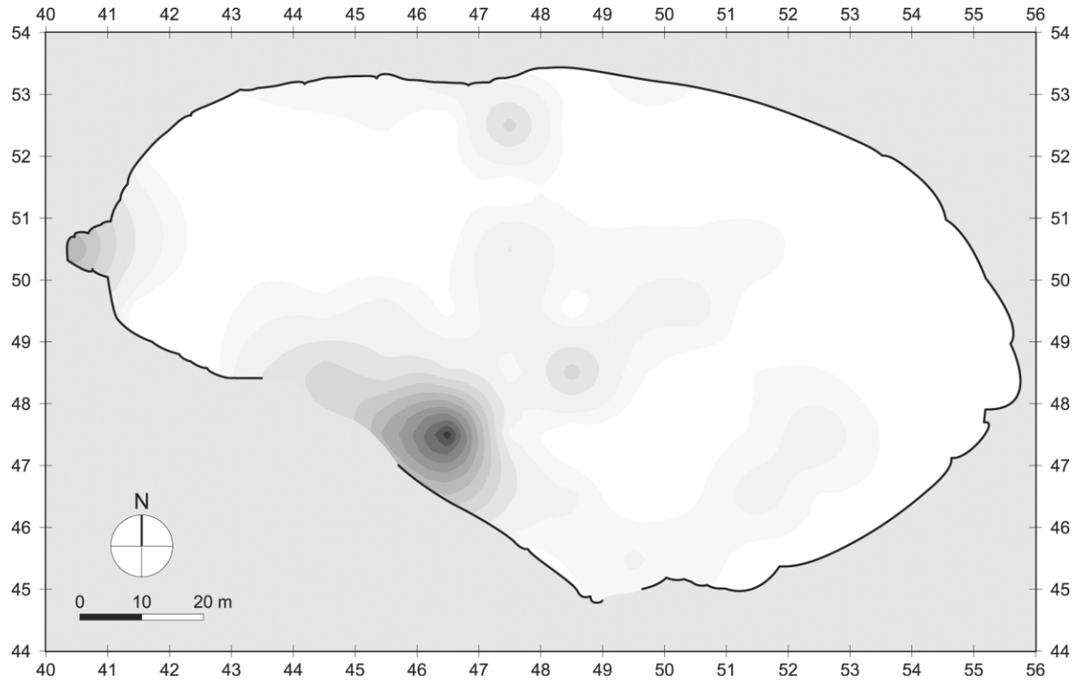

Fig. 3. Spatial distribution of Bronze Age/Iron Age potsherds.

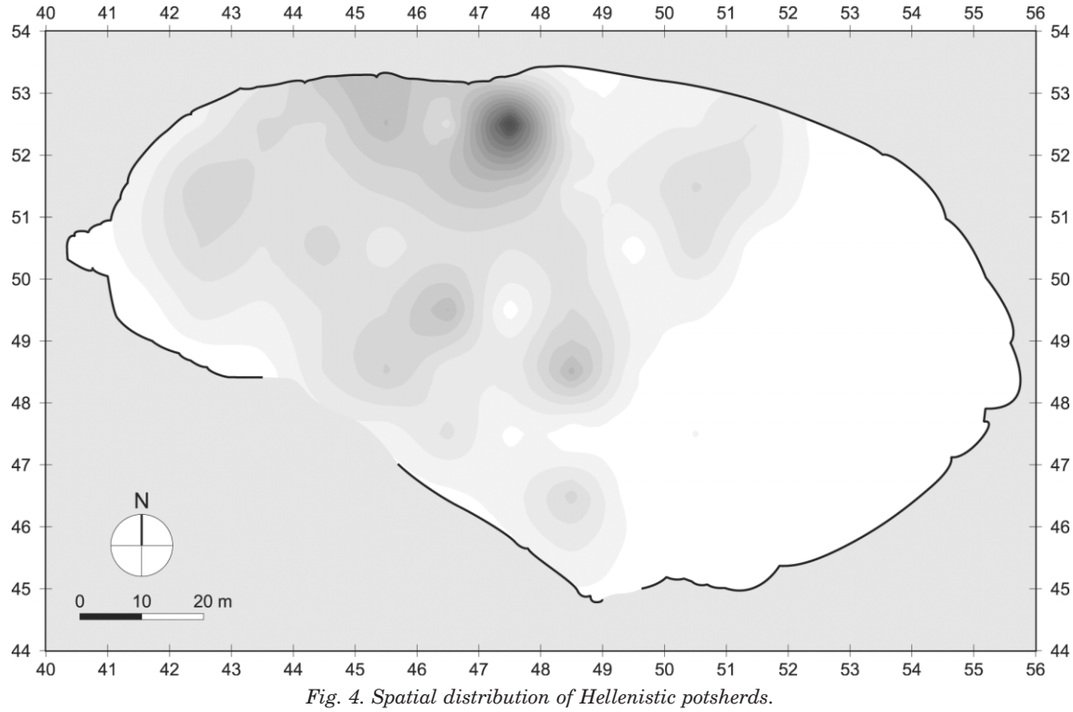

Fig. 4. Spatial distribution of Hellenistic potsherds.

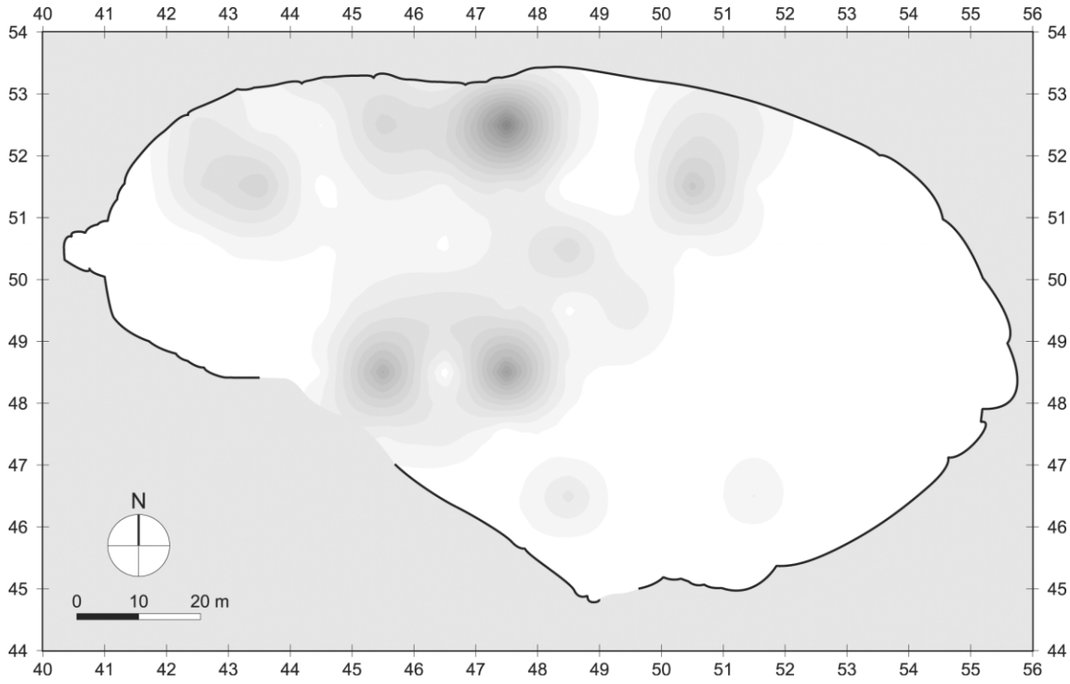

Fig. 5. Spatial distribution of thick-walled hand-made vessel sherds with red-gray-red wall section.

among the earliest finds recovered by the survey. One may add to them a single typologically early Hvar-style potsherd, published by Petrić[20]. These finds indicate that the hill was occasionally visited or ephemerally occupied already during the Late Neolithic or the Copper Age (fifth or fourth millennia B.C.).

A considerable assemblage of later prehistoric pottery was recovered, containing mostly small, non-diagnostic fragments of coarse, hand-made vessels. Occasional fragments of everted-rims, strap handles, massive horizontal handles, and flat bases, suggest that the greatest part of this assemblage belongs to the Late Bronze Age and the Early Iron Age[4,21], and indicate that Grad was more permanently occupied during the last two millennia B.C.

Coarse Hellenistic potsherds represent the majority of all collected finds (two thirds by number, three fourths by weight). Among them, amphorae fragments are the most common, especially those belonging to the type Lamboglia II[22]. Only eight fragments of the fine, black--slipped Hellenistic pottery known as »Gnathia ware« were recovered[23], closely similar to the examples found in great quantity at the nearby Nakovana Cave. Abundant Hellenistic finds indicate that intensive human activity on Grad was contemporaneous with the use of Nakovana Cave as a sanctuary during the last few centuries B.C[15].

Extremely rare post-Hellenistic finds suggest that there was little activity at Grad after the first century B.C. Among the scarce evidence for later building activities are a few lumps of mortar that almost certainly post-date the Hellenistic period. Three sherds of glazed pottery, each from a different vessel, probably belong to recent historic times. We also collected, recorded, and appropriately discarded a relatively small quantity of modern waste, such as plastic and glass bottles.

Spatial Distribution of the Surface Finds

Distribution diagrams were produced for all classes of recovered archaeological materials. Of particular interest are spatial distributions of the three main classes of pottery. The plots indicate relative weight densities of potsherds collected from the surface, calculated in g/m^2 and corrected for ground visibility.

A thin scatter of Bronze Age/Iron Age pottery extends across much of the central, southern and eastern parts of the surveyed area, with a minor concentration at its western end, and a major concentration in the south, near the main point of access to the plateau (Figure 3). Hellenistic pottery is distributed quite differently. It is spread across the central, western and northern parts of the surveyed area, roughly coinciding with the visible structural remains, with a major concentration in the north, but it is virtually absent from its highest, eastern part (Figure 4). Finally, the distribution of thick-walled hand-made sherds with red-gray-red wall section is much closer to that of the Hellenistic pottery then to the Bronze Age/Iron Age pottery (Figure 5). This distinctively fired ware thus seems to be associated with, and contemporary to, the Hellenistic finds.

Spatial distributions of these three classes of pottery suggest that most of the structural remains visible at the surface should be associated with the Hellenistic period – either the structures were built during that period, or were still being used at the time. Differential distributions of Bronze Age/Iron Age and Hellenistic finds may indicate that different parts of the high plateau were used differently during those two periods. Alternatively, they may indicate that Bronze Age/Iron Age deposits are capped by a Hellenistic layer in the central and northern parts of the site, while in its southern part, the Hellenistic layer has been eroded away.

While it is too early to discuss the function and internal organization of Grad, a few general remarks can be made. Character and quantity of the remains (mostly, coarse pottery, including many large vessels suitable for cooking and storage) hints at generalized residential activities. So do the lumps of burned clay, scattered almost everywhere across the site. On the other hand, the location itself suggests a defensive purpose, which is further supported by the remains of an enclosing structure that controlled the only feasible access to the high plateau. A fairly large drystone structure of elongated rectangular ground plan (13 x 7 m) occupies a central location and faces west; its purpose, for the moment, remains unknown. Other recorded structural remains are even less informative, consisting mainly of collapsed drystone walls. We collected only a very few fragments of ceramic roof tiles, which suggests that these were not widely used for covering roofs.

Grad is a large and complex site, and each one of its parts probably was characterized by a specific set of functions and activities. A particularly interesting issue is the relationship between the elevated, naturally protected central part of the site and its peripheral parts. Discussion of that issue must be postponed until intensive survey is extended to the surrounding slopes. For the moment, one can only speculate about a »special role« of the central plateau – its possible use as a fortress, a local elite residence, an area assigned for ceremonies, or a combination of those uses.

Conclusion

Analysis of the data recovered by the intensive survey of Grad indicates that the central area of the hillfort was intensively used during the last few centuries B.C., at the time when Dalmatia was being incorporated into the world of the classical Mediterranean civilization. Its origins can be traced back to the Bronze Age, while a few artifacts testify of an even earlier episode of occupation. The recorded structures and the recovered finds hint at a residential and defensive function of this large site, while its central, elevated area may have been a focus of special activities. A continuation of intensive survey beyond that central area, augmented by test excavation at selected locations, is a prerequisite for resolving some of the issues raised in this report.

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PRETPOVIJESNA GRADINA GRAD NA PELJEŠCU U DALMACIJI – PRELIMINARNI REZULTATI INTENZIVNOG PREGLEDA POVRŠINE

S A Ž E T A K

Intenzivni pregled površine proveden na Gradu, gradini na Pelješcu u južnoj Dalmaciji, obuhvatio je otprilike 10.000 m^2. Cilj pregleda bio je prikupiti podatke koji bi omogućili određivanje veličine nalazišta, njegove funkcije, razdoblja naseljenosti, stupnja očuvanosti, te potencijala za daljnja istraživanja. Pregled je obuhvaćao kartiranje vidljivih ostatka arhitekture i sustavno sakupljanje svih površinskih nalaza. Većina nalaza su ulomci grube helenističke keramike, te nešto manji broj ulomaka brončanodobne i željeznodobne lončarije. Središnji dio gradine intenzivno se koristio u zadnjim stoljećima prije Krista, dok njeni počeci sežu u brončano doba. Vidljivi ostaci arhitekture i prikupljeni nalazi ukazuju na stambenu i obrambenu funkciji nalazišta, dok su se na njegovom uzdignutom središnjem dijelu vjerojatno odvijale posebne aktivnosti.

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Original scientific paper

An Example of Demographic Anthropology, the Study of Matrimonial Exchanges – Endogamy, Choice of Spouse and Preferential Marriage

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A B S T R A C T

The development of demographic studies in anthropology is directly linked to the success of population genetics. The anthropodemographic or anthropogenetic approach is thus underpinned by questions of genetics. While demographers focus on population dynamics and renewal in quantitative terms, population geneticists refer not to individuals but to the sets of genes carried by individuals in a population. Their aim is to detect the factors and processes which influence the genetic evolution of a group, i.e. which modify gene frequencies from one generation to the next. Among them are the factors which affect modes of reproduction. To illustrate the association of these three approaches, i.e. demographic, anthropological and genetic, I use here the example of matrimonial exchanges – which lie at the heart of the population renewal process – among the Dogon of Boni, a Malian ethnic group living in the southern Sahel. We can see how successive analyses – starting with endogamy at macroscopic level and moving down to the individual with choice of spouse and preferential marriage – combining both quantitative and qualitative approaches, can be used to obtain a detailed description of matrimonial exchanges which shed light upon and complement the three different viewpoints.

Key words : *demographic anthropology, genetic anthropology, matrimonial exchanges, endogamy, preferential marriages, Dogon of Roni, Mali*

Introduction

The development of demographic studies in anthropology is directly linked to the success of population genetics (sometimes qualified as »qualitative demography«, notably when it concerns the study of hereditary diseases). The anthropodemographic or anthropogenetic approach is thus underpinned by questions of genetics. While demographers focus on population dynamics and renewal in quantitative terms, population geneticists refer not to individuals but to the sets of genes carried by individuals in a population. Their aim is to detect the factors and processes which influence the genetic evolution of a group, i.e. which modify gene frequencies from one generation to the next. Alongside population size and migrations, these are the factors which affect modes of reproduction. Hence, for example, endogamy, family size distribution, choice of spouse, the existence or otherwise of preferential marriages, formation of marriage cycles, etc. are important parameters to be taken into account. And the concepts of anthropology are often borrowed for the study of these practices.

To illustrate the association of these three approaches, i.e. demographic, anthropological and genetic, I will use the example of matrimonial exchanges – which lie at the heart of the population renewal process – among the Dogon of Boni, a Malian ethnic group living in the southern Sahel. The topography of their territory is highly specific, comprising a series of raised massifs around 15 km apart that emerge above the plain. The Dogon population totalled around 5,000 at the time of the study, distributed between 4 massifs, each comprising 3 to 4 villages.

Matrimonial Exchanges among the Dogon

Endogamy

Various levels of analysis are possible. *Exogamy* (or its opposite *endogamy*) are located at the macroscopic level and call for a quantitative approach. Endogamy may exist in varying degrees, at the scale of the ethnic group or

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on a finer, intra-ethnic scale. Among the Dogon of Boni for example, strong ethnic endogamy is observed[1] : 96% of marriages take place between Dogon, and only 4% with a different ethnic group. If the analysis is pursued within the ethnic group, by cross-tabulating the origins of spouses by their village of birth, we obtain a table in which the majority of marriages are on the diagonal. We observe that 84% of marriages take place within the same massif, and only 16% between massifs (Table 1).

It is interesting to look for an explanation for this massif endogamy, using log-linear models for example, which can be used to test, from a statistical viewpoint, the pertinence of certain criteria[2].

Among the Dogon, these endogamy criteria are variable[3]. When the village is large enough (as is the case for Tabi, a village of 1,200 inhabitants), significant lineage endogamy is observed. On the Sarnyéré massif, where customs are strongly adhered to, spouses are sought either in the same lineage or, failing that, in the same village. On the two other massifs, Ella and Loro, where the villages are smaller and much further apart, the criteria of geographical distance alone is sufficient to explain the exchanges.

This type of macroscopic analysis, though specific to demography, also seeks to interpret the phenomena observed. It highlights the importance of factors such as population size, geographical location or the weight of tradition in the practice of endogamy. The demographic and genetic consequences of this endogamy are important, since it divides the population into »islands«, thus implying that the population may be biologically heterogeneous.

Choice of Spouse

Beyond endogamy, there is a second level of analysis involving the study of preferential marriage. This is a much finer »microscopic« level. Do matrimonial customs recommend the choice of spouse based on a criterion of kinship?

Since most Dogon marriages take place within the massifs, the second analysis level focuses on a local level[4]. Within the massif, do people marry by chance (i.e. with no constraints other than those linked to age difference between spouses or to the existence of durable unions in the population)? Or does preferential marriage exist?

TABLE 1
DISTRIBUTION OF MARRIAGES CONCLUDED SINCE THE FOUNDATION OF THE GROUP BY SPOUSES' VILLAGE OF BIRTH

Wife 's village	Husband's village														
	Sarnyéré massif				Tabi massif			Ella massif				Loro massif			
	Nem	Dja	Tan	Koyo	Tabi	Tup	Téga	E.Bu	E.Bo	Mom	Ban	Loro	Yuna	K.Bo	Prin
Nemgéné	349	72	67	72	12	2	1	5	0	1	0	0	0	0	1
Djamaga	60	124	60	40	0	0	0	0	2	1	0	0	0	1	0
Tandi	54	56	98	29	1	0	1	2	1	0	0	0	0	0	0
Koyo	41	26	20	34	0	0	0	0	0	0	0	0	0	0	0
Total Sarnyéré	504	278	245	175	13	2	2	7	3	2	0	0	0	1	1
Tabi	10	0	2	1	746	39	60	1	1	0	0	0	1	0	10
Tupéré	0	1	2	0	31	164	19	0	0	0	0	2	1	3	5
Téga	0	0	6	0	49	35	276	0	0	0	0	4	1	3	1
Total Tabi	10	1	10	1	826	238	355	1	1	0	0	6	3	6	16
Ella-Buli	1	2	4	0	0	0	0	10	6	6	0	0	0	1	1
Ella-Boni	2	0	0	0	3	1	1	6	20	13	10	0	0	4	1
Momni	2	1	1	0	1	0	0	8	11	22	10	5	0	4	0
Banaga	0	0	0	0	0	1	0	3	10	11	20	3	0	4	1
Total Ella	5	3	5	0	4	2	1	27	47	52	40	8	0	13	3
Loro	0	0	0	0	0	0	1	1	7	3	5	85	9	39	1
Yuna	0	0	0	0	1	2	3	0	0	1	0	15	7	10	5
Koyo-Boni	0	0	0	1	0	0	0	2	2	3	1	26	2	35	0
Pringa	0	0	0	0	11	5	2	0	2	0	0	1	4	0	88
Total Loro	0	0	0	1	12	7	6	3	11	7	6	127	22	84	94
All massifs	519	282	260	177	855	249	364	38	62	61	46	141	25	104	114
Non dogon vill.	0	0	0	0	0	0	0	0	2	2	5	3	0	0	0
Unknown vill.	60	36	22	17	57	19	26	9	24	17	24	41	4	9	42
TOTAL	579	318	282	194	912	268	390	47	88	80	75	185	29	113	156

TABLE 2
DISTRIBUTION OF MARRIAGES BETWEEN COUSINS OR OTHERWISE AMONG THE DOGON OF BONI

Marriage type	Marriages between first cousins					Other marriages			TOTAL
	MoBrDa	FaSiDa	MoSiDa	FaBrDa	Total	Unkn. A	N. C.	Total	
First marriages	57	25	9	36	127	264	259	523	650
Next marriages	31	15	17	31	94	352	329	681	775
All marriages	88	40	26	67	221	616	588	1,204	1,425

MoBrDa – mother's brother's daughter (matrilateral cross-cousin), FaSiDa – father's sister's daughter (patrilateral cross-cousin), MoSiDa – mother's sister's daughter (matrilateral parallel cousin), FaBrDa – father's brother's daughter (patrilateral parallel cousin), Unkn.A – unknown ancestry, N. C. – non cousin spouses

This question is an anthropological one, though geneticists are also highly interested in the answers obtained. For them, the most important point is to know whether preferential marriage rules are actually applied or whether they are no more than »theoretical«. If preferential marriage is indeed widely practiced, this will affect the genetic structure of the group.

Analysis of this kind brings in anthropological concepts, taking into account the population's kinship terminology (Iroquois among the Dogon) but also, in many cases, its history. Traditionally among the Dogon, preferential marriage was with the matrilateral cross-cousin (mother's brother's daughter, MoBrDa), and this tied in with the traditional system of inheritance whereby Ego inherited from his maternal uncle. But over the last century, the introduction of Islam has transformed the customary system. In certain villages, the preferential spouse has become the patrilineal cross-cousin (FaSiDa).

From a methodological viewpoint, the possibility of obtaining the *family trees of the population* over several generations is a considerable advantage. A database of this kind makes it possible to compare the types of marriage actually concluded with the rules laid down by custom.

Thus calculations drawn from Dogon family trees show that, for a young man who wishes to marry, only an average of 6% of young women of the right age are cousins. Yet Table 2 shows that 19.5% of marriages are between first cousins for first marriages, though this proportion falls to 15.5% for all marriages.

If the four types of marriage between cousins are ranked in order of decreasing proportion among first marriages, the following result is obtained:

45% MoBrDa 28% FaBrDa 20% FaSiDa 7% MoSiDa

The most frequent marriage is indeed that recommended by traditional custom (MoBrDa) and not the type of marriage newly »officialized« over the last century (FaSiDa) which arrives in third position. The type of marriage classically recommended by Islam (FaBrDa) ranks in second position only.

So these observations contradict the declarations relating to the »new« preferential marriage. For geneticists, the key question is to know whether the observed distribution of marriages differs from that which would be obtained in the case of random marriage distribution. This is why researchers always seek to compare actual observations with what one would expect to observe in the case of random unions.

Moreover, we know that the mean age difference between spouses is in itself a structural constraint which may be decisive in the choice of spouse. Among the Dogon, wives are, on average, 6 years younger than their husbands. In a pioneering article in 1963, Hajnal showed that certain marriages between cousins reproduced the traditional age differences between spouses more easily than others (Figure 1).

Here again, genealogical data, taking account of individuals' age, were used to calculate the theoretical percentages of marriage with each type of cousin in the Tabi village, *taking account of the age difference between spouses* (Table 3, 1st line).

They can be compared with the proportions observed in the population (Table 3, 2nd line). We observe a very high proportion of marriages with the matrilateral cross--cousin (MoBrDa) compared with the expected average and strong avoidance of the matrilateral parallel cousin (MoSiDa), probably linked to the incest taboo.

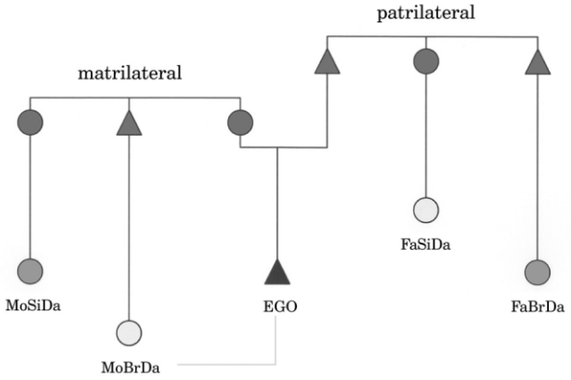

Fig. 1. The different types of Ego's cousins. The age difference observed between Ego's mother and father is observed again between Ego and his matrilateral cross-cousin only. MoBrDa – mother's brother's daughter (matrilateral cross-cousin), FaSiDa – father's sister's daughter (patrilateral cross-cousin), MoSiDa – mother's sister's daughter (matrilateral parallel cousin), FaBrDa – father's brother's daughter (patrilateral parallel cousin).

TABLE 3
COMPARISON BETWEEN EXPECTED AVERAGE PROPORTIONS UNDER THE ASSUMPTION OF RANDOM UNIONS, TAKING ACCOUNT OF AGE DIFFERENCE BETWEEN SPOUSES, AND OBSERVED PROPORTIONS

Marriages with	Cross-cousins		Parallel cousins		Total
	MoBrDa	FaSiDa	MoSiDa	FaBrDa	
Average proportions expected under the assumption of random unions	0.32	0.18	0.27	0.23	1
Proportions observed	0.49	0.23	0.03	0.25	1

MoBrDa – mother's brother's daughter (matrilateral cross-cousin), FaSiDa – father's sister's daughter (patrilateral cross-cousin), MoSiDa – mother's sister's daughter (matrilateral parallel cousin), FaBrDa – father's brother's daughter (patrilateral parallel cousin)

Hence, family trees can be used to control for the divergence between »words« and »deeds«. Among the Dogon, the most frequently observed form of marriage is with the matrilateral cross-cousin. And, given the age difference between spouses, this is the only cousin who structurally reproduces the right customary age difference. It is also the spouse who was recommended by traditional custom before the arrival of Islam. So Islamization in the early 20th century has not modified matrimonial practices, despite what the Dogon say.

Conclusion

We can see how successive analyses, starting at macroscopic level and moving down to the individual, combining both quantitative and qualitative approaches, can be used to obtain a detailed description of matrimonial exchanges which shed light upon and complement the three different viewpoints: demographic, but also and above all, anthropological and genetic. They simultaneously highlight the complexity of these exchanges, which are always very difficult to interpret.

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PRIMJER DEMOGRAFSKE ANTROPOLOGIJE, ISTRAŽIVANJE ODABIRA BRAČNOG DRUGA – ENDOGAMIJA I ODABIR SUPRUŽNIKA

SAŽETAK

Razvoj demografskih istraživanja u antropologiji izravno je povezan s uspjehom populacijske genetike. Tako su antropodemografski ili antropogenetički pristup poduprti pitanjima iz genetike. Dok su demografi usmjereni na dinamiku populacije i reprodukciju u kvantitativnim određenjima, populacijski genetičari ne okreću se pojedincima, već skupu gena čiji su nosioci pojedinci u populaciji. Njihov cilj je otkriti faktore i procese koji utječu na genetičku evoluciju grupe, tj. one koji modificiraju frekvencije gena od jedne do druge generacije. Među njima su faktori koji utječu na reprodukciju. Kako bi se prikazala povezanost ova tri pristupa, tj. demografskog, antropološkog i genetičkog, bit će korišten primjer odabira bračnog druga – koji predstavlja središte procesa produženja populacije– između Dogona iz Bonija, malijske etničke skupine koja živi u južnom Sahelu. Možemo vidjeti kako uzastopne analize koje kombiniraju kvantitativne i kvalitativne pristupe i to počevši od endogamije na makroskopskoj razini do odabira supružnika te poželjnog braka na individualnoj razini mogu biti korištene za postizanje preciznijeg opisa bračnih izmjena, što dodatno rasvjetljava i upotpunjuje tri pristupa problemu.

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Trend of Mortality Observed in a Cohort of Drug Addicts of the Metropolitan Area of Bologna, North-Eastern Italy, During a 25-Year-Period

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A B S T R A C T

The aim of our study is to evaluate the temporal trend of deaths in a cohort of i.v. drug users (IVDU) followed in a city of Northen Italy (Bologna), and to assess its relationship with HIV infection and AIDS, and availability of potent antiretroviral therapy. One thousand and 214 IVDUs (mainly heroin addicts), 916 males and 298 females, attending an outpatient service for treatment and prevention of substance abuse between 1977 and November 1996, were enrolled into our observational cohort, and their vital status was ascertained up to December 31, 2002. The large majority of enrolled subjects were born in the Bologna metropolitan area and surroundings; no extra-European immigrants were present. During the observation period, 271 IVDUs (22.3%) died, 211 males (23.0%), and 60 females (20.1%). No death was recorded before 1984. Main death causes result as follows: AIDS (52.8% of episodes), heroin overdose (22.1%), street accidents (7.4%), decompensated liver cirrhosis (6.3%), and suicide (2.9%). The highest absolute number of deaths was observed between years 1991 and 1996. Crude mortality rate caused by AIDS was 10.0 per 1000 for males and 13.2/1000 for females; the rate of death due to other causes proved 11.1/1000 among males and 5.2/1000 among females. In most recent years, a sharp decrease in the number of AIDS-related deaths, attributable to the increased use of potent antiretroviral regimens, was recorded among IVDUs, although overall mortality rate remained appreciable.

Key words: *epidemiology, drug users, HIV infection, overdose, mortality, temporal trend, antiretroviral therapy*

Introduction

A remarkable decrease of HIV-associated disorders, as well as of notified cases of AIDS, has been observed in industrialized countries during the last seven years. Such significant drop of HIV-related morbidity and mortality (mostly occurred thanks to the introduction and diffusion of highly active antiretroviral therapy, or HAART), was also observed among i.v. drug users (IVDUs)[1–3]. However, a significant number of potential Italian patients, still unaware of their HIV serostatus[4], do not yet make use of antiretroviral treatment: in our previously reported series regarding the year 1997, 29% of active or prior IVDUs were never tested for HIV infection[5].

A European multicentre study[6] shows how IVDUs with recent HIV seroconversion are subject to an increased risk of mortality due to pneumonia, endocarditis, sepsis, meningitis, encephalitis, and decompensated liver cirrhosis. Before the availability of HAART, an increased rate of mortality due to heroin overdose and suicide was also recorded among HIV-infected IVDUs[7,8]. Just the use of elevated heroin dosages associated to a suicidal behavior could be partially responsible for this phenomenon in patients with a newly diagnosed HIV disease and lacking of any social and psychological support, especially before the introduction of effective antiretroviral drug combinations (HAART)[9–11].

In a cohort study of 4,962 IVDUs carried out in Bologna (Italy) between years 1980 and 1990, 332 deaths were observed: 150 of them occurred because of AIDS, 64 of drug overdose, and 39 of accidents[8]. The HIV infection serostatus (28.2% of 1214 subjects were positive) proved to be significantly related also to deaths caused by trauma, heroin overdose, bacterial infections, and acute and

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chronic liver disease. The mortality rate of IVDUs proved significantly higher among those subjects who did not receive a HIV serodiagnosis with respect to those subjects who underwent a HIV infection test with negative result (12.1% *versus* 2.5%)[8]. Again in Bologna, at the end of nineties the risk of a fatal heroin overdose remained remarkably important[12], while between years 1996 and 1998 Italian national cumulative data showed a progressive decrease of deaths due to this last cause[13].

In a cohort of 11,432 IVDUs enrolled in Rome (Italy) between years 1980 and 1995, 1,734 deaths were registered (15.2%). The peak of AIDS-associated mortality was observed in 1991 and 1992 (13.2 per 1000 person-years), followed by a decrease occurred in both male and female population since 1993–1994, even earlier than expected according to the availability of (before HAART became available in mid-1996)[14].

In the Emilia-Romagna region of Italy (of which Bologna is the main administrative centre and largest town), a retrospective study conducted on 4,260 IVDUs followed in Piacenza, Modena and Ferrara during two decades (1975–1995), pointed out a dramatic increase in the rate of deaths caused not only by AIDS; but also by overdose and other causes, especially accidents. The mortality rate observed in this last IVDUs cohort proved particularly high: the calculated standard mortality rate (SMR) was 16.7 for males, and 33.4 for females. The overall probability of survival after 15 years of follow-up was 65%. After drug overdose and AIDS, the other relevant causes of deaths were street accidents, decompensated liver disease, other infectious disorders and malignancies among male IVDUs, while accidents (in particular, homicide), and gastrointestinal tract disorders had a greater prevalence among females[15].

Finally, in an Italian multicentre study carried out in Turin, Rome, Naples, and Cagliari on IVDUs enrolled between 1980 and 1992, a highly variable mortality rate was detected, ranging from 11.0 per 1000 persons-years in Naples, up to 20.5 per 1000 persons-years in Rome. Again, the most frequent causes of death were represented by drug overdose, AIDS, and accidents, although relevant differences were observed among the four considered cities. A higher mortality rate among IVDUs compared with that of the general population was consistently observed in all considered geographical cohorts[16,17].

In a very recent 7-year survey from the US, active drug use proved temporally linked to HIV disease progression and overall mortality[17].

Our present study aims at:

i) investigating the past and present mortality trend of a cohort of IVDUs based in Bologna, in an attempt to identify its causes;

ii) investigating whether HIV infection plays any influence on the rate of mortality due to causes other than AIDS;

iii) evaluating the AIDS-associated fatality rate among subjects with missed or delayed HIV serodiagnosis, hence with a possible late diagnosis of the infection only at an advanced stage of the condition.

Materials and Methods

Patients

An open cohort of 1,214 IVDUs has been assessed for a number of epidemiological and clinical variables. The people involved were mainly i.v. heroin addicts, and included 916 males (75.5%) and 298 females, referring to a specialized outpatient Centre for the treatment and prevention of substance abuse of Bologna (Italy), from the year 1977 up to November 1996. The great majority of enrolled patients at the time of their first visit lived in South-Western districts of the Bologna conurbation (400,000 inhabitants in the city, half a million with the suburbs). The place of birth is a missing datum for one minor part of the people reached by our study; as to the others, most of them were born within the Province of Bologna and no one comes from abroad.

By matching anagraphical data with the registries of the abode or birth municipalities, a retrospective research focusing on whether subjects were still alive on December 31, 2002, has been carried out.

In some cases, neither the year of birth nor the subject's age were available due to patients' refusal to provide their own personal data. Referring to population registries allowed us to retrieve missing information regarding almost all individuals in our cohort: in 60 cases, still assisted by our outpatient services, data are still lacking, but for deceased patients figures were adjusted when causes of death were researched.

HIV infection

At the time of patients' first contact with our dedicated outpatient centre, HIV testing and specific counselling were always offered, but 355 subjects out 1,214 (29.2%) refused this examination. HIV serology became recommended in late 1984, so that the first recognition of a possible underlying HIV infection is related to the first visit at our outpatient Centre, starting from 1984–1985. IVDUs enrolled before 1985 had HIV testing performed starting from this year either at the outpatient units, or at one of the two Infectious Diseases Divisions of Bologna, or local prison facilities. At the time of their next contact with the above-mentioned health care structures, HIV testing and specific counselling were always offered. Data regarding HIV serostatus for IVDUs enrolled after 1985 dates back mainly to the time of first access to IVDA outpatient Centres. Usually, the seroconversion time for patients who were HIV-negative at their first control is not definable, because of the limited availability of patients to be tested or to furnish results.

Statistical analysis

As to the calculations of person-years and rates, the beginning of observation is identified with the year of the first visit at our outpatient Centre for IVDUs. The first

access date of 41 individuals is unknown; for each of them, the enrollment was identified with the first date available in his/her clinical records. The end of observation period was matched with that of death for deceased patients, while it was fixed at December 31, 2002 for living individuals; for two persons who were lost to follow-up, the end-of-observation time is identified with 1997: the year of their last access to the Centre. The cause of death of deceased subjects was drawn by local registries, as expressed by the ICD-IX code[18,19]. Population data were obtained from the official annual statistical update of Bologna; mortality figures were drawn by public health notification registries.

Statistical analysis included specific mortality rates per age, gender and HIV-status, and standardized mortality rate (SMR)[20], obtained by indirect standardization performed on Bologna rates per each considered year. Chi-square has been used for the analysis of frequency distribution; Student's t test and Analysis of variance were employed to evaluate differences among mean values. The rates per HIV serostatus were calculated by attributing the years of observation of each single subject to his/her own category (HIV-positive, HIV-negative, HIV--unknown), without focusing on the time of seroconversion (which was not always available). As a consequence, not each person's time of HIV-positive or HIV-negative serology, but only the patient serostatus was considered.

The considered study time was analyzed according to a wide range of variables, by dividing it into four different periods, characterized by prominent epidemiologicals, treatment, prognosis, type of medical assistance, and outcome. The first period (years 1977–1983) encompassed the initial service offer, opposition and resistance by patients, no deaths, and absence of HIV-AIDS cases; the second period (1984–1990) was characterized by the rapid spread of AIDS, spontaneous patient access, and early HIV-associated deaths; during the third period (year 1991–1996) the patient access remained sustained, AIDS had the greatest fatality rate, and many deaths occurred; the fourth period (years 1997–2002) was characterized by the availability of highly active antiretroviral therapy (HAART), followed by a sharp drop of AIDS mortality. Our cohort was closed to enrollments at the end of previous period.

Results

Series description – general features

Overall, 1,214 patients were considered: 916 males and 298 females. The distribution per age classes at the time of enrollment is summarized in Table 1A, where also enrollment period is reported. The age of 60 subjects was not available. Table 1B shows the subjects' enrollment age in different periods. One hundred and 10 IVDUs were enrolled from year 1977 to 1983. At the end of year 1990, patients still alive in our cohort were 607, and in the period 1991–1996 enrolled and alive patients became 1,174. The characterizing features of the above- -mentioned time periods are recognizable in the cumulative Table 6.

Age and sex

Overall follow-up accounted for 10,030.50 person--years for male subjects, and 3,249.75 person-years for females: cumulated follow-up accounted for 13,280.25 person-years. Mean age did not differ between enrolled males and females, when considering both the entire follow-up, and single time periods. A temporal trend to increased patients' age occurred through time: the difference among mean age at enrollment during different time periods turned out to be significantly different ($p<.0001$; Table 1B). The age at which each person started to take up IVD was asked, but not provided for nearly one half of involved subjects;.

HIV serostatus

When assessing HIV serostatus, IVDUs HIV-positive were 426, and HIV-negative subjects were 433 (Table 2). In particular, HIV infection was found in 297 men (32% of males), and in 298 women (43% of females). Moreover, among tested individuals, HIV-positive male patients accounted for 46%, while females were 61%, leading to a statistically different distribution ($\chi^2=14{,}2667$; $p=0.0002$).

The record of HIV positive serostatus was documented in 8% of patients early HIV-negative; the great majority of them was deducted from death certifications.

Analysis of deaths

Overall IVDUs subjects dead on December 31, 2002 were 271 (22.3% of all enrolled individuals): 211 males (23% of enrolled males), and 60 females (20.1% of the female cohort). The mean patient age at the time of death was comparable by gender: 33.2 ± 6.6 years among males, and 33.5 ± 6.0 years among females ($\chi^2=1{,}091$, p=n.s.). The overall 1.214 enrolled IVDUs accounted for 13,280.25 years-person (a mean of nearly 11 years per patient), while the 271 deceased individuals contributed for 1,258 years-person (an average of less than five years per patient). Table 3 resumes all data concerning deaths, classified per age group and sex, with person-years and rates per 1000. The overall death distribution does not show any significant difference between males and females. The time of initial i.v. drug addiction, when available, does not provide evidence of significant relation between age of start of IVDUs and eventual lethal outcome.

The cause of each single death has been retrieved for 267 of the 271 deceased patients (98.5%). No deaths were registered in this cohort in the years preceding 1984. Figure 1 shows the different causes of death per year, in the period 1984–2002. Overall AIDS-related deaths total 143 (52.8%), followed by those due to heroin overdose (60: 22.1%), liver cirrhosis (17: 6.3%), road accidents (20: 7.4%), suicide (8: 2.9%), homicide (3: 1.1%), infectious endocarditis and other heart disorders (6: 2.2%), neoplasm (lung's or lymphoma, 5 deaths, 1.8%), and pneumonia (2: 0.7%). Three causes of death occurred only once: wasting syndrome, bleeding endocranic aneurism, and fire accident. The cause of death could not be found

TABLE 1
BOLOGNA IVDU COHORT. CASE DISTRIBUTION ACCORDING TO SEX, ENROLLING PERIOD, AND AGE AT ENROLLMENT (SECTION A); COMPARISON OF DIFFERENT PERIODS AND ENROLLMENT AGES (SECTION B)

Section A

	Enrolling	Period			
Sex	Age groups	I	II	III	All
Women	0–14	1	1		2
	15–19	6	9	5	20
	20–24	7	45	33	85
	25–29	4	38	49	91
	30–34	2	20	26	48
	35–44	1	10	27	38
	45–49			2	2
	50–54			1	1
	Nn	1	3	7	11
Total women		22	126	150	298
Mean age		22.71	26.06	29.01	27.29
Standard error		1.342	0.487	0.557	0.377
Men	0–14		2		2
	15–19	21	18	10	49
	20–24	41	130	95	266
	25–29	16	125	143	284
	30–34	6	55	97	158
	35–44	1	22	67	90
	45–49		2	10	12
	50–54		1	3	4
	>55	1		1	2
	nn	2	19	28	49
Total men		88	374	454	916
Mean age		22.88	26.17	29.28	27.37
Standard error		0.609	0.287	0.324	0.219

Section B

	Enrolling	Period			
Sex	Age groups	I	II	III	All
Total		110	500	604	1214
Mean age		22.85	26.14	29.21	27.35
Standard deviation		5.7196	5.4080	6.6763	
Standard error		0.553	0.247	0.280	0.189
95% confidence limits	lower	21.7542	25.6562	28.6629	
	upper	23.9467	26.6283	29.7624	
Median		22	25	28	
Minimum		14	10	17	
Maximum		56	53	62	

	Anova variation	Deviance (SS)	gf	Variance (MS)	p (F)
	among periods	4837.661	2	2418.831	
	within periods	42735.203	1151	37.129	
	total	47572.864	1153	41.260	<0.001

in four patients only. Table 4 reports the causes of death stratified according to gender, with the associated rate per 1000 person-years.

When examining deaths, according to whether they are due to AIDS or not, it can be highlighted that 111 fatal episodes non AIDS related occurred to males (41% of

TABLE 2
BOLOGNA IVDU COHORT: SUBJECT DISTRIBUTION ACCORDING TO INITIAL HIV SEROSTATUS AND SEX

HIV at enrollment	Sex				Total
	Females		Males		
Negative	83	27.9%	350	38.2%	433
Positive	129	43.3%	297	32.4%	426
(not available)	86	28.9%	269	32.3%	355
Total	298	100%	916	100%	1214

TABLE 3
BOLOGNA IVDU COHORT. DISTRIBUTION OF DEATHS OVER THE FOUR GIVEN AGE GROUPS, CLASSIFIED BY GENDER. PERSON-YEARS (P-YS) FOLLOW-UP, AND MORTALITY RATE PER 1,000 P-YS SUBJECTS ARE ALSO PRESENTED

Age groups	Males			Females			Total
	Deceased	P-ys	Rate/ 1000 p-ys	Deceased	P-ys	Rate/ 1000 p-ys	Deceased
< 25	11	943.5	11.66	1	334.0	2.99	12
25–34	130	485.5	26.68	39	1590.0	24.53	169
35–44	57	3109.0	18.33	16	1015.75	15.75	73
≥ 45	13	608.0	21.38	4	195.0	20.46	17
Total	211	10030.5	21.04	60	3249.75	18.46	271

overall men), while only 17 to females (28.3% of deceased women). The general distribution of causes of death was significantly different according to patient gender ($p < 0.001$): AIDS-related deaths proved significantly more frequent among females, while overdose-related ones occurred predominantly among males ($\chi^2=11{,}042$; $p < 0.001$). AIDS-related deaths have been compared with all other cumulative causes of death, and disease caused deaths with violence caused ones, in Table 5.

When analyzing the entire time span split into four different periods (as mentioned above), not a single death occurred in the period 1977–1983. Between 1984 and 1990, 40 deaths were registered among 459 enrolled subjects (8.7%): 13 of them were attributed to AIDS, and 27 due to other causes. Between 1991 and 1996, of 1,174 IVDUs 172 deceased (14.6%): 109 dead because of AIDS, and 63 due to all other possible causes. Between 1997 and 2002, among 1002 living subjects we observed 59 deaths (5,9%): 21 because of AIDS, and 38 due to all other cumulative causes (Table 6).

Table 7 presents the mortality rate per 1000 person-years according to gender and grouped age of death, as well considering HIV/AIDS, and all the other causes of death.

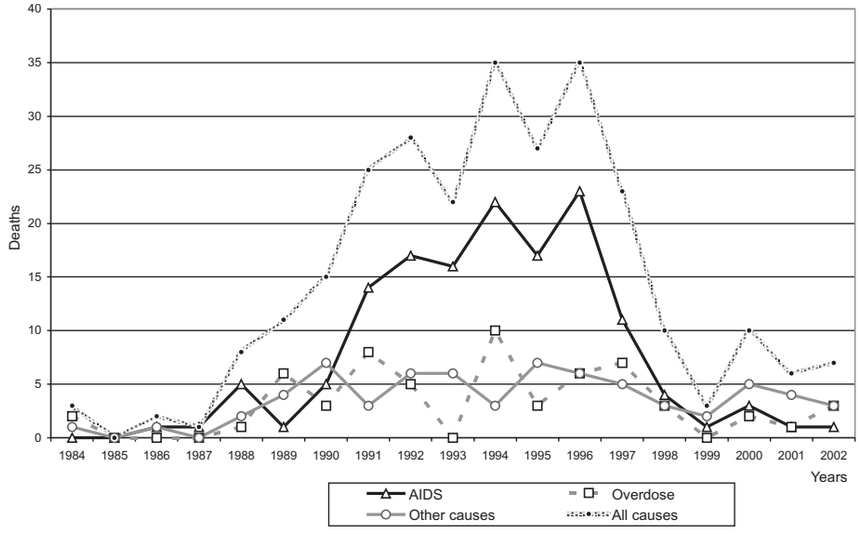

Fig. 1. Bologna IVDU cohort. Deaths per main causes during the whole time of observation. The cohort had no deaths until 1983.

TABLE 4
BOLOGNA IVDU COHORT. ALL DEATHS OCCURRED IN OUR COHORT ARE REGISTERED ACCORDING TO THEIR RECOGNIZED CAUSES. BOTH ABSOLUTE FIGURES AND MORTALITY RATE PER 1,000 PATIENTS-YEARS (P-YS) ARE SHOWN

Sex	Females		Males		Total	
Cause of death	Deceased	Rate/1000 p-ys	Deceased	Rate/1000 p-ys	Deceased	Rate/1000 p-ys
AIDS	43	13.231	100	9.970	143	10.768
Overdose	6	1.846	54	5.384	60	4.518
Hepatitis, cyrrhosis	3	0.923	14	1.396	17	1.280
Heart diseases		0	6	0.598	6	0.452
Road accidents	5	1.539	15	1.495	20	1.506
Suicide	1	0.308	7	0.698	8	0.602
Neoplasm	1	0.308	4	0.399	5	0.377
Respiratory pathologies		0	2	0.199	2	0.150
Undefined cause		0	2	0.199	2	0.150
Other violent causes		0	4	0.399	4	0.301
Unknown causes	1	0.308	3	0.299	4	0.301
Total	60	18.463	211	21.036	271	20.406

TABLE 5
BOLOGNA IVDU COHORT. DEATH DISTRIBUTION ACCORDING TO SETS OF DEATH CAUSES: AIDS, NON-AIDS, AND DISEASE/VIOLENT DEATH

Sex	Females		Males		Total	
	Deceased	Rate/1000 p-ys	Deceased	Rate/1000 p-ys	Deceased	Rate/1000 p-ys
AIDS	43	13.231	100	9.970	143	10.768
Non-AIDS	16	4.923	108	10.767	128	9.337
Unknown	1	0.308	3	0.299	4	0.301
Total	60	18.463	211	21.036	271	20.406
Diseases	47	14.463	126	12.562	173	13.027
Violent causes	12	3.693	80	7.976	92	6.928
Unknown or undefined causes	1	0.308	5	0.499	6	0.452
Total	60	18.463	211	21.036	271	20.406

TABLE 6
BOLOGNA IVDU COHORT. DYNAMIC TRENDS OBSERVED IN OUR PATIENTS: ENROLLMENTS AND DEATHS OCCURRED DURING THE FOUR GIVEN TIME PERIODS

Periods	I	II	III	IV	Total
Years	1977–1983	1984–1990	1991–1996	1997–2002	1977–2002
Survivors at prior time interval	–	110	570	1002	
Newly enrolled subjects	110	500	604	0	1214
IVDUs alive in the period	110	610	1174	1002	
IVDUs deceased in the relevant period	0	40	172	59	271
Fatality rate (%) in the relevant period	0%	6.6%	14.6%	5.9%	22.3%
Subjects lost at follow-up	0	0	0	2	2
Subjects alive at the end of follow-up	110	570	1002	941	941

When analyzing the separated time periods (as mentioned above), the mortality rate per 1000 patients-year was 0/1000 (no deaths), 19.5/1000, 32.2/1000, and 10.2/1000 respectively: in the overall period 1977–2002, the mean mortality rate proved 20.4/1000. The highest fatality index was reached in the period 1991–1996, when it accounted for 33.5/1000 among women, and 31.8/1000 among men.

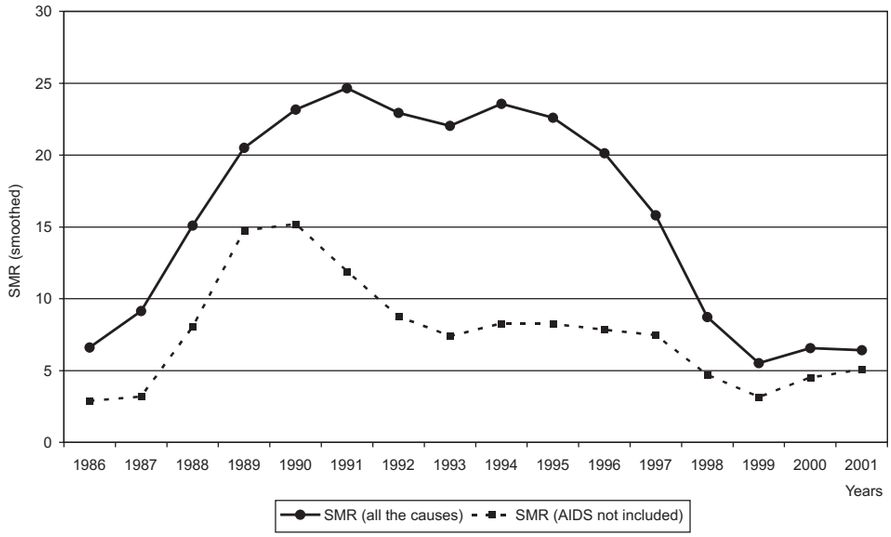

Fig. 2. Bologna IVDU cohort. Mortality trend from 1996 to 2001. Yearly SMR for all death causes. Indirect standardization based on Bologna population rates for each year (upper line), smoothed using weighted adjacent year values. Lower line shows the contribution to SMR of death causes other than AIDS; the area comprised between the two slopes identifies AIDS contribution to deaths.

Figure 2 shows the smoothed temporal trend of standardized mortality rate (SMR) for all causes, 1986 to 2002 (upper line). The lower line shows the SMR calculated without considering AIDS-associated deaths, so that the area between the two curves represents the absolute contribution of AIDS to excess mortality during the relevant period. The decrease of deaths due to other causes shows a drop paralleling that of AIDS-related fatality events, but excess mortality remains higher than the values observed in the pre-AIDS era.

When considering our series according to first HIV serostatus, we obtained a rate of 37,00/1000 for HIV-infected patients, compared with 17,00/1000 for non-HIV--infected IVDUs, and 7,85//1000 for individuals with undetermined HIV serostatus. In the whole time period, the mortality index was higher for male HIV- positive patients compared with HIV-positive females, but women aged 25–44 years, as subgroup, had the greatest risk of death for AIDS. When excluding AIDS, all other causes of death largely predominated among males, especially for people aged 25–44 years. In more detail, the highest risk of death was found in the group aged 25–34 years, while only a few deaths were observed Table 9 presents mortality rate analyzed according to HIV serostatus and subdi-

TABLE 7
BOLOGNA IVDU COHORT. DEATH DISTRIBUTION PER CAUSES (AIDS/NON-AIDS), AGE GROUPS, AND SEX

Sex		Non AIDS		AIDS		Unknown	Total	
	Death age	Deceased	Rate/ 1000 p-ys	Deceased	Rate/ 1000 p-ys	Deceased	Deceased	Rate/ 1000 p-ys
Females	<25	1	2.99	0	0.00		1	2.99
	25–34	8	5.03	28	17.61	1	37	24.53
	35–44	5	4.92	14	13.78		19	15.75
	>44	2	10.23	1	5.12		3	20.46
	Total	16	4.92	43	13.23	1	60	18.46
Males	<25	11	11.66	1	1.06		12	11.66
	25–34	60	12.37	61	12.57	2	123	26.80
	35–44	30	9.65	32	10.29	1	63	18.33
	>44	7	11.51	6	9.87		13	21.38
	Total	108	10.77	100	9.97	3	211	21.04
Females+ Males	All together	124	9.34	143	10.77	4	271	20.41

TABLE 8
BOLOGNA IVDU COHORT. MORTALITY RATE PER 1,000 PERSONS-YEARS ANALYZED ACCORDING TO GENDER, SUBDIVIDED INTO THREE GROUPS: HIV-INFECTED PATIENTS, HIV-NEGATIVE SUBJECTS, AND SUBJECTS WITH UNKNOWN HIV SEROSTATUS

Gender	HIV-infected patients	HIV-negative subjects	Subjects with unknown HIV serostatus	Total
Males	39.26	18.06	8.47	21.04
Females	32.08	12.42	5.86	18.46
Total	37.00	17.00	7.85	20.41

TABLE 9
BOLOGNA IVDU COHORT. MORTALITY RATE ACCORDING TO HIV SEROSTATIS AND MOST RELEVANT CAUSES OF DEATH. »MISCELLANEOUS« REFERS TO THE LESS FREQUENT CAUSES CONSIDERED AS A WHOLE. HIV-INFECTED SUBJECTS INCLUDE PATIENTS WHOSE HIV SEROSTATUS WAS IDENTIFIED ONLY FROM THEIR DEATH CAUSE CERTIFICATE

Causes of death	HIV-infected subjects		HIV-negative subjects		Subjects with unknown HIV serostatus	
	Deceased subjects	Rate Per 1000 p-ys	Deceased subjects	Rate Per 1000 p-ys	Deceased subjects	Rate Per 1000 p-ys
AIDS	143 (*)	107.7	–	–	–	–
Heroin overdose	20	15.1	26	19.6	14	10.5
Liver cirrhosis	11	8.3	3	2.3	3	2.3
Road accidents	8	6.0	6	4.5	6	4.5
Miscellaneous	12	9.0	11	8.3	8	6.0
Total	194	146.1	46	34.6	31	23.3

(*) The subjects known as HIV-negative and the subjects with unknown HIV serostatus who died because of AIDS are reported in this group, as mentioned in the text.

vided following the four more frequent causes of death; the »miscellaneous« patient group includes the four IVDUs whose death cause is unknown.before 25 years of age, and after the age of 45 (Tables 7 and 8).

Table 9 presents mortality rate analyzed according to HIV serostatus and subdivided following the four more frequent causes of death: the »miscellaneous« patient group includes the four IVDUs whose death disease in unknown. Among the 143 patients deceased because of AIDS, we registered 32 IVDUs who were HIV-negative upon enrollment, and 3 subjects who did not undergo HIV testing, but who became known as HIV-infected when death cause was obtained. Two more HIV-unknown subjects, who resulted HIV-positive at death, died because of liver diseases.

Among IVDUs who deceased because of overdose, 33.3% had HIV infection, 43.3% was HIV-negative (not statistically significant difference), while the HIV serostatus of the remaining 23.3% was not ascertained. At death, the prevalence of HIV infection is significantly higher among females (81.7%) than among males (68.7%; $p<0.001$).Mortality rates per 1000 person-years for HIV--infected, HIV-negative, HIV-unknown patients, stratified according to gender, are shown in Table 8. Among 271 deceased IVDUs, those infected with HIV were 194 (71.6%), including 145 males and 49 females. HIV-negative IVDUs were 46 (16.9%), including 41 males and five females; deceased IVDUs with unknown HIV serostatus were 31 (11.4%), including 25 males and six females. The patients, whose serostatus was negative or unknown, and who resulted HIV infected from death cause, are 37. As already mentioned, 35 of them died because of AIDS; as well two HIV-unknown, proved HIV-positive at death, died because of liver diseases. At the time of death, this last patient group was aged 25 to 34 years in 66% of cases, while the remaining IVDUs of this subgroup were aged 35–44 years. All these patients died between 1991 and 1997.

Discussion and Conclusion

In a 25-year-long observation study, the wide time span of our follow-up allows us to assess the mortality trend along well distinguished periods: the pre-AIDS era, the HIV pandemic era, and the HAART era (with the introduction and consolidation of HAART as the standard of antiretroviral care for HIV disease).

Several Authors point out that, if the availability of potent HAART regimens has improved the prognosis of HIV disease since end-1996 or early 1997, this effect applies only to those IVDU who have undergone serodiagnosis turning out positive to the test, then have accepted antiretroviral treatment, and have followed it with satisfactory adherence levels[17,21–25].

Our data show that an initally slow, but later progressive increase in the number of deaths has taken place since 1984 (the year when the first death of our cohort was registered). After the first AIDS-related death was

reported, in 1986, the increase in the mortality rate of our cohort became increasingly important until 1994, with the last peak observed in the year 1996 (immediately before the introduction of HAART). Since 1997, with the implementation of potent HAART regimens, a dramatic drop of AIDS-associated deaths has been observed in our cohort of IVDUs. In more details, exceptionally high standardized mortality rates (SMR) were reached between 1988–1997, followed by a significant reduction from 1998. A limited increase observed in 2000, was however not confirmed over the following two years. As to the causes of death in our extensive observational cohort, death due to AIDS and drug overdose were remarkably predominant among males, applying to HIV positive, HIV negative and HIV unknowkn alike.

Previous experiences carried out by us[12], proved that overdose-associated deaths in Bologna since 1994 have accounted for a steadily increasing fraction of the overall Emilia-Romagna regional reported cases. Based on all the above-mentioned comprehensive data, we don't think we can read a decrease in the risk of death from heroin overdose in our cohort until 1997, while in Italy some cumulative data have shown a progressive reduction of this cause of death already since 1996[13]. We would like to stress that death in the female component of our cohort is largely related to AIDS-associated causes, all other causes showing a lower frequency among women. For a correct interpretation of these data, it is necessary to consider the higher prevalence of HIV serostatus among women with respect to men, in our cohort.

Since 1998, in our observational study, we witnessed a significant reduction of both AIDS-related deaths (still quite high in 1997), and overdose-caused deaths, which dropped to a half in 1998 compared to 1996 and 1997. Such a notable reduction in frequency could be related to a concurrent increase of substitutive methadone treatment, carried out in most of IVDUs outpatient services of Bologna over the same period of time. In the Emilia-Romagna region, the implementation of the so-called »*damage reduction strategy*« had already significantly contributed to foster a decrease in overdose-related deaths in such cities as Modena or Ferrara, where methadone treatment had been massively introduced among IVDUs since early 90s[15]. Since 1997 the global mortality trend for all non-AIDS-related deaths has paralleled the decrease in AIDS-associated fatalities. A very recent study from Baltimore (USA) longitudinally followed i.v. drug abusers with a concurrent HIV infection since 1998 (when HAART was already available since three years. The authors observed that active drug use was temporally linked to HIV disease progression and overall mortality[17].

Over two thirds of all deceased subjects in our cohort were HIV-infected. Nearly half of all deaths were related to AIDS. Awareness of the infection can be regarded both as a possible concurrent reason for adopting risky behaviour or even for suicide (from overdose to car accidents, suffered or committed aggressions, reported self-damage), and as a cause of increased caution which can lead to approach and trust in outpatient health care services. In our series, the progressively increasing patient's age at enrollment observed throughout the study period, is linked to the ageing of IVDU population who refer to dedicated outpatient services. These data are not related with any evidence that proportionally younger patient population is at modified risk of IVDU.

During the whole analyzed period, the mortality rate for all causes is higher for male HIV-infected IVDUs than for female HIV-infected IVDUs, but women aged 25 to 44 years represented the subgroup at higher risk of AIDS-related death, as confirmed by a very recent study from Canada[24]. When excluding AIDS, all diseases involved as a cause of death in our cohort were predominantly present in the male component of our patients group, notably in the age interval ranging from 25 to 44 years. In more details the highest mortality risk applies to the 25–34 age group, while it is at its lowest before 25, and after 45 years of age.

Should HAART maintain its remarkable effectiveness in future years, a further increase of life expectancy of HIV-infected subjects could be foreseen[17,24–26]. This favorable condition may induce the IVDU population to refer to dedicated outpatient centres, and adhere to both HAART and substitutive pharmacological strategies aimed at »drug damage containment«, and may therefore benefit from a further reduction of overdose-related deaths. On the other hand, we cannot exclude that a progressively lowering HIV morbidity may allow a long-term increase of incidence of chronic concurrent diseases commonly affecting the same IVDU population, as causes of mortality (i.e. decompensated liver cirrhosis, cancer)[6]. Moreover, we also cannot exclude that part of these patients will continue and/or resume the use of drugs while undergoing treatment for HIV disease, hence remaining prone to incur in lethal overdose episodes.

In conclusion, we would strongly recommend repeated HIV serology for all IVDUs and persons adopting HIV-risky sexual practices, who have undergone previous HIV tests with negative results. This is meant to avoid that patients regarded as HIV negative, who then got infected while carrying on their risky practices through the exchange of syringes or through sexual contacts, may end up with their infection not being diagnosed until their HIV infection progresses into advanced clinical signs and symptoms, related to a more severe immunological and neurological deterioration, more unlikely to be treated successfully. The favorable perspective offered by the large-scale availability of multiple HAART combinations for HIV-infected IVDUs makes it absolutely necessary nowadays to apply a more strict and effective epidemiological monitoring of the population at-risk, especially of IVDUs[27]. It is in fact worth reminding that in Italy there is wide recorded evidence of general lack of awareness of the HIV serostatus of current and past sexual partners in many patients who have been later tested and resulted positive for HIV and/or AIDS.

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SMANJENJE SMRTNOSTI U SKUPINI OVISNIKA O DROGAMA NA PODRUČJU GRADA BOLONJE, ITALIJA

SAŽETAK

Cilj ovog istraživanja je ocijeniti trenutni trend smrtnosti u skupini intravenoznih ovisnika o drogama (eng. intra venous drug users, IVDU) praćenih u gradu sjeverne Italije, Bolonji i procijeniti povezanost s HIV infekcijama i AIDS-om, te dostupnost učinkovite antiretroviralne terapije. 1214 IVDUa (uglavnom ovisnika o heroinu), 916 muškaraca i 298 žena, koji su dolazili u ambulante radi liječenja i prevencije ovisnosti u razdoblju od 1977. godine i studenog 1996. godine, ili su uključeni u našu promatranu skupinu te je njihov vitalni status bio proučavan sve do 31. 12 2002. godine. Velika većina ispitanika uključenih u istraživanje bili su rođeni na području grada Bolonje i njegovoj okolici, imigranti izvan područja Europe nisi bili uključeni. Tijekom perioda promatranja 271 IVDU (22.3%) je umro, 211 muškaraca (23.0%) i 60 žena (20.1%). Niti jedan smrtni slučaj nije zabilježen prije 1984. godine. Glavni uzroci smrti su slijedeći: AIDS (52.8% slučajeva), predoziranje heroinom (22.1%), ulične nesreće (7.4%), ciroza jetre (6.3%), i samoubojstvo (2.9%). Najveći sveukupni broj smrtnih slučajeva primijećen je između 1991. i 1996. godine. Gruba stopa smrtnosti uzrokovana AIDS-om bila je 10.0 na1000 za muškarce i 13.2 na 1000 za žene, a utvrđena stopa smrtnosti iz drugih razloga 11.1/1000 među muškarcima i 5.2/1000 među ženama. Posljednjih je godina zabilježen nagli pad u broju smrtnih slučajeva povezanih s AIDS-om kod IVDUa, što se pripisuje učinkovitim antiretroviralnim režimima, iako je sveukupna stopa smrtnosti i dalje ostala značajna.

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Original scientific paper

Do We Need the »Adolescent Crisis« Diagnosis?

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A B S T R A C T

The aim of the study was to examine patients in adolescent crisis at the beginning of treatment and after a period of 12 months in order to evaluate the relative diagnostic and therapeutic validity. The study included 153 Split University students in adolescent crisis; 90 of them were treated by counseling and 63 served as controls. For diagnosis, Hampstead index and Diagnostic and Statistical Manual of Mental Disorders Fourth Edition (DSM-IV) multiaxial evaluation were used, allowing a wider insight into personal functioning. The study sample was split in 7 significantly different diagnostic subgroups. The counseling-treated examinees had better personality functioning after 12 months, but did not differ significantly from the control group. Some of their single functions were more severely disturbed at the very beginning. Counseling is a valuable therapeutic and diagnostic tool for adolescent crisis. The assessment must evaluate the entire person, because looking at only one aspect, due to different development and its place, a wrong conclusion may be reached. The »adolescent's crisis« entity is clinically relevant.

Key words: *students, adolescent crisis, counseling*

Introduction

The term »adolescent crisis« does not exist in the International classification of diseases and related health problems (ICD-10)[1] nor in Diagnostic and statistical manual of mental disorders (DSM-IV)[2], but is widely used in clinical practice and in the literature[3–7]. Adolescent crisis is a developmental storm, which blows through adolescent's emotional and existential space[3]. Even with no deeper emotional disturbance, developmental crisis animates that something has to be balanced, held or treated in the adolescent[4–6]. In this developmental period every aspect of self- representation is questioned and an effort in achieving physical, instinctive, sexual, emotional and intellectual development is present[4,5,8]. The tasks of adolescence, such as the choice of a partner, of profession and of individual life style[9], in some adolescents trigger emotional storming and some of them experience real depressive breakdown[10–12]. The behavioural manifestations during such a crisis include neurotic elements, psychotic reactions, or antisocial outbursts: the differentiation between an adolescent crisis and real, deep, ominous psychopathology is very difficult, particularly at the beginning. How someone will solve the adolescent tasks depends on her/his prior development and ego abilities, family and social circumstances, and chance events[7,13]. The feelings of alienation, anxiety, and depression accompany that period and adolescent defends her/himself by intellectualism, rationalization, asceticism, and refusal of compromise, trying to find the balance between unacceptable instinct pressures and superego demands.

The personality development takes place in social environment by continued and discontinued advancements of maturity, experience, and their interactions. Indirect connections between maturity and experience lead to complex manifestations evoked by interactions of a person and her/his environment[14]. Understanding of abnormal adolescent functioning stems from knowledge of »normality« in the culture to which a particular adolescent belongs. Normal adolescents in various cultures differ in experience of self-representations; in many cultures girls show poorer self representation than boys[15].

The most demanding diagnostic problem in the work with such adolescents is to establish whether the present phenomenon is a transitional disorder in development,

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in solving the new relations toward parents and own sexuality, a permanent disorder, or stagnation in development with the possibility for nascent psychopathology. So, it is necessary to assess ego ability in solving inner demands.

The crisis in adolescence is a »break point« during which the adolescent comes to senses of him/herself as a complete person with own conscience and responsibility, and relations towards his/her partner and environment. Such course leads to »normal« adult, but various difficulties in development, mental or physical illnesses, social circumstances and chance events can disturb it and lead to pathologic formations or functions, or opposite, my also be positive in development of the adolescent, at this stage of life.

The aim of this study was to analyze undergraduate students in adolescent crisis at the beginning, after 1 month, and after 12 months of treatment, to evaluate the diagnosis and to assess the treatment results. Our hypothesis was that adequate diagnosis cannot be set at the very beginning, because the crisis phenomena cover and interact with the personality structure. We supposed that a presumptive, working diagnosis during the crisis is sufficient enough, and after its resolution a definitive diagnosis can be established.

Patients and Methods

In the period of late adolescence, 18–22 years, 153 Split University undergraduates in adolescent crisis, were diagnosed, included, and treated in the Student′s Outpatient Clinic in Split, Croatia, between January 1992 and December 1997. Ninety out of them, 50 female and 40 male students were randomly allocated to a counseling program, while the remaining 63 (42 women and 21 men) were allocated to a control group, having only 1–2 initial consultations, later being just followed-up and revaluated after 12 months in order to control the effects of counseling, the self-healing phenomenon in transitory disturbances, and persisting maladjustments in untreated vs. treated subjects. The patients were 18 to 21 years old; the mean age in the intervention group was 20.1±0.94 years (20.1±1.02 women and 20.1±0.84 men), and 19.9±0.86 for female and 20.1±0.91 years for male examinees in the control group, whose mean age was 20.0±0.86.

The events during the 12 months of study were classified using Hampstead index[16] and Multiaxial assessment in DSM-IV[2]. The Hampstead index discloses the reason for help seeking, the adolescent′s aspect, personal and family history, possibly important environmental impact, and the development of instincts (libido and aggression), ego (ego functions, defenses, identifications, partnership, affections) and superego, general outlines of regression and fixations, dynamic and structural aberrations: conflicts (inside /ego-superego/, outside /from surroundings/ and ambivalence), and some general characteristics, such as anticipation of the future, relation towards achievements, self introspection, verbalization, and diagnosis. In late adolescence, the adolescents with anxiety, the anxieties and worries often concern the quality of their performance or competence at university, partner relations, or sporting events, even when their performance is not being evaluated by others. They are typically overzealous in seeking approval and require excessive reassurance about their performance and their worries. The anxiety is often seen as somatoform disorder: gastrointestinal, pseudo neurological, sexual or cardiovascular. Depression in late adolescence, more common in women[17], leads to decrements in social and academic performance. Usual mood is dominated by dejection, gloominess and unhappiness. Self-concept centers on beliefs of inadequacy, worthlessness and low self-esteem. Adolescent crisis was working diagnose for students who were too anxiety and depress, with problems in academic achievements, partner or other social relations. The Multiaxial assessment detects the following: Axis I – clinical disorders; Axis II – personality disorders; Axis III – general medical conditions; Axis IV – psychosocial and environmental problems; Axis V – general assessment on a functional scale.

The counseling intervention was diagnostic and therapeutic at the same time, defined as an interacting process in understanding oneself and ones own environment. The changes in patient are expected in aim which decides alone. During the counseling process, the patient is familiarized with emotional verbalization, defining the essential problem, and after that, through reflection, confrontation, and sometimes interpretation, the person is allowed to look into what helps in problem solving[18,19]. The sessions were held once weekly, lasting 45 minutes, during which the patient achieved introspection, help and support in solving his/her trouble, and the final diagnosis was established with more confidence

Psychomotor and social functioning was estimated at the inception, after one month, and at the end of this study according to a global assessment (GAF) scale, where higher point score denotes better functioning: 100 superior functioning in a wide range of activities, 80 if symptoms are present, they are transient and expectable reactions to psychosocial stressors, 60 moderate symptoms, or moderate difficulty in social, occupational, or school functioning, 40 some impairment in reality testing or communication, 20 some danger of hurting self or others, 0 inadequate information[2].

Statistical significance of the observed differences was assessed using the chi-square test, Stuart-Mexwell test or Pearson χ^2 test, as appropriate, and $p<0.05$ was considered significant[20] using statistical package Statistica 6.0[21].

Results

All subjects either treated or controls, entered the study in a very anxious and depressed state, covering the students' personality. The initial diagnostic groups (after 1 month) among the treated and the control subjects were later (after 12 months) refined in seven subgroups, as

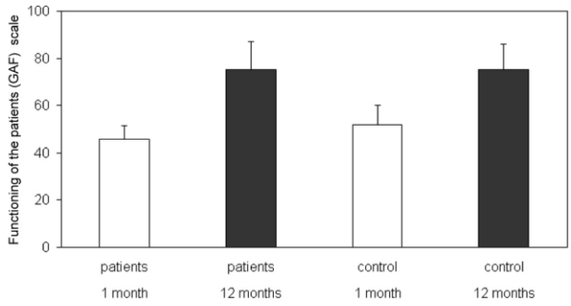

Fig 1. Treated patients and control according personality functioning after 1 and 12 months observation. p>0.5.

shown on Table 1. The differences between the intervention and the observation group were minor; while the observation group had 7 subsets, the intervention group had 6, lacking the 7th one (permanent psychosis, one patient).

In the intervention group, the initial diagnosis was unchanged after one year in 58 patients (64.7%); 23 (25.6%) patients passed from a more severe to a milder diagnostic subset, and 9 (10.0%) patients had the opposite switch. These changes, i.e. amelioration in 23 and worsening in 9 were marginally significant: Stuart-Mexwell test Q=6.26, df2, p=0.05.

As shown on Table 2, in the control group the diagnosis was unchanged in 53 (84.1%) examinees, in 9 (14.3%) it ameliorated, while it worsened in 1 (1.6%). These changes were statistically significant: Stuart-Mexwell test Q=8.00, df2, p=0.05

As can be seen from Figure 1, the examinees from either interventional or observational group, were functioning much better after 12 months. Among the treated patients the maximal achievement was 96 points, similar to the controls (89 points). At the end of the study 63 (70.0%) subjects from the intervention group, and 40 (63.5%) from the observation group were functioning well. Moderate functional problems had 24 (26.7%) patients from the treatment group, and 22 (34.9%) from the control group, while severe problems had 3 (3.3%) and 1 (1.6%) patient, respectively. According to personality functioning, treated patients did not differ significantly from those in the control group (χ^2=0.448, df1, p>0.5).

Neither at the beginning nor at the end of this study, the diagnostic spectrum differed significantly between the interventional and the observational group (χ^2=0.105, p=0.079).

Sorting our intervention patients according to diagnostic fluctuation over time, 6 subgroups may be formed, as shown on Table 1. Summing up subgroup 1 (n=33, 36.7%) and subgroup 4 (n=25, 27.7%), there were 58 (64.7%) patients that did not change the diagnostic class in the analyzed period (12 months). However, subgroups 2 (n= 6, 6.7%) and 5 (n= 3, 3.3%), totaling 9 (10.0%) patients, had milder diagnosis at the beginning than at the end of the study. Conversely, our subgroups 3 (n=17, 18.9%) and 6 (n=6, 6.7%), totaling 23 (25.6%) patients had more favorable diagnosis at the end.

The control group patients could be subdivided again in 6 subsets (group 2 was not represented, and group 7 was introduced). Among these individuals the diagnosis did not change in 53 or 84.1% (sum of group 1 with 38,

TABLE 1
INTERVENTION (TREATMENT) GROUP DIAGNOSIS AFTER 1 AND AFTER 12 MONTHS (TREATMENT)

Diagnosis after 1 month	Diagnosis at 12 months						Total	
	»Variation of normal«		Constant regression		Hindered psychosexual development			
»Variation of normal«	33	(36.7%)	6	(6.7%)			39	(43.3%)
Constant regression	17	(18.9%)	25	(27.7%)	3	(3.3%)	45	(50.0%)
Hindered psychosexual development			6	(6.7%)			6	(6.7%)
Total	50	(55.6%)	37	(41.1%)	3	(3.3%)	90	(100.0%)

TABLE 2
CONTROL (OBSERVATION) GROUP DIAGNOSES AFTER 1 AND AFTER 12 MONTHS

Diagnosis after 1 month	Diagnosis at 12 months						Total	
	»Variation of normal«		Constant regression		Hindered psychosexual development			
»Variation of normal«	38	(60.3%)	0	(0%)			38	(60.3%)
Constant regression	8	(12.7%)	14	(22.2%)	1	(1.6%)	23	(36.5%)
Hindered psychosexual development			1	(1.6%)	1	(1.6%)	2	(3.2%)
Total	46	(73.0%)	15	(23.8%)	2	(3.2%)	63	(100.0%)

group 4 with 14, and group 7 with one participant). After one year of follow-up 9 examinees (14.3%) passed to a less severe diagnostic level (8 from group 3, 1 from group 6), and 1 (1.6%) from group 5 passed to a worse level.

Adolescent crisis evolutionary subgroups after the 12 months' follow-up period were formed as follows:

- Subgroup 1 (G1, C1) – patients who after 1 and after 12 months had the same diagnosis of adolescent crisis
- Subgroup 2 (G2, C2) – patients with initial diagnosis of adolescent crisis, and after 12 months were reclassified as personality disorder
- Subgroup 3 (G3, C3) – patients who after 1 month were diagnosed as personality disorder, and after 12 months reclassified as adolescent crisis.
- Subgroup 4 (G4, C4) – patients who after 1 and after 12 months had the same diagnosis of personality disorder.
- Subgroup 5 (G5, C5) – patients who initially had the diagnosis of personality disorder, resulting in frank psychosis after 12 months.
- Subgroup 6 (G6, C6) – patients who after 1 month were classified as psychosis, and after 12 months a
- personality disorder was recognized
- Subgroup 7 (G7, C7) – patients classified as psychotic all the time

Discussion

The treated and the control patients differed significantly in terms of diagnostic subgroups, but the diagnoses at the beginning and at the end of the study period did not differ significantly between the arms. The variegated clinical presentations of adolescent crisis, changing with developmental alterations, resulting either from serious pathology or from developmental difficulties (which, because of ego weakness, induce regression mimicking deep pathology), makes the diagnosis of adolescent′s crisis a practical clinical necessity.

Authors[7,22–24] have reported similar data; these authors consider such results important for psychodynamic theory of adolescence, illustrating a major diagnostic problem in these persons and highlighting all the difficulties in the field: initially it is often impossible to distinguish serious pathology from mild crisis[25–28]. Like studies[29–32] suggest that complex personal adaptation processes with developmental changes have to be carefully examined through the patient′s main symptoms and environmental challenges in order to get appropriate insight into the pathogenic mechanisms. The time spent to get acquainted with the patient′s personality and assiduous attending thereafter, during the crisis, is invaluable in the diagnostic-supportive-therapeutic process.

Our patients′ functioning in adolescent crisis had a legion of starting problems but improved quite a lot over time, both in the intervention and in the control arm. The crisis phenomenology produced such difficulties in all the examinees, supporting the introduction of diagnostic subgroups. The adolescent′s crisis is often a self-healing process. However, the counseled examinees showed a marginally better personality functioning. These results could not be compared, because no similar investigation was found. It would be good to plan such investigations on national, and probably on international adolescent population.

The development of programs bridging academic and scientific communication between mental health professionals and educators in making the best possible knowledge transmission about psychosocial and behavioral problems, and offering the mental health assistance to students who need it is highly recommended[33]. High school mental health consultants, as educational team members, offer the best assistance to students in such a need [33–35], and personal interaction/conversation is invaluable[7,22,36–38]. This study shows therapeutic and diagnostic difficulties in psychological procedures with adolescents, what is showed by assessments of diagnostic groups, and investigation, of all personality aspects of adolescents is original.

We conclude that counseling is an effective adjuvant therapeutic approach to adolescent's crisis. Because of the crisis phenomenology it is a diagnostic tool as well, helping in selection of patients who need it most and showing the path to further, complementary or different treatment. In adolescent's assessment it is necessary to view the whole person: looking at only one aspect, and due to different development and its velocity, a wrong conclusion may be reached. More than one diagnostic assessment is a must and the diagnosis of adolescent crisis is mandatory for good clinical practice.

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TREBA LI NAM DIJAGNOZA »ADOLESCENTNA KRIZA«?

S A Ž E T A K

Cilj rada bio je pratiti pacijente u adolescentnoj krizi na početku liječenja i nakon 12 mjeseci, kako bi se procijenila dijagnostička i terapijska postignuća. U studiju su uključena 153 studenta u adolescentnoj krizi, koji studiraju na Sveučilištu u Splitu. 90 od njih liječeno je savjetovanjem, a 63 studenta su bili kontrolna grupa. Pri dijagnostici poremećaja rabili smo Hampstead index i Multiaksijalnu procjenu po Dijagnostičkom i Statističkom Priručniku za Mentalne Poremećaje – četvrta revizija (DSM-IV), što je omogućilo širi uvid u unutarnje funkcioniranje osobe. Ispitivana grupa podijeljena je u 7 dijagnostičkih podgrupa, koje su se statistički značajno međusobno razlikovale. Ispitanici liječeni savjetovanjem, pokazivali su bolje funkcioniranje osobnosti nakon 12 mjeseci liječenja, premda razlika prema kontrolnoj grupi nije statistički značajna. U pojedinim funkcijama osobnosti, liječeni pacijenti na početku liječenja, imali su teže poremećaje. Savjetovanje je dobra terapijska i dijagnostička metoda u liječenju adolescentne krize. Pri procjeni, potrebno je obuhvatiti čitavu osobu, budući da promatranje pojedinih parametara, zbog različitog razvoja i brzine tijeka, može zavesti na pogrešno zaključivanje. Dijagnoza adolescentna kriza je klinički važna.

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Original scientific paper

Structure of Visits Persons with Diabetes in Croatian Family Practice – Analysis of Reasons for Encounter and Treatment Procedures using the ICPC-2

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A B S T R A C T

The reasons for encounter and the procedures conducted during the visit persons with diabetes to family practice have been investigated. Five family practitioners located in two Croatian counties took part in this study. In this study patients with diagnoses E10–E14 according to International Classification Disease – 10 (ICD-10), were involved. There were 543 persons with diabetes (women 324) in the total population of 10,150 patients Data were registered according to the International Classification Primary Care-2 (ICPC-2) (components 1–7 for reasons of encounter, and components 2–6 for procedures during the visit), in period october till december 2005. 871 visits of persons with diabetes (average age 65.7±12.5 were registered. Patients presented in total 1921 reasons for encounter or 2.1±1.1 per visit. Family practitioner made in total 2,341 procedures or 2.6±1.5 procedures per visit. 85.0% of patients had 1 to 3 reasons for encounter, 78.4% of patients had 1 to 3 procedures per visit. 64.4% of patients with diabetes presented at least one reason for encounter connected to diabetes. The most common reasons for encounter were prescriptions of medication 46.4 per 100 reasons for encounter,the second was dignostic procedure 19.9, request for analysis of findings 11.1, symptoms complaints 11, request for referrals to diagnostic procedures or specialist consultation 8.9 and administrative reuqests 1.6 per 100 reasons for encounter. Family practitioner performed procedure prescriptions of medication 47 per 100 procedures. The second was dignostic procedure 32.8 per 100 procedures, referrals to diagnostic procedures or specialist consultation 14.7 and administrative procedures 1.7 per 100 procedures. From the total number of 100 referrals to specialist, 23 were to diabetologist, 15 to ophtalmologist, 13 to cardiologist. The largest proportion of procedure belong to diabetics 33.8%, followed by the circulatory system 25.4%, musculosceletal 6.9%, symptoms 5.1%, respiratory 4.5%. The reasons for encounter and the procedures conducted during the visit have direct influence to the quality of care for persons with diabetes. It is necessery collecting the data and research in the field of reasons for encounter and procedures during the visit of person with diabetes. The results then can be compared to the results already found in literature.

Key words: *visits, persons with diabetes, family practice, Croatia*

Introduction

Diabetes is a prototype of chronic disease that imposes a large public health burden[1].

New perceptions about the role of family practitioner in managing chronic patients came along. Care of person with diabetes is being transferred from hospital and specialistic care to family practice[2,3]. A person suffering from diabetes being a chronic patient is presented in a complex comorbidity form[4–6].

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Changes in therapeutic approaches in diabetic patients have been noticed during the last decade. The number of their visits to family practitioner and diabetologist is constant while the number of their consultations is increasing because of diabetic complications. Duration of visits of diabetic patients in family medicine is becoming longer[5]. Management of chronic patient, persons with diabetes, in family medicine is an indicator of quality care in family medicine. Indicators for monitoring the quality of care were arranged, mostly connected with the procedures conducted by the fixed guidelines and the procedures' results[7,8]. There were not many studies investigating the type and amount of problems that general practitioner was facing during the patients' visits and reasons for their encounter. The reasons for encounter and the procedures conducted during the visit have direct influence to the quality of care, application of curing guidelines, patient's education, support in the management of the disease, record management, research[4,9]. It is well known that family practitioner is solving most of the problems during the visit of person with diabetes in comparison with all other chronic patients. Family practioner, as a part of the treatment of persons with diabetes, conducts the biggest proportion of prescriptions and referrals[3,9].

The new role of the family practitioner in the management of the chronic diabetic patient raised the number of concerns whether the visit of diabetic patient is »a traditional visit» of a chronic patient and how much time is there for preventive procedures concerning complications, early detection of other diseases, education, for patient support, and for the further studies as well[4,10–12].

Such analyses were not conducted in Croatia so far, within it's transitional health care system.

It is a complex task to code the verbal content of the patients' requests along with practioner's care description. The structure of International Classification Primary Care-2 (ICPC-2) has the possibility of transcription and coding the reasons for encounter and procedures conducted on the patient during the visit[13,14].

The objective of this study is a prospective analysis of reasons for encounter and the procedures conducted during the persons with diabetes visits to family practitioner in Croatia using ICPC-2.

Subjects and methods

Study population

Five family practitioners located in two Croatian counties (Zagreb county and Brodsko-posavska county) took part in this study. This investigation involved patients with diagnoses, E10–E14 according to International Classification Disease – 10 (ICD-10) out of the total population in care of those practices. The data were collected prospectively for each patient with diabetes for every visit during the follow-up period from October till December 2005. Visits of person with diabetes in Croatia to family practitioners are unlimitted and are defined as meetings of patients and practitioners in practice. This study analysed visits during the regular practices working day.

Instrument for measures

Data were registered according to the ICPC-2 components (components 1–7 for reasons of encounter, and components 2–6 for procedures during the visit). Reasons for encounter of person with diabetes were registered according to the statements and requests of the patients. Procedures done during the visits were registered independently by each other member of the team (nurse): diagnostic procedures, therapeutic and prescribed medications, preventive procedures, referrals to diagnostic procedures outside the practice, referrals to specialists by diagnoses, administrative procedures. Data were registered in electronic form other than the regular practice program.

Statistical analysis.

Data of reasons for encounter, procedures during the visits were analysed by descriptive statistical analysis.

Results

There were 543 persons with diabetes (women 324) in the total population of 10,150 patients. During the three month follow up period 871 visits of persons with diabetes were registered in range of 1–11 visits, average 4.2± 12.5 visits per day. Average age of diabetic patients who visited family practitioner in the follow-up period was 65.7±12.5). Women did made 583 visits or 66.9% of all visits (Table 1).

Persons with diabetes presented in total 1921 reasons for encounter or 2.1±1.1 per visit. Family practitioner made in total 2341 procedures or 2.6±1.5 procedures per visit according to ICPC-2. 85.0% of patients had 1 to 3 reasons for encounter, and 78.4% of patients had 1 to 3 procedures per visit. 64.4% of patients with diabetes presented at least one reason for encounter connected to diabetes.

The most common reasons for encounter of person with diabetes were prescriptions of medication or other therapies 46.4 per 100 reasons for encounter. The second was dignostic procedure and prevention 19.9 per 100 reasons for encounter, request for analysis of findings 11.1, symptoms complaints 11, request for referrals to diagnostic procedures or specialist consultation 8.9 and administrative reuqests 1.6 per 100 reasons for encounter.

Family practitioner performed procedure prescriptions of medication or other therapies 47 per 100 procedures. The second was dignostic procedure and prevention 32.8 per 100 procedures, referrals to diagnostic procedures or specialist consultation 14.7 and administrative procedures 1.7 per 100 procedures performed during visits of persons with diabetes (Table 1).

The largest proportion of procedure belong to endocrine metabolic (diabetics) 33.8%, followed by the circu-

latory system 25.4%, musculosceletal 6.9%, symptoms 5.1%, respiratory 4.5% (Table 2).

Diabetic patients mentioned component 6 as a reason for the encounter in 171 cases. The physician made 357 procedures included in the componet 6. 197 referrals for consultation to specialists were given to the patients. In total, the number of 100 referrals, 23 were to diabetologist, 15 to ophtalmologist, 13 to cardiologist.

According to morbidity reasons for all referal the diseases were ranked as follows: metabolic diseases, cardiovascular diseases, muskulosceletal,eye diseases,urinary diseases (Table 3).

TABLE 1
REASONS FOR ENCOUNTER AND PROCEDURES OF PERSONS WITH DIABETES BY ICPC-2 CHAPTERS AND COMPONENTS

All visits N = 871	Reasons for encounter	Procedures	Per 100 visits Per 100 reason/procedure
Total number of reasons/procedures	1921	2431	
Reasons/procedures per visit (X±SD)	2.1±1.1	2.6±1.5	
Visits with 1 to3 reasons/procedures	740	683	*85 / 78.4
Visits with 4 and > reasons/procedures	131	188	*15 / 21.6
Visits with diabetes as reasons/procedures	561	598	*64.4 / 68.8
Symptoms, complaints	212		**11
Diagnostic, screening,prevention	384	799	**19.9 / 32.8
Treatment,procedures, medication	892	1143	**46.4 / 47
Test results	214		**11.1
Administrative	30	42	**1.6 / 1.7
Referal and other	171	357	**8.9 /14.7

N – number of visits, * per 100 visits, ** per 100 reason/procedure

TABLE 2
PROCEDURES AND DISTRIBUTION OF CHRONIC CONDITIONS OF PERSONS WITH DIABETES BY ICPC-2 CHAPTERS AND COMPONENTS

Components	A	B	D	F	H	K	L	N	P	R	S	T	U	W	X	Y	Z	
Symptoms, complaints																		
Diagnostic, screening prevention	112	1	13	6	10	242	31	16	3	53	20	214	17		7	5	49	799
Treatmant, procedures, medication	7	2	25	26	12	304	88	14	78	36	50	462	25		6	8		1,143
Test results																		
Administrative	1		2	3		1	2	3				21	2		2		5	42
Other		1	17	37	10	48	41	20	9	16	12	96	30		12	8		357
Diagnoses,disease																		
Total number of procedures	120	4	57	72	32	595	162	53	90	105	82	793	74		27	21	54	2,341

A – general, B – blood, D – digestive, F – eye, H – ear, K – circulatory, L – musculosceletal, N – neurological, P – psychological, R – respiratory, S – skin, T – metabolic, endocrine, U – urinary, W – pregnancy, family planning, X – female genital, Y – male genital, Z – social

TABLE 3
ANALYSIS OF COMPONENT 6 OF ICPC-2. ANALYSIS REFERRALS TO DIAGNOSTIC PROCEDURES OR SPECIALIST CONSULTATION

	A	B	D	F	H	K	L	N	P	R	S	T	U	W	X	Y	Z	
*67		1	9	30	9	25	18	13	9	10	10	46	8		4	5		197
**68			8	7	1	23	19	5		6	2	50	21		8	3		153
***66							4	2										6
69													1					1
	0	1	17	37	10	48	41	20	9	16	12	96	30	0	12	8		357

* – referral to consultant, ** – other referral (laboratory findings, x-ray, ultrasound, physiotherapy), *** – home care, help at home, dietetitian, A – general, B – blood, D – digestive, F – eye, H – ear, K – circulatory, L – musculosceletal, N – neurological, P – psychological, R – respiratory, S – skin, T – metabolic, endocrine, U – urinary, W – pregnancy, family planning, X – female genital, Y – male genital, Z – social

Discussion

According to our study there were 4 to 5 visits per day to the family practitioner by persons with diabetes.

Diabetic patient presented 2.1 reason for encounter per visit according to components I–VII, ICPC – 2. Family practitioner made 2.6 procedures according to components II–VI, ICPC-2.

By using other methods, there were 2.5 problems solved during the visit of person with diabetes in comparison to 2.1 problem of other chronic patients without diabetes[9]. The »defansive« relation between a number of reasons for encounter and performed procedures was seen in our study. The analysis of reasons for encounter to family practitioner showed that it is a complex item. Patients perception of reasons for encounter is multifactorial, frequently considering biological, social, cultural, as well as psychological influences. There is a difference in perception of patients and practitioners in content of procedures during the visit. Patients usually expected less procedures than the practitioner performed[10–12]. Chronic patients does not talk about his expectations but he trusts the physicians who is treating him for a long time[15].

Patient requested 1921 procedures in comparison to 2341 that were actually performed. This study didn't investigate whether all the requested procedures were really performed. The factor related to health care system could have the influence to the number of reasons for encounter and procedures conducted in this investigation[16]. There is a big proportion of frequent attenders in Croatia and it is well known that chronic patients make 50% of frequent attenders[17]. Chronic patient with diabetes is frequent visitor in family practice in Croatia in comparison to other European countries[18].

Prescription is the most common reason for the encounter in our study. Literature shows that reason for encounter of chronic patients who are older than 65 are mostly request for prescription. Chronic patients and their physician mostly agreed on the prescription[11,12]. In the last decade the biggest changes happened especially in therapeutic approach to person with diabetes. The number of persons with diabetes that use 5 medications and more is raising. Studies shows the constant number of prescribed antidiabetic drugs, but the number of prescribed antihypertensive and hypolypemic drugs is raising[4,19]. It influenced the reasons for encounter and content of procedures during the visit.

There are dillemas in some studies which find »danger» in the fact that education, support and early detection of other diseases can suffer because of the prescription especially because of the time limit of the visit. The other opinion is in the fact that new prescription needs individual coordination and adaptation because it motivates the patient for compliance and strengthen the trust between the physician and the patient. New investigations need to be targeted in that direction[3–5,15].

According to literature and this study physician is oriented more towards diagnostic and therapeutic procedures and results control than towards the patient's requests. On the other hand, patients are expecting more explanations and advices about their illness, ways of its curing, possibilities of care and future health[10–12]. This does not exclude one another but on the contrary the evaluation of the patient's condition gives the basis for further recommendations.

In the last decade there is a constant number of diagnostic procedures performed during the person with diabetes visit such as blood pressure measurement, blood sugar measurement, lipids, body weight[4].

The limitation factor for our results was that we didn't know if diagnostic procedures were connected with unexplained symptoms of the new episode of the disease or with the acute exacerbation of chronic disease. There were 212 symptoms and complains recorded according to ICPC 2 or 11.0% reason for encounter. The onset of the new episode of the disease, should be separated from the reasons for the encounter and procedures.

197 referrals were issued for specialist consultations. From the total number of 100 referrals, 23 were for diabetologist, 15 for ophtalmologist, 13 for cardiologist. The literature showed the constant number of visits to diabetologist and increase in the number of visits to specialists – consultants for diabetes complications in the last decade[4].

Patient with diabetes in our study is presented in a more complex comorbidity form. Most frequent comorbidity diseases in the seleced group of patients with diabetes were cardiovascular diseases and locomotor diseases. Nowadays the most dynamic changes in the management of chronic diseases especially in their therapy are happening in diabetes and cardiovascular diseases[4,5,19].

Conclusion

Is the person with diabetes visit in Croatia a traditional visit of a chronic patient older than 65 to family physician? During one visit of such patient the physician is in fact managing several chronic diseases. He prescribes several different drugs and gives referrals to various consultants. The largest part among those procedures is prescription and other therapeutic procedures followed by diagnostic procedures in practice and referrals where physician takes the active part. Every visit of diabetic patient should be elaborated with its duration. This study was performed on the the selected group of chronic patients. Data should be compared with chronic patients in total population in the care of Croatian health care system, Croatia being a transitional country[14].

The results of our study points to the necessity of collecting the data and research in the field of reasons for encounter and procedures during the visit of person with diabetes. The results then can be compared to the results already found in literature. The results should be translated into mechanisms of treating person with diabetes their visits to achive the best outcome.

Educational programes for family medicine should be targeted toward chronic comorbid patient rather than to a solitary disease.

Limitation Factors

The most serious limitation factor is dependance on physicians self-report. The physicians were aware of the study hypothesis and its goal and they could have aggerated or exaggerated the number of problem seen at each encounter. Collection of data according to ICPC-2 is not a routine collection of data in everiday family practice in Croatia. The other limitaton factor is a period when data were collected and seasonal respiratory symptoms could influence the results especially to the reasons for encounter.

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STRUKTURA POSJETA OSOBA OBOLJELIH OD ŠEĆERNE BOLESTI U OBITELJSKOJ MEDICINI U HRVATSKOJ. ANALIZA RAZLOGA DOLASKA I POSTUPAKA TIJEKOM POSJETA UPORABOM ICPC-2

SAŽETAK

Istražili smo razloge dolaska osoba oboljelih od šećerne bolesti i postupke tijekom posjeta obiteljskom liječniku. U studiji je sudjelovalo 5 obiteljskih liječnika iz 2 županije u Hrvatskoj. Prema Međunarodnoj klasifikaciji bolesti -10 (MKB-10), izdvojeni su pacijenti s E10–E14: 543 pacijenta (324 žene) od 10 150 ukupno opredijeljenih pacijenata u skrbi obiteljskog liječnika. Podatci su bilježeni prema International Classification Primary Care-2 (ICPC-2), komponente 1–7 za razloge dolaska, a komponente 2–6 za postupke tijekom posjeta. U razdoblju listopad-prosinac 2005. Zabilježen je 871 posjet bolesnika oboljelih od šećerne bolesti prosječne dobi 65.7±12.5. Pacijenti su iznijeli 1,921 razlog dolaska ili 2.1±1.1 razloga po posjetu. Obiteljski liječnik je učinio 2,341 postupak ili 2.6±1.5 postupaka po posjetu. 85.0% pacijenata iznijeli su od 1 do 3 razloga dolaska. 78.4% pacijenata imali su od 1 do 3 postupka u posjetu. 64.4% bolesnika oboljelih od dijabetesa prezentirali su najmanje 1 razlog dolaska vezan uz dijabetes. Najčešći razlog za dolazak bilo je propisivanje lijekova ili druge terapije u ordinaciji, i to 46.4 na 100 razloga dolaska. Na drugom mjestu s 19.9 su dijagnostički postupci. Ostali razlozi su: nalazi na uvid 11.1; neobjašnjeni simptomi i tegobe 11; traženje uputnica za dijagnostiku i specijalističke konzultacije 8.9; administrativni postupci 1.6 na 100 razloga dolaska. Obiteljski liječnik je propisao lijekova ili druge terapije u ordinaciji 47 na ukupno 100 postupaka. Na drugom mjestu s 32.8 su dijagnostički postupci u ordinaciji. Potom slijede: slanje na dijagnostiku i konzultacije specijalistima 14.7; administrativni postupci 1.7 na 100 postupaka u ordinaciji. Od 100 slanja specijalistima 23 su dijabetologu, 15 očnom, 13 kardiologu. Najveći udio postupaka odnosi se na za dijabetes, i to 33.8%, zatim kardiovaskularne bolesti 25.4%, lokomotorne 6.9%, neobjašnjene simptome i tegobe 5.1%, za respiratorne bolesti 4.5% postupaka. Razlozi dolaska i postupci tijekom posjeta obiteljskom liječniku imaju izravan utjecaj na kvalitetu zaštite osoba oboljelih od šećerne bolesti. Potrebno je stoga podatke bilježiti, istraživati i uspoređivati s podatcima iz literature.

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Original scientific paper

Cerebrovascular Insult Hospital Cases in the Clinical Hospital Mostar (Bosnia and Herzegovina) From 1999 to 2003 – An Example of an Institutional Register

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A B S T R A C T

The analysis of a cerebro-vascular insult hospitalized cases in the Clinical Hospital Mostar as a retrospective epidemiological study was done in the Clinical Hospital Mostar for the period from 1999 to 2003. The major source of data was medical documentation of this hospital (an institutional register), the only hospital for the treatments of 457,491 inhabitants who gravitate by a health insurance for the treatment in this hospital. The study included a total of 1,555 cerebro-vascular insult cases treated in the Clinical Hospital Mostar. Among them 727 (46.8%) were male patients, while 828 (53.2%) cases were female. The majority of the cases were above 50 years of life. Majority of treated female patients were older than 61 (45.6% of all cases), as well as among male patients (31.3%). The least number of cases was under 41 years in both groups (1.2%). Prevalence of risk factors was 2,035 cases (74%). During the same period risk factors research for entire Federation of Bosnia and Herzegovina (FBiH) was performed on the sample of 2,750 national insurance holders, out of which 852 gravitate for treatment in CB Mostar. Out of them 1.7% was found to suffer of cerebro vascular insult.

Key words: *cerebro-vascular insult, hospital morbidity and lethality, Clinical Hospital Mostar*

Introduction

Epidemiological studies worldwide showed that the incidence of cerebro-vascular insult cases (brain insult) ranges from 300 to 500 cases per 100,000. The incidence is higher among male than female population, and it is doubling with each decade after 55 years of life[1]. Cerebro-vascular (brain) insult is defined as a sudden focal neurological deficit, caused by a cerebro-vascular disease lasting more than 24 hours. Cerebro-vascular insult in general is divided as ischemic and hemorrhagic (bleeding brain insult). Ischemic insult is more often, while around 10% of all brain insults are manifested as brain bleeding[2].

Together with cardiac and malignant diseases, cerebro-vascular diseases are the most common mortality and invalidity cause of modern humans. Each year about 5.5 million of people all around the Globe suffer from the brain insult. Because of that fact, this disease is the leading cause of disability[3–5]. Complications occur among 25–40% of the cases; the most common are pneumonia, hearth decomposition, and pulmonary embolia[6]. Brain insult occurs because of brain blood circulation problems, which results in insufficiency in oxygen supply for some parts of the brain. That leads to partial brain damages resulting in functional disability for the function

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performed by that part of the brain[3,7,8]. It is estimated worldwide that as much as 46% of brain insult cases are among people between 45 and 59 years of life, while from 30 to 45 years the incidence is about 3%. The major cause in etiology of brain insult is blood vessels damaging due to atherosclerotic processes by mechanical (blood pressure) and/or chemical (hypercholesterolemia) factors; inflammation process of blood vessels; or some immune diseases (lupus erithematodes)[2,6,9]. In the literature major risk factors for developing of brain insult are: arterial hypertension, cardiac diseases, diabetes mellitus, adiposity, smoking, stress, high cholesterol, and rare hyper-uric diathesis[10,11].

Prevention is the most relevant strategy in the brain insult approach, especially influencing of the changeable risk factors such as: smoking, dietary regime, alcohol intake, obesity etc., as well as influencing and treating some diseases as hypertension, heart diseases, diabetes, hypercholesterolemia. Blood pressure decrease of 5% among hypertonic population leads to the decrease in the brain insult frequency for more than 40 %[12]. Together with the treatment, preventing the risk factor (except for the unchangeable) is the best way to avoid insult.

The objective of this paper was to analyze the hospital morbidity and lethality of cerebrovascular insult in the Clinical Hospital Mostar from 1999 to 2003 from the Neurology department institutional register as well as research on hospitalized cases according to the sex and age distribution, hospital treatment outcome (lethality) and complications by epidemiological characteristics /risk factors.

Materials and Methods

This is a retrospective epidemiological study of hospitalized brain insult patients which by health insurance gravitate for treating to the Clinical Hospital Mostar. During 2003 that was a total of 457,491 people. This hospital is the major hospital for the treatment of health insured patients. There are other hospitals for these populations, but majority of these cases were treated in this hospital considering that this hospital is the only one with the neurology department and the intensive care unit. There are 29 beds, 8 neurologists and 26 nurses at this department. As a source of data we used medical documentation – an institutional register – of the neurology department. For each of the cases we analyzed: age, sex, risk-factors, place of living and clinically confirmed cerebro-vascular insult diagnosis, as well as hospital treatment results and complications.

From 1 January 1999 until 31 December 2003 a total of 1,555 insult cases were hospitalized. Among them, 727 (46.7%) were male and 828 (53.3%) were female patients. The cases were divided in three age groups: less or equal 40, 41–50, 51–60, 61–70 and 70 and more years of life by sex. For the analyzing of table data, standard methods of descriptive statistics were performed.

Results

The structure of cerebro-vascular insult treated cases in the Clinical Hospital Mostar according to the age and gender was shown in Table 1.

A total of 1,555 cases were treated of brain insult in the period from 1999 through 2003 in the Clinical Hospital Mostar. Out of that number 727 (46.7%) were male patients, while 828 (53.3%) were female patients (Figure 1). Among both sexes a total of 20 cases (1.2%) were younger than 40 years old, 87 patients (5.6%) were from 51 through 60 years old, and the majority of patients treated because of the brain insult were 61 and over. Among all male and female patients observed separately by gender as a cluster, majority of the treated male cases were in the oldest group (over 61 years) of cases 67.0% (487 patients) as well as it was observed among female patients where registered percent in this group was even higher – 85.7% or 709 patients. The lowest number of cases according to the age structure was treated among younger patients – younger than 50 years old among either sexes – 9.2% (or 16 cases) of male cohort, and only 0.4% (or 4 cases) of female cohort (Table 2, Figure 2).

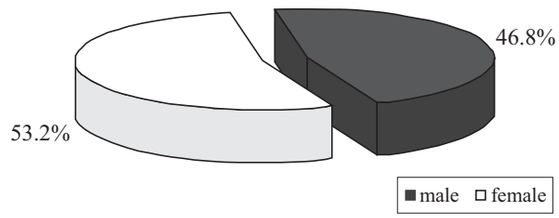

Fig. 1. The structure of total number of brain insult cases by gender treated in the Clinical Hospital Mostar from 1999 to 2003.

TABLE 1
BRAIN INSULT CASES ACCORDING TO THE AGE AND GENDER DISTRIBUTON TREATED IN THE CLINICAL HOSPITAL MOSTAR FROM 1999 TO 2003

Gender	Total number of cases 1999–2003		Age									
			< 40		41–50		51–60		61–70		> 70	
	N°	%	N°	%	N°	%	N°	%	N°	%	N°	%
Male	727	100.0	16	2.2	67	9.2	157	21.6	231	31.8	256	35.2
Female	828	100.0	4	0.4	20	2.4	95	11.5	301	36.4	408	49.3
Total	1,555	100.0	20	1.2	87	5.6	252	16.2	532	34.2	664	42.8

TABLE 2
BRAIN INSULT CASES HOSPITAL LETHALITY IN THE CLINICAL HOSPITAL MOSTAR 1999–2003

Gender	Treatment outcome	Total number of cases 1999–2003		Age									
				W 40		41–50		51–60		61–70		> 70	
		Nº	%	Nº	%	Nº	%	Nº	%	Nº	%	Nº	%
Male	Successfully treated	479	66,8	13	81.2	48	71.6	107	68.1	147	63.6	164	64.1
	Died	248	34,2	3	18.8	19	28.4	50	31.9	84	36.4	92	35.9
Female	Successfully treated	508	61,3	3	75.0	13	65.0	66	69.5	171	60.1	25	50.3
	Died	320	38,7	1	25.0	7	35.0	29	30.5	130	39.9	153	37.5
Total	Successfully treated	987	63,5	16	80.0	61	70.1	173	68.6	328	61.6	419	63.1
	Died	568	36,5	4	20.0	26	29.9	79	31.4	204	38.1	245	36.9

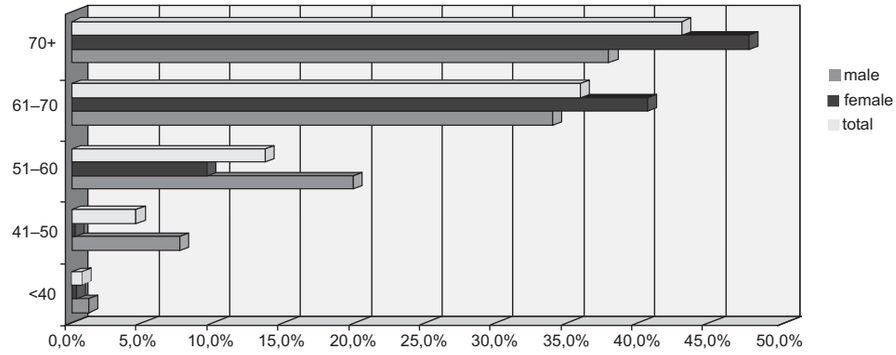

Fig. 2. Brain insult hospital lethality by age groups in the Clinical Hospital Mostar 1999–2003.

A research on risk factors among 213 patients during 2003 was conducted as well, either searching through available medical documentation or directly checking with patients wherever was possible regarding patient's conditions. The results were: 57.2% (122 patients) had hypertension, 32.3% (69 patients) had myocardiopathy, 22.3% (47 patients) had diabetes, 16.1% (34 patients) had atrial fibrillation, 17.8% (38 patients) had hypercholesterolemia, 41.3% (88 patients) had increased triglycerides, 7.1% (15 patients) had triglycerides + cardiac diseases,

TABLE 3
TREATMENT OUTCOME OF BRAIN INSULT IN 2001

Gender	Treatment outcome	Republic of Croatia*		Tuzla Canton**		West Herzegovina Canton ***	
		Nº	%	Nº	%	Nº	%
Male	Treated in hospital	4,642	100.0	322	100.0	38	100.0
	Successfully treated	3,415	73.5	218	67.7	25	65.8
	Died	1,267	27.3	104	32.3	13	34.2
Female	Treated in hospital	6,706	100.0	375	100.0	44	100.0
	Successfully treated	4,621	68.9	244	65.1	27	61.4
	Died	2,045	30.5	131	34.9	17	38.6
Total	Treated in hospital	11,348	100.0	697	100.0	82	100.0
	Successfully treated	8,036	69.8	462	66.3	52	63.5
	Died	3,312	29.2	235	33.7	30	36.5

*Republic of Croatia (all hospitals) = 4,381,352 inhabitants

**Canton Tuzla (KB Tuzla) = 500,503

***Canton West Herzegovina (KB Mostar) = 88,257 inhabitants

5.6% (12 patients) had triglycerides + hypercholesterolemia, 3.8% (8 patients) had hypertension + triglycerides, 3.3% (7 patients) had triglycerides + diabetes, 2.8% (6 patients) had diabetes + hypertension and 1.3% (3 patients) had hypercholesterolemia + diabetes. Some patients had more than one risk factor present in their status.

Discussion

During the previous, recent period, population of Bosnia and Herzegovina was exposed to certain level of exposure to different risk factors influencing health status of individuals. This was particularly the case with the chronic non-communicable diseases, gastrointestinal problems as well as some viral diseases. Psychosocial factors and changed life styles, dietary regime, psycho stresses and social and economic factors have been linked with greater number of brain insult cases[13,14]. Cardiovascular diseases are the leading cause of mortality worldwide. Cerebro-vascular diseases are the leading cause of mortality among female population in neighboring countries in transition (Croatia), while the same diseases are in the second place among male population, just following cardiovascular diseases[15]. The same is characteristic for Bosnia and Herzegovina, and particularly for Federation of BiH[16]. If we compare our results with results of a similar study done in Tuzla Canton[17] we can see that hospital lethality among our patients was 36.5% while among Tuzla patients were 33.5%. The difference can be explained by different population age structure – Herzegovina has in general ageing population out of 457,491[2] and it is exposed to many risk factors that are combinative. More than 23% patents had 2 or more risk factors that result in higher lethality. This rather high lethality rate in both B&H regions is higher than in Western European Countries[18] but it is similar to the hospital lethality of other countries in transition[19]. In the Republic of Croatia (counting all hospitals) the hospital lethality is lower than in B&H, and that can be explained as a result of better organized prevention and early diagnosis[16,20]. One of the recommendations for decreasing the hospital lethality by up to 18% in B&H, according to the studies in developed countries, is establishing intensive care units for brain insult treatment[21]. Among our patients high value of triglycerides was observed in almost half of our patients (41.3%) – maximum referral values for male is 1.9 mmol/L and 1.6 mmol/L for female; and 17.8% of our patients had cholesterol above normal values (referral from 6.7–10.1 mmol/L). For them a treatment with *statins* was recommended as one kind of brain insult preventions, because *statin* treatment leads to the increased risk for clinical manifesting of brain insult for 19–31% of cases according to the other authors research's results[22]. Significance of establishing of an intensive care unit is greater when one bears in mind that among all treated cases in the Clinical Hospital Mostar over 70% (72.3%) are brain insult cases. According to Poeck[13] there is no statistically significant difference in brain insult morbidity between men and women. The same finding was a result of our study.

According to the available literature the greater part of hospital treated patients according to the age is older than 50 year of life. That is confirmed in our study as well, where 93.2% of patients belong to that age group. We observed 77.0% patients older than 61 years. If we look to the sex distribution, 46.8% were male, and 53.2% were female patients among hospitalized. This data is similar to other authors' findings[6,14]. Our findings also showed that greater number of treated women were older than 61–59.3% compared to 40.7% of men among our hospitalized patients in that age group (45.6% female to 31.3% men of all cases in that age group). According to the same findings, looking to the younger patients, that ratio is slightly different – 66.9% of men vs. 33.1% of treated women. That is understandable having in mind that women have some hormone protection until after of menopause, and this finding is matching the other authors' findings[8]. Some authors' estimation considering age and brain insult is that in between 45 and 59 years of life should be about 46.0% of all brain insult cases[11]. Among our cases that percentage is lower for that age group (from 41 to 60) – 21.8%. Brain insult incidence according to the literature until 45 years of life is 3%[11], while we found 1.2% younger than 40 years among all our observed hospitalized patients. Stress has significant role in increasing brain insult incidence and lethality as it was proved during the Gulf War in Jerusalem and during the recent War in Bosnia and Herzegovina[22, 23].

If we calculate our total number of cases as an incidence rate per existing population gravitating to the Clinical Hospital and per 1,000 people the incidence rate for this population is $3.4^{0}/_{00}$.

Cerebro-vascular/brain insult as a last stage of cerebro-vascular diseases is among top leading mortality causes worldwide, so research on risk factors for developing that disease is of the highest importance. Such a research study was done among 2,750 insured in FBiH using questionnaires and measurements. Among that population 46 (or 1.7%) were found to have brain insult. Regarding standard risk factors 18.5% were found to have hypertension, 15.8% hypercholesterolemia, and 5.9% diabetes. In 2002 in all Cantons of Federation of B&H research on chronic non-communicable diseases risk factors was conducted on a sample of 2,750 populations. A part of that study was 852 inhabitants of Herzegovina from 25 to 64 years gravitating for a medical treatment to Clinical Hospital Mostar. Among them 1.7% were found to had brain insult (2.0% male, 0.5% female). Some of the risk factors analyzed and found among them were: hypertension had 41% (35.5% male, 44.8% female), smoking 37.6% (49.2% male, 29.7% female), overweight was 21.5% (16.5% male, 25.0% female), diabetes has 5.4% (4.8% male, 5.8% female)[20].

In this retrospective epidemiological study including the Clinical Hospital Mostar patients from 1999–2003, a total of 1,555 brain insult patients were hospitalized. The greater part of hospitalized patients age 61 and more were women; in the age group from 51 to 60 years majority were men, same as among patients below 41 which

was the smallest patient cohort. Regarding the objective, this study follows other neighboring countries in transition. Data obtained for the neurology department from an institutional register as a very reliable source of information proved to be very useful for our study. Using these information public health care system can organize and undertake targeted brain insult risk factors preventive measures. Better organization of primary health care settings and capacities planning is easier to achieve if collected data are reliable. Establishing population screening programs through family medicine physicians/practice will contribute significantly to early diagnosis and adequate on-time treatment. If more efforts will be given to the prevention programs and population based activities including screening and its results, brain insult lethality rate in B&H should be reduced.

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HOSPITALIZACIJA OBOLJELIH OD CEREBROVASKULARNOG INSULTA U KLINIČKOJ BOLNICI MOSTAR OD 1999. DO 2003. GODINE – PRIMJER INSTITUCIJSKOG REGISTRA

SAŽETAK

Analiza oboljelih od cerebrovaskularnog inzulta u Kliničkoj bolnici (KB) Mostar, kao epidemiološka retrospektivna studija, učinjena je u Kliničkoj bolnici Mostar u vremenskom razdoblju od 1999 do 2003 godine. Glavni izvor podataka je bila medicinska dokumentacija (institucionalni registar) pacijenata ove bolnice, kao jedine bolnice za liječenje 457 491 stanovnika koji gravitiraju ovoj bolnici prema zdravstvenom osiguranju. Studijom je obuhvaćeno 1 555 pacijenata, koji su liječeni zbog cerebralno-vaskularnog inzulta u KB Mostar. Od sveukupnog broja, 727 (46.8%) su bili muškarci, dok je 828 (53.2%) bilo žena. Većina oboljelih je bila starija od 50 godina. Većina liječenih ženskih pacijenata je bila starija od 61godine života (45.6% svih oboljelih), isto kao i muškaraca (31.3%). Najmanji broj oboljelih je imao manje od 41 godine u obje grupe (1.2%). Prevalencija čimbenika rizika je 2,035 (74%). Tijekom istog razdoblja čimbenici rizika su istraženi na 2,750 nacionalnih osiguranika cijele Federacije Bosne i Hercegovine, od kojih 852 je gravitiralo liječenju u KB Mostar. Od njih 1.7% je imalo cerebrovaskularni inzult.

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Pulmonary Function in Persons Who are Professionally Exposed to Formaldehyde Fumes

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A B S T R A C T

The present study examines long-term effects of occupational exposure to formaldehyde fumes on lung function. Forced spirometry and diffusing lung capacity were measured in 16 health-service professionals (8 medical doctors and 8 laboratory technicians) working at the pathoanatomic laboratory for at least 4 years with daily exposure 8±1 hours. Control group employed 16 males, which were matched by age and stature to members of the exposed group. Only non-smokers were included in the study. Spirometric parameters in study participants exposed to formaldehyde fumes compared to control group were not significantly different indicating absence of restrictive and/or obstructive deterioration of lung function in exposed group. The only parameter differing in two groups was blood volume of pulmonary capillaries (Vc') which was significantly larger in a group exposed to formaldehyde fumes. The possibility that the hyperemic lung reaction is the consequence of the exposure to formaldehyde fumes should be further explored.

Key words: *formaldehyde, pulmonary function, diffusing capacity, occupational health hazards*

Introduction

Formaldehyde is widely used as a preserving, disinfecting and embalming agent. In addition to its technical benefits, formaldehyde has eliminated many health hazards during histological procedures in anatomy and pathology laboratories. Paradoxically, formaldehyde itself is a noxious chemical, highly unpleasant to the user, and a well-recognized occupational health hazard[1–4]. Formaldehyde has been reported to produce allergic contact dermatitis[5], neurobehavioral changes[6] and carcinogenesis[7].

Symptoms of respiratory irritancy and effects on pulmonary function have been examined in studies of both indoor and ambient air exposure to formaldehyde[8–12]. Exposure to formaldehyde fumes is almost exclusively occupational, and has been investigated in workers in the production of resinembedded fibreglass[13–14], chemicals, furniture, and wood products[15–18] or through employment in the funeral services industry[19]. Short-term effects of formaldehyde exposure included symptoms of irritation of the eye and respiratory tract. Workers in these studies were exposed to mean formaldehyde concentrations of 0.16 ppm (0.10–0.12 mg/m^3) and greater. In a survey in which the dosage of exposure was considered[10,20] formaldehyde was a statistically significant predictor of symptoms of eye, nose and throat irritation, phlegm, cough and chest complaints. Short-term effects of occupational exposure to formaldehyde fumes on lung function were also investigated but with controversial conclusions. Some studies reported decreased lung function in workers exposed to formaldehyde[9–11] others concluded that the formaldehyde was not associated with decrements in lung function[8,12]. Asthmatic symptoms and lowered FEV_1, MEFs in studies by Fransman et al.[9] and by Bender[11] showed dose-response effects but in both studies authors concluded that lung function changes are re-

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versible. Since the long-term effects of professional exposure to formaldehyde fumes on lung function are not well established, the aim of the present study is to examine lung function parameters in persons working 4–20 yrs. in pathoanatomic laboratory.

Materials and Methods

The present study was designed to examine long-term effects of professional exposure to formaldehyde fumes on lung function. Exposed group encompasses 16 male health-service professionals (8 medical doctors and 8 laboratory technicians) working at the University of Split. All exposed examinees were working in the pathoanatomic laboratory for at least 4 years with daily exposure 8±1 hours. Control group employed 16 males that were matched by age and stature to exposed group. To avoid possible additive or synergistic effects of smoking on lung function[21–23], only non-smokers were included in the study. All 32 examinees voluntarily participated in the present study.

The following pulmonary function measurements were considered: forced vital capacity (FVC), forced expiratory volume in 1st second (FEV_1), forced midexpiratory flow rates (PEF, MEF_{25}, MEF_{50}), diffusing lung capacity for carbon monoxide (DLCO), Kroch constant (DLCO/VA), blood volume of pulmonary capillaries (Vc') and membrane diffusion capacity (Dm).

Forced spirometry measurements were recorded by Masterlab from Jaeger instrument. The testing was performed in a sitting position without a noseclip. Each examinee was instructed to perform acceptable maneuvers. The FVC maneuver was repeated three times and the best values were taken from any of the acceptable tracings.

For a single breath (10 s breathhold) DLCO measurements an inspired concentration of 0.3% CO, 0% He, 21% O_2, and balance N_2 was used. Before the DLCO test was performed each subject was instructed in all required maneuvers, emphasizing the importance of giving a sign when at the level or residual volume (maximal expiration) inhaling the test gas mixture rapidly to vital capacity, continuing to hold the breath (assisted by the valve system) for 10 s, and exhaling volume of at least 2.0 L rapidly. The effective breathhold time included two-thirds of the inspiratory time and the portion of the expiratory time until one half of the alveolar sample was obtained. This procedure is automatically built into the instrument. Two satisfactory tests were carried out with an interval of at least 4 minutes between the two tests and their average was taken. The subjects rested for at least 30 minutes before the start of each test. One trained technician did 94% of the tests. All tests were performed between 9 a.m. and 1 p.m.

The reference values for DLCO, DLCO/VA where VA is the alveolar volume, Dm and Vc' were those of Cotes and Hall[24] and for forced spirometry parameters those of Cotes[25]. These reference values agreed well with values for healthy population tested in our laboratory[26].

Dm and Vc' estimates were done according to the Roughton and Foster's formula:

$$1 / TLCO = 1 / Dm + 1 / Vc'\Theta$$

Where TLCO is carbon monoxide transfer factor, Θ is the rate of CO binding to hemoglobin and is generally dependent on the examinee's mean oxygen tension in the pulmonary capillaries ($Pc'O_2$), Hb and COHb, as follows:

$$1 / \Theta \text{ (ml blood min mmHg ml}^{-1} \text{ CO)} = [0.006 (Pc'O_2(\text{mmHg}) + 0.33)] \, 14.6 / Hb \text{ (g/dl}^{-1}) \, (1 - \% \text{ COHb} / 100)$$

$Pc'O_2$ may be assessed as:

$$Pc'O_2 \text{ (mmHg)} = PAO_2 \text{ (mmHg)} - 10$$

or more accurately, from oxygen consumption and Dm. In order to calculate Dm and Vc' from equations (1) – (3), measurements were made at two levels of alveolar oxygen tension, room air and after breathing 100% oxygen for 10 min, providing two equations with two unknowns: Dm and Vc'. The explicit solutions are:

$$Vc' \text{ (ml)} = (Hb / 14.6) \frac{0.006 \, [PA\,O_2 \text{ (oxygen)} - PA\,O_2 \text{ (air)}]}{1 / DLCO \text{ (air)} - 1 / DLCO \text{ (oxygen)}}$$

$$Dm \text{ (ml x CO/mmHg x min)} = \frac{1 / .(\text{oxygen}) - 1 / .(\text{room air})}{1 / .(\text{oxygen}) \; 1 / DLCO \text{ (air)} - 1 / .(\text{air}) \; 1 / DLCO \text{ (oxygen)}}$$

The pulmonary function test results were interpreted using the criteria of Konig et al.[27]:

(a) normal finding: FVC ≥ 80% predicted; $FEV_1/FVC \geq 70\%$; MEF_{25}, MEF_{50}, and $MEF_{75} \geq 60\%$ predicted and DLCO ≥ 80% predicted;

(b) restrictive impairment: FVC < 80% predicted and $FEV_1/FEC \geq 70\%$;

(c) obstructive impairment: $FEV_1/FVC < 70\%$;

(d) isolated DLCO reduction: DLCO < 80% predicted, as a sole finding and

(e) DLCO > 120% predicted was described as isolated DLCO increase.

Comparison between study participants exposed to formaldehyde fumes and control group in diffusing lung capacity and in lung function parameters was carried out by means of Student's t-test for independent samples. The difference between groups at $p < 0.05$ was considered as statistically significant.

Results

Descriptive statistics of age, height, weight, body mass index (BMI = weight (kg) / height $(m)^2$), years of professional exposure to formaldehyde fumes and lung function parameters (percentage of expected values) in 16 exposed participants is presented in Table 1. Table 2 provides the results of the comparison of spirometric parameters in study participants exposed to formaldehyde

TABLE 1
AGE, HEIGHT, WEIGHT, BMI, YEARS OF EXPOSURE AND LUNG FUNCTION PARAMETERS (% EXPECTED) IN STUDY PARTICIPANTS EXPOSED TO FORMALDEHYDE FUMES (N=16)

	X	SD	Min	Max
Age (yrs)	38.06	8.16	25	52
Height (cm)	166.5	7.4	156	182
Weight (kg)	68.88	12.04	55	104
BMI (kg/m^2)	24.78	3.44	19.71	33.28
Exposure (yrs)	12.38	5.21	4	20
FVC (%)	111.43	14.35	90.1	139
FEV_1 (%)	111.95	13.09	91.7	137
FEV_1/VC (%)	107.81	5.69	93.9	117
PEF (%)	99.03	19.02	62.1	124
MEF_{50} (%)	104.27	22.95	71.9	139
MEF_{25} (%)	102.18	22.32	69.2	143
DLCO (%)	101.13	27.44	63	141
DLCO/VA (%)	95.94	11.13	70	112
Vc' (%)	103.38	18.18	76	132
Dm (%)	88.38	11.12	72	111

BMI – weight (kg) / height (m)^2, FVC – forced vital capacity, FEV_1 – forced expiratory volume in 1st second, PEF, MEF_{25}, MEF_{50} – forced midexpiratory flow rates, DLCO – diffusing lung capacity for carbon monoxide, DLCO/VA – Kroch constant, Vc' – blood volume of pulmonary capillaries, Dm – membrane diffusion capacity

TABLE 2
LUNG FUNCTION PARAMETERS IN STUDY PARTICIPANTS EXPOSED TO FORMALDEHYDE FUMES COMPARED TO CONTROL GROUP

	Exposed group (N=16)		Control group (N=16)		t-test p
	X	SD	X	SD	
FVC (%)	111	14	106	11	ns
FEV_1 (%)	112	13	102	9	ns
FEV_1/VC (%)	108	6	96	6	ns
PEF (%)	99	19	92	13	ns
MEF_{50} (%)	104	22	110	20	ns
MEF_{25} (%)	102	22	105	25	ns

FVC – forced vital capacity, FEV_1 – forced expiratory volume in 1st second, PEF, MEF_{25}, MEF_{50} – forced midexpiratory flow rates, ns – nonsignificant

fumes compared to control group. Exposed group did not show any difference in mean values of either examined forced spirometry parameters – FVC, FEV_1, PEF, MEF_{25}, MEF_{50} and MEF_{75}. However, 25% of exposed participants showed incipient changes in small respiratory airways.

The comparison of diffusing lung capacity for carbon monoxide (DLCO), Krogh constant (DLCO/VA), blood volume of pulmonary capillaries (Vc') and membrane diffusion capacity (Dm) in participants exposed to formaldehyde fumes compared to control group is shown in Table 3. The measured values of diffusing lung capacity for carbon monoxide (DLCO) and membrane diffusion capacity (Dm) in exposed group fell within expected referent values. While, blood volume of pulmonary capillaries (Vc') showed to be significantly higher in a group exposed to formaldehyde fumes in comparison with control group.

TABLE 3
DIFFUSING LUNG CAPACITY PARAMETERS IN STUDY PARTICIPANTS EXPOSED TO FORMALDEHYDE FUMES COMPARED TO CONTROL GROUP

	Exposed group (N=16)		Control group (N=16)		t-test
	X	SD	X	SD	p
DLCO (%)	101	27	105	6	ns
DLCO/VA (%)	95	11	90	5	ns
Vc' (%)	103	18	81	9	<0.001
Dm (%)	88	11	89	11	ns

DLCO – diffusing lung capacity for carbon monoxide, DLCO/VA – Krogh constant, Vc' – blood volume of pulmonary capillaries, Dm – membrane diffusion capacity, ns – nonsignificant

Discussion

Occupational hazards of formaldehyde were thoroughly investigated but the studies were primarily dealing with its possible carcinogenic effects[28–32]. Only small proportion of studies were oriented towards inflammatory reactions of respiratory system relating exposure to formaldehyde fumes inhalation to dynamic changes (short--term and long-term) in bronchial and pulmonary symptoms and function.

Various studies provided the evidences that formaldehyde is an irritant of the respiratory tract[13,17,19,33] that causes nonproductive cough, breathing problems, eye tears and nose dripping. There is a correlation between clinical symptoms and concentration of formaldehyde in the workplace[20]. Usual concentration provoking symptoms being from 10 to 20 ppm.

The possibility of the occurrence of asthmatic reactions, or even asthma itself was also suggested by some authors[34]. The inhalation of formaldehyde fumes causes bronchial hyperactivity[35] and – according to some authors – the reductions of air circulation speed as well[15]. However, the increases of respiratory values were reported in some other studies[16].

Results of investigations of effects on pulmonary function in occupationally exposed populations are somewhat conflicting. Pre-shift reductions (considered indicative of chronic occupational exposure) of up to 12% in parameters of lung function (e.g., forced vital capacity, forced expiratory volume, forced expiratory flow rate) were reported in a number of smaller studies of chemical, furniture and plywood workers[15–18,36]. In general, these

effects of lung function were small and transient over a work shift, with a cumulative effect over several years that was reversible after relatively short periods without exposure (e.g. 4 weeks); effects were more obvious in non-smokers than in smokers[37,38]. In the subset of these investigations in which exposure was monitored for individuals (i.e., excluding only that of Malaka and Kodama 1990[18]), workers were exposed to mean concentrations.

The present study examines lung function parameters in laboratory technicians and medical doctors professionally exposed to formaldehyde. The long-term effects of exposure were examined by comparing their spirometric indicators and diffusing capacity parameters with non-exposed subjects. The study showed that flow/volume curves were within reference values in all 16 examined subjects (Table 1).

The clinical symptoms of nonproductive cough and eye tears were present in 80% of exposed subjects, whereas 20% of the examinees have subjectively present breathing complaints.

When compared to unexposed controls the only lung function parameter significantly differing in exposed group was the blood volume of pulmonary capillaries, which was significantly higher in exposed group. The increase of the blood volume of pulmonary capillaries in persons exposed to formaldehyde is also radiologically confirmed[39,40]. One of the explanatory hypotheses includes the hyperemic lung reaction as the consequence of the exposure to environmental irritants including formaldehyde.

Although mean values of all other parameters were within normal ranges it should be mentioned that the lungs' diffusion capacities in 16 examinees professionally exposed to formaldehyde showed to be rather divergent. Increased diffusion capacity has been recorded in eight examinees (50%), a decrease of diffusion capacity has been recorded in three examinees, whereas only in five examinees the recorded values fall within normal range. The diffusion capacity of the lungs showed a tendency to be related with the years of exposure: exposure to formaldehyde fumes inhalation up to ten years causes an increase of the diffusion capacity of the lungs; exposure to formaldehyde fumes inhalation more than ten years causes a decrease of the diffusion capacity of the lungs. A larger sample is needed to provide sufficient evidences to confirm above findings.

Conclusions

The present study of lung function in 16 persons working in pathoanatomic laboratory being daily professionally exposed to formaldehyde fumes showed that:

- No respiratory function impairments, either of the obstructive or restrictive type, were detected;
- No relation between clinical symptoms and pulmonary function tests has been found;
- When compared to unexposed controls the only lung function parameter significantly differing in exposed group was the blood volume of pulmonary capillaries, which was considerably higher in exposed group. The possibility that the hyperemic lung reaction is the consequence of the exposure to formaldehyde fumes should be further explored.

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PLUĆNA FUNKCIJA U OSOBA PROFESIONALNO IZLOŽENIH PARAMA FORMALDEHIDA

S A Ž E T A K

Dinamička spirometrija i difuzijski kapacitet pluća određen je u 16 ispitanika koji rade u patoanatomskom laboratoriju. U istraživanje su uključeni samo nepušači. Minimalna dnevna izloženost formaldehidu je bila osam sati, a svi ispitanici rade najmanje četiri godine u laboratoriju. Kontrolnu skupinu sačinjavalo je 16 ispitanika slične dobi koji nisu bili izloženi udisanju formaldehida. Nisu pronađene značajne smetnje u ventilacijskim vrijednostima kao ni restrikcijski, a ni opstrukcijski poremećaji ventilacije. Povećan difuzijski kapacitet zabilježen je u 50%, a smanjen u 18,8% ispitanika skupine izložene formaldehidu. Ova studija je pokazala da je među provedenim testovima plućne funkcije samo razina volumena krvi plućnih kapilara statistički značajno različita između dviju skupina. Ispitanici profesionalno izloženi parama formaldehida pokazali su povećanje u odnosu na kontrolnu skupinu u razini volumena krvi plućnih kapilara. To je po mišljenju autora posljedica hiperemijske reakcije u plućima, što je radiološki i potvrđeno.

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Original scientific paper

Epidemiological Characteristics of Sarcoidosis Patients Hospitalized in the University Hospital for Lung Diseases »Jordanovac« (Zagreb, Croatia) in the 1997–2002 Period

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A B S T R A C T

The aim of our study was to explore the characteristics of hospitalized patients with sarcoidosis concerning age, gender, clinical forms and staging, seasonality, geographical distribution, smoking habit and profession, familial clustering and mortality. We included 476 biopsy-proven sarcoidosis patients who were diagnosed at the University Hospital for Lung Diseases »Jordanovac« in the period from 1997–2002. Most of the patients (44%) were in the group of age between 20 and 40 years. The ratio of women to men was 1.4:1. The onset of the disease usually appeared in spring and summer, especially in the patients presenting with erythema nodosum, with majority of patients hospitalized in the period from May to August (51%). More patients came from urban, than from rural areas (1.5:1), and they were mostly nonsmokers (3.3:1). In 2% of sarcoidosis patients we found familial clustering. Although these data are biased regarding the selection of patients they give new insights into characteristics of sarcoidosis patients in Croatia.

Key words: *sarcoidosis, epidemiology, erythema nodosum*

Introduction

Sarcoidosis is a systemic multiorgan disease of unknown etiology[1]. It occurs most frequently among younger adults between 20 and 40 years of age and most of the studies show that it is more frequent among women[2]. Its clinical and histological representation does not depend on climatic conditions although the disease peaks with its clinical appearance in spring months[3].

The epidemiological studies show great variability of results in which prevalence of sarcoidosis varies from 0.2 in Portugal to more then 50/100 000 in black people in USA, depending on the studied population and diagnostic methods used[4]. In the USA, sarcoidosis occurs 10–17 times more frequently among African Americans and Puerto Ricans then in white people. In Europe it is more frequent in the north than in the south. Sweden was once considered as the country with the highest incidence of 64/100000, but recent studies showed the incidence of about 19/100000. Spain with 1.2 and Italy with 0.5/100,000 have some of the lowest incidences in Europe[5]. The prevalence of sarcoidosis in Croatia is about 4.1/100,000 persons according to the National Registry data[6]. Profession or social status do not predispose to sarcoidosis although it is more frequent in non-smokers[7].

In this article we have presented the patients hospitalized from 1997 to 2002 at the University Hospital for Lung Diseases »Jordanovac« in relation to age, gender, clinical forms and staging, seasonality, geographical distribution, smoking habit and profession, familial clustering and mortality.

Patients and Methods

The data available from case history documentation, with sarcoidosis as a discharge diagnosis from our hospital, for the period from 1997 until the end of 2002 were

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analyzed retrospectively. All medical history documents were analyzed in relation to age and gender, seasonal occurrence, geographical distribution, life style and familial occurrence of the disease. We found that in the period from 1997 until the end of 2002 sarcoidosis as discharge diagnosis was present in 711 cases representing 59.4% of all hospitalized patients for sarcoidosis in Croatia for the designated period of time. Since some patients were hospitalized more than once there were all together 476 persons who suffered from sarcoidosis. In all patients diagnosis was confirmed by histopathology of a biopsy samples recovered from sarcoidosis lesions. Family history data were analyzed for familial clustering relating the family cases and non-family contacts, relation to living together, time elapsing between diagnoses of pairs. In all patients data were available for clinical forms and staging of the disease.

All statistical analyses were done using STATISTICA version 6 (StatSoft, Inc., Tulsa, USA). As it is almost impossible to define the population from which these patients were recruited as they come from different parts of Croatia no standardized prevalence was calculated. Statistical significance was set at $p<0.05$.

Results

Age and gender

Among 476 patients, treated for sarcoidosis in the period from 1997 until the end of 2002 there were 276 women and 200 men. The ratio of women to men was 1.4:1 ($p<0.001$). Among the total number of patients treated for sarcoidosis, 206 patients were between 20 and 40 years of age, which represents 44%, with the peak between 35 and 39 years of age (20%) as shown in Figure 1. The increase in incidence of sarcoidosis was different in women and men with »second peak« between 50 and 54 years of age for woman and between 45 and 49 years of age for men, respectively. For all age groups the incidence was higher in women ($p=0.03$), except between 45 and 50 years of age when the incidence is higher in men (RR=1.551, 95% CI 1.225–1.956, $p=0.001$).

Clinical forms and staging

Lung sarcoidosis was present in 370 (77.7%) and extra-pulmonary forms in 106 (22.3%) patients. In 41.6% (N=198) of patients radiographic stage I was confirmed, stage II in 39.7% (N=189), stage III in 18.1% (N=86), and stage IV in 0.6% of patients (N=3).

Seasonal occurrence

Sarcoidosis patients were hospitalized throughout the whole year, but the highest rate was in May, June, July and August when 243 (51%, 95% CI 46.4%–55.6%) patients were treated with the rate ratio of 2.09 (95% CI, 1.743–2.497; $p<0.001$) compared to patients hospitalized during the rest of the year. The increase in the occurrence of the disease in spring and summer months showed the same pattern in women and men (rate ratio, women 2.035, 95% CI, 1.569–2.641, rate ratio, men 1.705, 95% CI, 1.236–2.346, $p<0.001$ for both; Figure 2). The seasonality of the occurrence was especially pronounced in patients presenting with the erythema nodosum (rate ratio 4.333, 95% CI, 2.828–6.757, $p<0.001$). Erythema nodosum was found in 18.7% patients (N=89).The majority of patients (N=75) had EN in the period from April till August (81.3%).

Geographical distribution

Among the patients treated in our hospital for sarcoidosis, 282 persons were from urban areas while 194 were from rural areas. The ratio of urban to rural population is 1.5:1. The highest number of the persons affected was from Zagreb (N=142). It is difficult to establish their origin since Zagreb is a metropolis with a significant immigration of rural population in the last 10 years.

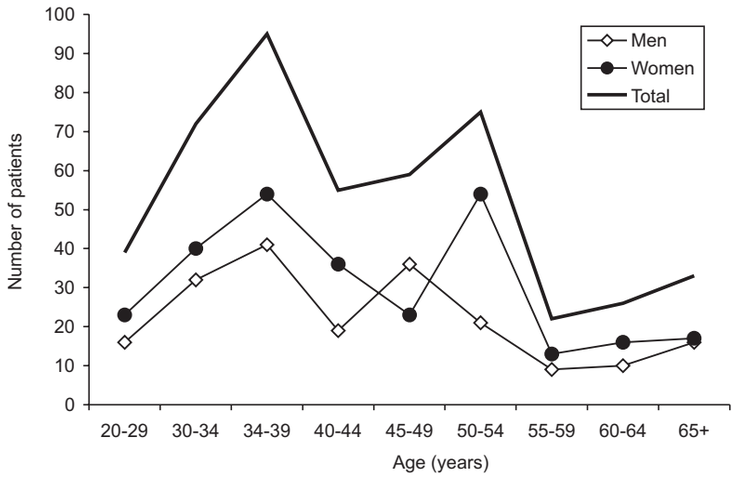

Fig 1. Distribution of sarcoidosis by age and sex.

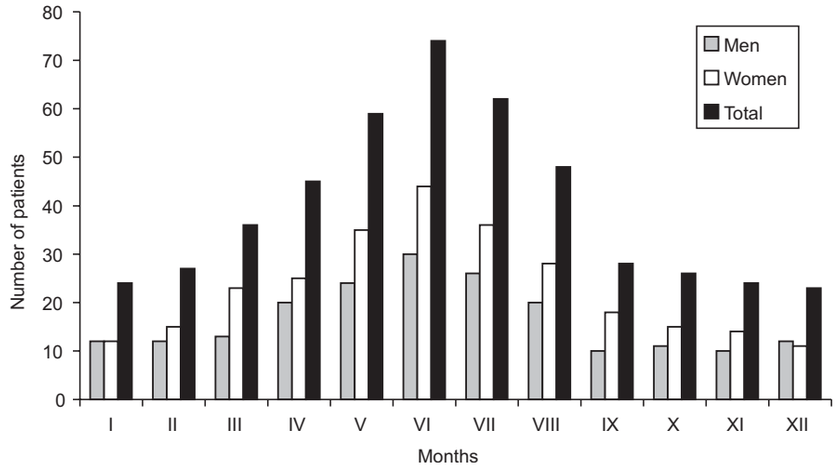

Fig. 2. Seasonal distribution of sarcoidosis.

Life style and profession

There are numerous studies which discuss the correlation between sarcoidosis and non-smoking. Out of 476 patients in our sample 362 (76%) of them were non--smokers whereas 114 (24%) were smokers. The ratio of non-smokers to smokers in this sample was 3.3:1.

As for the professions, 2/3 of men did manual jobs (construction, drivers, fitters) while 1/3 did white-collar jobs. Among women there was roughly about the same number of white–collar jobs and housewives on one hand and jobs involving certain manual labor (cleaners, hair--dressers, shop-assistants) on the other. The number of smokers in men and women does not depend on profession. We found roughly about the same number of smokers among white-collar workers, manual workers and housewives.

Familial clustering

Among the patients treated in our hospital in the six-year period, we found familial occurrence within four families (mother and son, two sisters, a mother and a daughter, two brothers) which makes 2% of the total number of 476 patients. The occurrence of the disease in these cases was from 4 to 14 years apart and in three families it was pulmonary sarcoidosis in both family members but with different staging and/or additional organs affected. In one familial setting sarcoidosis affected different organs (mother, spleen; son, lungs). The age of onset was different in all but one family where two sisters had an onset of the disease at almost the same age (38 and 40 yrs) but 4 years apart.

Mortality

Among all the patients hospitalized for sarcoidosis, two of them (0.42%) died, one at the age of 72 and the other at the age of 78. The cause of death was heart failure duo to chronic cor pulmonale and respiratory failure, respectively.

Discussion

Sarcoidosis is a systemic disease which occurs all around the world and among all nations. It is considered as an infrequent disease but numerous studies in the last twenty years provided evidence that sarcoidosis is significantly more frequent than previously thought[8].

Sarcoidosis is the disease affecting mostly adults between 20 and 40 years of age. In literature we can find data on children affected by the disease, mostly between 9 and 15 years of age. The incidence in children below 15 years of age is between 0.06 and 1.02 per 100,000 children[9,10].

The highest number of patients treated in our hospital was between 20 and 40 years of age which is in accordance with the findings in the literature. According to numerous studies in Japan and Scandinavia an increase in incidence in women after the age of 50, i.e. »the second peak«, is repeatedly found[11]. The same was the case in our study for the age group between 50 and 54 years.

Sarcoidosis occurs in both sexes but according to the results of most of the studies it is more frequent in women. According to the published results the ratio of women to men in Japan is 3:1 and in Spain 2:1. In some countries sarcoidosis is almost equally frequent among both sexes[12]. Among patients treated at our hospital from 1997 until the end of 2002 the ratio of women to men was 1.4:1 which corresponds to our previously published results based on National Registry data[6].

Studies from Finland and Japan in biopsy-proven cases of sarcoidosis show relatively high proportion of extra-pulmonary sarcoidosis (45–53.4%) which is substantially different from our series of patients (22.3%). In 41.6% of our patients radiographic stage I was confirmed, stage II in 39.7%, stage III in 18.1%, and stage IV in 0.6% of cases. Studies in Finland showed similar results (43.7% stage I, 42.6% stage II, 13.3% stage III, and 0.4% stage IV)[13].

The proportion of patients presenting with erythema nodosum in our study was similar to the Finnish data (around 20%), as it was present in 18.7 % of cases[14]. Most often it occurs in the period from April till August (81.3%).

Many studies state more frequent occurrence of sarcoidosis from April to August and suggest some causative agent as trigger[15]. In England it is most frequent in the period from March to May, in Japan from April to August, in Spain from April to June and in Scandinavia from January to June[16]. Our results show similar pattern, even more pronounced in patients presenting with erythema nodosum.

Sarcoidosis has a variable incidence and prevalence depending on various environmental factors and numerous hypotheses about the causes of these differences have been suggested but none of them has been confirmed. One of them is that sarcoidosis is more frequent among people who are living or lived in childhood in rural households[17]. In our population, although it is more frequent among urban population, it might possibly be due to the significant migration of rural population to urban areas during the last war (1991–1995). According to data published by Đurić B.[18] in ex-Yugoslavia, more cases of sarcoidosis in urban areas were registered. This was explained by the fact that sarcoidosis was diagnosed more frequently in large medical centers.

According to numerous studies sarcoidosis is mostly the disease of non-smokers which was also confirmed among our patients[19]. Profession and social status are not predisposing factors for the occurrence of sarcoidosis.

According to epidemiological studies in Japan (region Furano on the island of Hokkaido) and Finland, sarcoidosis frequently occurs within the family. Studies in Finland show familial clustering in 3.6% of patients, Hokkaido 4.3% and in Japan in 8.7%[20]. Among patients treated in our hospital familial clustering was recognized in 2% of our patients.

The mortality among sarcoidosis patients is 2–4%. The cause of death usually is respiratory failure, arrhythmias due to sarcoidosis of the heart, renal failure due to nephrocalcinosis or sarcoidosis of central nervous system[21]. The mortality rate in our series was substantially lower (0.42%) probably due to the fact that it represents only the hospital mortality rate and not the global mortality rate for sarcoidosis.

Although these data are biased regarding the selection of patients and chance of determining the populations they arrive from, they give new insights into epidemiologic characteristics of sarcoidosis patients in Croatia. They fill up the gaps regarding our already published data on the subject.

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EPIDEMIOLOŠKE OSOBINE BOLESNIKA SA SARKOIDOZOM HOSPITALIZIRANIH U KLINICI ZA PLUĆNE BOLESTI »JORDANOVAC« (ZAGREB, HRVATSKA) U RAZDOBLJU OD 1997–2002. GODINE.

S A Ž E T A K

Cilj naše studije bio je istražiti osobine hospitaliziranih bolesnika sa sarkoidozom prema dobi, spolu, kliničkom obliku, stadiju proširenosti, sezonskom pojavljivanju, geografskoj rasprostranjenosti, navikama pušenja, profesiji, obiteljskoj povezanosti i smrtnosti. Uključena su 476 bolesnika sa sarkoidozom dokazanom biopsijom u Klinici za plućne bolesti »Jordanovac« u razdoblju od 1997.–2002. godine. Većina bolesnika (44%) bila je u dobi između 20–40 godine života. Omjer muškaraca i žena je bio 1.4:1. Znakovi bolesti su se kod 51% bolesnika pojavljivali u proljeće i ljeto, posebno kod bolesnika koji su imali nodozni eritem. Više je bolesnika iz gradske nego seoske sredine (1.5:1), a više je bilo nepušača nego pušača (3.3:1). Kod 2% bolesnika sa sarkoidozom utvrdili smo obiteljsku povezanost. Premda su ovi podaci bazirani na selekciji bolesnika daju nam novi pogled na osobine bolesnika sa sarkoidozom u Hrvatskoj.

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Original scientific paper

Esomeprazole Versus Pantoprazole for Healing Erosive Oesophagitis

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A B S T R A C T

The aim of this study was to compare the efficacy of esomeprazole and pantoprazole with regard to healing and relief from gastroesophageal reflux disease-related symptoms. I this multicentre, randomized, single-blind study 180 patients (ITT population) diagnosed with endoscopically proven GERD grade A,B,C received esomeprazole (40 mg once daily (o.d.), n=90) or pantoprazole (40 mg o.d., n=90). Healing and relief from GERD-related symptoms were assessed at first and final visit (after 4 or 8 weeks of treatment). Esomeprazole 40 mg provided significantly greater healing than pantoprazole 40 mg after 4 weeks of treatment in patients with EE (77.8% vs. 72.2%). Esomeprazole-treated patients were healed after up to 8 weeks of treatment similar those treated with pantoprazole (92.2% vs. 91.1%). The proportion of heartburn-free days was similar in patients treated with esomeprazole and to those treated with pantoprazole.

Key words: *gastroesophageal reflux disease, GERD, esomeprazole, pantoprazole*

Introduction

Gastroesophageal reflux disease (GERD) is an extremely common clinical problem accounting for a large proportion of physician visits regarding gastrointestinal problems.

GERD is associated with severe and frequently life-long symptoms that lead to a marked reduction in normal function and well-being[1]. Up to 50% of patients with chronic GERD develop erosive oesophagitis (EE)[2]. GERD and/or EE are associated with complications such as peptic stricture, bleeding and Barrett's oesophagus; the latter is a risk factor for oesophageal adenocarcinoma[3]. Patients with GERD have a diverse range of symptoms, the most common of which is heartburn[4]. The goals of management in patients with GERD are healing of EE, resolution of symptoms and prevention of complications[5]. The most effective and established drugs to inhibit gastric acid secretion are proton pump inhibitors (PPIs), which are nowadays recommended as the treatment of choice for GERD[6].

The aim of this study was to compare esomeprazole 40 mg with pantoprazole 40 mg for healing and symptom relief in patient with EE. The results presented here relate to the acute treatment phase of a management study.

Methods

Patients with EE were enrolled into this randomized, single blind, multi-centre study. The study was undertaken in accordance with the Declaration of Helsinki and with the prior approval of local ethics committees. Informed consent was obtained from all patients prior to study entry. The study was conducted at 3 centres in Croatia.

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Inclusion criteria included: history of GERD symptoms for at least 6 months immediately prior to enrolment, confirmed by endoscopy and graded using the LA grading system[7].

Exclusion criteria included: other significant upper gastrointestinal disorders (including Zollinger-Ellison syndrome, gastric or duodenal ulcer, oesophageal stricture, history of dysplasia in Barrett's oesophagus); intake of medication liable to affect the outcome of the study (including non-steroidal anti-inflammatory drugs); pregnancy, childbearing potential (unless taking suitable precautions) or lactation; alcohol and/or drug abuse; PPI use within 4 weeks prior to the first endoscopy.

At visit 1 (baseline) physical examination was carried out and the investigator assessed GERD symptoms. These symptoms were on a fourpoint severity scale: none, mild, moderate or severe. The number of days with symptoms of heartburn over the previous 7 days was also recorded. Patients were then randomized to receive esomeprazole 40 mg or pantoprazole 40 mg once daily for up to 8 weeks. Treatment compliance was determined by counting unused capsules at the end of the study. Patients taking 75–110% of prescribed doses were deemed to have been compliant with the dosing protocol.

At 4 weeks, patients underwent a further endoscopy and their GERD symptoms were assessed in the same way as at visit 1. Patients with unhealed EE and/or with moderate or severe heartburn or acid regurgitation in the prior 7 days, as assessed by investigators, continued treatment for a further 4 weeks, after which EE and GERD symptoms were re-assessed. From baseline to the 4-week visit, patients were instructed to record the severity of heartburn on daily diary cards. Heartburn was assessed using a four-graded scale: none, mild, moderate or severe. Adverse events were recorded on each control visit.

TABLE 1
BASELINE DEMOGRAPHIC AND CLINICAL CHARACTERISTICS OF THE INTENT-TO-TREAT POPULATION (180 PATIENTS TO TREATMENT WITH EITHER ESOMEPRAZOLE 40 mg OR PANTOPRAZOLE 40 mg)

Characteristics	Esomeprazole 40 mg (N=90) n (%)	Pantoprazole 40 mg (N=130) n (%)
Gender – male	57 (63.3%)	59 (65.6%)
Age (years)		
< 65	77 (85.6%)	79 (87.8%)
X (SD)	51.2 (14.5)	49.4 (13.9)
LA grade oesophagitis		
A	37 (41.1%)	35 (38.9%)
B	40 (44.4%)	39 (43.3%)
C	13 (14.4%)	16 (17.8%)
Barrett s oesophagus,		
Absent	84 (93.3%)	85 (94.4%)
Present	6 (6.7%)	5 (5.6%)
Helicobacter pylori status		
negative	68 (75.6%)	70 (77.8%)
positive	22 (24.4%)	20 (22.2%)

Statistical analyses

The study was planned to include 180 patients calculated by assuming 8 week healing rates of 96% and 92% for esomeprazole and pantoprazole, respectively, using a two-sides chi-squared test with 5% significance level and a power of 95%. The assumptions about healing rates for esomeprazole and pantoprazole were based on data from previous studies[8–12]. Time to sustained resolution of heartburn symptoms (defined as a period of seven consecutive days without heartburn) based on patient daily diary cards, with differences analysed by log-rank test and the difference between treatment groups for the proportion of heartburn-free days analysed by analysis of variance (ANOVA).

No formal statistical analysis was planned on adverse event reports.

Results

In total, 180 patients were randomised to treatment with either esomeprazole 40 mg or pantoprazole 40 mg. 2 patients were excluded from the intent-to-treat (ITT) population because of intake of an unknown study drug, and a further 2 patients because of study protocol violations. The baseline demographic and clinical characteristics of the ITT population are shown in Table 1. There were no clinically relevant differences between the two treatment groups. Overall treatment compliance rates were similar for the two treatment groups (esomeprazole 40 mg: 87.6%, pantoprazole 40 mg: 88.2%).

Esomeprazole 40 mg provided significantly greater healing than pantoprazole 40 mg after 4 weeks of treatment in patients with all grades of EE severity at baseline (Table 2).

Esomeprazole-treated patients were healed after up to 8 weeks of treatment similar those treated with pantoprazole. Healing rates at 8 weeks, by LA grade at baseline, are provided in Table 3.

TABLE 2
HEALING RATES OF EROSIVE OESOPHAGITIS (EE) AFTER 4 WEEKS TREATMENT WITH EITHER ESOMEPRAZOLE 40 mg OR PANTOPRAZOLE 40 mg BY BASELINE LOS ANGELES (LA) GRADE SEVERITY (INTENT-TO-TREAT POPULATION). χ^2 TEST (ESOMEPRAZOLE VS. PANTOPRAZOLE)

LA grade	Esomeprazole 40 mg n (%)	Pantoprazole 40 mg n (%)
A	31 (83.8%)	29 (82.8%)
B*	31 (77.5%)	28 (71.8%)
C**	8 (61.5%)	8 (50.0%)
All patients*	70 (77.8%)	65 (72.2%)

*p<0.05, **p<0.01

TABLE 3
HEALING RATES FOLLOWING UP TO 8 WEEKS TREATMENT WITH ESOMEPRAZOLE 40 mg (N=130) OR PANTOPRAZOLE 40 mg (N=130) BY BASELINE LOS ANGELES (LA) GRADE OF EROSIVE OESOPHAGITIS (EE) SEVERITY. χ^2 TEST (ESOMEPRAZOLE VS. PANTOPRAZOLE)

LA grade	Esomeprazole 40 mg n (%)	Pantoprazole 40 mg n (%)
A	35 (94.6%)	33 (94.3%)
B	38 (95.0%)	37 (94.9%)
C	10 (76.9%)	12 (75.0%)
All patients	83 (92.2%)	82 (91.1%)

The proportion of heartburn-free days was similar in patients treated with esomeprazole 40 mg and to those treated with pantoprazole 40 mg (mean values – esomeprazole: 70.2%; pantoprazole: 69.8%). Time to sustained heartburn resolution (the first of seven consecutive days with no heartburn) was equally short for patients treated with esomeprazole 40 mg and with pantoprazole 40 mg (median days – 6).

Safety

A total of 12% patients in the esomeprazole group and 11% in the pantoprazole group had adverse events. The most commonly reported of these were, in esomeprazole group, nausea, dizziness and headache. In the pantoprazole group, headache, diarrhoea and nausea.

All these adverse events were considered mild or moderate in intensity and none were considered treatment--related.

Discussion

Comparative studies with PPIs are relatively few, but some have shown advantages, albeit small, for esomeprazole and pantoprazole. In a study comparing esomeprazole (20 mg and 40 mg daily) with omeprazole (20 mg daily), both esomeprazole doses proved significantly superior to omeprazole in terms of oesophagitis healing after 8 weeks[13]. In terms of daytime symptom resolution, esomeprazole 40 mg was superior to both esomeprazole 20 mg and omeprazole 20 mg. However, in terms of night-time heartburn symptom relief both doses of esomeprazole were significantly better than omeprazole. Compared with lansoprazole (30 mg daily), esomeprazole (40 mg daily) proved superior in terms of both healing of oesophagitis and night-time symptom resolution[14].

In another comparative study, pantoprazole (40 mg daily) was compared with omeprazole (40 mg daily) in terms of healing of oesophagitis and symptom relief. No significant differences were noted between the two treatment groups. No distinction was made between daytime and night-time heartburn[15]. Pantoprazole (40 mg daily) was compared with lansoprazole (30 mg daily) and omeprazole (20 mg daily) in the resolution of heartburn symptoms. Both omeprazole and pantoprazole were superior to lansoprazole in the relief of heartburn symptoms[16]. Using continuous intra-gastric pH-metry, it was demonstrated that equal doses of pantoprazole and omeprazole have similar potency to inhibit gastric acid secretion[17,18].

In a study pantoprazole (40 mg daily) and esomeprazole (40 mg daily) have an equivalent effect on intra-oesophageal pH after repeated intake. Both drugs were safe well tolerated[19]. Gillessen et al.[20] and Scholten et al.[21] have reported similar effectiveness for esomeprazole 40 mg and pantoprazole 40 mg, or even greater effectiveness for latter drug in terms of speed of symptom resolution. In these studies however »GERD-related symptoms» included gastric complaints, feeling of satiety and flatulence. As these symptoms are not generally accepted as specifically related to GERD, and the studies lacked statistical power to detect differences between treatments, the studies added little information of the two treatments in resolving classical GERD symptoms.

Crossover studies in healthy subjects and patients with symptoms of GERD have shown that esomeprazole is more effective than all other PPI for providing greater time with pH>4[22,23]. The results of large comparative study demonstrate a therapeutic advantage of esomeprazole 40 mg over pantoprazole 40 mg for healing of EE and providing resolution of associated heartburn[12]. This result may be predicted, as healing of EE is inversely related to gastric acidity[24], and esomeprazole has been shown to provide greater suppression of gastric acidity than standard doses of all other PPIs[22].

The results of this study demonstrate a therapeutic advantage of esomeprazole 40 mg over pantoprazole 40 mg for providing healing of EE after 4 weeks, but not after 8 weeks.

The proportion of heartburn-free days was similar in patients treated with esomeprazole 40 mg and to those treated with pantoprazole 40 mg (mean values – esomeprazole: 70.2%; pantoprazole: 69.8%). Time to sustained heartburn resolution (the first of seven consecutive days with no heartburn) was equally short for patients treated with esomeprazole 40 mg and with pantoprazole 40 mg (median days – 6).

Treatment with esomeprazole and pantoprazole was well tolerated. Similar rates of adverse events occurred in both treatment groups.

In conclusion, the present study demonstrated that esomeprazole 40 mg provides more effective healing of EE than pantoprazole 40 mg after 4 weeks of treatment. But, after 8 weeks of treatment esomeprazole and pantoprazole 40 mg daily are equally effective in the treatment of GERD.

Similar rates of adverse events occurred in both treatment groups. Both study drugs were well tolerated, safe and had high patient compliance.

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ESOMEPRAZOL NASUPROT PANTOPRAZOLU U CIJELJENJU EROZIVNOG EZOFAGITISA

SAŽETAK

Cilj ovog rada je bio komparirati učinkovitost esomeprazola i pantoprazola u cijeljenju erozivnog ezofagitisa (EE) i nestanku simptoma gastroezofagealne refluksne bolesti (GERB). U ovu multicentričnu, randomiziranu, jednostruko slijepu studiju je bilo uključeno 180 bolesnika s endoskopski dijagnosticiranim GERB-om i dobivali su esomeprazol 40 mg/dan (90 bolesnika) ili pantoprazol 40 mg/dan (90 bolesnika). Nakon 4. i 8. tjedna terapije kontrolirani su radi dokaza cijeljenja EE i nestanka simptoma GERB-a. Esomeprazol bio je statistički značajno učinkovitiji od pantoprazola u cijeljenju EE nakon 4 tjedna liječenja (77.8% nasuprot 72.2%). Nakon 8 tjedana liječenja učinkovitost im je bila podjednaka (92.2% nasuprot 91.1%). Postotak bolesnika bez simptoma GERB-a je bio podjednak nakon 4. i 8. tjedna liječenja u obje skupine.

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Original scientific paper

Serum Concentration of Zinc, Copper, Manganese and Magnesium in Patients with Liver Cirrhosis

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A B S T R A C T

The role of trace elements in the pathogenesis of liver cirrhosis and its complications is still not clearly understood. Serum concentrations of zinc, copper, manganese and magnesium were determined in 105 patients with alcoholic liver cirrhosis and 50 healthy subjects by means of plasma sequential spectrophotometer. Serum concentrations of zinc were significantly lower (median 0.82 vs. 11.22 μmol/L, p<0.001) in patients with liver cirrhosis in comparison to controls. Serum concentrations of copper were significantly higher in patients with liver cirrhosis (median 21.56 vs. 13.09 μmol/L, p<0.001) as well as manganese (2.50 vs. 0.02 μmol/L, p<0.001). The concentration of magnesium was not significantly different between patients with liver cirrhosis and controls (0.94 vs. 0.88 mmol/L, p=0.132). There were no differences in the concentrations of zinc, copper, manganese and magnesium between male and female patients with liver cirrhosis. Only manganese concentration was significantly different between Child-Pugh groups (p=0.036). Zinc concentration was significantly lower in patients with hepatic encephalopathy in comparison to cirrhotic patients without encephalopathy (0.54 vs. 0.96 μmol/L, p=0.002). The correction of trace elements concentrations might have a beneficial effect on complications and maybe progression of liver cirrhosis. It would be recommendable to provide analysis of trace elements as a routine.

Key words: *zinc, copper, manganese, magnesium, liver cirrhosis, trace elements*

Introduction

The role of trace elements in pathogenesis of liver cirrhosis and its complications is still not clearly understood. In fibrogenesis the initial occurrence is hepato-cellular necrosis. In the early phase, inflammation cell products, proteinases and reactive oxygen radicals, may initiate hepato-cellular necrosis with consecutive releasing of numerous cytokines. Following hepatic injury, there is the increase in extracellular matrix, the activation of stellate cells, the increase in rough endoplasmatic reticulum and expression of smooth muscle specific α--actin[1]. Activated stellate cells are influenced by numerous cytokines. Some of them have proliferative effect on stellate cells while others stimulate fibrogenesis[2].

Zinc, copper, manganese and magnesium are essential trace elements whose role in liver cirrhosis and its complications is still a matter of research. There are contrary reports about their serum concentrations in patients with liver cirrhosis. Zinc is associated with more than 300 enzymatic systems[3]. Zinc augments the natural defense of reactive oxygen radicals by Zn-enzyme Cu-Zn superoxide dismutase[4]. Zinc acts as an antioxidant, a membrane and cytosceletal stabilizator, an anti-apoptotic agens, an important co-factor in DNA synthesis, an anti-inflammatory agens etc[5].

Copper is an essential trace element which participates in many enzymatic reactions. Its most important role copper has in redox processes. Reactive copper can participate in liver damage directly or indirectly, through Kupfer cell's stimulation[6]. Scientists agree that copper's toxic effects are related to oxidative stress[7].

Manganese is a structural part of arginase, which is an important enzyme in the urea metabolism. Manganese acts as an activator of numerous enzymes in Krebs cycle, particularly in the decarboxilation process.

Magnesium is important for the protein synthesis, enzyme activation, oxidative phosphorilation, renal potassium and hydrogen exchange etc.

Since zinc, copper, manganese and magnesium have a possible role in the pathogenesis of cirrhotic complica-

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tions**,** the aim of this study was to investigate the serum concentrations of mentioned trace elements in patients with liver cirrhosis and compared them with concentrations in controls.

Material and Methods

Subjects

The study included 105 patients with diagnosed liver cirrhosis of ethylic etiology who were hospitalized from 2000 to 2005 in the Division of Gastroenterology at Dubrava University Hospital, with median age 55 years. Seventy eight (74%) of them were male and twenty seven (26%) were female. According to the Child-Pugh classification patients with liver cirrhosis were divided in Child--Pugh A, B and C group. There were 35 subjects in every Child-Pugh group.

Inclusion criteria were liver cirrhosis (diagnosed by anamnestic data of alcohol consumption, laboratory and pathohistological findings, negative markers of viral hepatitis and normal values of ceruloplasmine), ability to sign the Informed consent and age 18 to 70.

Exclusion criteria were vegetarianism, Wilson's disease, malign disease, acute liver failure, impaired renal function (creatinine clearance <60 ml/min), multiorganic failure and inability to sign the Informed consent.

The control group consisted of 50 healthy subjects (median age 52 years) who were performed laboratory analysis as part of systematic medical examinations. There were 35 (70%) males and 15 (30%) females.

The Informed consent was obtained from all study subjects. The study protocol was approved by the Ethics Committee of Dubrava University Hospital. The protocol was carried out in accordance with the ethics guidelines of the Helsinki Declaration.

Methods

Blood samples were collected without anticoagulans and serum was stored in a freezer on –20°C until processing. In processing 1 ml of serum was taken, 1.5 ml of concentrated nitric acid and 0.5 ml 30% H_2O_2 were added on account of the digestion. After the digestion the sample was cooling for 20 minutes. The solution was transferred into a 10 ml container and was supplemented with ultra clean water. The concentrations of trace elements were determined by means of plasma sequential spectrophotometer TraceScan (Thermo Jarrell Ash, USA). Data were presented with median and 5–95 percentile range and compared using Wilcoxon and Kruskal-Wallis non--parametric tests. Statistics was done using MedCalc software (MedCalc Software, Mariakerke, Belgium). Only $p<0.05$ was considered significant.

Results

The serum levels of zinc, copper, manganese and magnesium in patients with liver cirrhosis and controls are presented in Table 1. The levels of zinc were significantly lower in patients with liver cirrhosis in comparison to controls (0.82 μmol/L vs. 11.22 μmol/L, $p<0.001$). The serum concentration of copper was significantly higher in patients with liver cirrhosis in comparison to controls (21.56 μmol/L vs. 13.09 μmol/L, $p<0.001$) as well as manganese concentration (2.50 μmol/L vs. 0.02 μmol/L, $p<0.001$). The concentration of magnesium was not significantly different between patients with liver cirrhosis and controls (Table 1, $p=0.132$). There were no differences in the concentrations of zinc, copper, manganese and magnesium between male and female patients with liver cirrhosis (Table 2).

The data in Table 3 show that the serum levels of manganese were significantly different between Child--Pugh groups ($H=9.21$, $p=0.036$). An additional analysis showed that the serum levels of manganese were significantly higher in patients with Child-Pugh C liver cirrhosis (6.30 μmol/L) in comparison to patients with Child--Pugh A (2.00 μmol/L, $z=–3.09$, $p=0.002$) and B liver cirrhosis (2.10 μmol/L, $z=–2.06$, $p=0.039$). The concentrations of zinc, copper, and magnesium did not differ significantly between Child-Pugh groups (Table 3).

The serum concentrations of zinc, copper, manganese and magnesium in cirrhotic patients with and without hepatic encephalopathy are represented in Table 4. The concentration of zinc was significantly lower in patients with hepatic encephalopathy in comparison to cirrhotic patients without encephalopathy (0.54 μmol/L vs. 0.96 μmol/L, $p=0.002$). There were no differences in serum concentrations of other trace elements between patients with or without encephalopathy. The serum concentrations of zinc, copper, manganese and magnesium in cirrhotic patients with and without ascites are represented

TABLE 1
SERUM CONCENTRATIONS OF ZINC, COPPER, MANGANESE AND MAGNESIUM IN PATIENTS WITH LIVER CIRRHOSIS AND CONTROLS

Trace elements	Subjects (N=105) median and 5–95 percentiles	Controls (N=50) median and 5–95 percentiles	Statistics	
			z	p
Zinc (μmol/L)	0.82 (0.24–1.74)	11.22 (9.23–15.10)	10.05	<0.001
Copper (μmol/L)	21.56 (11.17–30.60)	13.09 (11.17–19.95)	–7,66	<0.001
Manganese (μmol/L)	2.50 (0.01–29.65)	0.02 (0.01–0.40)	–8,21	<0.001
Magnesium (mmol/L)	0.94 (0.63–1.36)	0.88 (0.56–1.12)	–1.51	0.132

TABLE 2
SERUM CONCENTRATIONS OF ZINC, COPPER, MANGANESE AND MAGNESIUM IN MALE AND FEMALE PATIENTS WITH LIVER CIRRHOSIS

Trace elements	Male (N=78) median and 5–95 percentiles	Female (N=27) median and 5–95 percentiles	Statistics	
			z	p
Zinc (μmol/L)	0.84 (0.25–1.70)	0.74 (0.20–1.99)	–0.32	0.750
Copper (μmol/L)	21.18 (9.86–30.07)	23.56 (15.49–32.12)	1.58	0.113
Manganese (μmol/L)	2.10 (0.01–31.20)	3.70 (0.08–29.55)	1.45	0.146
Magnesium (mmol/L)	0.96 (0.58–1.40)	0.88 (0.71–1.36)	–1.05	0.293

TABLE 3
SERUM CONCENTRATIONS OF TRACE ELEMENTS IN CHILD-PUGH GROUPS

Trace elements	Child-Pugh A (N=35) median and 5–95 percentiles	Child-Pugh B (N=35) median and 5–95 percentiles	Child-Pugh C (N=35) median and 5–95 percentiles	Statistics	
				H	p
Zinc(μmol/L)	1.06 (0.38–1.49)	0.78 (0.26–1.94)	0.54 (0.14–1.45)	19.24	0.053
Copper (μmol/L)	19.98 (13.75–29.84)	22.30 (10.51–31.65)	23.20 (9.75–29.76)	1.00	0.608
Manganese (μmol/L)	2.00 (0.12–9.42)	2.10 (0.01–27.62)	6.30 (0.01–35.75)	9.21	0.036
Magnesium (mmol/L)	0.93 (0.65–1.18)	0.96 (0.65–1.38)	0.88 (0.40–1.53)	5.34	0.084

TABLE 4
SERUM CONCENTRATIONS OF TRACE ELEMENTS IN CIRRHOTIC PATIENTS WITH AND WITHOUT HEPATIC ENCEPHALOPATHY

Trace elements	Without encephalopathy (N=83) median and 5–95 percentiles	With encephalopathy (N=22) median and 5–95 percentiles	Statistics	
			z	p
Zinc (μmol/L)	0.96 (0.25–1.77)	0.54 (0.19–1.11)	–3.07	0.002
Copper (μmol/L)	21.56 (13.07–31.43)	21.31 (9.85–29.31)	–1.21	0.227
Manganese (μmol/L)	2.20 (0.01–31.38)	4.90 (0.01–26.94)	0.66	0.506
Magnesium (mmol/L)	0.95 (0.63–1.36)	0.90 (0.53–1.37)	–1.72	0.086

TABLE 5
SERUM CONCENTRATIONS OF TRACE ELEMENTS IN CIRRHOTIC PATIENTS WITH AND WITHOUT ASCITES

Trace elements	Without ascites (N=45) median and 5–95 percentiles	With ascites (N=60) median and 5–95 percentiles	Statistics	
			z	p
Zinc (μmol/L)	0.97 (0.36–1.57)	0.69 (0.18–1.78)	1.77	0.077
Copper (μmol/L)	20.25 (11.84–30.47)	22.42 (10.74–30.82)	–0.28	0.778
Manganese (μmol/L)	1.80 (0.01–11.20)	4.10 (0.01–31.80)	–3.43	<0.001
Magnesium (mmol/L)	0.92 (0.64–1.13)	0.94 (0.54–1.46)	–0.58	0.564

in Table 5. Only manganese concentration was significantly different between patients with and without ascites. Namely, serum manganese concentration was higher in cirrhotic patients with ascites in comparison to cirrhotic patients without ascites (4.10 μmol/L vs. 1.80 μmol/L, $p<0.001$).

Discussion

Mechanisms linked on ethanol metabolism, especially oxidative stress, redox potentials and acetaldehyde, participate in the emergence of liver damage. Trace elements play an important role in oxidative stress and redox potentials. A possible role of zinc, copper, manganese and magnesium in pathogenesis of liver cirrhosis and its complications is still subject of researches.

In our research the serum levels of zinc were significantly lower in patients with liver cirrhosis in comparison to controls (Table 1, median 0.82 μmol/L in patient with liver cirrhosis and 11.22 μmol/L in controls, $p<0.001$). The results confirm Kugelmans' research[8], who explained low zinc levels with low ingestion due to pro-

tein reluctance, increased loss in gastroenterological system due to diarrhea or intestinal malabsorption and increased urinary losses. The assumption is also based on the research of McClain[9] and Extremera[10]. Protein deficiency occurs frequently due to poor dietary intake. Our results confirm findings of decreased serum concentrations of zinc in patients with liver cirrhosis. Possible explanations for the decreased zinc levels in cirrhotic patients are mentioned above.

In Celik's research[11] the decrease in both serum and ascites zinc content was found in patients with liver cirrhosis. The interaction between zinc and copper in their intestinal absorption and their competition for binding sites on the carrier proteins and cellular uptake may be regulators of their homeostasis. Maybe this can explain inverse concentrations of zinc and copper**.** Zinc binds on albumin, transferrin and metalloproteins in the cell, so relative concentrations of these proteins might regulate the serum concentration of zinc[11,12].

The serum copper content was found significantly increased in patients with liver cirrhosis in comparison to the control group (Table 1, median 21.56 μmol/L in patient with liver cirrhosis and 13.09 μmol/L in controls, $p<0.001$). It could be explained with copper's role in the redox process. Redox cycling between Cu^{2+} and Cu^{1+} can catalyze the production of toxic hydroxyl radicals[13,14]. It is a well known fact that redox processes and oxidative stress play an important role in the pathogenesis of liver cirrhosis.

Serum concentrations of manganese were significantly higher in cirrhotic patients in comparison to controls (Table 1, median 2.50 μmol/L in cirrhotic patients and 0.02 μmol/L in controls, $p<0.001$). Higher serum levels of manganese in Krieger's research[15] as well as in research of Layrargues and co.[16] were also found in cirrhotic patients.

Moscarello[17] did not find any significant difference in the concentrations of manganese between cirrhotic patients and controls. After all, it seems that serum levels of manganese are higher in patients with liver cirrhosis than in healthy people. Manganese is secreted in bile so the concentration of manganese increases in cholestatic liver disease, which could be one of the possible explanations why manganese accumulation is common in liver cirrhosis[15,18].

It has been suggested that a possible mechanism responsible for manganese accumulation in the pallidum of patients include a decrease in biliary excretion and increased systemic availability due to portosystemic shunting.

Intrahepatic shunting or portosystemic shunting also have an additional effect on manganese accumulation. In the study of Rose et al.[19] pallidal manganese concentrations were the highest in shunted rats, which confirms that shunting is a major determinant of manganese accumulation in the brain. Manganese accumulation in the brain was confirmed by several clinical studies[15,19–21].

The difference between serum concentrations of magnesium in cirrhotic patients and controls was not significant (Table 1, median 0.94 mmol/L in cirrhotic patients and 0.88 mmol/L in controls, $p= 0.132$). Results are opposite to Kosch's research[22]. In that research serum levels of magnesium were lower in patients with liver cirrhosis in comparison to patients with liver steatosis and controls. In addition, the research of Rocchi[23] and Suzuki[24] confirmed the same. Our research did not confirm lower concentrations of magnesium in patients with liver cirrhosis. That partially could be explained with influence of spironolactone on magnesium levels. Namely, in Stergiou's research[25] spironolactone in health subjects decreased urine excretion of magnesium and in cirrhotic patients antagonized magnesiuric effect of furosemide. Our patients with liver cirrhosis mostly have spironolactone in their standard therapy, but there were no differences between patients who were taking spironolactone and those who were not taking spironolactone.

There was a slight decrease in serum zinc concentrations in patients with more severe clinical state of liver cirrhosis according to Child-Pugh classification but these differences in our research were not significant.

As zinc is bound to albumin in the serum, it has been thought that the serum zinc concentration would decrease with advancing grades of hepatic fibrosis[26]. Yoshida[27] found that patients with decompensated liver cirrhosis have lower levels of zinc than patients with compensated cirrhosis. However, in Hatano's research[26] serum zinc levels did not differ significantly between grades of hepatic fibrosis.

Copper levels in our research were similar in all three Child-Pugh groups (Table 3), as well as in Hatano's research.

Serum levels of manganese were higher in patients with Child-Pugh C liver cirrhosis in comparison to those in Child-Pugh A and B cirrhosis. Our results are contrary to Spahr's research[21] who found similar concentrations of manganese in all three Child-Pugh groups. It seems that manganese concentrations are higher in patients with severe liver cirrhosis possible due to the advanced intrahepatic and portosystemic shunting.

In our study magnesium levels were similar in all Child-Pugh groups. Moscarella's research[17] also confirms similar levels in compensated and decompensated liver cirrhosis. However, Wang[28] found that magnesium deficiency occurs more frequently in severe liver disease**.**

Significantly lower zinc levels were found in cirrhotic patients with hepatic encephalopathy (Table 4, median 0.54 μmol/L in patients with encephalopathy and 0.96 μmol/L without encephalopathy, $p= 0.002$), which was confirmed in other studies[29,30]. There are some findings that zinc supplementation can cause increased releasing of glutamine from sceletal muscle and also activate glutamine synthetasis, which can decrease the level of ammonia and improve hepatic encephalopathy[29]. That can be explained with the fact that zinc supplementation increases the hepatic activity of ornithine transcarbamoy-

lase, key enzyme of the urea cycle, which consecutively increases urea formation and decreases ammonia levels[30]. The rationale for use of zinc is also its ability to induce intestinal and hepatic metallothionein synthesis. Zinc decreases copper absorption by increasing the formation of Cu-metallothionein in intestinal epithelial cells[31]. However, Riggio found that short-term zinc supplementation has no influence on hepatic encephalopathy[32].

Considering all, zinc supplementation could have a positive influence on hepatic encephalopathy but before the implementation of this result in the treatment, further researches are necessary.

The levels of manganese were not significantly different between patients with liver cirrhosis and hepatic encephalopathy and patients without encephalopathy (Table 4, $p=0.506$), which is opposite to the researches of Hauser[20] and Krieger[15]. They found increased concentrations of manganese and suggested a beneficial effect of prevention of accumulation or decreasing manganese concentration in patients with liver cirrhosis. Rose[19] and Layrargues[16] found increased concentrations of manganese in basal ganglia of cirrhotic patients in comparison to controls.

Manganese concentrations in our research were significantly higher in cirrhotic patients with ascites in comparison to those without ascites (Table 5, median 4.10 μmol/L in patients with ascites and 1.80 μmol/L in patients without ascites, $p<0.001$). The levels of zinc, copper and magnesium were within reference range. Our results are contrary to the research of Pasqualetti[33] who found significantly lower magnesium concentrations in patients with ascites. Therefore, it is necessary to research the possible role of manganese in emergence of ascites in patients with liver cirrhosis.

Finally, decreased serum concentrations of zinc and increased levels of manganese in patients with liver cirrhosis could have an important role in the pathogenesis of liver cirrhosis and its complications, especially in hepatic encephalopathy. The supplementation of zinc could improve hepatic encephalopathy. The decrease in manganese levels could also have a beneficial effect on the neurological status in patients with liver cirrhosis and hepatic encephalopathy. Increased concentrations of manganese in cirrhotic patients with ascites inspire further researches about a possible role of manganese in the pathogenesis of ascites in patients with liver cirrhosis. Maybe, decreasing of manganese levels might also have beneficial effect on prevention or volume of ascites.

Considering all that, the correction of serum trace elements concentrations would have a beneficial effect on some complications of liver cirrhosis and maybe on progression of the disease, so it would be recommendable to provide laboratory analysis of trace elements as a routine.

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SERUMSKE KONCENTRACIJE CINKA, BAKRA, MANGANA I MAGNEZIJA U BOLESNIKA S JETRENOM CIROZOM

S A Ž E T A K

Cilj istraživanja bio je odrediti serumske koncentracije cinka, bakra, mangana i magnezija u bolesnika s jetrenom cirozom i usporediti ih s koncentracijama u zdravih ispitanika. Serumske koncentracije navedenih elemenata u tragovima bile su određivane u 105 bolesnika s jetrenom cirozom i 50 ispitanika kontrolne skupine pomoću plazma sekvencijskog spektrofotometra. Serumske koncentracije cinka bile su statistički značajno niže u bolesnika s jetrenom cirozom u odnosu na ispitanike kontrolne skupine (medijan 0.82 prema 11.22 μmol/L, $p<0.001$). Koncentracije bakra bile su značajno povišene u bolesnika s jetrenom cirozom u odnosu na kontrolnu skupinu (medijan 21.56 prema 13.09 μmol/L, $p<0.001$) kao i koncentracije mangana (medijan 2.50 prema 0.02 μmol/L, $p<0.001$). Koncentracije magnezija nisu bile značajno različite između bolesnika s jetrenom cirozom i ispitanika kontrolne skupine ($p=0.132$). Koncentracije cinka, bakra, mangana i magnezija nisu bile različite u muških i ženskih bolesnika s jetrenom cirozom. Serumske koncentracije mangana bile su statistički značajno različite između Child-Pughovih skupina ($p=0.036$), dok koncentracije cinka, bakra i magnezija nisu bile značajno različite između Child-Pughovih skupina. Značajno niže serumske koncentracije cinka pronađene su u bolesnika s jetrenom cirozom i portalnom encefalopatijom u usporedbi s bolesnicima bez portalne encefalopatije (0.54 prema 0.96 μmol/L, $p=0.002$). Korekcija koncentracija elemenata u tragovima mogla bi imati pozitivan učinak na komplikacije a moguće i tijek jetrene ciroze, stoga bi bilo preporučljivo u bolesnika s jetrenom cirozom određivati serumske koncentracije elemenata u tragovima kao dio rutinske laboratorijske obrade.

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The Place and Role of Serologic Methods in Detecting *Helicobacter Pylori* Infection

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A B S T R A C T

The aim of the study was to determine the place and role of serologic methods in detecting Helicobacter pylori *(*H. pylori*) infection, on the basis of estimated enzyme-linked immunosorbent assay (ELISA) and complement fixation test (CFT) sensitivity and specificity. A total of 549 patients were included in the study. ELISA and CFT as serologic methods were compared with invasive methods (rapid urease test – CLO test, culture, histology). The sensitivity of serologic methods was above 90%, and their specificity was around 80%. Study results confirmed the value, reliability and usefulness of serologic methods in the detection of* H. pylori *infection.*

Key words: H. pylori*, serology tests, sensitivity, specificity*

Introduction

Helicobacter pylori (*H. pylori*), a bacterium that marked the 20th century, is the most common etiologic factor of peptic ulcer, especially in duodenum[1–6]. It is associated with non-cardiac carcinoma of the stomach (diffuse and intestinal type)[7], and its association with some extraintestinal diseases has also been postulated[8–10]. Diagnostic methods for the detection of *H. pylori* infection are divided into two groups: invasive and noninvasive[11]. All invasive methods are based on endoscopy with biopsy samples of gastric mucosa obtained for direct (histology and culture) or indirect (rapid urease test) diagnosis.

Rapid urease test or CLO test has a sensitivity of 90–95% and specificity of 98%. In 90% of patients with negative CLO test gastric mucosa is usually unchanged. However, 5–10% of tested samples can be CLO negative because of inadequate number of the bacteria present in the sample[12–14].

Histology is a rapid, reliable and reproducible method. This method can also be used to determine the morphological characteristics of gastritis. The sensitivity and specificity of the method are around 95%[13,15,16]. Culture requires a gastric mucosa biopsy sample; however, at least two samples (antrum/corpus) are needed due to uneven colonization of gastric mucosa. This is particularly important on taking samples for the control of *H. pylori* eradication. The sensitivity of culture is 90–95% and specificity around 100%[13,17]. In addition to identifying the strain of *H. pylori,* molecular methods are used to determine the genes responsible for different factors of virulence[6,13].

Noninvasive methods are based on the detection of urease activity (urea breath test), presence of specific antibodies in serum and/or saliva of infected person (serology), and in recent time on antigen detection in stool.

Urea breath test detects the presence of *H. pylori* in stomach by detecting the *H. pylori* urease. This test has a high sensitivity and specificity (95–98% both)[13,18,19]. Urea breath test is usually used to prove *H. pylori* eradication at 4 weeks of antimicrobial therapy completion.

H. pylori induces inflammatory reactions in gastric mucosa, thus activating specific humoral immunity response, which in turn results in the production of specific IgM, IgA and IgG antibodies. Specific IgM antibodies are produced in a minority of infected persons. They are specific but difficult to detect. The sensitivity of tests for the detection of specific IgA antibodies, which are bound to the surface of the bacteria and prevent their adhesion to the cells, is 60–80%. Specific IgG antibodies, subclasses

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IgG1, IgG2 and IgG4, are most commonly present in the serum of infected individuals. The tests used for their detection have a high sensitivity (94%) and specificity (98%), and are most commonly used in the diagnosis of *H. pylori* infection. During the course of infection, the levels of antibodies are insignificantly changed[20,21]. Different serologic tests are used to detect *H. pylori* infection: agglutination, latex agglutination, passive hemagglutination, complement fixation test (CFT), enzyme-linked immunosorbent assay (ELISA) and immunoblot test[22]. Serologic diagnosis has a special place in epidemiological studies[23].

Since recently, immunoenzyme procedures have been used for direct detection of *H. pylori* antigen in stool sample. These procedures are used to detect active infection as well as its eradication. The procedure sensitivity is 80–90% and specificity around 100%[17–23].

The aim of this study was to determine the place and role of serologic methods in the diagnosis of *H. pylori* infection, on the basis of estimated ELISA and CFT sensitivity and specificity.

Materials and Methods

The study was performed during the 1994–2002 period at Merkur University Hospital and Prison Infirmary in Zagreb, and included 549 patients (Table 1): 436 patients (M/F 250/186, mean age 53.4 years) regularly attending Endoscopy Laboratory, Merkur University Hospital, and 113 patients (M/F 102/11, mean age 41.9 years) from Prison Infirmary. All patients suffered pain in the upper abdomen with dyspeptic symptoms. Prior to entering the study, the patients signed the informed consent form for gastroscopy. The study design was approved by the Hospital Ethics Committee.

TABLE 1
PATIENTS GENERAL CHARACTERISTICS

	N	Mean age (χ) (yrs)
Merkur University Hospital patients	436	53.4
Men	250	53.2
Women	186	54.1
Prison Infirmary patients	113	41.9
Men	102	42.2
Women	11	40.0
Total	549	51.8

Study patients underwent clinical examination and gastroscopy. During gastroscopy 7 histological samples of gastric mucosa were obtained (3 from the corpus and 4 from the antrum). One sample was taken for rapid urease test (CLO test, Delta West, Bentley, Western Australia), two samples were obtained for culture (Skirrow agar, Mueller-Hinton agar, E-test), and four samples for histology (Giemsa modified technique and Warthin-Starry stains; Sydney classification system of gastritis).

Patients were included into *H. pylori* positive group if the result of histology and urease test and in some cases of culture were positive for *H. pylori*. The *H. pylori* negative group included patients in whom histology, urease test and culture were negative. Histology[16], urease test[14] and culture for *H. pylori*[17] were done according to the previously described methodology. The patients who had been taking any kind of antibiotic therapy or a combination of antisecretory and antibiotic therapy for one month before endoscopy were excluded from the study.

Serum samples were tested with commercial ELISA (Eurospital, Trieste, Italy) and CFT (Institute Virion, Zurich, Switzerland). The tests were performed according to the manufacturer's instructions. Borderline test values were established in line with the manufacturer's instructions, to interpret the results obtained.

ELISA: each serum sample diluted 1:200 was applied onto a microtiter plate with previously bound *H. pylori* antigen. The antigen-antibody complex was proven by sheep antihuman IgG antibodies labeled with alkaline phosphatase and incubated with chromogen substrate. The substrate absorption was determined by ELISA reader (Multiscan, Titertek, MCC/340, Finland). An index of IgG antibodies equal or higher than 40% was considered as a positive result.

CFT: complement fixation antibodies (IgM, IgG) were proven by *H. pylori* strain Lior type 1. Each serum sample was diluted with a 1:10 Veronal buffer and incubated for 30 minutes at 56 °C to inactivate the complement present in the serum. Then serum sample as well as positive and negative serum controls were diluted from 1:10 to 1:160, edging certain dilution of antigen and complement. The test included controls to detect anticomplementary activity in each sample tested as well as control for the complement used (0.5, 1.0, 1.5 and 2.0 units of complement). The result of CFT was assessed on the basis of hemolysis inhibition. The inhibition of 50% or more was considered positive, indicating the presence of antibodies in the respective dilution. Antibody titer of less than 1:30 was considered negative.

To determine the specificity of the serologic methods used we tested sera of 227 patients with pain in the upper abdomen, free from dyspeptic symptoms and without *H. pylori* in the gastric mucosa biopsy samples (histology, rapid urease test, cultures were negative) (Table 2).

Statistics

The χ^2 test for dependent and independent samples, and the test of proportions were used. Statistical analysis was done by use of the Microstat software. Statistical significance was set at $p<0.05$.

Results

Sensitivity and specificity of ELISA and CFT

The sensitivity of ELISA and CFT was evaluated by testing serum samples of 276 patients with dyspeptic symptoms. Patients underwent gastroscopy, and *H. pylori* was detected in biopsy samples by culture, CLO test and histology. The sensitivity of serologic methods was above 90%, i.e. 94.9% for ELISA and 93.1% for CFT (Table 2).

The specificity of ELISA and CFT was assessed by testing serum samples of 227 patients free from dyspeptic symptoms and without *H. pylori* detected in biopsy samples of gastric mucosa (histology, rapid urease test, cultures were negative). The specificity of serologic methods was around 80%, i.e. 80.1% for ELISA and 78.4% for CFT (Table 2).

Evaluation of invasive and noninvasive serologic methods in patients with dyspeptic symptoms

On the basis of gastroscopy findings, 549 patients were divided into two groups: group 1 including patients without endoscopically verified ulcer and/or ulcer scar (168 patients with nonulcer dyspepsia), and group 2 including patients with ulcer and/or ulcer scar (381 patients).

In all patients, biopsy samples of gastric mucosa were tested for the presence of *H. pylori* (culture, CLO test, histology). Serum samples were tested by ELISA and CFT to detect specific antibodies against *H. pylori*. Results obtained in patient sera by use of invasive and noninvasive methods and their evaluation are shown in Table 3.

A statistically significant difference between ELISA and invasive methods was only recorded in the group of patients with ulcer (scar) (χ^2=6.45, p=0.09), however, only at a 90% level. Comparison of CFT and invasive methods showed no statistically significant difference in either group of patients (χ^2=6.02, ns). Comparison of ELISA and CFT results with the results of each individual invasive method produced a statistically significant difference in both groups of patients only between positive ELISA results and positive culture results (χ^2=4.57, p<0.05). Proportion testing showed a statistically higher number of *H. pylori* infection detected in the group with ulcer (scar) by both serologic and invasive methods: ELISA (Z=4.59, p<0.001), CFT (Z=5.70, p<0.001), histology (Z=3.09, p<0.001), rapid urease test (Z=2.7, p< 0.005) and culture (Z=5.23, p<0.001).

On analysis of overall results obtained by serologic and invasive methods (Table 3) using the test of proportions, there was no statistically significant difference between ELISA and CFT (Z=0.82, ns), or between ELISA and histology (Z=1.02, ns). However, ELISA showed a statistically significantly higher sensitivity than either urease test (Z=1.9, p<0.05) or culture (Z=7.27, p<0.001). CFT was statistically significantly more sensitive only compared with culture (Z=6.36, p<0.001), whereas the sensitivity of histology (Z=0.19, ns) and urease (Z=1.06, ns) yielded no statistically significant difference.

Discussion

A variety of methods have been used in the diagnosis of *H. pylori* infection. Most of the methods are invasive because they require gastroscopy to obtain biopsy samples of gastric mucosa for further analysis and detection of *H. pylori* infection. Culture is necessary to test for antimicrobial susceptibilities. The other group of methods are noninvasive because they do not require gastroscopy and *H. pylori* infection can be detected by the

TABLE 2
EVALUATION OF SENSITIVITY AND SPECIFICITY OF ELISA AND CFT

	N	ELISA		CFT		Sensitivity		Specificity	
		n +	n –	n +	n –	ELISA (%)	CFT (%)	ELISA (%)	CFT (%)
H. pylori (+)	276	262	14	257	19	94.9	93.1	–	–
H. pylori (–)	227	45	182	49	178	–	–	80.1	78.4

N – total number of tested patients, (+) – *H. pylori* positive patients, (–) – *H. pylori* negative patients, ELISA – enzyme-linked immunosorbent assay, CFT – complement fixation test

TABLE 3
COMPARISON OF ENDOSCOPY FINDINGS WITH RESULTS OF SEROLOGIC AND INVASIVE METHODS IN STUDY PATIENTS

Endoscopy finding	N	ELISA n (%)	CFT n (%)	Histology n (%)	CLO n (%)	Culture n (%)
Non-ulcer dyspepsia	168	142 (84.5)	127 (75.5)	134 (79.7)	126 (75.0)	65 (38.6)
Ulcer (scar)	381	365 (95.8)	354 (92.9)	341 (89.5)	323 (84.7)	244 (64.0)
Total	549	507 (92.3)$^{*/**}$	481 (87.6)***	475 (86.5)	449 (81.7)*	309 (56.2)$^{**/***}$

N – total number of patients, ELISA – enzyme-linked immunosorbent assay, CFT – complement fixation test, CLO – rapid urease test, *p<0.05, **p<0.001, ***p<0.001

presence of antibodies in serum samples (serology), by the presence of labeled CO_2 in exhaled breath upon ingestion of labeled urea, and by the bacterial urease activity (urea breath test).

ELISA is most widely used in the detection (qualitative) and measurement (quantitative) of the level of specific antibodies in serum samples. The previously used non-purified antigens have been replaced by purified products of urease and/or proteins of great molecular mass extracted from glycine. Immunoblot (Western blot) has recently been used as the method of choice for evaluation of immunity response against different *H. pylori* antigens (VacA, CagA). Antibodies against these antigens indicate an increased risk of ulcer and gastric adenocarcinoma, and are used as a confirmation test for the results obtained by other serologic methods[24–26]. ELISA detects the presence of individual classes of specific antibodies and can also determine the level of these antibodies in serum samples. ELISA tests for the detection of IgG antibodies have a more than 90% sensitivity and specificity[27]. The sensitivity of serologic methods used in the present study was more than 90%. The sensitivity of ELISA was 94.9%, exceeding the sensitivity of CFT of 93.1%. The specificity was slightly lower: 80.1% for ELISA and 78.4% for CFT, which is consistent with the results reported elsewhere for commercial serologic procedures[28,29].

The sensitivity and specificity are important parameters which show the purpose of using serologic methods in the diagnosis of *H. pylori* and evaluation of the methods employed. Different values of the sensitivity and specificity reported from various studies could be explained by the use of different normal values and »standard« methods. Some studies employed only one invasive method (culture, histology or rapid urease test) as a standard method, whereas others employed a combination of two or more methods. In our study, we chose histology, culture and rapid urease test as standard methods.

The sensitivity and specificity of the superior serologic tests are the same as the sensitivity and specificity of urea breath test[30]. The sensitivity of serologic tests is slightly higher than the sensitivity of invasive methods[31,32], as confirmed by our results. In the group of patients with ulcer and/or ulcer scar, a statistically significant difference was recorded in the detection of infection between ELISA (at 90% level) and invasive methods. The difference in sensitivity between serologic and invasive methods may be caused by difficulty in obtaining biopsy material due to the poorly visible site of *H. pylori* colonization on the gastric mucosa[32–36] and the effect of antibacterial therapy. Some authors[37,38] emphasize a disproportion between the grade of infection and the degree of immune response, pointing to inter-individual differences in the immune response to infection. There are literature reports on cases of *H. pylori* infection detected by invasive methods yet not accompanied by corresponding antibody levels, which results from a weak or absent response of the immune system[39,40]. In atrophic gastritis, serology may be the only tool to detect *H. pylori* infection[41–43]. In addition, invasive tests do not perform well in patients with bleeding ulcers[44–46]. It should be noted that the sensitivity and specificity of serologic methods are reduced in persons above 60 years of age as the result of weak immune response[47].

The incidence of *H. pylori* negative »nonspecific gastritis« is higher in the elderly, which may be due to the small number of bacteria present in gastric mucosa, previous infection treated with antibiotics, gastric mucosa atrophy, and gastritis of other etiology (autoimmune gastritis, prolonged therapy with nonsteroidal anti-inflammatory drugs). Like serologic methods, histologic methods also are less reliable in detecting *H. pylori* infection in the elderly. Tests for antibody detection in saliva samples have a lower sensitivity and specificity than tests for the detection of serum antibodies[48,49].

In spite of these shortcomings associated with serologic methods, simultaneous usage of a serologic method with one or more invasive methods will significantly increase the overall sensitivity of the diagnostic work-up. This is important in patients with clinical signs of severe infection and in those aged >45, who are at a higher risk of developing serious complications. Some authors suggest that patients younger than 45 without alarming symptoms can be screened for the presence of infection using only serologic methods[50]. Today, the recommendation is to use more methods for detecting *H. pylori* infection because all known methods yield some 5–10% of false positive or false negative results[51].

Our results showed the use of serologic methods in the detection of *H. pylori* infection (primary infection) with commercial CFT and ELISA tests to be helpful, reliable and fully justified. Commercial products were evaluated by testing the sera from a selected patient population. The sensitivity, specificity and reference values were determined, as they differ from population to population. Three standard methods, i.e. histology, culture and urease test, were used on evaluation of the serologic method sensitivity and specificity. The sensitivity of serologic methods exceeded 90%; however, ELISA showed higher sensitivity and specificity than CFT (94.9% *vs* 93.1% and 80.1% *vs* 78.4%, respectively).

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MJESTO I ULOGA SEROLOŠKIH METODA U UTVRĐIVANJU INFEKCIJE *HELICOBACTER PYLORI*

S A Ž E T A K

Cilj nam je bio na temelju utvrđenih vrijednosti osjetljivosti i specifičnosti metoda ELISA (imunoenzimski test) i reakcije vezanja komplementa odrediti mjesto i značenje seroloških metoda u otkrivanju infekcije *Helicobacter pylori (H. pylori)*. U ispitivanje je bilo uključeno 549 bolesnika, a navedene serološke metode su uspoređene s invazivnim metodama (CLO test, izolacija, histološki pregled). Osjetljivost seroloških metoda premašila je 90%, dok je specifičnost bila približno 80%. Ovim radom je dokazana vrijednost, pouzdanost i opravdanost uporabe seroloških postupaka u otkrivanju infekcije *H. pylori*.

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Duplex Sonography of Arteriovenous Fistula in Chronic Hemodialysis Patients

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A B S T R A C T

Duplex sonography was used to assess functional features of arteriovenous fistula (AVF) for hemodialysis (HD). Internal diameter (ID), resistance index (RI) and blood flow (BF) velocity in feeding artery and in vein of AVF, and venous BF volume were analyzed with purpose to determine the normal values. Presumed normal BF velocities are those of clinically well functioning shunts, allowing BF through HD lines of minimally 250 ml/min. Study included 66 nondiabetic HD patients (30 women, 36 men), mean age 52±13 years, treated by HD for median 61 (4–252) months. Measurements in 47 patients with clinically well functioning AVF were as followed: mean arterial ID 5.2±1.4 mm, median arterial RI 0.3 (0.3–0.9), median arterial BF velocity 1.5 (0.6–3.6) m/s, mean venous ID 7.6±2.2 mm, median venous RI 0.3 (0.3–0.9), mean venous BF velocity 1.6±0.7 m/s, and median venous BF volume 530 (120–1890) ml/min. Patients with poor functioning AVF had significantly less arterial ID, higher arterial RI, less venous ID, less venous BF velocity and volume. Duplex sonography findings obtained for clinically estimated well functioning shunt should be considered as normal Doppler values. Blood vessels' morphologic features depend upon age, and older patients have more pronounced changes.

Key words: *arteriovenous fistula, duplex sonography, blood flow, hemodialysis*

Introduction

Patients in need for chronic hemodialysis (HD) mostly undergo arteriovenous fistula (AVF) surgical creation. Native AVF is the most frequent form of vascular access for HD, and to date the optimal one. The communication is commonly established in forearm between radial artery and cephalic vein, usually on left (nondominant) arm[1]. Anastomosis can be terminoterminal (T-T) (the end of radial artery is connected with the end of cephalic vein), terminolateral (T-L) (end vein-to-side artery) and laterolateral (L-L) anastomosis. Communication between artery and vein can also be established by using artificial materials (polytetrafluoroethylene) and native grafts (Figure 1)[2–4].

Spectral analysis by duplex sonography technique shows that there are regular triphasic spectra in intact arteries in forearm. Hemodynamic changes occur following shunt creation between artery and vein, rendering

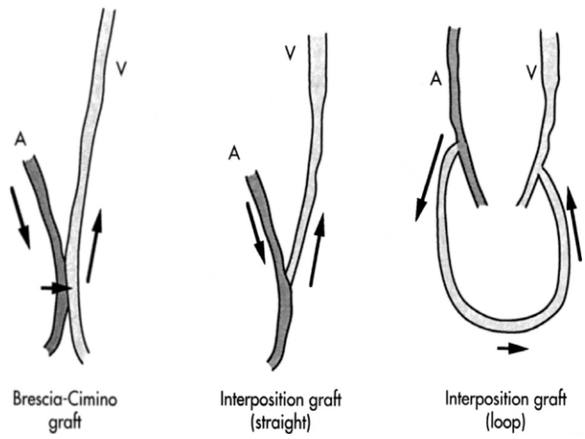

Fig. 1. Artificial communications between artery and vein.

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normal arterial triphasic spectra of high resistance to be replaced by low-resistant biphasic spectra (Figure 2 and 3)[5–8].

Complications associated with AVF include development of small and large aneurysms, pseudoaneurysms, stenoses, and total occlusion (thrombosis) of lumen at arterial or venous segment or of the shunt. The changes are more frequent in the venous segment; namely, more than 80% of all stenoses occur in the venous segment. Hemodialysis procedure requires certain flow volume in HD system. Minimum flow volume required is about 250 ml/min. Flow volume in AVF can be measured by various methods, such as duplex sonography, ultrasound dilution and magnetic resonance (MR). Analyzed values show great variability in flow volumes in the range of minimum 450± 214 ml/min to 2,131±565 ml/min according to various authors[13–16].

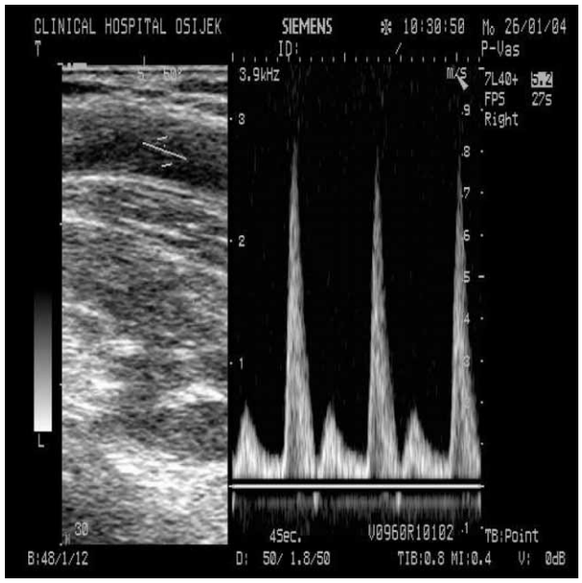

Fig. 2. Example of normal triphasic spectrum in arm or leg artery.

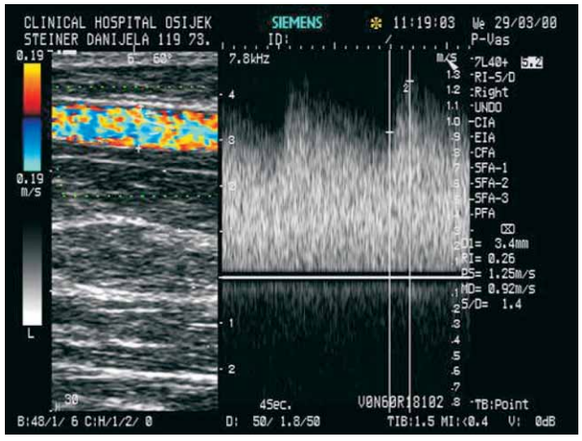

Fig. 3. Typical biphasic spectrum in arteriovenous fistula.

Duplex sonography still does not provide firm quantitative finding that could discriminate between well and poor function of the shunt. The aim of the study was to show the usefulness of the Doppler technique in imaging stenosis or imminent thrombosis, once clinical dysfunction occurs. The study should give the rationale for standardizing the procedure.

Materials and Methods

The study design was cross sectional. The research included 66 nondiabetic HD patients (37 men and 29 women), mean age 52±13 years, treated by HD for median time of 61 (4–252) months, with mean serum hemoglobin concentration 92±16 g/l. The patients gave informed consent for the participation in the study.

The patients underwent AVF examination by CD ultrasound prior to middle-week HD session. A greater proportion of patients had laterоterminal and the rest of the patients had terminoterminal AVF, but there was no reliable documentation regarding this issue. Examination was performed using Siemens Versa plus device with the probe at frequency of 7.5 MHz. The point of ultrasonographic measurements in feeding artery was the one immediately preceding the communication, and the point with less turbulence in the venous part proximal to the anastomosis. Artery was examined by producing images in B mode. Internal diameter (ID), resistance index (RI), and blood flow (BF) velocity in the artery and the vein, and venous BF volume were measured. Resistance index is calculated by formula RI=A-B/A, where A is the highest BF velocity during systole and B is the lowest diastolic BF velocity. Aneurysmatic changes and calcifications were recorded.

The sonographic data were analyzed in relation with achieved BF volume in HD lines, history of AVF thrombosis, erythropoietin treatment, smoking habits, findings of aneurysmatic changes and calcifications, age, HD duration and hemoglobin concentration. They were divided in the two subgroups: 49 patients with BF volume of at least 250 ml/min during HD in dialysis lines and 17 patients with less than that. The BF volume of at least 250 ml/min allowed optimal HD without clotting in lines under standard heparinization (from 3,500 to 5,500 UI per session). History of AVF thrombosis was taken from the patients' medical records. The AVF thrombosis was considered the accident that occurred after the AVF was used for HD. There were 32 patients without history of AVF thrombosis, 19 patients with 1, 8 patients with 2 and 7 patients with 3 thrombotic accidents of AVF. Twenty two patients received human recombinant erythropoietin treatment (from 2,000 to 8,000 UI weekly). Smoking habit was found in 17 patients.

Data were entered onto a personal computer. Statistical tests were performed using SPSS for Windows. Normal distribution was considered when skewness was less

than 1. Normally distributed variables were expressed as means±SD, and the others as median (range). For determination of difference between two independent samples Student's t-test was used as a parametric method, and Mann-Whitney U-test as nonparametric method. Parametric ANOVA and post hoc Scheffe's test and nonparametric Kruskal-Wallis H test accompanied by Mann-Whitney post hoc test were used for differences estimation between more than two independent variables. Pearson's r was calculated for determination of correlation between normally distributed variables, and nonparametric correlation was measured with the Kendall's tau-*b* coefficient. Chi- square test and Odds ratio were used for analysis of nominal variables. Results were considered significant at $p<0.05$.

Results

Values of sonographic measurements in all patients are presented in the table 1.

Arterial ID was significantly greater in the subgroup of patients with clinically well functioning AVF, their arterial RI was less, and venous ID, BF velocity and volume significantly greater (Table 2). Those subgroups did not differ in age neither in hemoglobin concentration.

According to the history of AVF thrombosis, the patients were divided in the 4 subgroups: without thrombosis (n=32), with 1 (n=19), with 2 (n=8), and with 3 thrombotic accidents (n=7). Differences in rheologic parameters were examined using ANOVA test (for arterial ID, venous ID and venous BF velocity) and Kruskal-Wallis H test (for arterial RI, arterial BF velocity, venous RI, and venous BF volume). The subgroups differed significantly in arterial ID ($F=3.443$, $p=0.022$) and venous BF volume (Chi-square=10.350, $p=0.016$), and not in other examined parameters. Post hoc Scheffe's test revealed that the difference in arterial ID was between the subgroup without and those with 1 thrombosis (Mean Difference=–1.143, $p=0.024$), and the same pair of subgroups was the one with significant difference in venous BF volume, as was found using post hoc Mann-Whitney test ($z=-3.186$, $p=0.001$). The subgroups based on the history of AVF thrombosis also differed significantly in age (ANOVA test, $F=6.979$, $p<0.001$) and in HD duration (Kruskal-Wallis H test, Chi-square=23.486, $p<0.001$). Post hoc Scheffe's test found the difference in age between subgroups with 1 and 2 thrombosis (Mean Difference=–17.38, $p=0.009$) and subgroups with 1 and 3 thrombosis (Mean Difference=–18.86, $p=0.006$). The difference in HD duration (post hoc Mann-Whitney test) was found between the subgroup without AVF thrombosis and with 1 AVF thrombosis ($z=-3.362$, $p=0.001$), with 2 ($z=-3.756$, $p<0.001$) and with 3 thrombotic accidents ($z=-2.820$, $p=0.005$). The subgroups did not differ in hemoglobin concentration.

Regarding the human recombinant erythropoietin substitution, 22 patients who underwent the treatment differed from those 44 untreated in arterial ID, RI, venous ID, RI and venous BF volume (Table 3). At the same time, they differed significantly in age ($t=2.463$, $p=0.012$), but neither in HD duration nor in hemoglobin concentration.

Subgroups with (n=17) and without (n=49) smoking habit differed significantly in arterial ID ($t=2.157$, $p=0.035$), and not in arterial RI, BF velocity, venous ID, RI, BF velocity and volume. The smokers were significantly younger ($t=-2.212$, $p=0.031$). The differences in HD duration and hemoglobin concentration between the subgroups were not significant.

TABLE 1
DOPLEX ULTRASONOGRAPHIC FINDINGS OF ARTERIOVENOUS FISTULA IN 66 CHRONIC HEMODIALYSIS PATIENTS

Parameter	Feeding artery*	Vein†
Internal diameter (mm)	5.0±1.3	7.1±2.1
Blood velocity (m/s)	1.5 (0.5–3.6)	1.6±0.7
Resistance index	0.4 (0.3–0.9)	0.4 (0.3–0.9)
Blood flow volume (ml/min)		500 (120–1,890)

* measured at the point proximal to the anastomosis
† measured at the point of minimal turbulence close to anastomosis

TABLE 2
COMPARISON OF DOPLEX ULTRASONOGRAPHIC FINDINGS OF ARTERIOVENOUS FISTULA BETWEEN TWO SUBGROUPS OF HEMODIALYSIS PATIENTS DIVIDED ACCORDING TO THE ACHIEVED BLOOD FLOW THROUGH HEMODIALYSIS LINES (N=66)

Parameter	Patients with sufficient blood flow* (n=47)	Patients with insufficient blood flow† (n=19)	Test value	p
Arterial internal diameter (mm)	5.2±1.4	4.4±0.8	t=2.436	0.018
Arterial blood velocity (m/s)	1.5 (0.6–3.6)	1.4 (0.5–2.2)	z=–1.655	0.098
Arterial resistance index	0.3 (0.3–0.9)	0.4 (0.3–0.9)	z=–2.400	0.016
Venous internal diameter (mm)	7.6±2.2	6.0±1.2	t=3.617	0.001
Venous blood velocity (m/s)	1.7±0.7	1.3±0.7	t=2.100	0.040
Venous resistance index	0.3 (0.3–0.9)	0.4 (0.3–0.9)	z=–1.230	0.219
Venous blood flow volume (ml/min)	530 (120–1,890)	320 (140–950)	z=–2.897	0.004

*blood flow through hemodialysis lines ≥ 250 ml/min
†blood flow through hemodialysis lines < 250 ml/min

TABLE 3
COMPARISON OF DOPLEX ULTRASONOGRAPHIC FINDINGS OF ARTERIOVENOUS FISTULA BETWEEN TWO SUBGROUPS OF HEMODIALYSIS PATIENTS DIVIDED ACCORDING TO HUMAN RECOMBINANT ERYTHROPOIETIN TREATMENT (N=66)

Parameter	Patients treated with erythropoietin (n=22)	Patients not treated with erythropoietin (n=44)	Test value	p
Arterial internal diameter (mm)	5.7±1.3	4.7±1.2	t=3.122	0.003
Arterial blood velocity (m/s)	1.5 (0.7–3.5)	1.5 (0.5–3.6)	z=–0.198	0.843
Arterial resistance index	0.3 (0.3–0.9)	0.4 (0.3–0.9)	z=–1.521	0.128
Venous internal diameter (mm)	8.1±2.0	6.6±2.0	t=2.861	0.006
Venous blood velocity (m/s)	1.7±0.8	1.6±0.7	t=0.874	0.385
Venous resistance index	0.3 (0.3–0.9)	0.4 (0.3–0.9)	z=–2.068	0.039
Venous blood flow volume (ml/min)	735 (210–1,760)	400 (120–1,890)	z=–2.823	0.005

TABLE 4
COMPARISON OF DOPLEX ULTRASONOGRAPHIC FINDINGS OF ARTERIOVENOUS FISTULA BETWEEN TWO SUBGROUPS OF HEMODIALYSIS PATIENTS DIVIDED ACCORDING TO PRESENCE OF CALCIFICATIONS (N=66)

Parameter	Patients with calcifications (n=12)	Patients without calcifications (n=54)	Test value	p
Arterial internal diameter (mm)	6.0±1.4	4.8±1.2	t=3.067	0.003
Arterial blood velocity (m/s)	1.6 (0.7–3.5)	1.5 (0.5–3.6)	z=–0.584	0.560
Arterial resistance index	0.4 (0.3–0.9)	0.4 (0.3–0.9)	z=–0.042	0.967
Venous internal diameter (mm)	8.3±2.2	6.9±2.0	t=2.161	0.034
Venous blood velocity (m/s)	1.7±0.8	1.6±0.7	t=0.732	0.467
Venous resistance index	0.3 (0.3–0.9)	0.4 (0.3–0.9)	z=–0.476	0.634

Aneurysmatic changes in AVF were found in 12 patients. They did not differ from those free of the finding (n=54) in arterial ID, RI, BF velocity, venous ID, RI and BF velocity, while the difference in venous BF volume was significantly different (z=–2.478, p=0–013, Mann-Whitney test). The two subgroups did not differ in age, HD duration and serum hemoglobin concentration.

Calcifications were found in AVF of 12 patients. They significantly differed from the 54 patients without the finding in arterial ID, venous ID and venous BFV (Table 4). The two subgroups were similar in age and serum hemoglobin concentration. The patients with calcifications were treated by HD for median 85 (42–169) months, while the subgroup without calcifications spent 55 (4–252) months under the treatment (z=–2.083, p=0.037, Mann-Whitney test).

Age was found to be significantly related to the most examined ultrasonographic features. Age was in negative correlation with arterial ID, venous ID and venous BFV, and in positive correlation with venous RI (Table 5). Age was not related to HD duration. Age and serum hemoglobin concentration were in significant negative correlation (r=–0.244, p=0.048).

Table 6 presents bivariate correlations between the examined sonographic parameters. All except three pairs of variables (arterial ID and arterial BV, arterial BV and venous ID and arterial BV and venous RI) were significantly correlated.

TABLE 5
CORRELATION BETWEEN DOPLEX ULTRASONOGRAPHIC FINDINGS OF ARTERIOVENOUS FISTULA AND AGE IN 66 HEMODIALYSIS PATIENTS

Parameter in correlation with age	Correlation coefficient*	p
Arterial internal diameter	r=–0.351	0.004
Arterial blood velocity	τ=0.045	0.605
Arterial resistance index	τ=0.138	0.117
Venous internal diameter	r=–0.327	0.007
Venous blood velocity	r=–0.163	0.191
Venous resistance index	τ=0.271	0.002
Venous blood flow volume	τ=–0.184	0.033

*r – Pearson correlation coefficient, τ – Kendall's Tau-b

Chi- square and Odds ratio were calculated to assess if smoking, aneurysmatic changes, calcifications and insufficient BF through HD lines each increases the risk for the other one. Smokers were not at increased risk for calcifications, aneurysmatic changes and insufficient BF through HD lines. Patients with calcifications were not at increased risk for aneurysmatic changes and for insufficient BF through HD lines. Patients with insufficient BF through HD lines were not at increased risk for aneurysmatic changes and calcifications.

TABLE 6
BIVARIATE CORRELATION BETWEEN DOPLEX ULTRASONOGRAPHIC FINDINGS OF ARTERIOVENOUS FISTULA IN 66 HEMODIALYSIS PATIENTS

Parameters in correlation	Correlation coefficient*	p
Arterial internal diameter and arterial blood velocity	τ=–0.070	0.939
Arterial internal diameter and arterial resistance index	τ=–0.213	0.026
Arterial internal diameter and venous internal diameter	r=0.740	<0.001
Arterial internal diameter and venous blood velocity	r=–0.347	0.004
Arterial internal diameter and venous resistance index	τ=0.420	<0.001
Arterial internal diameter and venous blood flow volume	τ=0.662	<0.001
Arterial blood velocity and arterial resistance index	τ=–0.204	0.021
Arterial blood velocity and venous internal diameter	τ=0.102	0.265
Arterial blood velocity and venous blood velocity	τ=0.272	0.002
parArterial blood velocity and venous resistance index	τ=0.126	0.155
Arterial blood velocity and venous blood flow volume	τ=0.289	0.001
Arterial resistance index and venous internal diameter	τ=0.251	0.006
Arterial resistance index and venous blood velocity	τ=0.313	<0.001
Arterial resistance index and venous resistance index	τ=0.535	<0.001
Arterial resistance index and venous blood flow volume	τ=0.172	0.047
Venous internal diameter and venous blood velocity	r=0.338	0.006
Venous internal diameter and venous resistance index	τ=0.304	0.001
Venous internal diameter and venous blood flow volume	τ=0.498	<0.001
lmult1Venous blood velocity and venous resistance index	τ=0.351	<0.001
Venous blood velocity and venous blood flow volume	τ=0.222	0.011
Venous blood velocity and venous blood flow volume	τ=0.338	<0.001

*r – Pearson correlation coefficient, τ – Kendall's Tau-b

Discussion

The sonographic findings obtained for the patients with achieved sufficient blood flow through HD system differed significantly from those measured in AVF of patients with suboptimal blood flow during HD. These results indicate that limits of normal standard values range at the level of the findings obtained for the well functioning AVF. Our findings were close to those found by Malovrh in AVF measured 12 weeks after surgical creation and irrespective of function in the terms of our criteria[21].

Certain examined sonographic features of AVF differed significantly between the groups of patients divided according to the history of AVF thrombosis. The found differences referred to the feeding artery internal diameter and blood flow volume through the venous limb of AVF, between the group without history of AVF thrombosis and the group with a single accident. Presumably, patients with thrombosis might be older and such differences could be ascribable to the age, but the groups did not differ in age, so the other reasons should be considered. They could be a consequence of an already impaired circulation, while the second AVF were mostly created at the same forearm as the first, now clotted shunt.

Those patients with repeated AVF thrombosis mostly have currently functioning fistula at the forearm other than the one with clotted shunts. That fact explains the absence of sonographic difference between the group without AVF thrombosis and the group with history of repeated thrombosis.

Subgroups of patients according to the erythropoietin treatment did not differ in the observed sonographic parameters. They also did not differ in hemoglobin values, i.e., the treated group had well corrected anemia due to the treatment itself, while the untreated group, in fact, had no reason to be treated regarding a good hemoglobin level. Consequently, the two subgroups did not differ in blood viscosity that could alter AVF blood flow velocity. There was a significant difference in internal arterial diameter between those subgroups that could be explained by a significant difference in age. Smokers were found larger arterial diameter than nonsmokers, but the smokers were significantly younger than nonsmokers.

Patients with aneurysmatic changes of AVF did not have significant differences in diameters, RI nor blood flow velocity through AVF at the points outside the aneurysmatic area, as it was expected. Calcifications were found irrespective of patient's age, but occurred more often in those treated with HD for a longer time. Patients with calcifications had larger AVF vessels' di-

ameters as well as greater blood flow volumes through AVF. Therefore, the examined rheologic features do not reflect vessel wall consistence and normal values of the measured parameters do not exclude serious pathology of vessel wall. Thorough examination of AVF quality should include sonographic vessel wall assessment and native x-ray examination of the extremity.

Correlations between age and the examined sonographic features of AVF confirmed the already alleged association of age at one side and vessel diameter and blood flow at the other side. Therefore, older patients are expected to have *a priori* less functional AVF due to narrower vessel diameters and higher RI. An enlarged flow volume from artery into the vein through the shunt produces high pressure onto venous wall. However, older people are not expected to stretch and dilate veins sufficiently under that pressure. These sonographic and statistic results quantitatively confirm clinically well known difficulties with vascular approach for HD in older patients.

It would be useful to perform duplex sonography examination before surgery of AVF construction, and to predict success or failure based on the sonographic finding. Such assessment would be really relevant if standard values of the examined parameters could be determined. Malovrh et al. found predictive role of preoperative CD features of artery and vein of future AVF for time needed for AVF to dilate enough to achieve sufficient blood flow. Significant correlations between venous blood flow volume and all the other examined sonographic parameters indicate that final AVF function depends on the arterial and the venous features, and also both the diameters and RI in their walls. Beside the need to establish referent values of the examined sonographic parameters, the examination technique should be also standardized, especially the points of measurements of the arterial and the venous part of AVF. Small mistakes in luminal measurement result in great misestimating of blood flow volume in AVF.

Conclusion

Doplex sonography should be used as a useful tool for the evaluation of arteriovenous fistula function in hemodialysis patients. However, normal values of sonographic parameters remain to be standardized. Sonographic features obtained for well functioning AVF could be used by creating standards for the method.

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DUPLEKS DOPLER ULTRAZVUK ARTERIJSKOVENSKIH FISTULA U BOLESNIKA NA HEMODIJALIZI

SAŽETAK

Funkcijske osobine arterijskovenskih fistula (AVF) za hemodijalizu ispitivane su obojenim dupleks dopler ultrazvukom s ciljem utvrđivanja normalnih doplerskih vrijednosti. Određivani su unutarnji promjer, indeks otpora i brzina krvnog protoka u arteriji i veni AVF, te volumen protoka krvi kroz njezin venski dio. Normalnim vrijednostima smatrane su one koje su dobivene kod bolesnika koji ostvaruju protok krvi kroz sustav za HD od najmanje 250 mL/min. U

studiji je ispitano 66 bolesnika (30 žena i 36 muškaraca) liječenih hemodijalizom (medijan duljine liječenja 61 mjesec, od 4 do 25) koji nemaju šećernu bolest, prosječne dobi 52±13 godina.. U 47 bolesnika s dobrom funkcijom AVF nađen je prosječan arterijski unutarnji promjer ID 5.2±1.4 mm, medijan arterijskog indeksa otpora 0.3 (0.3–0.9), medijan brzine krvnog protoka kroz arteriju 1.5 (0.6–3.6) m/s, prosječni venski unutarnji promjer 7.6 ±2.2 mm, medijan venskog indeksa otpora 0.3 (0.3–0.9), prosječna brzina krvnog protoka kroz venu 1.6±0.7 m/s, i medijan volumena krvnog protoka kroz venski dio AVF 530 (120–1890) ml/min. Bolesnici s nezadovoljavajućom funkcijom AVF imali su značajno manji unutarnji arterijski promjer, veći arterijski indeks otpora, manji venski unutarnji promjer te manju brzinu i volumen krvnog protoka kroz venski dio AVF. Vrijednosti nalaza dobivenih obojenim dopler ultrazvukom za bolesnike s klinički procijenjenom dobrom funkcijom AVF može se smatrati približnom razinom prema kojoj će se odrediti normalne vrijednosti. Osobitosti krvnih žila bile su značajno ovisne o dobi, boljih vrijednosti u mlađih bolesnika.

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Original scientific paper

The Supply of Blood in the Skin Territory Above the Lower Part of the Serratus Anterior Muscle

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A B S T R A C T

At present, the putative clinical use of the musculocutaneous and ostomusculocutaneous serratus anterior flaps has been compromised by the risk of partial or total necrosis of the skin overlying the lower part of the serratus anterior muscle. Therefore, the aim of this study was to delineate a skin area vascularized by perforant musculocutaneous branches of arteries stemming from the lower segment of the anterior serrated muscle. Black ink was injected in thoracodorsal artery branches for the serratus anterior muscle in 50 human cadavers before the autopsies (the study was approved by the Institutional Review Board). The surface area of the labeled skin was determined and its borders delineated by means of transparent millimeter grid. Planimetry data were subsequently analyzed with the aid of PC computer program. The results show that the calculated mean surface area (143.79±2.68 × 2.077; range 138.22–149.36 cm^2) of the skin vascularized by perforant musculocuaneous branches stemming from the lower segment of the anterior serrated muscle, can serve as a reliable guide for taking serratus anterior flap in any patient. Therefore, appropriately sized musculocutaneous or osteomusculocutaneous serratus anterior flap can be safely and efficiently used in plastic and reconstructive surgery.

Key words: *serratus anterior flap, skin island, musculocutaneous perforators, microsurgery*

Introduction

The surgeons' desire to use the serratus anterior muscle in reconstructive surgical procedures as much as possible is limited because the area of the skin that can be lifted together with the muscle and/or the muscle and the bone segment, the front part of the ribs, in the process of shaping the serratus anterior flap, is unknown[1–15].

The same problem is mentioned in all the studies that describe the use of serratus anterior flap in plastic and reconstructive surgery. Due to frequent partial or complete necrosis of the skin island over the lifted muscle, the serratus anterior flap is most commonly used as a muscle or muscle-bone graft without the accompanying skin cover[16–20].

According to the research conducted by Taylor and Palmer the vascular territory of the skin over the serratus anterior muscle is formed as a juncture of the angiosomes of the lateral thoracic, thoracodorsal and the internal thoracic artery. The exact area of the skin that receives blood by way of the musculocutaneous perforators of the lower part of the serratus anterior muscle is unknown[4–15].

It was the above mentioned anatomical and clinical reasons that prompted us to conduct an anatomical research of the blood supply of this specific territory of the skin.

Materials and Methods

Our research was conducted at the Institute of Anatomy »Drago Perović«, the Institute of General Pathology and Pathological Anatomy of the Medical School at the University of Zagreb and the Department for Plastic and Reconstructive Surgery at the University Clinic of Surgery, University Hospital Center Zagreb.

The research was conducted on 50 cadavers prior to the autopsy with the approval of the Ethics Commission of the Medical School.

The research consisted of isolating the thoracodorsal artery and vein, injecting ink into the thoracodorsal artery above the branching point for the lower part of the serratus anterior muscle, and measuring the colored

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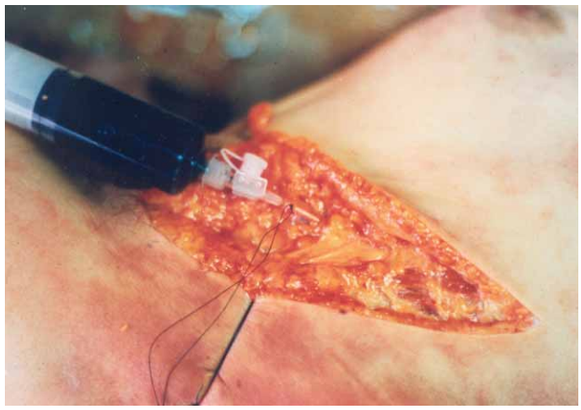

Fig. 1. Injection of Indian ink into the thoracodorsal artery.

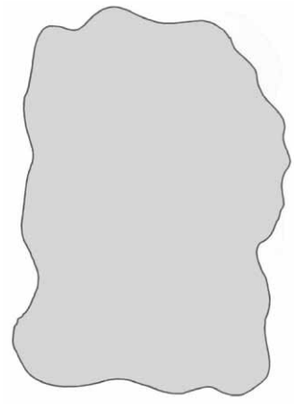

Fig. 3. Computer analyzed colored skin area.

area of the skin vascularized by the musculocutaneous perforators of the lower part of the serratus anterior muscle (Figure 1).

After the cadaver was placed laterally on the autopsy table and the brachium was completely abducted with the front margin of the latissimus dorsi muscle visualized, a 10 centimeter incision was made three centimeters from the center of the skin fold of the armpit along the front margin of the latissimus dorsi muscle, and the thoracodorsal artery and vein, as well as the accompanying nerve, were visualized. Using microsurgical instruments the above mentioned vascular structures were isolated and the metal clamp placed on the thoracodorsal artery branch for the latissimus dorsi muscle. The thoracodorsal artery was cut four centimeters above the branching point for the lower part of the serratus anterior muscle, a one millimeter-inner-diameter plastic tube attached to syringe was inserted in the artery and 40 milliliters of black ink were injected. Once the coloration was observed, the area of the skin that receives blood by way of the musculocutaneous perforators of the lower part of the serratus anterior muscle was measured (Figure 2). The maximum skin area showing coloration was established once no change occurred following additional injection of black ink, that is, the size of the already colored area of the skin did not change. Transparent graph paper was than placed on the skin area showing coloration and the exact area delineated. After the procedure was completed, the plastic tube was removed and the incision closed with intradermal suture.

The results obtained on 50 graph paper sheets were computer analyzed and the area of the colored skin calculated employing the computer-assisted planimetric method (Figure 3). Microsoft Excel 4.0.a. was used for statistical analysis of the data.

Results

The results of our research are shown in Figure 4. The statistical data analysis showed the largest and the smallest measured skin area of skin showing coloration in the sample, the range, the arithmetic mean, the standard error in the arithmetic mean, the median, the standard deviation, and the probability coefficient.

The largest measured area of skin showing coloration in the sample was 211.40 cm^2, and the smallest 131.83 cm^2. The range in the sample was 79.60, the arithmetic

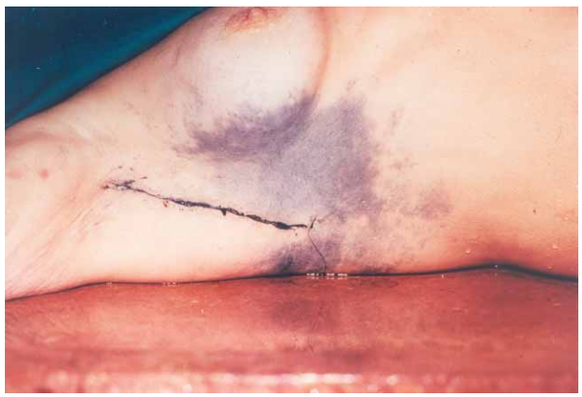

Fig. 2. Coloration of skin area following injection of Indian ink.

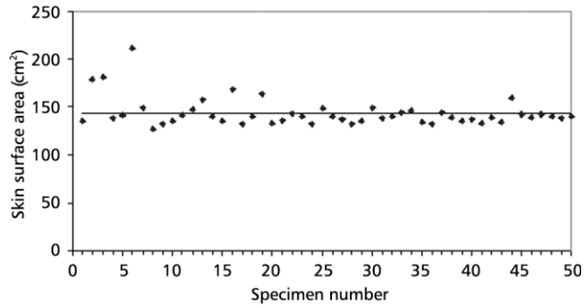

Fig. 4. Skin surface area for each specimen with arithmetic mean.

mean 143.79, the standard error in the arithmetic mean 2.077, the median 139.90, the standard deviation 14.70, and the probability coefficient 10.21.

The analysis of the results obtained by the statistical analysis shows that there is 99 percent probability of the mean value of the area of the skin that receives blood by way of the musculocutaneous perforators of the lower part of the serratus anterior muscle being within 143.79-+/–2.68 x 2.077 interval, that is, between 138.22 cm^2 and 149.36 cm^2.

Discussion

In our research, we applied the injection method to show the extent of blood supply in the skin as did Taylor, McGregor, Cormack, Lamberty, Rees, Timmons and Corlett[1–16]. The injected ink travels through the artery supplying blood to a certain area through musculocutaneous or fasciocutaneous perforators to the skin, just as blood does, and shows coloration on the skin surface. In this way, the area of the skin to which blood is supplied by the feeding artery or by perforators becomes visible and is easily interpreted. The above mentioned studies report various types of colorants that can be used in skin perfusion studies, such as black, dark blue and red ink, methylene blue and Indian ink[1–16.]. In our research preceding this study we used Indian ink, methylene blue and black ink. In the process of injecting Indian ink, we noted powerful resistance to injection, which we attributed to the greater density of the Indian ink in comparison to the density of the other two colorants.

Although the coloration of the skin cover was satisfactory, we had to apply greater force while injecting because of the mentioned resistance, tearing the fragile artery wall in two cases, and damaging the inner layer of the blood vessel due to rough handling of the syringe to which the plastic tube was attached in three cases.

Methylene blue traveled through the blood vessels without any resistance, but the coloration of the skin was rather pale (light blue), providing no guarantee that the area of the skin supplied by blood will be clearly shown. Black ink proved to be the most satisfactory and was therefore chosen as the basic colorant for showing the extent of blood supply in the skin in our study, as Taylor, Palmer and Cormack also used in their research[4–14].

While injecting, no resistance was noted, which facilitated gentle operation with the syringe, and the color that appeared on the surface of the skin was intense enough to make it possible for us to clearly delineate the colored area, which is the area of the skin supplied by blood.

We used 40 milliliters per sample, the same quantity used by Taylor, Cormack, Lamberty and Palmer[4–14].

In order to ensure that 40 milliliters of colorant were sufficient to show the area of the skin supplied by blood over the lower segment of the serratus anterior muscle, in our preliminary research we injected the same quantity of black ink twice to observe whether the area of skin showing coloration would increase. We noticed no change in the area of the skin showing coloration in any of the twenty samples in our preliminary experiments following the second injection.

Following our research standard autopsy was conducted on each of the cadavers. While opening the thoracic wall we found black ink in the intercostal arteries, the internal mammary artery and the internal thoracic artery, which are the blood vessels that anastomose with the system of the thoracodorsal artery supplying blood to the lower part of serratus anterior muscle.

This finding clearly shows that the colorant travels in the direction of the lowest resistance, following into all the branches of the arteries that anastomose with the branches of the artery into which the colorant had been injected, through so-called choke connections through the border between the two neighboring skin territories. [6–11]. In measuring the area of the skin showing coloration on the cadavers, we employed the reliable planimetric method, as described in literature, with the aid of transparent millimeter graph paper which we pressed onto the colored area[4–11].

The computer-processed diagrams, that is, the drawings on the millimeter graph paper, show a black line tracing the outline of a gray irregular plane figure. The inner margin of the black line represents the border of the area of the skin showing coloration. By adding up the square centimeters (millimeters) on the transparent millimeter graph paper it is possible to accurately determine the area.

The computer-processed drawings of the areas obtained facilitate a more plastic description of the researched skin area, that is, an acceptable graphical representation of the skin area. We would particularly like to emphasize that the computer planimetric method facilitates accurate delineation of the colored area and hence accurate determination of the irregular plane figures.

Figure 4 contains the values of the areas of the skin below the lower part of the serratus anterior muscle showing coloration following the injection of black ink in the thoracodorsal artery branch for the lower part of the serratus anterior muscle. The area of skin showing coloration shows the territory of the skin to which blood is supplied by the musculocutaneous perforators of the lower part of the serratus anterior muscle. The largest measured area was 211.40 cm^2, and the smallest 131.83 cm^2. The arithmetic mean in the sample was 143.79.

In seven cases the measured area of the skin showing coloration following the injection of the colorant was considerably larger (178.42 cm^2, 180.55 cm^2, 211.40 cm^2, 157.56 cm^2, 157.56 cm^2, 168.33 cm^2, 159.41 cm^2) than the areas of the skin over the lower part of the serratus anterior muscle in other specimens. A possible explanation for this phenomenon may lay in the fact that all these seven cases belonged to a group of cadavers younger then 40 years, and all seven had prominent osteomuscular constitution.

It is a well-known fact that exercise or great physical strain result in muscle hypertrophy which leads to an increase in the muscle vascular system due to increased metabolic needs of the muscle[13–15]. This increase in the number of musculocutaneous perforators in the hypertophic musculature with the resulting increase of the area of the skin supplied by blood was also described by Taylor and Palmer in their study of the angiosomes in the human body[8,15].

During our research, low values of measured areas, between 85 cm^2 and 105 cm^2 in size, were recorded on several cadavers who were affected by diabetes and generalized arteriosclerosis. Having checked the case histories of each of the subjects, we excluded those who had suffered from these chronic diseases from our research. In this selection we were guided by the postulate that a compromised state of vascular system, which is the case in diabetes as well as arteriosclerosis, presents a drawback in the reconstructive surgery procedures involving free flaps[16–20].

The area of the skin that can be lifted together with the other components of the serratus anterior flap, taking into account even the smallest measured skin area in our sample, which is 131.83 cm^2, represents the size of the of the skin cover sufficient for reconstruction of defects on the face neck, hand and foot.

The statistical data analysis in our research shows that there is 99 percent probability of the mean value of the area of the skin to which blood is supplied by the musculocutaneous perforators of the lower part of the serratus anterior muscle being within the 143.79±2.68 x 2.077 interval, that is, between 138.22 and 149.36 cm^2.

The results of our research show the surface of the skin area to which blood is supplied by the musculocutaneous perforators of the lower part of the serratus anterior muscle, measured for the first time.

This anatomical research facilitates the forming of a musculocutaneous and/or osteomusculocutaneous serratus anterior flap with the skin cover adequately supplied by blood.

Conclusion

The results of this study show the size of the area of the skin that receives blood by way of the musculocutaneous perforators of the lower part of the serratus anterior muscle. Considering the most frequent clinical indications for the use of the serratus anterior flap – reconstruction of defects on the face, hand and foot – the measured areas fully correspond to the required skin cover size.

The research results show a balanced distribution of the musculocutaneous perforators, which facilitates safe clinical application.

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PROKRVLJENOST KOŽNOG PODRUČJA IZNAD DONJEG SEGMENTA MIŠIĆA SERRATUS ANTERIOR

S A Ž E T A K

Svrha ovog istraživanja je prikaz površine kožnog područja koje je prokrvljeno muskulokutanim perforatorima donjeg segmenta mišića serratus anteriora. Istraživanje prokrvljenosti kožnog područja izvršeno je na pedeset svježih leševa, primjenom injekcijske metode. Za prikaz površine prokrvljenog kožnog područja upotrebljena je crna tinta koja je injicirana u arteriju torakodorzalis, odnosno njene ogranke za donji segment mišića serratus anteriora. Mjerenje površine obojene kože, odnosno područja prokrvljenog muskulokutanim perforatorima izvršeno je prislanjanjem prozirnog milimetarskog papira na područje obojene kože i crtanjem granice tog područja. Računanje površine obojenog kožnog područja učinjeno je upotrebom planimetrijske metode uz pomoć osobnog računala, scannera i odgovarajućeg programa za izračun površina. Statistička obrada podataka o mjerenjima u našem istraživanju pokazuje da se u populaciji sa 99% vjerojatnošću može očekivati srednja vrijednost površine kože prokrvljene muskulokutanim perforatorima donjeg segmenta mišića serratus anterior u intervalu 143.79 ± 2.68 x (2.077), odnosno između 138.22 i 149.36 cm^2. Rezultat našeg istraživanja omogućuje kliničku primjenu muskulokutanog i/ili osteomuskulokutanog režnja serratus anterior u plastično-rekonstruktivnoj kirurgiji, što do sada nije bilo moguće uslijed nepoznavanja površine kože koja se zajedno s mišićem može odignuti, odnosno zbog opasnosti od djelomične ili potpune nekroze prileţečeg kožnog segmenta.

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Association of Two Genetic Variations of Lipoprotein Lipase, S447X and Hind III, with Coronary Artery Disease and Hypertriglyceridemia

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A B S T R A C T

This study was performed to assess the effect of the S447X and Hind III lipoprotein lipase gene polymorphisms on development of coronary artery disease and hypertriglyceridemia. The study included 132 patients and 98 healthy control subjects of Croatian descent. The lipoprotein lipase S447X polymorphism was associated with coronary artery disease and hypertriglyceridemia, as indicated by the lower frequency of S447 allele in the patient group (p=0.005) and odds ratio (O.R=0.40, p=0.006). The patient and control groups also showed a significant difference in the distribution of Hind III/S447X genotype combinations (p=0.013). There were no significant associations with lipid parameters for any genotype or genotype combination in the patient group. Frequencies of the S447X polymorphism and S447X/Hind III combinations differed between the CAD/TG and control group, thus these polymorphisms may be associated with CAD and hypertriglyceridemia.

Key words: *coronary artery disease, hypertriglyceridemia, lipoprotein lipase, polymorphism, Croatia*

Introduction

Lipoprotein lipase (LPL) hydrolyzes the triacylglycerol component of chylomicrons and very low-density lipoproteins (VLDL), and indirectly participates in the reverse transport of cholesterol[1]. Abnormal LPL expression takes part in some pathophysiological processes, which include chylomicronemia, atherosclerosis, obesity, diabetes, etc.[2] It is also well known that hypertriglyceridemia itself can be a risk factor for the development of coronary artery disease (CAD), as well as of its lowering effect on the levels of high-density lipoprotein cholesterol (HDL-C)[3]. The genetic background in the interaction with many other factors determines some changes of the phenotype characteristics and influences some epidemiological events[4]. Several common genetic variants in LPL gene with different epidemiological effects have been reported[5–7]. Summary statistics of multiple studies have yielded statistically significant effects of some LPL polymorphisms on the development of CAD. These include a twofold increase in CAD risk associated with the lipoprotein lipase D9N and/or the –93T to G polymorphism, a marginal increase in CAD risk for N291S allele-S carriers among individuals from six different studies, and association of S447X allele-X carriers with a decrease in CAD in four different studies[7]. Polymorphisms affecting the noncoding region of LPL gene are also implicated in the development of hypertriglyceridemia and/or CAD. The most common and most widely investigated are the Pvu II and Hind III polymorphisms[6,8].

Over the last several years, many researchers have investigated allelic distribution of different LPL gene variants and their effect on lipid profiles in various patient populations. In a previous study, we also investigated the polymorphisms associated with CAD (–93T/G, D9N, N291S and S447X)[9]. Recent studies have demonstrated that LPL447X mutation was associated with higher postheparin LPL activity in patients[10]. The S447X and Hind III polymorphisms of the LPL gene represent two variant sites that are within 600 bp of each other in the gene[11]. It is also well known that these two polymorphisms are in the strong linkage disequilibrium[11]. Some European studies showed a favorable effect of the 447X allele on lipid traits, while some of them did not find any significant influence on lipid parameters[12]. The EARS

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study showed that H-X447 haplotype was associated with significantly lower concentrations of plasma triglycerides (TG)[11].

The aim of our study was to explore the possible effects of these two polymorphisms and their combinations in CAD patients with elevated TG levels (CAD/TG group). We therefore compared the frequencies of the S447X and Hind III polymorphisms individually and the frequencies of S447X/Hind III genotype combinations between the CAD/TG and control groups. We have also investigated the association between these two polymorphisms and levels of plasma lipids.

Materials and Methods

Study subjects

Study subjects were recruited among patients who underwent coronarography at Magdalena Specialized Hospital for Cardiovascular Surgery and Cardiology in Krapinske Toplice near Zagreb. The blood samples were collected from 2001 to 2004. The subjects were divided into two groups based on coronarography findings and TG concentrations. The CAD/TG group included 132 patients with at least 50% stenosis of any of the major coronary arteries and TG levels greater than 2.2 mmol/L (98 males, median age 59, range 34–82; and 34 females, median age 61.5, range 38–73). The control group included 98 subjects with <10% stenosis of major coronary arteries, TG levels lower than 2.0 mmol/L, and without evidence of any chronic disease (60 males, median age 52.5, range 18–76; and 38 females, median age 61, range 26–76). Patients who had a family history of acute or chronical pancreatitis were excluded from the study because of known relationships between changes in LPL activities and pancreatitis[6]. We used WHO criteria to define diabetes in both study groups[13]. We have also excluded patients with extremely high lipid concentrations, because they would complicate statistic analysis. Patients gave an informed consent to participate in the study, which was approved by the Ethics Committee of Magdalena Specialized Hospital for Cardiovascular Surgery and Cardiology in Krapinske Toplice and of the Zagreb University School of Medicine.

Determination of plasma lipid and lipoprotein subclass concentrations

Blood samples were collected after an overnight fast. The concentrations of total cholesterol (TC) and TG were measured by standard enzymatic methods on an Olympus AU-640 (Olympus, Tokyo, Japan). HDL-C was determined by selective precipitation (Immuno AG, Vienna, Austria), and also measured on an Olympus AU-640. Low density lipoprotein cholesterol (LDL-C) was calculated using Friedewald equation[14]. If TG concentration was greater than 3.0 mmol/l, HDL-C was measured by direct immunoinhibition method (Olympus Diagnostica GmbH, Lismeehan, Ireland), and LDL-C by homogeneous assay (Randox Laboratories, Crumlin, United Kingdom). Apolipoproteins (apo) A–I and B were measured by the nephelometric method (Dade Behring, Marburg, Germany).

PCR amplification and RFLP analysis

DNA was extracted from peripheral whole blood by the salting out method[15]. Polymerase chain reaction (PCR) for exon 9 of LPL gene was performed using primers previously described by Monsalve et al[16]. The reactions were performed in a DNA thermocycler (Eppendorf Mastercycler 3350, Hamburg, Germany), as described elsewhere[17]. S447X polymorphism was genotyped by digestion of exon 9 with Hinf I restriction endonuclease (Roche, Mannheim, Germany). Hind III locus was genotyped using Eppendorf Mastercycler 3350 and oligonucleotides, PCR and restriction conditions as previously described[17,18].

Statistical analysis

Statistical analysis was performed by use of StatSoft, Inc. (2003) STATISTICA (data analysis software system) version 6.1, and MedCalc 4.10 (Frank Schoonjans, Mariakerke, Belgium). Genotype frequencies were calculated by counting. Group comparisons of categorical variables were performed using Pearson's χ^2 or Fisher's exact test. Hardy-Weinberg equilibrium was tested by χ^2-test. Odds ratios were calculated for assessment of association between LPL gene polymorphisms and CAD. All continuous data were expressed as X±SD. Differences between the two groups studied were evaluated by Student's t-test or Mann-Whitney test, depending on distribution normality. Multivariate analysis (MANOVA) with age, gender and diabetes as covariates were used for testing of the effects of LPL gene polymorphisms on lipid parameters. TG values were not normally distributed and were therefore log-transformed. The level of significance was set at 0.05.

Results

The characteristics and lipid parameters of the two groups are presented in Table 1. There were more males, diabetics and smokers in the CAD/TG group. HDL-C was significantly lower and LDL-C higher in the CAD/TG group.

Table 2 shows genotype frequencies in the two study groups. The observed genotypes were in Hardy-Weinberg equilibrium for all the genetic variants examined. Carriers of the 447X allele were more frequent in the control group, while there was no significant differences between groups in genotype frequencies for the Hind III polymorphism. The odds ratio for the association of S447X polymorphism with CAD and hypertriglyceridemia (O.R.) was 0.40 (p=0.006). The frequencies of genotype combinations for the S447X and Hind III polymorphisms are also presented in Table 2. There are nine possible combinations but only six of them were observed. There was a statistically significant difference between the CAD/TG and control group in the distribution of different Hind III/S447X genotype combinations. As

TABLE 1
CHARACTERISTICS OF CAD/TG AND CONTROL GROUP[a]

Parameter	CAD/TG group (n=132)	Control group (n=98)	Significance, p
Age, years (range)	59 (34–82)	56.5 (18–76)	
Sex (male), n (%)	98 (74.2)	60 (61.2)	0.035
Hypertension, n (%)	68 (51.5)	51 (52.0)	0.937
Diabetes, n (%)	28 (21.2)	3 (3.1)	<0.001
Smoking, n (%)	44 (33.3)	22 (22.4)	0.071
Glucose, mmol/L (X±SD)	6.61±3.26	5.19±1.26	<0.001
TC, mmol/L (X±SD)	6.1±1.6	5.33±1.05	0.414
TG, mmol/L (X±SD)	3.09±0.85	1.24±0.35	Not done[b]
HDL-C, mmol/L (X±SD)	0.95±0.24	1.21±0.33	<0.001
LDL-C, mmol/L (X±SD)	3.98±1.27	3.35±0.90	0.002
Apo A-I, g/L (X±SD)	1.26±0.29	1.43±0.34	0.161
Apo B, g/L (X±SD)	1.25±0.31	1.02±0.25	0.244

[a] Differences between groups were evaluated by Student's t-test or Mann-Whitney test, depending on distribution normality.
[b] TG values were used to define the CAD/TG group (TG >2.2 mmol/l) and control groups

hypertriglyceridemia can be a consequence of diabetes, we examined the effect of excluding diabetic subjects. Without diabetic subjects, the S447X genotype frequencies, as well as the Hind III/S447X genotype combination frequencies were still significantly different between the CAD/TG and control groups.

To explore possible mechanisms for this protective effect, the associations between LPL genotypes and genotype combinations and some plasma lipid traits (TC, TG, HDL-C, LDL-C, apo A–I and apo B) were examined in the CAD/TG group. The results are presented in table 3. Due to the low frequency of the H-H– genotype, the H-H– and H-H+ genotypes were pooled together as a single subgroup (H–&+) and compared with the subgroup consisting of the H+H+ genotype. There was no significant genotype effect on lipid parameters for any of the genotypes or genotype combination.

Discussion

When investigating possible LPL genotype effects, one has to take into account that the LPL enzyme can have both pro- and anti-atherogenic roles[1]. The main role of the LPL is its catalytic activity that includes hydroly-

TABLE 2
FREQUENCIES OF GENOTYPES AND GENOTYPES COMBINATIONS IN CAD/TG AND CONTROL GROUP

Genotype	CAD/TG group	Control group	p	Odds ratio (95% C.I.)	p	Odds ratio (95% C.I.)
			Diabetics included		Diabetics excluded	
S447X				0.40		0.43
SS, n (%)	113 (85.6)	69 (70.4)		(0.21, 0.77)		(0.21, 0.85)
SX, n (%)+ XX, n (%)	19 (14.4)+0 (0)	28 (28.6)+1 (1)	0.005	p=0.006	0.015	p=0.016
Hind III						
H-H–, n (%) H-H+,	8 (6.1)+46 (34.8)	6 (6.1)+45 (45.9)		1.57		1.49
n (%)H+H+, n (%)	78 (59.1)	47 (48.0)	0.084	(0.93, 2.65)	0.163	(0.85, 2.61)
				p=0.094		p=0.164
Hind III/S447X						
H-H–/SS, n (%)	6 (4.5)	0 (0)				
H-H–/SX, n (%)	28 (21.2)	22 (22.4)				
H-H–/XX, n (%)	79 (59.8)	47 (48.0)	0.013		0.050	
H-H+/SS, n (%)	2 (1.5)	5 (5.1)				
H-H+/SX, n (%)	17 (12.9)	23 (23.5)				
H+H+/SS, n (%)	0 (0)	1 (1.0)				

[a] Comparison of study groups were performed by using Pearson's χ^2 or Fisher's* exact test.

TABLE 3
LIPID PARAMETERS ACCORDING TO GENOTYPES IN CAD/TG GROUP

	TC X±SD (mmol/L)	TG X±SD (mmol/L)	HDL-C X±SD (mmol/L)	LDL-C X±SD (mmol/L)	Apo A–I X±SD (g/L)	Apo B X±SD (g/L)
Hind III genotype						
H–&+	6.38±1.18	3.10±081	1.00±0.25	4.25±1.25	1.29±0.29	1.31±0.31
H+	5.92±1.32	3.07±0.87	0.93±0.22	3.79±1.26	1.24±0.29	1.21±0.31
Significance, p	0.813	0.813	0.176	0.080	0.063	0.065
S447X						
SS	6.05±1.30	3.09±0.84	0.96±0.23	3.92±1.23	1.27±0.29	1.25±0.31
SX	6.44±1.13	3.10±0.92	0.96±0.30	4.37±1.29	1.23±0.26	1.29±0.32
Significance, p	0.136	0.913	0.427	0.137	0.075	0.142
Genotype combinations						
SS/– –	6.30±0.57	2.72±0.50	1.04±0.13	4.50±0.88	1.33±0.24	1.33±0.15
SS/–+	6.36±1.32	3.19±0.78	1.02±0.24	4.14±1.30	1.33±0.31	1.32±0.34
SS/++	5.92±1.32	3.08±0.87	0.93±0.22	3.79±1.26	1.24±0.29	1.21±0.31
SX/– –	6.45±0.64	3.20±1.03	1.14±0.20	4.65±1.06	1.40±0.11	0.94±0.25
SX/–+	6.44±1.19	3.08±0.94	0.94±0.30	4.34±1.34	1.21±0.27	1.32±0.31
Significance, p	0.262	0.817	0.336	0.241	0.155	0.087

[a] Differences between groups were evaluated by multivariante analysis and TGs were log-transformed before testing.
[b]P-values were calculated after adjustment for age, sex and diabetes.

sis of TGs from TG-rich lipoproteins. This main catalytic activity is involved in antiatherogenic effects. Namely, diminishing TGs in blood can limit reduction of HDL-cholesterol mediated by cholesteryl ester transfer protein[19]. This leads to low HDL-C concentrations as reported in hypertriglyceridemic humans[1]. We have also reported on inverse correlations between concentrations of HDL-C and TGs[20]. In contrast to the anti-atherogenic role, pro-atherogenic effects have also been well defined in some animal models[21]. In the vessel wall, LPL activity may be associated with lipoprotein retention because of foam cell formation[22]. As a consequence of its transferase activity, LPL possibly induces cholesteryl ester accretion in smooth muscle cells during atherogenesis[21]. The anti-atherogenic role is mostly due to plasma LPL, whereas the pro-atherogenic role is mediated by LPL from vessel wall epithelial cells and macrophages.

The present study investigated the S447X and Hind III lipoprotein lipase polymorphisms and lipid profiles in patients with CAD and TGs greater than 2.2 mmol/l relative to control subjects. These two different polymorphisms proved relevant and comparable with many similar studies. It is well known that lowering of LPL activity can influence the development and progression of atherosclerosis[1]. Diabetes mellitus may be secondary cause of hypertriglyceridemia[23], because individuals with impaired glucose tolerance and with diabetes mellitus have a slower plasma reduction of TGs[24]. Consequently, we have calculated and analyzed the frequencies between the CAD and the control group with and without diabetics to exclude the influence of hyperglycemia. Many studies report on a decrease in CAD risk in individuals carrying 447X-allele, in European and American populations, as well as Japanese[7,25]. The frequency of the X447 gene variant was also significantly lower in over than 1,300 myocardial infarction survivors than in the same number of the control subjects from the Central Valley of Costa Rica[26]. We similarly observed a significantly lower frequency of 447X-carriers among those with CAD.

Studies of *in vitro* expression showed both increased or unchanged activity and mass of LPL-X447 relative to S447[12] and studies of *in vivo* activity yield inadequate data for any general conclusion[12]. Many lipid association studies indicated a lowering effect on TGs and/or higher HDL-C concentrations in X447 carriers[5, 27–31]; therefore this beneficial LPL 447-X gene variant was considered for a gene therapy investigation[32]. Other recent studies, which include English[33] and Welsh[34] populations, did not indicate significant changes of TGs or HDL-C. These latter findings are in agreement with our study results. A lower LPL-X447 allele frequency was found in the LPL deficient cohort compared to Quebec population-based cohort, suggesting that beneficial LPL-X447 allele in combinations with some mutant alleles do not provide protective effect against the risk to develop CAD[35]. The HERITAGE family study showed that the relationships between the X447 allele and beneficial lipid profile were observed in obese but not in normal-weight subject[36] probably due to a higher LPL activity in obese people. The association of LPL gene mutations with CAD appeared to be exacerbated by the presence of additional risk factors, gene-gene, gene-environmental interactions

and lifestyle habits. Those facts, as well as inclusion criteria in the design of our study, such as CAD+hypertriglyceridemia, may modify the effect of polymorphisms on the lipid traits. Therefore we were unable to show expected benefits on lipid profiles such as lower TG concentrations.

The LPL Hind III polymorphism may affect RNA splicing because lies in intron eight, 495 bp from the splice-donor site[6]. It has been proposed that the H-allele of the Hind III polymorphism acts as a genetic marker for a functional mutation that could cause either enhanced enzyme activity or more efficient lipid binding[11]. It is in strong linkage disequilibrium with S447X polymorphism[11,12]. Therefore S447X and Hind III LPL polymorphisms are candidates that might be involved together in the development of CAD and hypertriglyceridema. We have also documented in this paper that almost all Croatian subjects with the Hind III H+H+ genotype had the 447SS genotype. This was comparable with the results from the European Atherosclerosis Research Study, which included university students from 12 European countries and investigated haplotype effects[11]. Although Hind III genotypes alone did not differ in frequencies between our two subgroups studied, there was a statistically significant difference in gene combinations with S447X. The H+H+/SS genotype combination was more prevalent in CAD/TG than in the control group. Namely, H+H+/SS genotype combination seems to be unfavorable and may be involved in the development of CAD.

The LPL-H+ and LPL S447 alleles have been associated with an increased risk for the development of CAD or hyperlipoproteinemia in a number of other studies. The H+H+ genotype was associated with predisposition to myocardial infarction in Russian patients[37] and the Hind III polymorphism correlated significantly with cerebrovascular disease in Japanese subjects[38]. Among German patients included in the MONICA study there was no difference in the frequencies of Hind III genotypes between patients and controls, but there were observed unfavorable lipid levels in homozygotes for H+ allele[4]. The H+ allele was also connected with some unfavorable lipid profiles in Spanish and US-American women[39,40]. By comparison, as in the study presented here, there was no observed association between the Hind III polymorphism and lipid profile among overweight, postmenopausal, US-American woman[41] nor in the Quebec family study[29,42]. Although we expected that patients with CAD and high TGs might have unfavorable lipid profile with LPL polymorphisms mentioned above, we did not find any significant influence of these polymorphisms. Data from the literature suggests that this effect may depend upon the specific patient population studied.

In summary, the S447X polymorphism and genotype combination of S447X and Hind III polymorphisms were shown to differ in frequencies between CAD/TG and control groups, suggesting their association with CAD and hypertriglyceridemia.

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POVEZANOST DVIJU VARIJANTI GENA ZA LIPOPROTEIN LIPAZU S KORONARNOM BOLESTI SRCA I HIPERTRIGLICERIDEMIJOM

S A Ž E T A K

Cilj ovog rada je bio ispitati učinak polimorfizama S447X i Hind III u genu za lipoprotein lipazu na razvoj koronarne bolesti srca i hipertrigliceridemiju. U istraživanje je uključeno 132 ispitanika s koronarnom bolesti srca i triacilglicerolom iznad 2.2 mmol/L i 98 zdravih ispitanika iz Hrvatske. U grupi ispitanika s koronarnom bolesti srca i povišenim triacilglicerolom učestalost S447 alela u genu za lipoprotein lipazu značajno je manja nego li kod kontrolne skupine ($p=0.005$) što potvrđuje i statistički značajan omjer vjerojatnosti (O.R.=0.40, $p=0.006$). Također je statistički značajna razlika učestalosti kombinacija gena Hind III/S447X između ispitivanih skupina ($p=0.013$). Genotipovi kao ni kombinacije genotipova nisu se značajno razlikovale u lipidnom profilu nijedne od ispitivanih skupina. Kako se učestalost S447X polimorfizma i kombinacije polimorfizama Hind III/S447X u genu za lipoprotein lipazu razlikuju između pacijenata i kontrolnih ispitanika može se zaključiti da su navedeni polimorfizmi mogu imati utjecaja na razvoj koronarne bolesti srca i hipertrigliceridemije.

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Original scientific paper

Frequency of HFE Gene Mutations C282Y and H63D in Bosnia and Herzegovina

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A B S T R A C T

Genetic epidemiology studies of hereditary hemochromatosis (HHC) have shown a high prevalence of the C282Y mutation in individuals of the North Western European origin, whereas lower prevalence of HFE gene mutations was detected in the populations from southern European countries. However, no HFE mutation prevalence data have been provided for the population of Bosnia-Herzegovina so far. Therefore, the aim of this study was to determine the frequency of the C282Y and H63D HFE gene mutations in the population of Bosnia-Herzegovina. Among 200 analysed subjects 8 (4%) were C282Y heterozygotes; no C282Y homozygotes were found. The frequency of the H63D allele was 11.5%. There were 33 (16.5%) heterozygotes and 6 (3%) homozygotes for the H63D mutation. One (0.5%) compound heterozygote C282Y/H63D was identified. The observed C282Y and H63D allele frequency was 2.25% (95% confidence interval: 1.2–4.2) and 11.5% (95% confidence interval: 8.7–14.9), respectively. The prevalence of the C282Y and H63D mutations was estimated in Bosnia-Herzegovina, which fit well in the European northwest-to-southeast gradient of the C282Y mutation distribution. In addition, these results have an important implication for clinical evaluation of HHC in Bosnia-Herzegovina.

Key words: *hereditary hemochromatosis, HFE gene, C282Y and H63D mutation, allele frequency*

Introduction

Hereditary hemochromatosis (HHC) is one of the most common autosomal recessive inherited diseases in Caucasians with an estimated prevalence of 1:188 to 1:327[1]. In Caucasians the disease leads to progressive iron accumulation in various organs (liver, pancreas, heart and joints) with reduced lifetime-expectancy if phlebotomy treatment is not instituted[2].

The hemochromatosis gene (HFE) was identified by positional cloning and located on chromosome 6. Two most frequent missense mutations were identified, namely a G to A transition at nucleotide 845 which leads to a substitution of cysteine for tyrosine mutation at the amino acid position 282 (C282Y) and a C to G change at nucleotide 187 that results in a substitution of histidine for aspartate at the amino acid position 63 (H63D)[3]. Over 80% of HHC patients from North European populations are homozygous for the C282Y mutation. A significant proportion of HHC patients who are not homozygous for C282Y are C282Y heterozygotes, compound heterozygotes, H63D homozygotes, or H63D heterozygotes[4]. Hereditary hemochromatosis was initially described exclusively in individuals of the North Western European origin, whereas lower HFE gene mutations were detected in populations from southern Europe[5–9]. However, no HFE mutation prevalence data have been reported on the Bosnia-Herzegovina population so far.

Therefore, the aim of this study was to determine the frequency of C282Y and H63D HFE gene mutations in the Bosnia-Herzegovina population.

Participants and Methods

The study population included two hundred (100 males and 100 females) unrelated healthy blood donors from Bosnia and Herzegovina, in the period from 2003 to 2005. All participants subscribed the informed written consent.

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Genomic DNA was extracted from peripheral blood leukocytes using a standard procedure[10]. *HFE* mutation analysis was performed using PCR-RFLP analysis, as previously described[11]. The amplified PCR fragments were digested with restriction enzyme *Rsa* I to identify the C282Y mutation and with *Mbo* I to detect the H63D mutation, as recommended by the manufacturer (Promega, USA). Restriction enzyme digest products were analysed on a 3.0% agarose gel and visualized after ethidium bromide staining.

Chi-squared (χ^2) test was used to compare the frequency of C282Y and H63D genotypes. A 95% confidence interval (CI) was calculated for the frequency of the alleles.

Results

Frequencies of the C282Y and H63D genotypes are shown in Table 1. Among 200 analysed subjects 8 (4%) were C282Y heterozygotes, 33 (16.5%) were H63D heterozygotes and 6 (3%) were homozygotes for the H63D mutation. One (0.5%) compound heterozygote C282Y/H63D was identified and no C282Y homozygotes were found.

The genotype distribution of C282Y was within Hardy-Weinberg equilibrium ($p=0.74$; $\chi^2=0.11$), whereas the H63D distribution was not ($p=0.02$; $\chi^2=5.43$).

In Table 2, the allelic frequencies of the two mutations are presented. The overall C282Y allele frequency in the population of Bosnia-Herzegovina population was 2.25% (95% CI: 1.2–4.2) and the observed H63D allele frequency was 11.5% (95% CI: 8.7–14.9). Taking into account the allele frequency of C282Y, the prevalence of homozygous C282Y was calculated as approximately 0.5 per 1000.

TABLE 1
DISTRIBUTION OF HFE GENOTYPES IN BOSNIA-HERZEGOVINA

C282Y	H63D	N = 200	%
–/–	–/–	152	76.0
+/–	–/–	8	4.0
+/+	–/–	0	0
–/–	+/–	33	16.5
+/–	+/–	1	0.5
+/+	+/–	0	0
–/–	+/+	6	3.0
+/–	+/+	0	0
+/+	+/+	0	0

+ denotes the mutated allele, – denotes wild-type allele

TABLE 2
ALLELE FREQUENCIES AND 95% CONFIDENCE INTERVAL (CI) FOR C282Y AND H63D MUTATIONS IN BOSNIA-HERZEGOVINA

HFE gene mutations	Allele frequencies (%) and 95% CI
282Y	2.25 (1.2–4.2)
63D	11.5 (8.7–14.9)

Discussion

Hereditary hemochromatosis HFE mutations have been studied in several European populations. Initially HFE mutations were described exclusivelly in populations of North Western European origin[12]. It has been suggested that the C282Y mutation originates within the population of Celtic origin. On the other hand, there is evidence that the C282Y mutation originated from the Germanic Iron Age population in Southern Scandinavia and spread with the Vikings[13]. The allele frequencies are lowest in locations where the Vikings had been scarce such as Central Europe, the Balkans and the Mediterranean countries. As previously described, the frequency of the C282Y mutation declines from Northern to Southern Europe, thus allele frequencies of 6.5 to 8.2% was found in English blood donors[14], intermediate allele frequencies (3.1–4.8%) are seen in the populations in Central Europe[4], whereas low allele frequencies (0–3.1%) are recognized in populations in Southern Europe and the Mediterranean[6]. According to the geographic position in Europe, the prevalence of the C282Y mutation in Bosnia and Herzegovina (2.25%) fits in the observed north/south gradient. Recent studies have shown that in Slovenia the observed allele frequency of the C282Y mutation is 4.0%, in Croatia 3.3%, and in Greece 1.0%, which is comparable to the frequencies found in the Bosnia-Herzegovina population[9,15].

In the Bosnia-Herzegovina population, the H63D allele frequency is 11.5%. This frequency is comparable to European populations, in which the H63D mutation is present, and is more frequent than C282Y, ranging between 10% and 20%[4]. In the Slovenian and Croatian populations, the estimated prevalence of H63D is 14.5% and declines to Middle East where it is between 8% and 10%[4,9]. There is a moderate deviation from Hardy-Winberg equilibrium in the distribution of H63D genotypes, which might be explained as a consequence of genetic drift and/or migrational movements in the area of Bosnia and Herzegovina in the war and post-war period.

In conclusion, the estimated prevalence of the C282Y and H63D mutations in the Bosnia-Herzegovina population was lower than that in the Slovenian and Croatian populations, which supports the idea of the so-called gradient distribution of the C282Y mutation; a more definite proof on the origin of this mutation remains unclear. These results have an important implication to clinical evaluation of HHC in Bosnia and Herzegovina, and contribute to an easy identification of those at risk of developing the disease.

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FREKVENCIJA C282Y I H63D MUTACIJA GENA HFE U BOSNI I HERCEGOVINI

SAŽETAK

Genetičko-epidemiološka istraživanja nasljedne hemokromatoze (HHC, hereditary hemochromatosis) pokazala su visoku učestalost mutacije C282Y kod osoba porijeklom iz sjeverozapadne Europe, dok je niža učestalost mutacija HFE gena opažena u populacijama južnoeuropskih zemalja. Međutim, do sada nisu postojali podaci o učestalosti mutacija tog gena za populaciju Bosne i Hercegovine. Zbog toga je cilj ovog istraživanja bio odrediti frekvenciju mutacija C282Y i H63D HFE gena u populaciji Bosne i Hercegovine. Među 200 analiziranih ispitanika bilo je 8 (4%) C282Y heterozigota i niti jedan C282Y homozigot. Frekvencija alela H63D bila je 11.5%. Zabilježena su 33 (16.5%) heterozigota i 6 (3%) homozigota za H63D mutaciju. Zabilježen je 1 (0.5%) složeni heterozigot C282Y/H63D. Opažene frekvencije alela C282Y i H63D bile su 2.25% (95%-tni interval pouzdanosti: 1.2–4.2) i 11.5% (95%-tni interval pouzdanosti: 8.7–14.9). Procijenjena učestalost mutacija C282Y i H63D u Bosni i Hercegovini dobro se uklapa u europski gradijent distribucije mutacije C282Y od sjeverozapada prema jugoistoku. Uz to, ovi rezultati imaju važnu ulogu za kliničku evaluaciju HHC-a u Bosni i Hercegovini.

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Original scientific paper

Dermatoglyphs and Brachial Plexus Palsy

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A B S T R A C T

Perinatal brachial plexus palsy (PBPP) is a handicap quite commonly encountered in daily routine. Although birth trauma is considered to be the major cause of the defect, it has been observed that PBPP occurs only in some infants born under identical or nearly identical conditions. The aim of this study was to test the hypothesis of genetic predisposition for PBPP. It is well known that digito-palmar dermatoglyphs can be used to determine hereditary roots of some diseases. Thus, we found it meaningful to do a study analysis of digito-palmar dermatoglyphs in this disease as well, conducting it on 140 subjects (70 males and 70 females) diagnosed with PBPP. The control group was composed of fingerprints obtained from 400 adult and phenotypically healthy subjects (200 males and 200 females) from the Zagreb area. The results of multivariate and univariate analysis of variance have shown statistically significant differences between the groups observed. In spite of lower percentage of accurately classified female subjects by discriminant analysis, the results of quantitative analysis of digito-palmar dermatoglyphs appeared to suggest a genetic predisposition for the occurrence of PBPP.

Key words: *dermatoglyphs, brachial plexus palsy, perinatal, genetic predisposition*

Introduction

Perinatal brachial plexus palsy (PBPP) has been traditionally classified into three types: upper plexus palsy (Erb's) affecting the C5, C6, and +/– C7 nerve roots, lower plexus palsy (Klumpke's) affecting the C8 and T1 nerve roots, and total plexus palsy[1,2]. PBPP is a handicap quite commonly encountered in daily routine. The incidence of PBPP in Croatia is 3.4/1000 newborn [3]. In Sweden it increased significantly from 1.4/1000 in 1980 to 2.3/1000 in 1994, while in Netherlands it is estimated 4.6/1000 newborn[4,5]. Although the etiology of PBPP varies, birth trauma (caused by high birth weight, vertex presentation, shoulder dystorcia) is considered to be the major cause of the defect[6–9]. Gherman and collaborators noted that brachial plexus injury may be unrelated to manipulations performed at the time of delivery and can be associated with cesarean delivery. They concluded that such palsies appear to be of intrauterine origin and more likely to persist[10]. The incidence of Erb's palsy in Pennsylvanian population is similar to that of other studies and has remained unchanged over the past 30 years, even as cesarean rate has risen from 5 to 20 %[11]. This would suggest that there is some genetic or intrauterine influence for perinatal expression of brachial plexus palsy. Any analysis to confirm these suspicions could be helpful for reduction all factors that may possibly influence damage of the brachial plexus in intrauterine development.

Dermatoglyphs are patterns made by epidermis on fingers, palms and soles. They are completely formed by 21st week of intrauterine development. The dermatoglyphic pattern of human palms and soles are individually unique and unchangeable during the life time. These are highly hereditary determined, although the exact way of inheritance is still unknown. Therefore, dermatoglyphs are informative for understanding the genetic status, as well as early disturbances in intrauterine development[12–14].

It has been observed that PBPP occurs only in some infants born under identical or nearly identical conditions, giving rise to a hypothesis on the possible genetic susceptibility of the disorder. In a number of Egyptian families with high consanguinity rate several members in successive generations were found to have PBPP[15]. In

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some cases with PBPP the patient had a history of decreased right arm movement detected by fetal ultrasound at 18 to 20 week of gestation, which coincide with development of dermatoglyphs[12,16]. With all above mentioned there is reason to assume that it would be useful to test the hypothesis of genetic predisposition for PBPP. The circumstances the development of the peripheral nerves and the development of the dermatoglyphs are the same, leading to the situation where the disturbances in the phase of embryological development could have various influences on the evolution of the palsy, simultaneously be reflected on the dermatoglyphic patterns. This reflection can be tested using the comparative analysis of dermatoglyphs of the digito-palmar complex in all of PBPP patients and healthy control groups.

Materials and Methods

Analysis of digito-palmar dermatoglyphs was examined in 140 subjects (70 males and 70 females), with PBPP. Among our patients, there were number of close relatives (three pairs consisting of – two sisters, a brother and a sister, and a mother and a daughter)[17]. Fingerprints were obtained from 400 adult and phenotypically healthy subjects (200 males and 200 females) from the Zagreb area, was used as a control group[18]. The digito-palmar prints were taken and analyzed according to the Cummins and Midlo (1961), Schauman and Alter (1976), Miličić et al. (1989) methods[12,14,19].

The analysis comprised a total of 18 quantitative variables of digito-palmar dermatoglyphics: finger ridge-counts on the right and left hand: FRCR1, FRCR2, FRCR3, FRCR4, FRCR5, FRCL1, FRCL2, FRCL3, FRCL4, FRCL5; palmar ridge counts on the right and on the left hand: a–b rc R, b–c rc R, c–d rc R, a–b rc L, b–c rc L, c–d rc L and the atd angles: atd R and atd L. The quantitative traits of digito-palmar dermatoglyphs were analyzed using descriptive statistics, multivariate and univariate analysis of variance and discriminant analysis.

Results

The results of descriptive statistics based on the comparison 18 quantitative variables of digito-palmar dermatoglyphs from PBPP patients groups (male and female) with their control groups are presented in Table 1 for males and Table 2 for females.

Multivariate analysis of variance (Table 3) showed statistically significant difference between male control group and male patients with PBPP ($F=2.21064$; $p<0.004$), as well as statistically significant difference between healthy females and female patients with PBPP ($F=2.27947$; $p<0.003$).

Univariate analysis of variance (Table 4) enabled the identification of variables, tracing the greatest contribution to this heterogeneity between the investigated groups: in males: FRCL1 ($F=4.8679$; $p<0.05$), c–d rc L ($F=4.31446$; $p<0.05$), atd R ($F=12.182$; $p<0.001$) and in

TABLE 1
DESCRIPTIVE STATISTICS FOR QUANTITATIVE DERMATOGLYPHIC TRAITS IN PATIENTS WITH BRACHIAL PLEXUS PALSY – PBPP (N=70) AND HEALTHY CONTROLS (N=200) – MALES

	PBPP		Healthy control	
Variables	X	SD	X	SD
FRCR 1	19.79	6.44	19.37	5.63
FRCR 2	11.31	7.12	11.41	7.27
FRCR 3	11.83	6.41	11.99	6.58
FRCR 4	15.33	5.89	16.16	6.15
FRCR 5	13.21	4.97	13.63	5.16
a–b rc R	40.51	6.64	41.85	6.86
b–c rc R	27.77	6.00	28.59	5.78
c–d rc R	36.34	7.12	37.94	5.98
atd R	51.57	9.32	47.42	8.27
FRCL 1	18.10	6.44	16.19	6.14
FRCL 2	11.60	6.88	10.76	6.78
FRCL 3	12.53	6.25	11.78	6.37
FRCL 4	16.16	5.63	16.24	6.17
FRCL 5	13.46	4.45	13.49	4.60
a–b rc L	42.03	5.13	43.58	7.05
b–c rc L	28.51	6.10	28.73	5.72
c–d rc L	34.54	8.16	36.62	6.84
atd L	48.86	9.10	47.86	7.70

TABLE 2
DESCRIPTIVE STATISTICS FOR QUANTITATIVE DERMATOGLYPHIC TRAITS IN PATIENTS WITH BRACHIAL PLEXUS PALSY – PBPP (N=70) AND HEALTHY CONTROLS (N=200) – FEMALES

	PBPP		Healthy control	
Variables	X	SD	X	SD
FRCR 1	20.14	4.56	17.23	5.56
FRCR 2	13.89	6.09	11.62	6.55
FRCR 3	14.43	4.66	11.44	5.31
FRCR 4	16.96	4.65	15.78	5.72
FRCR 5	14.19	4.64	12.70	4.84
a–b rc R	41.00	5.93	41.03	6.02
b–c rc R	29.06	4.75	27.31	6.01
c–d rc R	38.84	5.92	36.70	6.43
atd R	49.07	10.71	46.87	8.67
FRCL 1	17.59	4.99	14.80	5.76
FRCL 2	13.16	5.60	10.87	6.88
FRCL 3	14.09	5.18	11.58	5.72
FRCL 4	16.70	4.20	15.13	5.25
FRCL 5	14.46	4.21	12.26	4.81
a–b rc L	42.40	4.87	41.82	5.90
b–c rc L	28.89	4.95	26.90	5.67
c–d rc L	37.66	8.54	36.34	6.86
atd L	50.23	11.95	47.70	8.39

females: FRCR1 (F=14.848; p<0.001), FRCR2 (F=6.010; p<0.05), FRCR3 (F=16.611; p<0.001), FRCR5 (F= 4.6109; p<0.05), FRCL1 (F=12.382; p<0.001), FRCL2 (F=5.668; p<0.05), FRCL3 (F=9.512; p<0.005), FRCL4 (F= 5.131; p<0.05), FRCL5 (F=11.558; p<0.001), b–c rc R (F= 5.002; p<0.05), c–d rc R (F=5.374; p<0.05), b–c rc L (F= 6.090; p<0.05).

TABLE 3
MULTIVARIATE ANALYSIS OF VARIANCE FOR THE QUANTITATIVE DERMATOGLYPHIC TRAITS BETWEEN PATIENTS WITH BRACHIAL PLEXUS PALSY – PBPP AND HEALTHY CONTROLS

	F	P	df
Males	2.21064	0.004	18; 251
Females	2.27947	0.003	18; 249

TABLE 4
UNIVARIATE ANALYSIS OF VARIANCE FOR THE QUANTITATIVE DIGITO-PALMAR DERMATOGLYPHIC TRAITS BETWEEN PATIENTS WITH BRACHIAL PLEXUS PALSY – PBPP AND HEALTHY CONTROLS

Variables	F-males	F-females
FRCR 1	0.255	14.848***
FRCR 2	0.010	6.010*
FRCR 3	0.029	16.611***
FRCR 4	0.955	2.640
FRCR 5	0.351	4.619*
a–b rc R	2.000	0.044
b–c rc R	1.031	5.002*
c–d rc R	3.339	5.374*
atd R	12.182***	2.908
FRCL 1	4.867*	12.382***
FRCL 2	0.798	5.668*
FRCL 3	0.733	9.512**
FRCL 4	0.010	5.131*
FRCL 5	0.003	11.558***
a–b rc L	2.855	0.526
b–c rc L	0.068	6.090*
c–d rc L	4.314*	1.554
atd L	0.796	1.703

*p<0.05, **p<0.01, ***p<0.005

We also analyzed the differences between investigated groups using discriminant analysis. It confirmed a correct classification in 68.52% of male patients (65.7% boys with PBPP and 69.5% healthy control), while in females 65.30% (60.3% girls with PBPP and 67% healthy control) were correctly classified (Table 5).

Discussion

With the knowledge of the existing literature we have found only few studies describing similar problematic. Loesch et al. (1990) compared hand locomotor function and body structure with epidermal ridge patterns[20]. Their data showed significant correlation between hand locomotor function and dermatoglyphic characteristics, especially in men. In the second study of Philpot et al. (1995) reported a case of an infant with congenital symmetrical weakness of the upper limbs and abnormal dermatoglyphs on both palms with poorly expressed transversal crease[21]. The finding of abnormal dermatoglyphs indicates a possibility of a prenatal start of extremity weakness. The authors linked this clinical finding to possible drug toxicity in the first trimester of pregnancy.

As far as we know, there are no other data connecting about the clinical state of PBPP with dermatoglyphs. Thus, our results are not further comparable to the other findings in the known literature The methods of descriptive statistics in our study did not show observable differences in ridge counts of boys, whereas girls were found to have a greater number of finger ridges. The total number of ridges for all ten fingers TRC in males was TRC= 141.03 for healthy controls and TRC=143.31 for PBPP, while in the case for females it was TRC=131.38 in healthy controls and TRC=155.59 in PBPP girls this difference being statistically significant (F=14.49; p< 0.001). The possible differences between male and female subjects with PBPP and their control groups were determined variables. As the multivariate analysis of variance produced statistically significant differences between the observed groups, each original variable was also subjected to a univariate analysis of variance. In the case of boys with PBPP, a statistically significant difference from the control group was found for the FRCL1, c–d rc L and

TABLE 5
THE RESULT OF DISCRIMINANT CLASSIFICATION BETWEEN THE GROUP OF PATIENTS WITH BRACHIAL PLEXUS PALSY – PBPP AND HEALTHY CONTROLS

Males	N	Correctly classified	%	Incorrectly classified	%
Patients PBPP	70	46	65.70	24	34.30
Healthy controls	200	139	69.50	61	30.50
Total of correctly classified			68.52		
Females	**N**	**Correctly classified**	**%**	**Incorrectly classified**	**%**
Patients PBPP	68	41	60.30	27	39.70
Healthy controls	200	134	67.00	66	33.00
Total of correctly classified			65.30		

atd R original variables. We wish to emphasize that in the case of girls with PBPP, a statistically significant difference from the control group was confirmed for almost all finger variables: FRCR1, FRCR2, FRCR3, FRCR5, FRCL1, FRCL2, FRCL3, FRCL4, FRCL5, as well as for the palmar variables: b–c rc R, c–d rc R and b–c rc L variables. Our results indicate that PBPP reflects more changes on dermatoglyphs of the girls than of the boys, and confirm the existence of genetic susceptibility to PBPP together with well known risk factors for development of the disease.

Literature data show that finger variables are mostly polygenically determined, while palmar variables are more susceptible to external effects[22,23]. Furthermore, it is documented that women are less susceptible to changes in dermatoglyphic characteristics in comparison to men[22,23]. Arrieta et al. (1991) investigated genetic component of variables a–b rc, b–c rc and c–d rc in healthy persons and concluded that in healthy men, concerning the c–d rc findings, there is a stronger influence of environment while in healthy women all variables a–b rc, b–c rc and c–d rc have a strong genetic component that affect their phenotypic expression[24].

Our findings also revealed significant differences in total ridge count TRC in the group of girls with PBPP in comparison to the control group for most variables.

Discriminant analysis allowed the allocation of individual entities to particular groups to be predicted. Accurate classification was recorded in 68.52% and 65.30% of male and female subjects, respectively. We believe that the percentage of accurately classified female subjects would bee higher if we were able to single out in the male and female subjects with real birth trauma as the cause of PBPP.

In spite of a lower percentage of accurately classified female subjects by discriminant analysis, the results of multivariate and univariate analysis of variance appeared to suggest a genetic susceptibility for the occurrence of PBPP, especially in girls. Furthermore, some epidemiological studies showed higher incidence for PBPP in girls. Mandić et al. (1957), Zancoli et al. (1981), Greenwald et al. (1984) and Stojčević-Polovina et al. (1986) showed in their studies that the percentage of female patients with PBPP is higher than male patients (55%, 61%, 56%, 55% respectively)[3,25–27].

It is a well known fact that the main cause of PBPP is obstetrical trauma. On the other hand, the empiric fact that PBPP is more frequent in some families seems to reveal that genetic predisposition could contribute to the development of PBPP [28]. Furthermore, it is important to acknowledge the fact that there were three pairs of relatives in our sample (two sisters, brother and sister, mother and daughter) with PBPP.

Many authors applied the analysis of dermatoglyphs of digito-palmar complex to estimate the hereditary foundation of some diseases, but they could not answer the question of the connectedness between the changes in dermatoglyphs and certain specific disease[29–34]. Schaumann and Kimura (1991) connected the distortions in whorl ridges with intrauterine disturbances in early pregnancy[35]. Knowledge of the embryological developmental stages clarifies this happening through the fact that the peripheral nervous system as well as epidermis develops from the ectoderm, while the supportable vertebral tissue, from the mesoderm. Consequently, the embryogenesis and morphogenesis of dermatoglyphs and peripheral nervous system happened simultaneously[36]. The formation of first spinal cord starts at the eighth and a half gestational week, which is the exact period in which the secondary volar pads on fingers vanish. With tenth and a half gestational weeks starts involution of primary volar pads and differentiation of epidermal ridges. At that time, the reflex of grasping has been already formed [37]. As the peripheral nervous system as well as dermatoglyphs develops at the same time, possible damages that led to aberrant dermatoglyphs could be the same stressors for the peripheral nervous system, as well.

Although our results indicate genetic predisposition in some patients, analysis of dermatoglyphs does not allow the allocation of individual entities to particular risk group of children.

However, our results are significant in the sense that the recognition of possible heredity in the background of PBPP might contribute to the prevention of this handicap in newborns by pointing to the need of more detailed family history anamnesis to be taken on routine check-ups of pregnant women. Above all it also orientates toward further investigations aimed at a targeted reduction of all factors that may possibly entail damage to the brachial plexus in intrauterine development.

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DERMATOGLIFI I KLIJENUT BRAHIJALNOG SPLETA U DJECE

S A Ž E T A K

Klijenut brahijalnog spleta u djece ulazi u red češćih hendikepa s kojim se susrećemo u svakodnevnoj praksi. Trauma u porodu smatra se najčešćim razlogom njenog nastanka. Činjenica da kod određenog broja novorođenčadi rođenih u istim ili približno istim uvjetima samo jedan njihov dio zadobije leziju brahijalnog spleta pobudila je pretpostavku o eventualnoj genetskoj predispoziciji oboljenja. Kako je analiza digito-palmarnih dermatoglifa već primjenjivana u procjeni nasljedne osnove nekih bolesti, u našem istraživanju izvršeno je ispitivanje digito-palmarnih dermatoglifa u 140 ispitanika s kljenuti brahijalnog spleta, i to 70 muškog i 70 ženskog spola. Kao komparativna skupina poslužili su otisci 400 odraslih i fenotipski zdravih osoba zagrebačke regije i to 200 muškaraca i 200 žena. Učinjene multivarijantna i univarijantna analizea varijance pokazale su da postoje statistički značajne razlike između promatranih skupina. Iako je diskriminacijskom analizom, kojom je moguće prognozirati pripadnost pojedinih entiteta pojedinim grupama, dobiven nešto niži postotak točno klasificiranih ispitanica, rezultati ukazuju da postoji određena genetska predispozicija za nastajanje kljenuti brahijalnog spleta.

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Bilateral MR Volumetry of the Amygdala in Chronic PTSD Patients

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A B S T R A C T

Posttraumatic stress disorder (PTSD) patients experience symptoms which implicate dysfunction of emotional memory circuits, and possible damage of the amygdala. Laterality differences in activity of the amygdala have been reported in PTSD patients, with presumed adaptive plasticity in the hippocampus and the amygdala. The aim of this study was to investigate possible interhemispheric differences of amygdalar volume in chronic PTSD patients, with calculation of right-to-left volume ratios. Bilateral magnetic resonance (MR) volumetry was applied in 11 chronic PTSD patients. The mean right amygdalar volume of our patients was significantly smaller than the left one (p=0.031), with the right-to-left volume ratio of 0.96±0.06. This tendency towards smaller right amygdala may be an acquired effect as a result of stress-induced plasticity, however we can not exclude the possibility of a predisposing condition.

***Key words**: MR volumetry, amygdala, PTSD*

Introduction

Patients suffering from posttraumatic stress disorder (PTSD) experience distortion and fragmentation of memory, declarative and non-declarative memory deficits and dissociative amnesia[1]. Such symptoms are commonly referred to as psychological problems, although they may be related to physical effects of extreme stress on specific brain structures. The amygdala, especially the amygdalar basolateral nucleus, is a prominent structure in emotional memory circuits and stress-induced aversive learning. Possible dysfunction of the amygdala can influence pathophysiology of PTSD at multiple levels[2,3]. It modulates the effect of emotional stimuli and stress hormones on encoding and/or consolidation of declarative memory, through direct and indirect influence on the hippocampus[4–6]. There are reports of laterality differences in activity of the amygdala in patients with PTSD[4,7,8]. Since chronic stress may cause adaptive plasticity, as well as dendritic remodeling in the amygdala in animal models[9–12], the purpose of this study was to explore possible asymmetry in the amygdalar volume in chronic PTSD patients, with calculation of right-to-left volume ratios. The volumes and the volume ratios were compared with healthy subjects used as control groups in studies of Szabo et al.[13] and Bower et al.[14].

Materials and Methods

The patients included in the study were 11 Croatian War veterans. They were diagnosed as chronic PTSD patients meeting the criteria stated in Diagnostic and Statistical Manual of Mental Disorders – Fourth Edition (DSM-IV), without other Axis-I and/or Axis-II diagnosis[15]. Their symptoms lasted for more than 5 years. They were receiving no psychotropic medication for at least six months preceding the study. Neurological examination excluded possible comorbid conditions, head trauma or loss of consciousness the year preceding the study. Unfortunately, at the time of inclusion of the patients in the study, there was no Croatian standardized version of Clinician Administered PTSD Scale (CAPS score) for PTSD severity. Patients with depressive symptoms were excluded from the study based on a psychiatric clinical interview, at the time and in our circumstances it was not

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possible to control these aspects in more details. All the patients were male, right-handed, with no history of alcohol or drug abuse. The age of the participants ranged from 32 to 52 (the average of 40). Comparison groups were healthy subjects matched for sex, handedness, and without history of alcohol abuse[13,14]. The control group, used for comparison from a study by Szabo et al., consisted of 9 healthy male participants, all right-handed, with a mean age of 27 years. The study by Bower et al. used the control group consisting of 31 male subjects without past head injury, medical or psychiatric history.

Magnetic resonance (MR) examinations were performed on a »Prestige Gyrex» General Electric/Elscint scanner with 2.0 Tesla field-strength. The imaging sequence used for volumetric analysis was a coronal three-dimensional (3D) T1 spoiled gradient-echo (SPGR) acquisition of the whole brain (repetition time 540 msec, echo time 20 msec, field of view 240 x 240 mm, Matrix 256 x 192). 1.1 mm thick slices were measured, without an interleave gap, with 1.0 x 1.0 mm in plane resolution. Both compared studies also used a coronal 3D T1 SPGR sequence without an interleave gap, Bower et al. used a 1.5 mm slice thickness, while Szabo et al. used a slice thickness of 1 mm. MR acquisition parameters have been comparable since minor differences in slice thickness do not lead to false estimation of volume[16].

The boundaries of the amygdala on individual slices were demarcated manually by an experienced neuroradiologist unfamiliar with the patients' diagnosis or the purpose of the measurement. The volume was measured using DicomWorks v 1.3.5 software (2000–2001. Philippe Puech, Loic Boussel). Both amygdalae in each subject were measured in three separate cycles. During each cycle both amygdala were measured in each subject once. The cycles were separated by one-week period. Mean values and standard deviation for each amygdala were calculated, and in such form used in statistical analysis. Amygdalar volume segmentation was performed in accordance with compared studies and previous studies on the subject of volumetry[13,14,17–19]. The posterior border of the amygdala was the slice where it first became visible as gray matter superior to the alveus and laterally to the hippocampus, overlying the temporal horn of the lateral ventricle. The anterior border of the amygdala was not clear in all patients, and for the purpose of consistency it was defined at the level of the opening of the endorhinal sulcus forming the lateral fissure. Superiorly, a straight line was drawn from the superolateral aspect of the optic tract to the fundus of the circular sulcus of insula, excluding parts of basal ganglia. Superomedially, the endorhinal sulcus separated the cortical amygdaloid nucleus from the substantia innominata. Inferolaterally, it was separated from the hippocampal head by the alveus and temporal horn of the lateral ventricle. Inferomedially, tentorial indentation served as a demarcation line between the amygdala and entorhinal cortex. Posteromedial border was delineated by the crural cistern and anteromedial by the angular bundle from entorhinal cortex. Amygdalar volumes were calculated by summing cross sectional areas and multiplying by slice thickness (Cavalieri's principle)[14].

For the purpose of statistical analysis, results were analyzed using Stat View software v. 5.0.1. Right-to-left amygdalar volume ratios were calculated for each patient. We used a paired t test for the assessment of any significant asymmetry in the volumes between right and left amygdala. The correlation between the patients' age and volume ratios was analyzed by simple regression. Volumes and right-to-left volume ratios were presented as arithmetic means ±SD. We compared our mean volume ratio (±SD) to the mean volume ratios (±SD) of healthy male subjects used as control groups in studies of Szabo et al.[13] and Bower et al.[14]. For the comparison of two means, unpaired t test was used.

Results

The mean right and left amygdalar volume of the patients in our study is presented in Table 1. The mean right and left volumes were significantly different ($p=0.031$). 8 of 11 patients had smaller right amygdalar volumes than left, the difference ranging from 0.14 to 14.51%. The mean right-to-left difference in these 8 patients was 7.8%. The right amygdala was larger in remaining 3 patients, in a range from 0.3% to 7%, with a mean difference of 2.9%. The right-to-left amygdalar ratio was 0.96±0.06. There was no correlation between the age of the patients and the inter-amygdalar ratios ($p=0.339$). The mean volume ratio (±SD) of our patients was directly compared to the mean volume ratio (±SD) of control groups in studies of Szabo et al.[13] and Bower et al.[14]. In a study by Szabo et al. a control group had a mean volume ratio of 1.07±0.06. The control group in a study of Bower et al. had the amygdala volume ratio of 1.04±0.06. The comparison of the mean volume ratio in our study with the ones from Szabo et al. and Bower et al. produced $t=4.88$ and $p<0.0001$ (degrees of freedom=29); and $t=3.80$ and $p=0.0005$, (degrees of freedom=40), respectively.

Discussion

Individuals with PTSD show hypothalamo-pituitary-adrenal (HPA) axis alterations[20] with attenuated feedback inhibition and release of epinephrine from the locus coeruleus to the amygdala. This is in line with reported hyper reactivity of the amygdala in these patients[21–23]. It modulates the effect of stress hormones on memory consolidation involving long-term potentiation (LTP) in hippocampus[5,24,25]. Prolonged stress with cortisol exposure impairs LTP in the hippocampus, while the same stress facilitates LTP in the amygdala[9–12]. Animal studies have shown dendritic growth in the amygdala during fear conditioning[26,27], as opposed to the effect in hippocampus where stress induced dendritic atrophy[12]. These data suggest volume alterations of the amygdala in PTSD, not necessarily in the same direction as hippocampal changes.

TABLE 1
CHARACTERISTICS OF THE PATIENTS AND AMYGDALAR VOLUMES

Subjects	Age (years)	Amygdalar volumes (cm^3)		Right-to-left volume ratios
		Right	Left	
1	32	2.33	2.53	0.918
2	41	2.35	2.35	0.999
3	42	2.28	2.37	0.962
4	36	2.74	3.03	0.906
5	33	1.91	2.09	0.915
6	52	2.23	2.37	0.941
7	43	2.13	2.12	1.003
8	41	2.60	2.84	0.915
9	40	2.43	2.26	1.075
10	42	1.74	1.72	1.014
11	38	2.50	2.87	0.873
X±SD	40±5.44	2.30±0.29	2.41±0.39	0.957

There are reports of asymmetric amygdalar activity in PTSD, in terms of greater relative cerebral blood flow (rCBF) and functional MR imaging (fMRI) activity in right amygdala[7,8], and in terms of correlation of affectively influenced memory with the activity of the right amygdala [4]. Therefore, there is reason to believe that the right amygdala may exhibit more pronounced volume changes compared to the left side in PTSD patients.

Most studies on amygdalar volume in healthy right-handed participants found larger right-than-left amygdalar volumes, while some found no interhemispheric differences[28]. Studies concerning brain volume changes in PTSD patients have primarily been focused on hippocampal volumetry, with little data on laterality differences of amygdalar volume. These studies also reported higher mean values of right amygdalar volumes compared to left[29–32]. Therefore, volume of the amygdala in PTSD patients in afore-mentioned studies did not differ significantly from the results in healthy subjects. However, Teicher et al.[33] found reduced size of the left amygdala related to stress, though it was in patients with history of sex abuse, accompanied by fear and terror, and not in PTSD patients. Bilaterally symmetric reduction of amygdalar volume in relation to stress was reported in patients with childhood maltreatment and borderline personality disorder[34].

The mean right amygdalar volume of the patients in our study was significantly smaller than left, with a right-to-left volume ratio of 0.96±0.06. This finding was interesting, and not easily explained, considering stress induced LTP and possible dendritic growth in right amygdala, which is hyper reactive in PTSD patients. When discussing the connection between hippocampal or amygdalar volume changes and effects of extreme stress, there are two confronted theories.

One is the possibility of stress-induced plasticity, with hippocampal volume reduction attributed to glucocorticoid toxicity[35,36]. The reduction of cortison level in patients with Cushing syndrome resulted in improved memory and increased hippocampal volume[37], which is in favor of this possibility. The same assumption may be applicable to amygdalar volume changes. However, one would expect stress-induced growth with an increase in volume of the right amygdala, which is not found in our patients. The patients in our study had a long duration of symptoms, more than five years, as opposed to one of the studies, where symptoms lasted 6 months[30]. The difference between our results and other, mostly Anglo-American studies in PTSD patients could possibly be caused by longer duty duration at battle field, in a homeland defensive war including multiple extreme stressor events, with lower rate of early trauma debriefing and professional psychological support. Therefore, it is likely that stress has a very gradual impact on amygdalar volume, so after a longer period of time hyper reactivity of the right amygdala could lead to noticeable cell loss. Alternatively, dendritic growth in amygdala, which would be responsible for stress-induced greater right amygdalar volume, has only been hypothesized according to studies in animal models, and may not be equivalent in human subjects.

The second possibility is that smaller volume of right-than-left amygdala represents predisposing factor which enlarges the risk of PTSD development after traumatic experience. This would be in line with hypothesized PTSD pathophysiology, which suggested that smaller hippocampal volumes may also be a preexisting condition in PTSD patients, since not all victims of trauma develop PTSD, and continued combat stress combined with already developed PTSD does not seem to produce further reduction of hippocampal volume[31]. However, smaller amygdala in the right hemisphere as a preexisting condition could hardly explain the higher risk of PTSD development, since it is the very side that is hyper reactive in these patients.

The role of amygdala in the pathophysiology of PTSD is substantial. Whether laterality differences reflect the vulnerability to PTSD development, or they represent a secondary event to traumatic experience, remains unclear. These volume changes may be, to a certain degree, related to the duration of PTSD. The limitations of our study were small sample size and the lack of PTSD symptom severity score. Unfortunately, there is no data on inter-rater reliability between morphometric raters in studies used for comparison groups and our study.

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OBOSTRANA VOLUMETRIJA AMIGDALA MAGNETSKOM REZONANCOM U BOLESNIKA S KRONIČNIM PTSP-OM

SAŽETAK

Bolesnici s posttraumatskim stresnim poremećajem (PTSP) imaju simptome koji upućuju na disfunkciju neuronskih krugova emocionalne memorije i na moguće oštećenje amigdala. Različita lateralizacija aktivnosti amigdala opisana je u bolesnika s PTSP-om, s pretpostavkom adaptivnog plasticiteta u hipokampusu i amigdalima. Cilj ove studije bio je istražiti moguće interhemisferične razlike volumena amigdala u bolesnika koji boluju od kroničnog PTSP-a, s izračunavanjem omjera volumena desnih i lijevih amigdala. Obostrana volumetrija magnetskom rezonancom (MR) provedena je u 11 bolesnika s kroničnim PTSP-om. Srednja vrijednost volumena desnih amigdala u naših bolesnika bila je značajno manja nego lijeve strane ($p=0.031$), s omjerom volumena desnih i lijevih amigdala 0.96 ± 0.06. Ta tendencija prema manjim desnim amigdalima može biti stečena kao rezultat plasticiteta induciranog stresom, međutim ne možemo isključiti mogućnost da se radi o predisponirajućem stanju.

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Original scientific paper

Denture Repairs in Different Regions of Croatia in Relation to Prosthodontic Teams

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A B S T R A C T

The purpose of this paper is to evaluate the incidence of denture repairs in different districts of Croatia through the year of 2002. and to analyse the percentage of different repairs (relinings, simple repairs up to 2 elements and complicated repairs-more than 2 elements) in relation to prosthodontic teams. Data on the number of dentures, and the number and types of denture repairs delivered in the Croatian regions of Zagreb, Rijeka, Split and Karlovac were obtained from the Croatian Insitute for Health Insurance for the whole of the year 2002. Information of the number of prosthodontic teams operating in those regions was also obtained. Proportionally more denture repairs were carried out in Karlovac (18%) than Split (5%). The smallest percantage of dentures that required relining was registered in Split and the highest in Rijeka ($\chi^2=36.7$, $p<0.01$). The smallest percentage of simple repairs was registered in Rijeka and the highest in Split ($\chi^2=24.3$, $p<0.01$).The smallest percentage of complicated repairs was registered in Split and the highest in Karlovac. In each region the proportion of denture repairs and types of repairs were correlated with a number of prosthodontic teams in that region. Karlovac had the smallest percentage of specialistic prosthodontic teams and the highest rate of denture repairs.

Key words: *removable denture repairs, prosthodontic teams, Croatia*

Introduction

Clinical follow-up or longitudinal studies of patients with removable dentures (RDs) are not commonly reported. It is likely that such reports are important in the study of factors considered to be significant for the longevity of removable dentures. Factors that are likely to be related to the survival rate (and failure) of removable dentures include technical, biological and »combined biological and technical« factors. A Swiss study by Studer et al.[1] assessed the survival rate and the reasons for failures of 130 combined fixed-removable dental prosthesis in 112 patients in Zurich and some 50 of these prostheses (38.5%) were categorised as failures. Of these 50 failures it was reported that 3 failed due to technical reasons, 36 due to biological reasons and 11 failed due to both, biological and technical reasons[1]. A study by Ettinger et al.[2] on 1,000 elderly people found that 53 of 1,000 persons needed a repair, reline, or replacement of an existing denture. A Finnish study by Peltola et al.[3] reported that 25% dentures needed a repair or replacement among 415 elderly RD wearers in Helsinki. Furthermore, a New Zealand study of dependent elderly people living in rest homes and geriatric hospitals in the Manawatu and Horowhenua regions reported that 18% of upper dentures and 26% of lower dentures needed replacement, while a further 24% of full lower dentures required relining[4].

It has been reported that acrylic resin partial dentures fracture on average about four times more frequently than metal-based partial dentures[5]. A fracture of metal frame appears in 10% to 20% of the removable partial dentures after 5 years and in 27% to 44% after 10

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years[6]. Quality of RDs is sometimes correlated with a lack of denture repairs[7–11].

According to this literature review, it is clear that many people currently use dentures, but that failure of dentures due to biological and technical reasons is a problem within the profession. In well-developed countries the proportion of the population that is partially or fully edentulous is declining[12,13]. In light of this finding some researchers have even raised the question as to whether denture training should be removed from the dental curriculum[14].

Little published information exists on denture deliveries and even less exists on how deliveries of new dentures relate the delivery of denture repairs.

Construction of partial or complete removable dentures is mostly covered by Croatian Health Insurance service once in a 5-year period. Both, specialists of prosthodontics and general dentists are allowed to construct removable dentures in Croatia.

Thus, the objectives of this study were to report on dentures (delivered under health insurance) in different regions of Croatia during the year of 2002. and to explore how the delivery of new dentures relates to the delivery of denture repairs in different districts of Croatia.

Materials and Methods

The Croatian Institute for Health Insurance provided the data about number of dentures delivered in the Croatian regions of Zagreb, Rijeka, Split and Karlovac during the whole of the year 2002., as well as about number and a type of repairs.

Information about number of prosthodontic teams in the same regions in relation to a population living there was obtained from the Croatian Prosthodontic Association.

Types of denture repairs included: relinings, simple repairs of up to 2 denture elements, and complex repairs of 3 or more denture elements. Denture repairs were expressed as percentage of denture deliveries during 2002. in each district. Information on each type of denture repair was also calculated for each district, and these data were related to the number of prosthodontic teams in each region.

Bivariate analyses using the χ^2 test were used to explore differences between region and percentages of each type repairs. The level of statistical significance was set at 0.05.

Results and Discussion

The percentage of denture repairs in different districts is shown in Figures 1a–d. The highest percentage of repairs, (18%) were registered in Karlovac (less than 100,000 inhabitants), followed by 13% in Rijeka (more than 250,000 inhabitants), 7% in Zagreb (more than 1000,000 inhabitants) and finally by Split (more than 350,000 inhabitants), where only 5% of denture repairs were registered (Figures 1a–d).

A statistically significant difference in the percentage of dentures receiving relines was observed between Split and Rijeka (χ^2=36.7; p<0.01), with the lowest percentage in Split and the highest percentage in Rijeka (Figures 2a–d).

The smallest percentage of simple repairs was registered in Rijeka and the highest in Split (χ^2=24.3; p<0.01) and the difference was statistically significant.

The smallest percentage of complicated repairs was registered in Split and the highest in Karlovac (Figures 2a–d).

Simple denture repairs such as fractures could happen during the procedure of maintaining denture hygiene if a denture is dropped down or during chewing

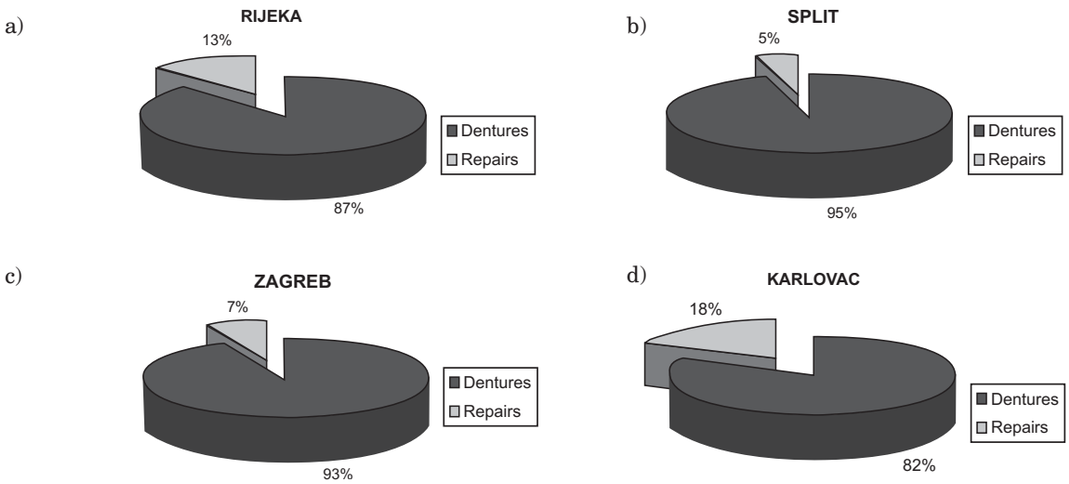

Fig. 1. Percentage of denture repairs in different districts of Croatia in relation to new denture deliveries: a) Rijeka, b) Split, c) Zagreb and d) Karlovac.

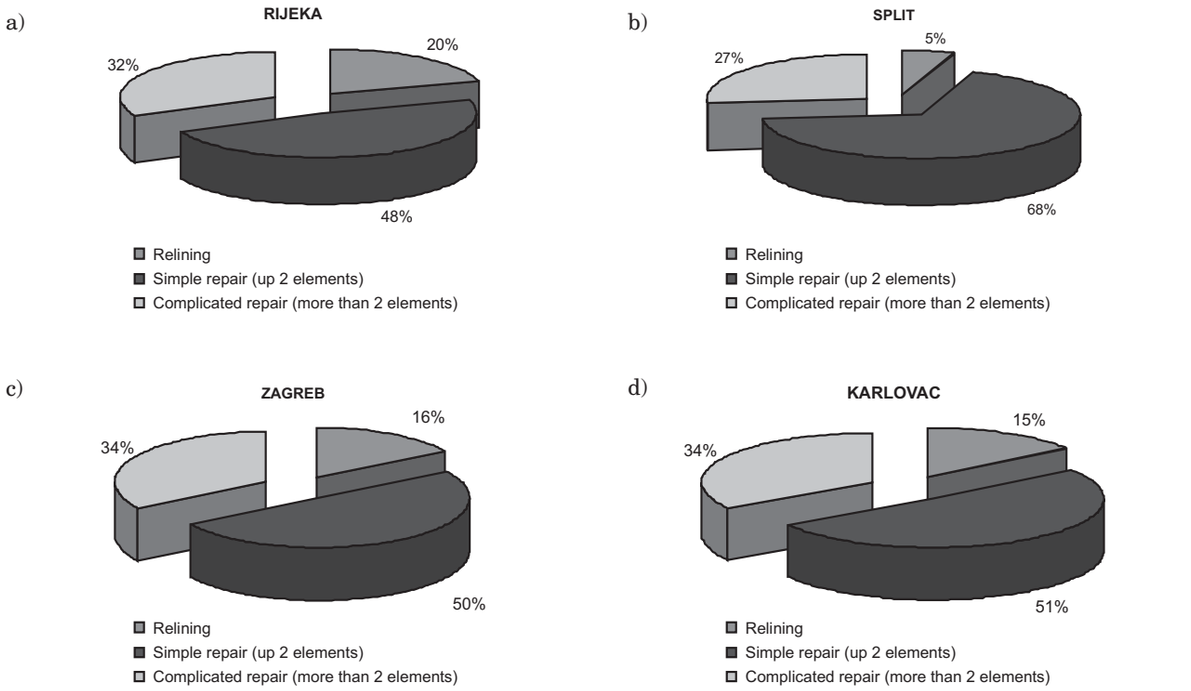

Fig. 2. Types of denture repairs in different districts of Croatia: a) Rijeka, b) Split, c) Zagreb and d) Karlovac.

tasks if a denture is miss fitting on a denture bearing area due to residual ridge resorption and a long time denture wearing[15].

Considering a need for relining two factors have to be considered: miss fitting denture due to its construction and residual ridge resorption due to long-term denture wearing[15,16].

Complicated repairs (broken clasp, fractures, tooth loss, and other examples with a repair of more than 2 elements) could be due to technical and/or biological reasons.

The likelihood that an acrylic resin RDs may, in future, require a further simple repair can be reduced using glass-fiber reinforcement during a repair procedure. This occurrence cannot be entirely prevented, however because some fractures appear due to misfit or suboptimal teeth arrangement or suboptimal jaw relationship and occlusion[16]. Midline fractures appear to be the most common type of failure of maxilllary complete dentures, these can be reduced also by reinforcement of the base material or use of a metal base[17–19].

Several investigations have concluded that a substantial number of patients show functional problems in wearing complete dentures after a certain period of time, this may be due to the gradual loss of alveolar bone, but many repairs are needed due to incorrect dentures delivered to a patient[20].

Investigations in Germany showed that 20% of all clasp-retained dentures had technical complications during the period of 4.2±1.7 years[21].

Percentage of repairs in each district, and the type of denture repairs are negatively correlated with the number of prosthodontic teams in relation to a population of the region. Karlovac had the smallest number of specialist prosthodontic teams and Split and Zagreb the highest number of specialists of prosthodontics in relation to a number of inhabitants of the region.

Due to changes in the amount of curriculum time available for teaching removable denture construction to undergraduate and postgraduate students, course content requires reconsideration and possible modification[20,22,23]. The suggestion to reduce or exclude training in the specialization of prosthodontics from dental education in Europe due to the reducing number of edentulous people in the population[24] and the trend to transfer the tasks of denture production to general dentists may not be advisable. Poor quality dentures are more likely to require repairs in future. The repair costs in a population with a high level of tooth loss may be greater than a cost of training specialists. The cost for the RDs and their repairs are normally covered by the Croatian Health Insurance, thus few patients use the service in a private practice where they are obliged to pay the whole cost. Therefore, it is likely to be reasonable to make an assumption that the data from the Croatian Health Insurance are representative of the denture wearing portion of Croatian population.

Removable dentures are a major part of dentistry, and will be for the foreseeable future.

Conclusion

Removable denture repairs were negatively correlated with the number of prosthodontic specialist teams in proportion to a number of inhabitants of each region, which means with the quality of dentures. Therefore education and training in prosthodontics (specialization) should not be eliminated from the dental curriculum.

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UČESTALOST REPARATURA ZUBNIH PROTEZA U ODNOSU NA BROJ SPECIJALISTIČKIH PROTETSKIH TIMOVA U HRVATSKOJ

SAŽETAK

Cilj ovog rada bio je utvrditi učestalost reparatura zubnih proteza u različitim regijama u Hrvatskoj u 2002. godini, kao i analizirati postotak različitih reparatura (podlaganje, jednostavne reparature do 2 elementa, kompliciranе reparature preko 2 elementa) u odnosu na broj specijalističkih protetskih timova. Informacije o broju predanih proteza, kao i o broju i vrsti reparatura za regiju Zagreb, Rijeka, Karlovac i Split dobivene su od strane hrvatskog Fonda za zdravstveno osiguranje. Također su prikupljene informacije o broju specijalista protetike u istim regijama. Najveći postotak reparatura u odnosu na broj predatih proteza zabilježen je u Karlovcu: 18%, a najmanji postotak u Splitu: 5%. Signifikantno najmanji postotak podlaganja proteza zabilježen je u Splitu, a najveći u Rijeci (χ^2=36,7; p<0.01). Najmanji postotak jednostavnih reparatura zabilježen je u rijeci, a najveći u Splitu (χ^2=24.3; p<0.01). Najmanji postotak kompliciranih reparatura zabilježen je u Splitu, a najveći u Karlovcu. Postotak reparatura, kao i vrsta reparature u korelaciji su sa brojem protetskih timova u regiji. Karlovac, sa namanjim brojem specijalista protetike ima najveći broj reparatura.

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Original scientific paper

Selection of Appropriate Artificial Frontal Teeth Size Using Dimensions of Hard Palate

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A B S T R A C T

Eighty dentate students participated as a study group and another 74 as a control group. The aim was to determine a possibility to reconstruct maxillary frontal teeth dimensions by use of certain hard palate dimensions. The height (IH) and the incisal (IW), contact point (CtW) and cervical width (CW) of maxillary central incisors (MCI), hamular width (HW) and the distance between the incisive papilla and the palatine foveas (IP-FP) were measured on the maxillary casts. CtW of maxillary lateral incisors and canines were measured too. In the study group the ratios were computed: HW/IW (5.71), HW/CtW (5.69), HW/CW (5.51) and IP-FP/IH (4.76). These ratios were multiplied by incisor's dimensions (obtained from the control group) to calculate the hard palate dimensions. No significant differences were obtained between the calculated and the measured (study group) hard palate dimensions. Furthermore, there was no significant difference between the HW and the Sum of contact-point widths of all maxillary frontal teeth ($p>0.05$) in the both groups. The results revealed: 1. MCI width and height might be calculated by dividing dimensions of a patient's hard palate and appropriate ratio; 2. hamular width dimension can be used as a selection guide for the sum of contact-point widths of six maxillary frontal teeth.

Key words: *angle class II, selection of artificial frontal teeth, hard palate*

Introduction

Esthetics is an important issue for both, dentists and their patients[1]. Esthetically acceptable dentures should not be different from the natural teeth[2–4]. Therefore, the selection of artificial teeth is an important concern in complete denture construction. Dimension, shape and color of artificial teeth are the most important factors in their selection[5–7].

Attempts have been made to find a method for selection of acceptable anterior teeth. Almost 90 years ago, Williams suggested that a correlation between the upside-down facial shape and the shape of the upper central incisors existed[8,9]. The dental outlines of the upper incisors were classified into three categories: tapered, ovoid and square-shaped. William's theory was the most accepted one throughout the literature, although data regarding the size of the teeth were lacking. Frush and Fisher introduced the dentogenic (SPA) theory[10]. Selection of artificial teeth was determined according to the sex, personality, and age (SPA) of each individual[11]. Lowery and Nelson proposed that a close relationship between face, tooth and tooth arch form (hard palate form) existed[12,13]. However, recent studies were neither able to confirm the relationship between the face form and the shape of the maxillary first incisor, nor between palatal shape and the shape of maxillary first incisor[14–17].

Appearance of artificial frontal teeth in dentures has often been unsatisfactory. It had been reported that artificial teeth were frequently too narrow and/or too long due to too narrow prosthetic moulds[18]. Therefore, attempts have been made to establish methods for selection of appropriate size of maxillary anterior teeth. Many investigators studied the relationship of dimensions between various landmarks on a subjects' face and a size of maxillary anterior teeth[19–21]. However, few attempts in

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order to correlate the dimensions of the hard palate and the maxillary incisors have been made.

The aim of this study was to assess a possibility to reconstruct maxillary first incisors and other frontal teeth dimensions using dimensions of various landmarks of the hard palate.

Materials and Methods

Study population

A total of 80 individuals (24 men and 56 women, 18–30 years old) participated as a study group, and another 74 individuals (23 men and 51 women) participated as a control group. All individuals had intact frontal teeth, Angle Class I occlussal relationship (minimal tooth rotations or compressions were allowed). Exclusion criteria were: one or more teeth missing (except the third molars), any restorations or visible tooth attrition on frontal teeth. Patients who had undergone orthodontic treatment or patients with any tooth size or shape abnormalities were also excluded from this study, as well as patients with marginal periodontitis and gingival recession.

Irreversible hydrocolloid impressions of the maxillary jaw were made (Alginoplast fast set, Heraeus Kulzer, Hanau, Germany) and casts were poured in the hard stone (ISO Type I, Vel-Mix Stone, Kerr Italia S. p. A., Salerno, Italy). The round end filling instrument was used for precise location of the hamular notch and indelible pencil (0.1 mm point) was used for demarcation prior to impressions.

All subjects were well-informed about the aim and the methods, and gave a written consent. The study was approved by the institutional ethic's committee.

Measurements

Measurements were made directly on the casts using a precise caliper (0.1 mm precision) (DKSH Switzerland Ltd. GPM Anthropological Instruments, Zurich, Switzerland). All measurements were made by one person.

Clinical crown height (IH) of the right and the left maxillary central incisor (MCI) are measured between incisal edge and the most apical point of marginal gingiva. The widths of the right and the left MCIs are measured at the incisal edge (IW), at the level of interdental contact points (CtW) and between the tips of interdental papillas (cervical width-CW). Contact point width of maxillary lateral incisors and canines were measured as well.

The hamular width (HW) was measured between the most mesial demarcation point of the left and the right hamular notch. Hard palate length (IP-FP) was measured between the palatine foveas (midline between left and right fovea palatina) and the centre of incisive papilla.

The ratios between the hard palate width (HW) and MCI widths (IW, CtW, CW) and ratio between the hard palate length (IP-FP) and the MCI height (IH) were calculated for the study group. The sum of contact-point widths of all maxillary frontal teeth (incisort and canines) was also calculated (SCtPW) for the both groups.

The dimensions of the maxillary first incisors were measured in the control group and the results were multiplied by the ratios (hard palate dimension / frontal tooth dimension) obtained from the study group. Then the calculated and the measured hard palate dimensions of the control group were compared.

Reliability

In order to test the reliability of measurement, 10 randomly selected casts were measured by five dental practitioners within a two-week period. Statistical analysis (ANOVA) revealed no significant differences between different subjects and between the first and the second survey ($p>0.05$).

Data analysis

Statistical analysis was made by SPSS 12 for Windows. Normality of the distribution was tested by the Kolmogorov-Smirnov test. Means and standard deviations were calculated. The significance of the differences were tested by the Student's t test.

Results

The distribution of the data was normal ($p>0.05$), as assessed by the one-sample Kolmogorov-Smirnov test.

Descriptive statistics for the study group (mean values, standard deviations, minimum and maximum values) is presented in Table 1.

There was no significant difference between men and women ($p>0.05$).

There was no significant difference between the dimensions of the left and the right MCI ($p>0.05$). Therefore, mean values between the left and the right MCI dimensions were calculated.

Descriptive statistics of the mean height and width of MCI, as well as SCtPW in the study group is presented in Table 2.

The ratios between palate dimensions and MCI dimensions were calculated in the study group and the results are presented in the Table 3.

Descriptive statistics and the significance of the difference between the measured hard palate dimensions and the calculated hard palate dimensions in the control group are presented in Table 4. The hard palate dimensions of the control group were computed by multiplying the MCI dimensions of the control group by the ratios obtained from the study group (Table 3). There was no significant difference between the measured and the calculated dimensions of the hard palate ($p>0.05$).

Furthermore, there was no significant difference between HW and the SCtPW in the both groups (study group: $t=1.69$, $df=79$, $p>0.05$; control group: $t=1.32$, $df=73$, $p>0.05$).

TABLE 1
MEASURED DIMENSIONS OF THE STUDY GROUP

	Min.	Max.	X	SD
Hamular width	36.0	55.0	47.1	4.71
Distance between incisive papila and palatine foveas	37.3	55.1	44.79	3.48
Height of left maxillary first incisor	7.0	11.4	9.58	0.89
Height of right maxillary first incisor	7.4	11.5	9.38	0.88
Cervical width of left maxillary first incisor	7.1	9.9	8.25	0.52
Cervical width of right maxillary first incisor	7.4	9.8	8.27	0.49
Contact points width of left maxillary first incisor	6.5	1..3	8.57	0.53
Contact points width of right maxillary first incisor	6.5	10.0	8.54	0.51
Incisal width of left maxillary first incisor	6.7	9.8	8.33	0.56
Incisal width of right maxillary first incisor	6.1	9.8	8.24	0.61
Contact points width of left maxillary second incisor	5.5	8.2	6.68	0.52
Contact points width of right maxillary second incisor	5.6	8.3	6.68	0.52
Contact points width of left maxillary canine	6.7	8.7	7.78	0.43
Contact points width of right maxillary canine	6.9	8.8	7.79	0.41

TABLE 2
CALCULATED VARIABLES FOR THE STUDY GROUP

	Min.	Max.	X	SD
Height of maxillary first incisor	7.30	11.45	9.48	0.857
Cervical width of maxillary first incisor	7.35	9.85	8.26	0.497
Contact points width of maxillary first incisor	6.50	10.15	8.55	0.514
Incisal width of maxillary first incisor	6.65	9.70	8.29	0.558
Contact points width of maxillary second incisor	5.65	8.25	6.68	0.508
Contact points width of maxillary canine	6.80	8.75	7.79	0.405
Sum of contact-point widths of all maxillary frontal teeth	36.9	53.8	46.04	0.223

TABLE 3
RATIOS BETWEEN THE HAMULAR AND THE MAXILLARY FIRST INCISOR DIMENSIONS

Ratios	Min.	Max.	X	SD
Hamular width / cervical width of maxillary first incisor	4.25	7.50	5.71	0.64
Hamular width / incisal width of maxillary first incisor	4.13	7.34	5.70	0.70
Hamular width / contact point width of maxillary first incisor	4.02	7.51	5.51	0.65
Distance between incisive papila and palatine foveoas / height of maxillary first incisor	3.63	6.90	4.76	0.58

Discussion

Proper selection of artificial teeth is very important in removable prosthodontics. When the maxillary anterior teeth have to be restored, clues gained from the natural dentition can be helpful in achieving an individual restoration[1,22,23]. However, when all teeth are missing and no photographs or cast documents of the original dentition are available, the choice of artificial teeth is more complex and other criteria have to be used.

Selection of artificial teeth has to be based on the proper shape and exact dimensions. Anterior position of the maxillary frontal teeth has the strongest influence to patients' esthetics[24–27]. The relationship of a shape and dimensions of various soft tissue landmarks on someone's face and a size and a shape of maxillary anterior teeth showed no significant correlation in previous studies[19–21]. Data correlating some dimensions of a hard palate and maxillary incisors are scarce in the literature.

The purpose of this research was to study a possibility to reconstruct maxillary frontal teeth dimensions using certain dimensions of a hard palate.

Measurements were made on the casts of maxillary jaws poured in a hard stone (ISO Type I). Although, hard stone expansion during setting might influence the precision of the results, possibility of such error is very small and of no clinical importance, as reported by Mack[28].

No significant differences between men and women for the height and the widths of the maxillary first incisors ($p>0.05$) were found. Lindemann found out that

TABLE 4
SIGNIFICANCE OF THE DIFFERENCE BETWEEN THE MEASURED AND CALCULATED HARD PALATE DIMENSIONS

	X	SD	t	df	p
Distance between incisive papillas and palatine foveas (study group)	44.79	3.48	–0.53	73	0.43 ns
Calculated hard palate length by multiplying the height of maxillary first incisor (control group) by the ratio (4.76)	45.07	4.08			
Hamular width (study group)	47.1	4.71	0.24	73	0.81 ns
Calculated hamular width by multiplying the cervical width of maxillary first incisor (control group) by the ratio (5.71)	47.14	2.84			
Hamular width (study group)	47.1	4.71	0.23	73	0.82 ns
Calculated hamular width by multiplying the contact points width of maxillary first incisor (control group) by the ratio (5.51)	47.13	2.84			
Hamular width (study group)	47.1	4.71	1.31	73	0.19 ns
Calculated hamular width by multiplying the incisal width of maxillary first incisor (control group) by the ratio (5.70)	46.98	4.41			

central maxillary incisors had the same width in both gender, but women had shorter incisors[16].

No significant differences were found between the dimensions of the left and the right maxillary first incisors ($p>0.05$). Therefore mean values for the height and the width of the maxillary first incisor were calculated (Table 2).

According to Brand and Isselhard and Berkovitz et al., maxillary first incisor was 8.5 mm wide, which is in agreement with the results of the present study[29,30]. Mavroskoufis reported only 0.03 mm difference between the dimensions of MCI on the left and the right side of dental arch[31].

When all teeth are missing, it is difficult to reconstruct the exact position of the maxillary frontal teeth, since the rate of alveolar bone resorption is individual in each subject. On the other hand, hamular notches, incisive papilla and foveae palatine have been considered to be reliable landmarks because they are not submitted to resorptive changes after teeth extraction[32]. Their position is determined by anatomical structures. Incisive papilla has been used as a guide for setting frontal maxillary teeth in the proper arch position during complete denture set-up procedure[33]. Therefore HW and IP-FP have been chosen as distance references in order to calculate MCI dimensions.

Ratio between the hard palate length (IP-FP) and the MCI height was calculated, as well as the ratios between the hard palate width (HW) and the MCI widths (Table 4). Appropriate ratios obtained from the measurements of the casts in the study group were then multiplied by the MCI height or appropriate width obtained from the control group in order to calculate the hard palate height and width of the control group. There was no significant difference between the measured and the calculated hard palate dimensions in the control group ($p>0.05$) (Table 4). Therefore the ratios calculated in this study seem to be relevant for a proper choice of the maxillary first incisor's dimensions.

Furthermore, there was no significant difference between HW and SCtPW ($p>0.05$). This suggests that the hamular width is an appropriate landmark for the choice of the widths of the six frontal maxillary teeth.

Conclusions

Hamular width (distance between the left and the right hamular notch), and the distance between the centre of incisive papilla and palatine fovea could be helpful landmarks in order to determine the maxillary first incisor's dimensions. Central maxillary incisor's height (IH) might be calculated by dividing IP-FP by 4.76. Central maxillary incisor's cervical (CW), contact-points (CtW) and incisal width (IW) might be calculated by dividing hamular width (HW) by 5.51, 5.69 or 5.71.

Hamular width could be used for selection of the sum of widths of the six frontal maxillary teeth.

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IZBOR PRIKLADNOG UMJETNOG PREDNJEG ZUBA POMOĆU DIMENZIJA TVRDOG NEPCA

S A Ž E T A K

U istraživanju je sudjelovalo 80 studenata kao studijska grupa te dodatnih 74 studenta kao kontrolna skupina. Cilj istraživanja je odrediti mogućnost rekonstrukcije dimenzija gornjih prednjih zuba pomoću određenih dimenzija tvrdog nepca. Na modelu gornje čeljusti je izmjerena visina (IH) gornjeg središnjeg sjekutića (MCI) te njegova incizalna širina (IW), širina u razini kontaktnih točaka (CtW) i cervikalna širina (CW). Izmjerena je i CtW bočnih sjekutića i očnjaka. Također je izmjerena i hamularna širina (HW) i udaljenost između papille incisive i fovea palatine (IP-FP). U studijskoj grupi izračunati su omjeri: HW/IW (5.71), HW/CtW (5.69), HW/CW (5.51) and IP-FP/IH (4.76). Omjeri su pomnoženi sa dimenzijama sjekutića kontrolne skupine da bi se izračunale dimenzije tvrdog nepca. Nije uočena statistički značajna razlika između izračunatih i izmjerenih dimenzija tvrdog nepca. Također nije uočena statistički značajna razlika između HW i Zbroja širina u razini kontaktnih točaka svih gornjih prednjih zuba ($p>0.05$) u obje skupine. Zaključci ovog istraživanja su: 1.širina i visina MCI se može izračunati dijeljenjem dimenzija pacijentovog tvrdog nepca i određenog omjera; 2. hamularna širina se može upotrijebiti za izbor Zbroja širina u razini kontaktnih točaka svih gornjih prednjih zuba.

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Original scientific paper

The Tai-Phake of Assam, India – A Morphometric Study and Population Comparison with Neighbouring Groups

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A B S T R A C T

Morphometric characters of the adult males of the Tai-Phake and the nature and extent of morphometric variation among five neighboring mongoloid groups of Assam have been examined in the present study. For the sake of investigation 12 anthropometric measurements have been taken and five indices have been calculated. In order to obtain the distance values size, shape and biological distance are calculated. It revels that the Tai-Phake maintains a far distance with other five neighboring groups (Ahom, Deuri, Chutia, Mishing and Moran). The Ahom also maintain far distance with other five groups. While, the Mishing and Moran, and Deuri and Chutia maintain close distance among themselves.

Key words: *anthropometrics, Tai-Phake, neighbouring populations*

Introduction

The Tai-Phake are one of the branches of the Tai groups who entered Assam in the later half of the 18th century. They are of mongoloid origin. Language of the Tai-Phake belongs to the Siamese-Chinese branch of the Sino-Tibetan linguistic group. They profess Buddhism and Lord Buddha is their supreme god. Their main occupation is agriculture. Among the Tai-Phake marriage must not take place within same clan and marriage outside the community is not allowed. Monogamy is the prevailing practices, while polygamy is rare.

The object of this paper is to present the results of an anthropometric survey undertaken among the Tai-Phake population. Chief concern of this study is therefore to reveal the physical characteristics of the people. Side by side, the most important aspect of it is to assess the nature and extent of biometrical variation among other five neighbouring endogamous mongoloid groups inhabiting in Assam, namely the Ahom, Deuri, Chutia, Mishing and Moran.

Materials and Methods

Anthropometric measurements

Material of the present study contains a collection of anthropometric data of 104 adult Tai-Phake males, aged between 21 to 55 years. The investigation was carried out by the first author (T.B.), during the month of November, 2003 to January, 2004 in two villages namely, Tipam Phakial and Nam Phakial situated in Dibrugarh district of Assam State. Average age of their subjects is 33.68±1.38. Altogether 12 anthropometric measurements were taken according to the definition and technique of Martin[1]. These are stature (St.), sitting height (S.H.), head length (H.L.), head breadth (H.B.), bizygomatic breadth (B.B.), head height (H.H.), nasal height (N.H.), nasal breadth (N.B.), total facial height (T.F.H.), upper facial height (U.F.H.), circumference of head (C.H.), and girth of thorax (G.T.). The abbreviations put within the brackets are used in the Tables. From these 12 anthropometric measurements 5 indices were calcu-

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lated following the technique of Martin and Saller[2] and Singh and Bhasin[3]. To find out the variability data of the present study were compared with the data of adult males from five neighboring mongoloid groups of Assam namely the Ahom, Deuri, Chutia Mishing and Moran. For this purpose data were taken from Das et al.[4].

Statistical consideration

Size and shape distance:

To find out the size and shape distances the measures of Penrose[5,6] have been followed. Its mathematical formula is as:

$$\text{Size distance} = c_q^2 = \left[(d_1 + d_2 + d_3 + \ldots + d_m) / m \right]^2$$
$$= \left[\sum_1^m (d) \right]^2 / m^2$$

$$\text{Shape distance} = c_z^2 = \sum_1^m (d)\, 2/m - \left[\sum_1^m (d) \right]^{2/m^2}$$

Where, d_1, d_2, d_3,…….d_m represent the difference between standardized means for m characters in two populations.

Biological distance:

The biological distances have been obtained according to the formula of El-Najjar[7] and its mathematical formula is as:

Biological distance (co-efficient of divergence)

$$\text{C. D.} = \sqrt{\frac{(a_1 - b_1)^2 + (a_2 - b_2)^2 + \ldots (a_k - b_k)^2}{K}}$$

$$\text{Where } a_1 = \frac{Ai}{Ai + Bi},\ b_1 = \frac{Bi}{Ai + Bi}$$

Ai – the mean of the i^{th} measurement in population 'A'
Bi – the mean of the i^{th} measurement in population 'B'
K – number of measurements

Results and Discussion

Descriptive statistics of 12 anthropometric characters of the Tai-Phake have been shown in Table 1. It reveals that the characters do not represent distinct physical polytypes. This justifies treating the material of the present study as a single breeding population. Exceptional cases in this respect are head height (13.65), nasal height (8.93), and total facial height (7.99). However, such departures from the general trend may be due to small sample size of the present study. Nevertheless, there are some determining differences of variation in greater or lesser order in the measurements. Considerably higher variation is noticed in case of nasal breadth (7.40) and girth of thorax (7.18). Upper facial height (6.98), Bizygomatic breadth (6.24) and circumference of head (5.59) also show a moderately high variation. Such a variation in these measurements, as explained by Pearson and Davin[8] is possible due to the spanning of cavities between the space of corresponding measuring landmarks and the varying degrees of thickness of the involved soft tissues of the subjects. In a normally distributed sample of a given population, however, the observed variability in different body measurements are not unexpected. Thus, on the whole, the Tai-Phake population represented by the present sample is homogenous.

In Table 2 percentage frequencies of different types of stature, cephalic index, length-height index, breadth--height index, total facial index and nasal index are presented. It appears that majority of the Tai-Phake have short stature (36.84%), followed by below medium stature (20.17%). In case of cephalic index hyperbrachycephalic head (40.35%) is found in the majority and in the latter brachycephalic head (28.95%) is found in the next highest frequency. Frequency of length-height and breadth-height indices occurs highest in hypsicephalic (89.47%) and acrocephalic head (77.19%) respectively. In case of total facial index the hyperleptoprosopic face (46.49%) and in nasal index the mesorhinae nose (50%) occurs in highest frequencies.

TABLE 1
BIOMETRIC DATA OF THE TAI-PHAKE MALES (n=104)

Measurements (cm)	Range	X±SE	SD±SE	CV±SE
Stature (St.)	137–180.0	162.54±0.81	8.26±0.57	5.08±0.35
Sitting height (S.H.)	63–99.0	84.73±0.41	4.21±0.29	4.97±0.54
Head length (H.L.)	16–19.9	17.94±0.06	0.58±0.04	3.23±0.22
Head breadth (H.B.)	11–17.9	15.15±0.04	0.41±0.03	2.71±0.19
Bizygomatic breadth (B.B.)	10–14.9	12.66±0.08	0.79±0.05	6.24±0.45
Head height (H.H.)	11–16.9	12.82±0.17	1.75±0.12	13.65±0.95
Nasal height (N.H.)	3–7.9	5.26±0.05	0.47±0.03	8.93±0.62
Nasal breadth (N.B.)	3–6.9	4.19±0.03	0.31±0.02	7.40±0.51
Total facial height (T.F.H.)	8–14.3	12.01±0.09	0.96±0.07	7.99±0.55
Upper facial height (U.F.H.)	5–8.9	7.73±0.05	0.54±0.04	6.98±0.48
Circumference of head (C.H.)	50–65.9	56.02±0.31	3.13±0.22	5.59±0.39
Girth of thorax (G.T.)	70–99.9	84.30±0.59	6.05±0.42	7.18±0.50

SE – standard error, CV – coefficient of variation

TABLE 2
DIFFERENT TYPES OF ANTHROPOMETRIC CHARACTERS IN THE TAI-PHAKE MALES (%)

Class	(%)
Stature	
Short	36.84
Below medium	20.17
Medium	14.91
Above medium	13.15
Tall	14.93
Cephalic index	
Hyperdolicocephalic	0.88
Dolicocephalic	1.75
Mesocephalic	28.07
Brachycephalic	28.95
Hyperbrachycephalic	40.35
Length-height index	
Chamaeocephalic	6.14
Orthocephalic	4.38
Hypsicephalic	89.47
Breadth-height index	
Tapeinocephalic	11.40
Metriocephalic	11.40
Acerocephalic	77.19
Total facial index	
Hypereuryprosopic	13.16
Euryprosopic	1.75
Mesoprosopic	14.91
Leptoprosopic	23.68
Hyperleptoprosopic	46.49
Nasal index	
Leptorhinae	17.54
Mesorhinae	50.00
Platyrhinae	32.46

Variability and classification of populations

It is rather difficult to assess the inter-group relationships based on univariate analysis of the data. In such case we face the problem of biological taxonomy. Multivariate distance analysis helps us in understanding such problem. For population classification it is necessary to find out the morphological affinities and differences between and among the groups. Thus, in estimating the numerical taxonomy and group divergence of some particular groups it is needed to perform the analysis of overall distance differences and mutual relationships among all possible pairs from a matrix of all multivariate distance between the groups obtained by utilizing a suitable measure of taxonomic distance[9].

Mean and standard deviation values of 12 anthropometric measurements of six mongoloid populations are shown in Table 3, while in Table 4, the means in terms of pooled standard deviation unit are presented. Size and shape distance values between any two groups are shown in Table 5 and 6 respectively. This has been performed for an overview on the size and shape factors used to find out the divergence among groups. Computed mean values for size and shape distance is 0.04 and 0.55 respectively between 15 pairs. This implies that the six mongoloid groups show a tendency to differ more in shape distance than in size distance. Thus, here, the shape distance plays a more important role than the size distance because of the morphological dissimilarity and differences.

On the basis of 12 anthropometric measurements biological distances (C.D.) are calculated among the 6 population groups. The distance values are furnished in Table 7. In Table 8 the values of biological distance are shown among these groups in an increasing order of magnitude. It reveals that the Mishing and Moran maintain a lowest distance (1.08). Side by side, the Deuri and Chutia also maintain a minimum distance (1.15). The Ahom and Tai-Phake in turn, maintain a far distance with those

TABLE 3
MEANS AND STANDARD DEVIATIONS OF ANTHROPOMETRIC CHARACTERS OF SIX MONGOLOID GROUPS

Measurements (in cm.)	Ahom (n=100)		Deuri (n=99)		Chutia (n=83)		Mishing (n=100)		Moran (n=100)		Tai-Phake (n=104)	
	X	SD	X	SD	X	SD	X	SD	X	SD	X	SD
Stature (St.)	162.83	6.20	163.86	4.78	164.11	5.01	161.13	5.00	162.97	6.20	162.54	8.26
Sitting height (S.H.)	84.72	4.00	83.95	3.18	84.13	3.28	84.05	2.90	84.59	3.30	84.73	4.21
Head length (H.L.)	18.24	0.80	18.38	0.50	18.38	0.64	18.63	0.60	18.80	0.60	17.94	0.58
Head breadth (H.B.)	14.88	0.70	14.63	0.50	14.68	0.64	14.78	0.68	14.46	0.50	15.15	0.41
Bizygomatic breadth (B.B.)	13.65	0.50	13.54	0.50	13.44	0.55	13.65	0.60	13.39	0.60	12.66	0.79
Head height (H.H.)	12.65	1.40	13.69	1.49	13.63	1.64	13.77	1.30	13.55	1.30	12.82	1.75
Nasal height (N.H.)	5.27	0.40	5.21	0.50	5.04	0.45	5.28	0.40	5.39	0.40	5.26	0.47
Total facial height (T.F.H.)	11.46	0.60	11.44	0.60	11.59	0.73	11.97	0.60	11.98	0.70	12.01	0.96
Upper facial height (U.F.H.)	8.98	0.50	7.00	0.60	6.76	0.55	7.19	0.50	7.29	0.50	7.73	0.54
Circumference of head (C.H.)	55.45	1.80	55.71	1.59	55.16	1.91	55.94	1.50	55.81	1.50	56.02	3.13
Girth of thorax (G.T.)	86.36	5.10	87.51	3.98	84.78	3.46	87.70	4.00	87.85	4.50	84.30	6.05

TABLE 4
MEANS OF ANTHROPOMETRIC CHARACTERS OF SIX MONGOLOID GROUPS (MALES) IN TERMS OF POOLED STANDARD DEVIATION UNIT

Population	St.	S.H.	H.L.	H.B.	B.B	H.H.	N.H.	N.B.	T.F.H.	U.F.H.	C.H.	G.T.
Ahom	26.78	24.00	28.95	26.57	22.75	8.49	11.98	13.50	16.14	16.94	27.59	18.61
Deuri	26.95	23.78	29.17	26.12	22.57	9.19	11.84	13.57	16.11	13.21	27.72	18.86
Chutia	26.99	23.83	29.17	26.21	22.40	9.15	11.45	13.50	16.32	12.75	27.44	18.27
Mishing	26.50	23.81	29.57	26.39	22.75	9.24	12.00	13.18	16.86	13.57	27.83	18.90
Moran	26.80	23.96	29.84	25.82	22.32	9.09	12.25	13.57	16.87	13.75	27.77	18.93
Tai-Phake	26.73	24.00	28.48	27.05	21.11	8.60	11.95	14.96	16.91	14.58	27.87	18.17
Pooled SD	6.08	3.53	0.63	0.56	0.60	1.49	0.44	0.28	0.71	0.53	2.01	4.64

St. – Stature, S.H. – Sitting height, H.L. – Head length, H.B. – Head breadth, B.B. – Bizygomatic breadth, H.H. – Head height, N.H. – Nasal height, T.F.H. – Total facial height, U.F.H. – Upper facial height, C.H. – Circumference of head, G.T. – Girth of thorax

TABLE 5
SIZE DISTANCES (C_Q^2) BETWEEN GROUPS

Population	Ahom	Deuri	Chutia	Mishing	Moran	Tai-Phake
Ahom	–	0.07	0.16	0.05	0.01	0.02
Deuri		–	0.02	0.02	0.01	0.01
Chutia			–	0.07	0.08	0.06
Mishing				–	0.00	0.00
Moran					–	0.00
Tai-Phake						–

TABLE 6
SHAPE DISTANCES (C_Z^2) BETWEEN GROUPS

Population	Ahom	Deuri	Chutia	Mishing	Moran	Tai-Phake
Ahom	–	1.17	1.41	0.16	1.06	0.96
Deuri		–	0.05	0.09	0.08	0.73
Chutia			–	0.14	0.18	0.73
Mishing				–	0.08	0.79
Moran					–	0.69
Tai-Phake						–

four populations. The distances between the Tai-Phake and the Moran, Mishing, Deuri, Chutia and Ahom are 2.53, 2.89, 2.99, 3.20 and 3.34 respectively. While, the distances between the Ahom and Tai-Phake, Moran, Mishing, Deuri and Chutia are 3.34, 3.38, 3.68, 3.78 and 4.64 respectively. For a clear view of the situation, the distance values of the Moran, Mishing, Deuri, Chutia and Ahom in relation to the Tai-Phake are plotted in Figure 1.

Following the methodology of Mardia et al.[10] a dendrogram has been computed (Figure 2) on the basis of the data given in Table 7. It appears that there are two distinct clusters. Cluster I: Mishing and Moran. Cluster II: Deuri and Chutia

The Tai-Phake and Ahom found to be maintaining a far distance with these clusters.

Interesting corroboration between anthropometric measurements and inter-group relationships has already been reported while dealing with numerical taxonomy and group divergence among some population groups of Assam and Meghalaya State of India[9, 11,12].

It may be mentioned that all these six populations are living in upper part of Assam for a long period of time. They came to Assam at different periods. Though all of them are mongoloid, each has its own identity. They differ with respect of their dialects/languages and many other cultural traits. The Deuri, however, form a sub-division of the Chutia, who are considered to be the priestly section of this population[13]. A close distance between the Deuri and Chutia corroborate this finding. The Mishing

TABLE 7
BIOLOGICAL DISTANCES (C.D.) BETWEEN GROUPS (%)

Population	Ahom	Deuri	Chutia	Mishing	Moran	Tai-Phake
Ahom	–	3.78	4.64	3.68	3.38	3.34
Deuri		–	1.15	1.65	1.53	2.99
Chutia			–	1.85	1.94	3.20
Mishing				–	1.08	2.89
Moran					–	2.53
Tai-Phake						–

TABLE 8
BIOLOGICAL DISTANCES (C.D.) BETWEEN ANY TWO GROUPS ARRANGED IN INCREASING ORDER OF MAGNITUDE

Ahom	Deuri	Chutia	Mishing	Moran	Tai-Phake
3.34 Tai-Phake	1.15 Chutia	1.15 Deuri	1.08 Moran	1.08 Mishing	2.53 Moran
3.38 Moran	1.53 Moran	1.85 Mishing	1.65 Deuri	1.53 Deuri	2.89 Mishing
3.68 Mishing	1.65 Mishing	1.94 Moran	1.85 Chutia	1.94 Chutia	2.99 Deuri
3.78 Deuri	2.99 Tai-Phake	3.20 Tai-Phake	2.89 Tai-Phake	2.53 Tai-Phake	3.20 Chutia
4.64 Chutia	3.78 Ahom	4.64 Ahom	3.68 Ahom	3.38 Ahom	3.34 Ahom

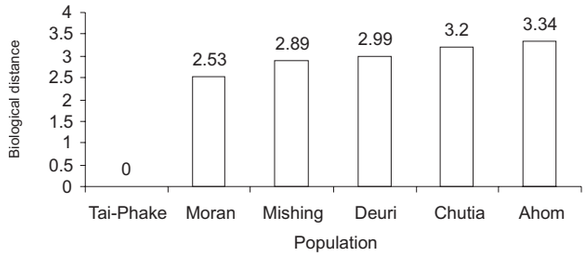

Fig. 1. Biological distance of five mongoloid groups in relation to the Tai-Phake.

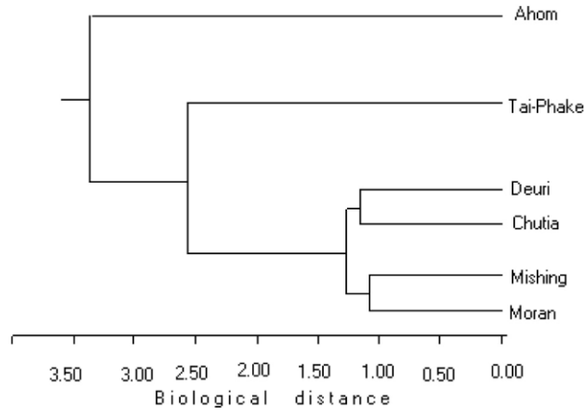

Fig. 2: Dendrogram based on biological distance of six mongoloid groups.

and Moran also speak a dialect belong to the Assam--Burma branch of the Tibeto-Burman family, like the Chutia and Deuri, though they form a separate cluster. The Ahom and Tai-Phake seem to be merely peculiar populations in this regard. They revealed remarkably higher biological distance among themselves, as well as with other four populations. It is interesting to note that linguistically, both the Ahom and Tai-Phake belong to the Siamese-Chinese branch of the Sino-Tibetan family. Thus, it may be the pattern of variability among the six mongoloid population groups of Assam. From the analysis of biological distance, however, it seems to be difficult to arrive at any conclusion regarding their inter-group relationship.

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TAI-PHAKE IZ ASSAMA, INDIA – MORFOMETRIJSKO ISTRAŽIVANJE I POPULACIJSKA USPOREDBA SA SUSJEDNIM GRUPAMA

SAŽETAK

U ovom istraživanju proučavane su morfometrijske značajke odraslih muškaraca Tai-Phake grupe te priroda i obim morfometrijske varijacije među pet susjednih grupa mongolskog podrijetla iz Assama. Za istraživanje je uzeto 12 antropometrijskih mjera i izračunato pet indeksa. Kao mjere udaljenosti, uzete su udaljenosti veličine, oblika i biološka udaljenost. Ustanovljeno je da Tai-Phake grupa pokazuje veliku udaljenost od pet susjednih grupa (Ahom, Deuri, Chutia, Mishing and Moran). Ahom skupina je također udaljena od ostalih pet grupa. Za razliku od toga, grupe Mishing i Moran te Deuri i Chutia pokazuju manju međusobnu udaljenost.

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Measures for Achieving Recruits' Enhanced Fitness – A Transversal Study

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A B S T R A C T

Because of 10.94% frequency in obese recruits in Rijeka in 2005 occupational medicine decided to study causality of that and other most frequent diagnoses: pedes plani, myopia and astigmatism, kyphosis and scoliosis, asthma, hypertension and branch block. Double monitoring of 1,311 recruits was carried out by a transversal study during 2005, 2000 and 1995 and within each year according to location: city, suburbs, islands. The differences in the three periods in the city were obesity ($p<0.05$) with highest frequency in 2005, asthenia ($p<0.05$) with lowest frequency 0.99% in 2005, and pedes plani ($p<0.05$) with highest frequency in 1995. Suburbs showed ($p<0.05$) for pedes plani, $p=0.054$ for obesity, and the islands obesity ($p<0.05$). Myopia and astigmatism frequency went up to 25%, kyphosis to 14.13% and asthma to 5.43%. Hypertension frequency was negligible. Occupational medicine decided to react by measures increasing recruit fitness cooperating with school medicine, teachers and parents, by check-ups, corrections, dieting and physical activities.

***Key words**: recruits, diagnoses, occupational medicine*

Introduction

Unhealty diet, lack of exercise and physical activities in the young presents a problem not only in Croatia but also in many West-European countries[1], and particularly in the USA[2]. Western life-style leads significantly to high prevalence of coronary arterial disease. Smoking, diet rich in fat, inactivity, obesity, alcohol abuse are conventional factors for 80% of cardiovascular incidences. Obesity is a lingering metabolic imbalance characterized by positive energy balance which leads to excessive fat deposits as compared to a normal organism[3]. The roots of the problem are multiple, from environmental, cultural, hormonal to physiological. Excessive weight predisposes developing diabetes type II and coronary heart diseases[4]. Obesity and sedentary life-style are increasingly becoming pandemic nationally and globally causing early death, invalidity and enormous expenses for health insurance[5]. Exposed to increased physical strain obese people react more rapidly to stress factors, dehydration, warming up, fatigue. This can be intensified by non-acclimatization, lack of practice, humidity, medication, respiratory and gastrointestinal diseases[6]. The lack of physical activities appears already in childhood and it progresses in adolescence[7]. Creating a healthy environment, which includes increased physical activity, would probably be the best intervention strategy to prevent weight gain in adolescents[8]. Visual impairment, besides obesity, may also be considered a major problem in the young. Modern civilization brings about many amenities, but it also causes damage to our health, particularly to most subtle eye structures. Exposure to microwaves, from those emitted by microwave ovens to professional exposure, may cause blurred lens, cataract[9]. The increase in illuminated signs especially in cities, too strong street lights but also increased daylight lead to phototototoxic visual impairment in the young[10]. Radiofrequency energies may also cause various eye effects, primarily cataracts, but changes in retina and cornea as well[11]. We also witness bad posture of the young that grow quickly. Enhondral spinal growth of the vertebral column does not stop after puberty. Although spinal growth is genetically determined the proper posture during puberty is very important for the right development of spine[12]. Environment and work place are becoming increasingly polluted and the populations most sensitive to such conditions are the young who often develop asthma and chronic bronchitis and who in big cities rather stay at home than go in

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for physical activities in cities in the open polluted environment[13].

When an occupational medicine specialist examines recruits, i.e. the youth exposed to present-day environmental stressful factors and who in most cases consequentially develop diagnoses mentioned in this study: obesity, asthenia, myopia and astigmatism, pedes plani, kyphosis and scoliosis vertebrae, asthma and chronic bronchitis, hypertension, ECG registered branch blocks, he can only assess their fitness for military service on the basis of the army book of rules.

To enhance such fitness occupational medicine, as a preventive branch of medicine, must co-ordinate emergency measures and programs in co-operation with vocational guidance teams, teachers of physical training and educators, school medicine, but also with parents of future recruits in order to prevent these diagnoses, particularly obesity as an internationally growing problem of public health[14,15].

Materials and Methods

The total of 1,311 recruits have been examined by double monitoring criterion at the occupational medicine in Rijeka, Croatia, at the Center for Prevention, Diagnostics and Assessment of Work Ability. The first criterion was grouping the recruits according to the time period of examination, that is grouping them as examinees in the year 2005, 2000 and 1995 so as to obtain a transversal study in order to monitor some of their somatotypic characteristics and most frequent somatic diagnoses to observe the expected changes and develop preventive medical measures to alleviate or eliminate them completely. The second criterion was grouping the recruits according to their residence within each analyzed year, i.e. grouping the recruits from the city, suburbs and islands.

In order to obtain a more precise comparison in all three years, in five-year intervals, the same number of 437 recruits were examined, i.e. 201 from the city, 144 from suburbs and 92 from islands.

In the course of the year 2005 in 437 recruits, aged 18.07±1.54 (17–27 range), the mean height was 179.59± 6.66 cm (157.00–199.00 range). The mean weight was 75.00±13.69 kg (50.00–130.00 range).

In the same number of recruits, five years before, i.e. in the year 2000, aged 18.59±1.58 (18–27 range), the determined mean height was 178.23±6.62 cm (158.00–199.00 range). The mean weight was 73.06±12.49 kg (49.00–124.00 range).

The earliest examined recruits, in the year 1995, aged 18.59±1.38 (18–27 range), showed the mean height of 178.13±7.00 cm (152.00–201 range). The mean weight was 70.99±11.41 kg (49.00–119.00 range).

Stat Soft statistical program was used, Statistics 6.0. Parameters besides height and weight were monitored and in that connection diagnoses Obesity and Asthenia, the most frequent somatic diagnoses in recruits Myopia and Astigmatism (simplex and mixtus), Pedes Plani, Kyphosis and Scoliosis, Asthma and Chronic Bronchitis, Hypertension and Incomplete and Complete Branch Block as ECG diagnoses.

To show relations between specific results Pearson Chi Square test and Kruskal-Wallis test were used as well as correlation matrices and single stream variance analysis, One-way ANOVA analysis.

Results

Recruits from the city

In the course of the years 2005, 2000 and 1995 were examined 603 recruits from the city (transversal analysis, N=603, Table 1). The obtained result shows that by diagnosis obesity, i.e. overweight 30% above normal, the recruits statistically differ significantly ($p<0.05$) in the city of Rijeka in the three different time periods. So in the year 2005 there were considerably more obese re-

TABLE 1
TRANSVERSAL SURVEY OF RECRUITS IN THE 2005, 2000 AND 1995 YEARS ON THE BASIS OF THE DIAGNOSES

Area	N	Year of examination	Age (years) $\bar{x}$	H (cm) $\bar{x}$	W (kg) $\bar{x}$	Ob N	As N	P.p. N	M N	KS N	A N	H N	RBB N
1 City	201	2005	18.20	179.86	76.58	22	2	31	48	20	9	3	10
2 Suburb	144	2005	18.08	179.29	75.15	14	6	7	31	8	2	0	11
3 Island	92	2005	17.91	179.46	71.28	4	8	9	21	12	5	1	5
4 City	201	2000	18.67	179.06	73.00	11	3	15	41	12	9	4	4
5 Suburb	144	2000	18.49	177.02	71.98	10	2	22	32	9	4	2	1
6 Island	92	2000	18.59	178.31	74.86	11	2	18	21	2	1	4	1
7 City	201	1995	18.50	179.30	72.41	10	10	34	51	24	7	2	9
8 Suburb	144	1995	18.43	176.82	68.93	4	6	20	36	14	4	3	10
9 Island	92	1995	19.06	177.61	71.09	3	4	18	24	13	2	2	3

N – number of examinees, H – height, W – weight, Ob – obesity, As – asthenia, P.p. – pedes plani, M – myopia and astigmatism simplex and combined, KS – kyphosis and scoliosis vertebrae, A – asthma and bronchitis, H – high blood pressure, RBB – right branch block

cruits, 22 (10.94%), compared to the year 2000 when there were 11 (5.47%), and particularly in relation to the year 1995 when there were only 10 (4.97%) obese recruits (Figure 1). By asthenia, statistically recruits also differ significantly ($p<0.05$), but here the situation is reverse, in 2005 there were few asthenic recruits in the city, 2 (0.99%), while in 1995 there were 10 (4.97%). At the level $p<0.05$ the recruits differ also by diagnosis pedes plani. The most frequent diagnosis with city recruits is myopia and astigmatism. In all three examined years the diagnosis reaches high percentage from 20.39% to 25.37% and there are no significant differences in the time of occurrence. By the presence of kyphosis and scoliosis recruits in the three time periods statistically do not differ considerably. That diagnosis occurs in 5.97% of the year 2000 recruits, 9.95% in 2005 and 11.94% in 1995. Asthma and chronic bronchitis range from 3.48% in 1995 to 4.47% in 2000 and 2005.

By right branch block ECG diagnosis recruits also do not differ significantly. The lowest frequency was in the year 2000 – 4 recruits (1.99%), while in 1995 and 2005 the frequency was much the same, 4.47% and 4.97%. There were few hypertensive recruits, 2 (0.99%) in 1995, 4 (1.99%) in 2000 and 3 (1.49%) in 2005. In the city the average height of recruits in the course of the analyzed years was 179.41 cm (158–201 range), while the average weight was 74.00 kg (49–130 range), (Figure 2 and 3). One-way ANOVA analysis showed that recruits do not differ significantly by height (Figure 4), while the weight shows considerable difference with the highest variability in the year 2005 (Figure 5). Generally, with age the recruits gain weight (Figure 6).

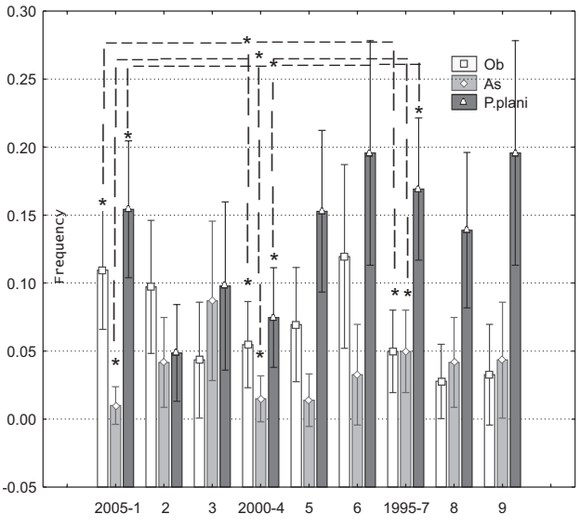

*Fig. 1. Changes in frequencies of obesity, asthenia and pedes plani in examinees from the city in the years 2005, 2000 and 1995. Years (2005: 1–city, 2 – suburb, 3 – islands; 2000: 4 – city, 5 – suburb, 6 – islands, 1995: 7 – city, 8 – suburb, 9 – islands). Abbreviations: Ob – obesity, As – asthenia, Pplani – pedes plani. *p<0.05.*

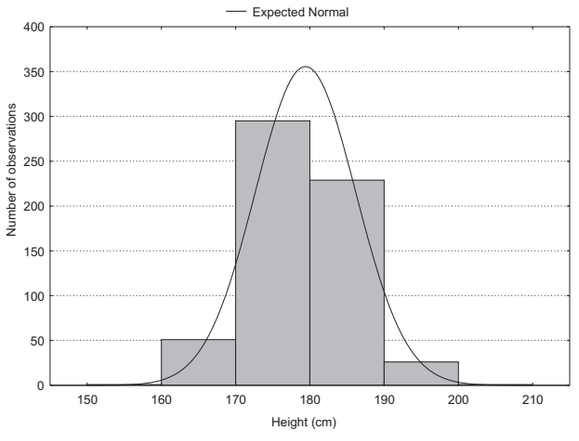

Fig. 2. Changes in the mean height of city recruits examined in the years 2005, 2000 and 1995.

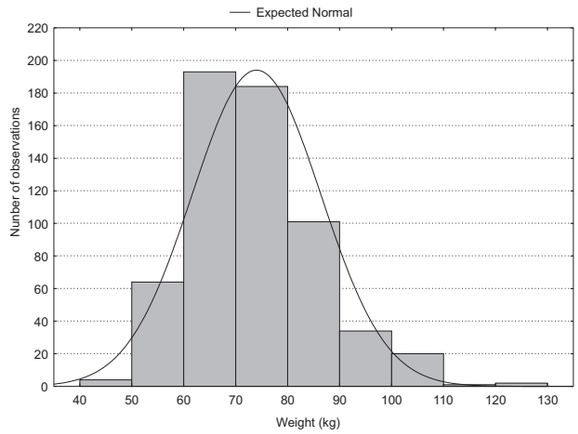

Fig. 3. Changes in the body weight of city recruits examined in the years 2005, 2000 and 1995.

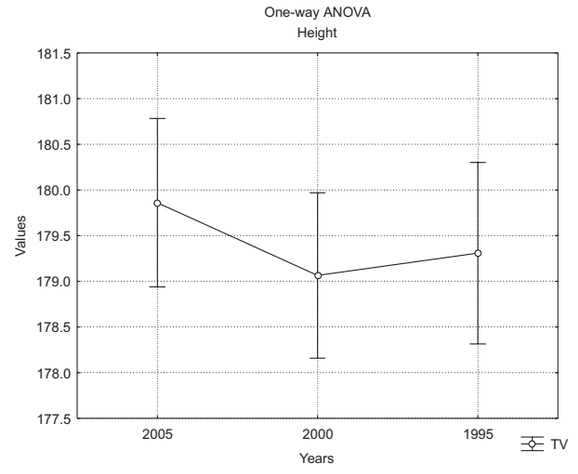

Fig. 4. Comparison in heights measured in different years in all recruits.

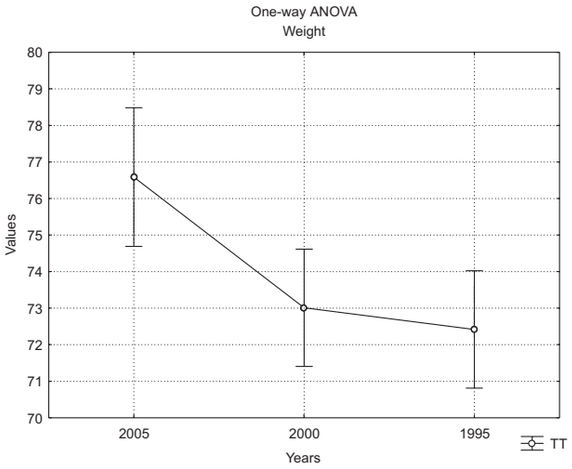

Fig. 5. Comparison in weights measured in different years in all recruits..

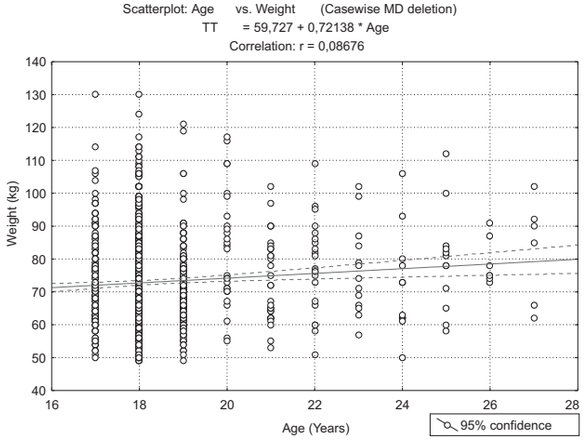

Fig. 6. Linear regression analysis and correlation coefficient between the age and weight of examinees.

Recruits from suburbs

During the years 2005, 2000 and 1995 we examined also 432 recruits from suburbs (transversal analysis, N=432, Table 1). Although the difference in recruits in the mentioned years is not statistically significant, the tendency of obesity increase is noticeable ranging from 2.77% in 1995, to 6.94% in 2000 and 9.72% in 2005. By asthenia the suburban recruits did not differ significantly. The year 1995 and 2005 showed the same frequency of 4.16%, while the lowest was in the year 2000 – 1.38%. The recruits from suburbs showed the highest percentage of pedes plani in the year 2000 – 15.27%, the lowest in 2005 – 4.86%, and the frequency of recruits with pedes plani in 1995 was 13.88%, which all shows a considerable statistical significance. Myopia and astigmatism are also the most frequent diagnoses in the recruits from suburbs, which with the frequency from 20-25% do not differ considerably when the years of occurrence are compared. With kyphosis and scoliosis when the years are compared there are no significant differences as the diagnoses show frequencies from 9.72 in 1995, 6.25% in 2000 to 5.55% in 2005. There were fewer cases of asthma and bronchitis in suburban recruits than in city recruits, from 2.77% in 1995 and 2000 to 1.38% in 2005.

The frequency of hypertension diagnoses was insignificant: from 0% in 2005 to 2.08% in 1995 and there were no major differences. The situation with right block branch diagnosis was different where the difference was considerable with highest frequency of 7.63% in 2005.

Recruits from islands

In the course of the years 2005, 2000 and 1995 were examined 186 recruits from the islands (transversal analysis, N=186). Recruits originated from islands by diagnosis obesity statistically differ significantly ($p<0.05$), the lowest frequency of the obese being 3.26% in 1995. Asthenia diagnoses increase considerably in 2005 so that frequency of very thin recruits grows to 8.69%. By diagnosis pedes plani in the three examined time periods islanders do not differ significantly. The diagnosis frequency was 19.56% for 1995 and 2000, and it diminished to 9.78% for the year 2005. By diagnosis kyphosis and scoliosis islanders differ significantly. The presence of the diagnosis with the year 2000 recruits was 2.17%, much higher in 1995 – 13.04%, and even higher in 2005 – 14.13%. Although the recruits do not differ considerably by diagnosis asthma and bronchitis, 2005 showed the increase of the diagnosis to 5.43% compared to 2.17% in 1995, and especially to 1.08% in 2000. The frequency of hypertension in island recruits is insignificant, while the presence of branch block is somewhat higher, reaching the highest frequency of 5.43% in 2005, so that by this diagnosis islanders do not differ statistically significantly.

Results comparing 437 recruits within the year 2005 regarding residence (city, suburb, islands)

Within the year 2005 the same parameters were examined in 201 city recruits, 144 suburban and 92 island recruits. They do not differ significantly by obesity, though the frequency of the obese in the city of 5.04% is noticeably higher than obesity frequency in the islands of 0.92% the same year. The percentage of thin recruits is the lowest in the city – 0.45%, while in the islands it is 1.83%, which makes a considerable statistical difference. Islanders have only 2.05% of pedes plani, which differs them notably from city youth with 7.09% of such diagnoses (Figure 7). By other somatic diagnoses the young regarding residence within the same year do not differ significantly.

Results comparing 437 recruits within the year 2000 regarding residence (city, suburb, islands)

Within the year 2000 recruits by the place of residence show fewer differences. As for obesity there are hardly any differences among islanders, suburban and

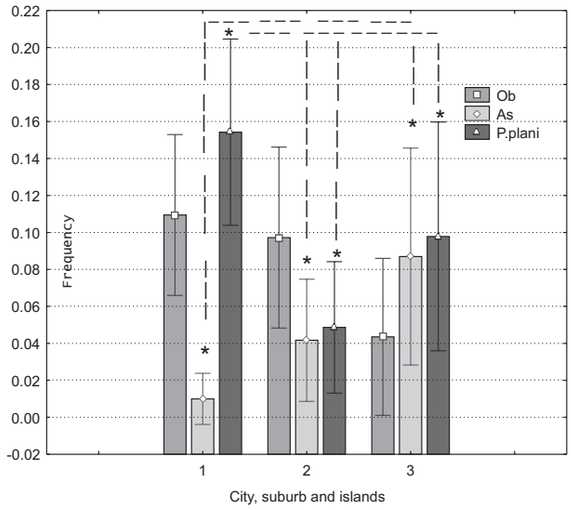

*Fig. 7. Changes in frequencies within of the year 2005 according to the diagnoses of asthenia and pedes plani showed significant difference, *p<0.05. Abbreviations: Ob – obesity, As – asthenia, P.plani – pedes plani.*

city recruits, and with asthenia the situation is similar. Only by pedes plani the examinees show statistical difference, the highest percentage of pedes plani – 5.04% show the recruits in suburbs.

Results comparing 437 recruits within the year 1995 regarding residence (city, suburb, islands)

Within the year 1995 there were no statistically significant differences in the examined parameters in recruits. Nevertheless, there was the highest percentage of obese recruits in the city – 2.28%, which is much higher but not statistically significant than in those from the islands – 0.4%).

Discussion

The study results have been almost identical by some parameters to the latest results obtained in developed West-European countries[16]. In our country since 1995 when the city examinee sample showed 4.97% of obese recruits till the year 2005 when it was 10.94% obesity has more than doubled. The Center for Disease Control and Prevention in USA published the results of their study, which reached back somewhat more than ours, and it found that the number of obese teenagers trebled in the last 20 years. Obesity is obviously becoming pandemic and the health system must oppose it first of all by introducing intensified physical activities, which will prevail over the sedentary life routine of the young[17]. Increased physical activity influences considerably diminishing of BMI and adiposity[18]. According to our study the largest number of obese recruits come from the city, beginning with 1995, then also in 2000, to the final analyzed year 2005 when their number is the largest and they statistically differ significantly from their peers within the same year, but also from those in suburbs and on the islands. It is hard to say why it is so. Namely, one can suppose that the young in the city have less physical activities than their peers in suburbs and islands, and it has been proved that the young who are physically more active in the city than their inactive city peers have substantially fewer obesity diagnoses[19]. Furthermore, »educational immigration« from small places and islands into city must also be considered. The young come to the city to school or university. That leads to changes in their life, discontinuation of physical activities, the new, unfortunately unhealthy diet, fried food, diet rich in fat with little vegetables and fruit, which way cause obesity, similar to the changes in the health of the workers immigrating into cities that had already been described[20]. On the other hand, neither the city dwellers are spared by obesity, especially in families regularly enjoying abundant dinners[21]. Therefore various programs are being designed in developed western countries instructing the whole families to go on diet, take healthy food, cut down on salt and exercise regularly[22,23].

Opposite to obesity, in the examined recruits asthenia, i.e. extreme thinness, 30% bellow normal weight, has almost disappeared as a diagnosis in the city, wit frequency of 0.99% in 2005, while in 1995 it used to be 4.97%. With island recruits the result was surprising. Contrary to our expectations the number of asthenic recruits in 2005 has doubled in comparison to the year 1995. The causes cannot be found in the changed economic status of the islands in 2005 compared to the situation 5 or 10 years back. Namely, the islands Krk, Cres, Lošinj and Rab are not far from the city of Rijeka and it has to be mentioned that there are lots of tourists so people gain from tourism, fishing, sheep farming and catering activities. The most probable cause of asthenia double frequency in island recruits is the increase in the temporary moving into the city for educational reasons. While a part of »educational immigrants« gain weight, the others develop asthenia so it could be commonly termed »eating disorders«. Namely, some people, especially young ones, are sensitive to certain sociodemographic factors like single life and leaving home early[24]. If some of them go in for a demanding sport, requiring intensive exercise and training, it may result in a considerable loss of weight[25,26].

Also the highest frequency of foot fallen arches occurs in city recruits, while the lowest is shown in islanders. It must be pointed out that the diagnosis pedes plani was the only one made approximately, i.e. on the basis of physician's experience, without the criterion of exact measurement, unlike all other diagnoses, so minor mistakes may appear but they do not influence the assessment of military fitness. That refers to pedes plani grades II, i.e. noticeably fallen, flat feet. It is supposed that the youth in the islands spend a lot of summertime on pebble beaches and they are ideal for forming young feet. The most dominating diagnoses in the examined recruits are myopia and astigmatism. The frequency of the said diagnosis in all three-time periods is above 20% without ma-

jor deviation in occurrence. Again, the high frequency of refractory anomalies in the young occurs in the city. Many of the performed studies found out that myopia is among the leading refractory anomalies and its frequency runs up to 87%[27]. Only a small number of high myopias are hereditary, connected to specific chromosomes[28] and gene mutations[29]. Majority of refractory anomalies may be influenced by preventive medical measures, like the early detection, prescribing optical aids, regular ophthalmologic checkup, the right lighting of classrooms, working premises and dwellings that are often not lit enough, while city streets are increasingly illuminated which is damaging and unnecessary. From ultraviolet radiation, which is also ever more present because of the damaged ozone layer and may harm the lens back pole, one can be best protected by less exposure[30]. Wearing sunglasses is also a preventive measure and as it is trendy the youth will accept it more readily than physical activities to fight obesity[31]. What the army really wants are the soldiers that are good marksmen, so what matters are not the degree and type of eye refractory anomaly but keen sight which can be attained by eye aids.

The transversal study of kyphosis and scoliosis frequency has shown that there are no statistically significant differences among city and suburb recruits, and the frequency of vertebral column deformations is relatively high especially in city recruits. Although spine deformations in recruits are not striking, and are not the cause for unfitness for the army, it is possible to prevent it by early detecting, education on proper posture, corrective exercise and in some cases by temporary wearing of spine correctors and fixators. The exceptions are serious spine deformations connected with neuromuscular disorders[32] and Scheuermann-like changes of the spine[33].

Asthma and bronchitis are not significantly widespread among recruits regardless of their residence and the examined time periods, and their frequency is always under 5%, with the exception of the year 2005 island recruits whose diagnosis frequency is 5.43%, which is surprising. The increased asthma frequency is a great surprise since the islands are known for pleasant Mediterranean climate, settlements by the sea etc. On the island of Lošinj there is also a health resort with hydroclimatothalassotherapy intended mainly for treating respiratory diseases. On the other hand, asthma increased frequency in island recruits is surprising because the results for the year 2005 show the highest number of asthenic youth on the islands, while it is known that asthma is in positive correlation with obesity and increased body mass index (BMI)[34,35.] The rise in asthma frequency in the islands in 2005 may be easily explained by the targeted health migrations of atopics from hinterland to the islands for the treatment of respiratory diseases. In spite of the favorable economic status of the islanders that had been mentioned, the migrations of inlanders to the islands causes if only temporary changes, crowdedness, looking for employment, newcomers' socio-economic problems, which may aggravate the existing atopy with asthma symptoms[36]. Crowded schools, dirty and unpainted classrooms and inadequate hygiene without measures for prevention of atopy bring about occurrence of asthma[37]. At recruits' examinations if a person complains of asthmatic or bronchial difficulties it is necessary to perform spirometry, paying particular attention to FEV_1 and FVC values which indicate lung respiratory obstructions[38]. In recruits with poorer FEV_1 and FVC values a more rapid relapse of respiratory difficulties may be expected[39]. $FEV_1 < 60\%$ may be considered a more severe form of asthma. In unclear cases when assessing fitness for military service radio allegro sorbent testing (RAST) to specific immunoglobuline E (IgE) is recommended[40]. By performing spirometry atopics could be identified during pupils' regular check-ups. By additional tests like allergy and immunotest, performing hypo sensitization, medicament therapy and climatological treatment, the number of recruits with asthmatic symptoms would surely be diminished. Very rarely asthma may be lethal or trigger acute metabolic illnesses[41]. Therefore extreme caution is required when assessing an asthmatics' fitness for military service.

As it was the young who were examined, hypertension as a diagnosis appeared in a small percentage of recruits, up to 1.99%, by a transversal analysis and the analysis of the relations within the years. Nevertheless, it takes a longer period of risk factors activity, among which is obesity, for the diagnosis to prevail. By incomplete and complete right branch block diagnosis the recruits also do not differ significantly. In our experience, right branch block is usually only a »cosmetic mistake«, and it is hardly ever that additional cardiology examinations find septal defect or other heart defect, so that such recruits free of subjective impediments and difficulties are completely fit for military service.

On the basis of the analyzed results of the most frequent diagnoses in examinees at occupational medicine, we suggest a set of preventive national and international measures to achieve recruits' enhanced ability for military service. Naturally, medical selection criteria depend on socio-demographic situation in the society, Ministry of Defense and health status of young generations[42]. Furthermore, books of rules for assessing military fitness must be such as not to exclude healthy people from military service. The importance of preventive examinations before joining the army is unquestionable, no matter whether it refers to conscripts or professional units[43]. Depending on financial means such examinations can be comprehensive or less so. Obviously, some diagnoses are possible only if additional diagnostic instruments are used. Particularly RTG spine examinations are needed when spondylolysthesis, knee disorders and back pains are suspected. Cycle ergometry estimation, Astrand oxygen uptake (VO_2 max) are recommended in all recruits, besides spirometry, but that requires more financial means[44].

Generally, books of rules should become stricter. Namely, if a recruit had overcome a serious disease, or even a malignant one, primarily in early childhood, and at the

time of recruitment there are neither consequences nor traces of illness nor possibilities of relapse, he should be declared fit for the army[45].

Then there is a question we will attempt to answer: why should a recruit be refused if his weight is somewhat below or above 30% of ideal body weight? It is known that good military training increases weight, decreases body mass and enhances locomotive performances of anaerobe depending activities[46]. So the answer and recommendation would be, if healthy, regardless of deviation in weight such recruits should be drafted. Some armies have military specialists and nutritionists who are to take care of adequate, energetic but at the same time dietetic food for their soldiers. Only the extremely obese recruits should be refused who because of their weight are prone to injuries but also to illnesses[47]. On the other side, at the professional selection for special services in the army, as in pilots and cadets, candidates must be of specific personal appearance and body built[48].

Finally, measures for achieving recruits' enhanced fitness for military service have to begin at the end of elementary education. In the final year of elementary school particular attention should be paid to the diagnoses mentioned in this study. Each youngster should be given his health card to be taken to vocational guidance before starting secondary education. Detailed medical check-ups every year of secondary education would monitor possible diagnoses, correct them by medical measures and they would be entered in the future recruit's health card. In such a program activities of many people have to be coordinated: from occupational medicine specialist (who at the very end of the process examines the recruit), school medicine specialists, gym and other schoolteachers, to parents responsible for their children's diet, control of habits, spare time and recreation. In that way the occupational medicine specialist, as head of recruiting board, would by means of a health card and case history get an insight into what had been done regarding the possible diagnoses. With little financial means, but by stricter monitoring of certain health parameters and timely corrections, a lot could be done at the national but also international level in view of enhancing recruits' fitness for military service.

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MJERE ZA POSTIZANJE VEĆE SPOSOBNOSTI REGRUTA NA PREGLEDIMA ZA VOJNU SLUŽBU – TRANSVERZALNA STUDIJA

S A Ž E T A K

S obzirom na 10.94% pretilih novaka u Rijeci 2005. godine na medicini rada odlučeno je ispitati uzročnost te i drugih najčešćih dijagnoza: spuštenih stopala, miopije i astigmatizma, kifoze i skolioze, astme, hipertenzije i bloka grane. To je učinjeno dvostrukim praćenjem 1311 novaka transverzalnom analizom kroz 2005, 2000 i 1995 godinu i unutar svake godine prema boravištu na: grad, okolna mjesta, otoke. Razlike u tri vremenska intervala u gradu su bile za pretilost ($p<0.05$) s najvećom učestalosti 2005. godine, za asteniju ($p<0.05$) s najmanjom učestalosti 0.99% 2005. godine i za spuštena stopala ($p<0.05$) s najvećom učestalosti 1995. godine. Kod novaka iz okolnih mjesta nađeno je ($p<0.05$) za spuštena stopala, $p=0.054$ za pretilost, te kod novaka s otoka ($p<0.05$) za pretilost. Učestalost miopije i astigmatizma bila je iznad 25%, kifoze do 14.13% i astme 5.43%. Učestalost hipertenzije bila je zanemariva. Medicina rada odlučila je reagirati mjerama za povećanje sposobnosti novaka surađujući sa školskom medicinom, nastavnicima i roditeljima, provođenjem pregleda, korekcija, dijeta i fizičkim aktivnostima.

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Quantification Model for Muscular Forces and Momentums in Human Lower Extremities

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A B S T R A C T

The possibility of calculating muscular forces and momentums and their influence on skeleton was evaluated in this study by means of computerized tomography performed on a living person. Through this, the surface and corrected surface for each muscle cross section area were obtained, the distance from muscular centroide to the neutral bone axis was measured, and muscular force and muscular momentum on the bone were determined. Muscular momentum on the bone was obtained by multiplication of the muscular force and the distance between muscular centroide and neutral bone axis. The use of computerized tomography, as a method for creating a model for quantification of muscular forces and momentum, was proven to be reliable according to exactness in evaluation of all human lower extremity structures which is the basis for muscular biomechanical characteristic calculations.

***Key words**: lower extremity biomechanics, muscular forces, muscular momentums*

Introduction

Evolution of the mankind, posing ever increasing demands on the human locomotor system, prompted a great number of biomechanical studies in the area of various components of the locomotor system[1,2]. Abundant research in this area most often analyzes separately isolated problems or isolated segments or components of the extremities such as bones, joints or muscles, while the integral data on analysis of passive and active parts of the human locomotor system are rarely found[3–8]. Separate observation of different components can't provide a comprehensive analysis because of the close interaction and dependence of various components and associated systems. The fact that the whole organism interacts to mechanical forces and that there is interdependence between the magnitude and method of action of these forces and the organism is best shown in the supportive tissue, bone and muscle reaction to the change in mechanical load[9,10]. Bone adapts to the mechanical load by adaptation of shape, rearrangement of the inner structure and redistribution of the structural material[10]. Muscles undergo hyper or hypotrophy parallel to the change in the proportion of different muscle fiber types. Important changes also occur in the tendons, ligaments and joint capsule. All of this applies when the magnitude of applied force is within a physiologically acceptable range, while the forces of a too great intensity will lead to tissue damage or destruction[10–12]. The basic function of the locomotor system is providing mechanical support to the organism, enabling movement and providing resistance and protection from the external forces. All these functions are maintained by the maximal adaptation of the locomotor system with the minimum materials employed, in accordance to the Roux's minimum-maximum principle[13,14].

Load distribution within the skeleton is a result of action of gravitational and other active and passive forces that have a direct force load on the skeleton, with different intensities and directions[14–16]. For a comprehensive understanding of kinematics and dynamics of motion, which includes the analysis of muscular forces in motion, it is important to bear in mind that the muscular forces act on bones and joints through pull, push and torque momentums with specific geometrical relationships between the muscle, bone and joint that results in three dimensional motion[14–16]. The magnitude of the muscular force is proportional to its cross sectional surface area, that is to the sum of the muscle fiber cross section area perpendicular to their axis[17–19]. Although we can estimate the activity of each muscle and forces in various

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joints in vivo through electromyography, dynamometry, and special force measuring platforms and through kinematical and dynamical analysis of various referral points marking specific body segments recorded by video cameras, the exact magnitude of muscular forces and geometrical properties of each muscle is very hard to determine[17–21]. This especially applies in pathological conditions, where the problem of exact quantification of muscular forces and momentums is of paramount importance in determining the appropriate treatment modality[17–21]. Having in mind the variation in forces within the locomotor system, as well as within the skeleton and muscular system, there is a justification and a necessity in an attempt to determine biomechanical properties in extremities through determination of muscular forces and momentums which would enable objective visualization based on computerized analysis of the data that can be obtained in a direct or indirect manner[17–21].

Material and Methods

The study was conducted at the Department of Anatomy Drago Perović, University of Zagreb, School of Medicine and at Department of Radiology, University Hospital »Sestre Milosrdnice« Zagreb. Subject of the investigation was the author of this article, male, age 35, 175 cm high. The thigh and the lower leg of the subject were scanned by computerized tomography, Siemens Somatom DR. Adequate radiation protection was used for the rest of the body. CT scan was done on the midpoint of the thigh, determined by measuring the distance between the mayor trochanter of the femur and genicular articular line, and on the midpoint of the lower leg, determined by measuring the distance between genicular articular line and medial tibial maleole.

Measurements of surfaces for each muscle and the distance between each muscle centroid and neutral bone axis were done on millimeter grid paper after copying the CT cross-section scans.

The origin of the coordinate system for particular bone cross section goes through the neutral axis of the bone (femur, tibia, and fibula). Muscular surface on CT scan, that is, on millimeter grid paper, was measured by use of planimetric method and divided with picture correction factor for getting the real surface value.

Surface and corrected surface for each muscle on the thigh and the lower leg was calculated as well as the distance between neutral bone axis and each thigh (fig.1) and lower leg (fig. 2) muscle centroid. In muscle force calculation we used, in literature known maximum, medium and the lowest force values of 30 N/cm^2, 60 N/cm^2 and 90 N/cm^2. The distance between bone centroid and muscle centroid was taken for determination of the momentum leaver. Muscular momentums on the bone were calculated as multiplication of muscle force and the distance of bone centroid which give us the moment of particular muscle on the bone in Nm. The similar method was used by McGill.

Results

Muscle cross-section surface and their distances from neutral bone axis

The surfaces and corrected surfaces of muscles cross--section shown on CT scans and the distance between neutral bone axes and muscle centroid in each thigh and lower leg muscle measured at the midpoint are shown in Tables 1 and 2.

The forces in each thigh and lower leg muscle are shown in Tables 3 and 4. In force calculations, we have used three variants for muscle force calculation – 30, 60 and 90 Ncm^{-2}

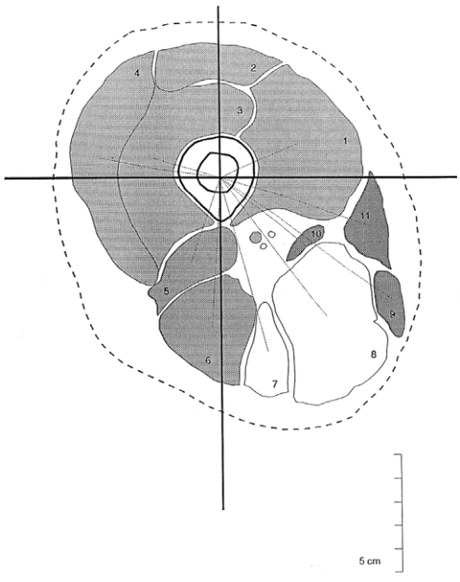

Fig. 1. Cross section through the right thigh.

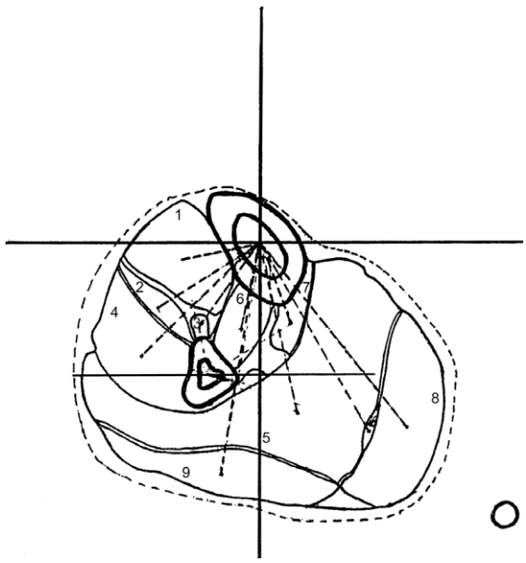

Fig. 2. Cross section through the right lower leg.

TABLE 1
RIGHT AND LEFT THIGH MUSCLE CROSS SECTION SURFACE AREA, CORRECTED AREA AND DISTANCE FROM THE FEMUR NEUTRAL AXIS

	Right thigh			Left thigh		
Muscle	Area (cm^2)	Corrected area (cm^2)	Distance from femur neutral axis (cm)	Area (cm^2)	Corrected area (cm^2)	Distance from femur neutral axis (cm)
M. vastus medialis	7.23	14.75	2.2	7.13	14.55	2.4
M. rectus femoris	1.52	3.10	3.0	1.62	3.30	3.0
M. vastus intermedius	6.48	13.22	1.8	6.39	13.04	1.6
M. vastus lateralis	4.75	9.69	3.2	4.81	9.81	3.1
M. biceps femoris (caput breve)	1.77	3.61	2.4	1.75	3.57	2.2
M. biceps femoris (caput longum)	3.85	7.85	4.0	3.88	7.91	4.0
M. semitendinosus	1.79	3.65	4.8	1.76	3.59	4.9
M. semimembranosus	3.98	8.12	4.6	3.90	7.95	4.8
M. gracilis	1.31	2.67	5.6	1.36	2.77	5.7
M. adductor magnus	0.39	0.79	2.8	0.38	0.77	3.0
M. sartorius	1.58	3.22	4.2	1.51	3.08	4.5

TABLE 2
RIGHT AND LEFT LOWER LEG MUSCLE CROSS SECTION SURFACE AREA, CORRECTED AREA AND DISTANCE FROM THE TIBIA AND THE FIBULA NEUTRAL AXIS

	Right lower leg				Left lower leg			
Muscle	Area (cm^2)	Corrected area (cm^2)	Distance from tibial neutral axis (cm)	Distance from fibular neutral axis (cm)	Area (cm^2)	Corrected area (cm^2)	Distance from tibial neutral axis (cm)	Distance from fibular neutral axis (cm)
M. tibialis anterior	3.00	8.33	1.9	2.2	3.17	8.80	2.20	2.2
M. extensor digitorum longus	1.03	2.86	2.9	1.4	1.11	3.08	3.0	1.4
M. extensor hallucis longus	0.22	0.61	2.3	0.8	0.20	0.55	2.5	0.9
M. fibularis longus	1.80	5.00	3.5	1.3	1.76	4.88	3.6	1.1
M. soleus	7.00	19.44	2.9	2.9	6.88	19.11	3.3	3.1
M. tibialis posterior	1.62	4.50	1.6	1.4	1.60	4.44	1.7	1.3
M. flexor digitorum longus	1.21	3.36	1.4	2.7	1.18	3.27	1.6	2.7
M. gastrocnemius (c.mediale)	5.00	13.88	4.6	5.0	5.09	14.13	5.1	5.2
M. gastrocnemius (c.laterale)	3.96	11.0	4.1	1.6	4.00	11.11	4.2	1.6
M. plantaris	0.16	0.44	3.5	4.1	0.15	0.41	4.1	4.5

Muscular forces and momentums

Muscular momentums on femur in right and left tight are shown in Table 5.

The greatest momentum on femur have m. vastus medialis and m. vastus lateralis as the extensor muscles, and m. biceps femoris (caput longum) as the flexor muscle.

M. soleus and m. gastrocnemius have the greatest momentum on tibia (Table 6) and fibula (Table 7) and extensor muscles, m. tibialis anterior and m. fibularis longus, have the greatest moment on leg bones. The difference between medial and lateral head of gastrocnemius muscle is so small that it can be ignored.

Results also show the existence of a near balance between flexor and extensor muscles, but leg extensors (the muscles of anterior leg), also like plantar flexors (the muscles of posterior part of the leg), have some greater muscular moment. That is in accord with a well known clinical fact such is extremity position in tetania (tetanus, hypoclaciemia, decerebration rigidity).

Discusion

The forces produced by muscles can cause various movements without resistance but they can also maintain static balance in certain position as well as dynamic balance during body movement, or enable lifting and transport of weight[1–6]. Muscular contraction can be realized as isotonic, with increasing or constant force or iso-

TABLE 3
RIGHT AND LEFT THIGH MUSCULAR FORCES CALCULATIONS

Right thigh				Left thigh			
Corrected area (cm^2)	Muscle force (n) 30	Muscle force (n) 60	Muscle force (n) 90	Corrected area (cm^2)	Muscle force (n) 30	Muscle force (n) 60	Muscle force (n) 90
14.75	442.5	885.0	1327.5	14.55	436.5	873.0	1309.5
3.10	93.0	186.0	279.0	3.30	99.0	189.0	297.0
13.22	396.6	793.2	1189.8	13.04	391.2	782.4	1173.6
9.69	270.7	581.4	872.1	9.81	294.3	588.6	882.9
3.61	108.3	216.6	324.9	3.57	107.1	214.2	321.3
7.85	235.5	471.0	706.5	7.91	237.3	474.6	711.9
3.65	109.5	219.0	328.5	3.59	107.7	215.4	323.1
8.12	243.6	487.2	730.8	7.95	238.5	477.0	715.5
2.67	80.1	160.2	240.3	2.77	83.1	166.2	249.3
0.79	23.7	47.4	71.1	0.77	23.1	46.2	69.3
3.22	96.6	193.2	289.8	3.08	92.4	184.8	277.2

TABLE 4
RIGHT AND LEFT LOWER LEG MUSCULAR FORCES CALCULATIONS

Right lower leg				Left lower leg			
Corrected area (cm^2)	Muscle force (n) 30	Muscle force (n) 60	Muscle force (n) 90	Corrected area (cm^2)	Muscle force (n) 30	Muscle force (n) 60	Muscle force (n) 90
8.33	249.9	499.8	749.7	8.80	264.0	528.0	792.0
2.86	85.8	171.6	257.4	3.08	92.4	184.8	277.2
0.61	18.3	36.6	54.9	0.55	16.5	33.0	49.5
5.00	150.0	300.0	450.0	4.88	146.4	292.8	439.2
19.44	583.2	1166.4	1749.6	19.11	573.3	1146.6	1719.9
4.50	135.0	270.0	405.0	4.44	133.2	266.4	399.6
3.36	100.8	201.6	302.4	3.27	98.1	196.2	294.3
31.88	416.4	832.8	1249.2	14.13	423.9	847.8	1271.7
11.00	330.0	660.0	990.0	11.11	333.3	666.6	999.9
0.44	13.2	26.4	39.6	0.41	12.3	24.6	36.9

metric contraction which is realized without movement, that is, without muscle fibers shortening but with tension increasing[6–9]. In both cases, the energy source is the chemical energy utilized by muscles and transformed into mechanical energy (elastic, potential, kinetic), and heat[6–9].

In our study we have used computer tomography imaging for muscle analysis.

Muscle surface shown on CT is not representing physiological cross-section of muscle. This surface is corrected surface, that is, real picture of horizontal muscle cross--section in particular CT cross section[10–13]. In muscles with longitudinal fibers, corrected surface is taken by measuring the cross section of the muscle in particular segment[14–16]. In feather-like muscles we have used Fick relation – when the muscular forces are at an angle with the tendon axis, tracking muscle force (Fm) will produce in tendon force component (Ft) depending on angle:

$$\cos \alpha = Ft/Fm$$

During the contraction the angle is changing. In feather-like muscles, fibers are running form their bone origin (length L) towards the tendon in a maximally short distance (a) muscle fibers layer thickness (b) fiber length in relaxed condition (l) and muscle fibers length in contraction (Δl)(14–16). The muscle fibers angle near tendon in relaxed condition (α) is changing during the contraction in angle ($\alpha 1$). During that, muscle fibers are contracting by contraction quotient $Cs = \Delta l/l$ into new length $l1 = l - \Delta l$, that is, by Alexander

$$l1x \sin \alpha 1 = a$$

$$\sin \alpha 1 = \sin \alpha / Cs$$

that is,

$$\cos \alpha 1 = (Cs - \sin \alpha)^{1/2}/Cs$$

TABLE 5
MUSCULAR MOMENTUMS ON THE RIGHT AND LEFT FEMUR

	Momentum on rifgt femur (nm)			Momentum on left femur (nm)		
Muscle	30	60	90	30	60	90
M. vastus medialis	9.75	19.47	29.20	10.47	20.95	31.42
M. rectus femoris	2.80	5.58	8.37	2.97	5.94	8.91
M. vastus intermedius	7.14	14.27	21.41	6.25	12.51	19.58
M. vastus lateralis	9.30	18.60	27.90	9.12	18.25	27.37
M. biceps femoris (caput breve)	2.60	5.19	7.79	2.35	4.72	7.10
M. biceps femoris (caput longum)	9.42	18.84	28.26	9.50	18.98	28.74
M. semitendinosus	5.26	10.51	15.76	5.27	10.55	15.73
M. semimembranosus	11.20	22.41	33.61	11.44	22.89	34.34
M. gracilis	4.48	8.97	13.45	4.73	9.47	14.21
M. adductor magnus	0.66	1.33	1.99	0.69	1.38	2.07
M. sartorius	4.05	8.11	12.17	4.15	8.31	12.47

TABLE 6
MUSCULAR MOMENTUMS ON THE RIGHT AND LEFT TIBIA

	Momentum on right tibia (nm)			Momentum on left tibia (nm)		
Muscle	30	60	90	30	60	90
M. tibialis anterior	4.74	9.50	14.24	5.28	10.56	15.84
M. extensor digitorum longus	2.48	4.97	7.46	2.77	5.54	8.31
M. extensor hallucis longus	0.42	0.84	1.26	0.41	0.82	1.23
M. fibularis longus	5.25	10.50	15.75	5.27	10.54	15.81
M. soleus	16.91	33.82	50.73	18.91	37.83	56.75
M. tibialis posterior	2.16	4.32	6.48	2.26	4.52	6.79
M. flexor digitorum longus	1.41	2.82	4.23	1.56	3.13	4.22
M. gastrocnemius (c.mediale)	19.15	38.30	57.46	21.61	43.23	64.85
M. gastrocnemius (c.laterale)	13.53	27.06	40.59	13.99	27.99	41.99
M. plantaris	0.42	0.92	1.38	0.50	1.00	1.51

The total muscle fibers volume $Vm = a \times b \times L$, physiological cross-section $A1 = b \times L$, the number of muscle fibers $bL/(a\sin\alpha)$, vertical force in the tendon will respond to:

$$Ft = Fm\cos\alpha 1$$

Ratio between vertical force caused by feather-like muscles and the vertical force of same volume muscle with parallel fibers can be lager or smaller than 1 which depends on the angle[14–16].

Our study model on human lower extremities introduce computerized tomography as a method for determination of muscular forces and momentums. Therefore, it is necessary to emphasize following details which are, by our opinion crucial understanding the procedure:

- the muscular momentums and estimated muscular forces which are acting on bones, shown in our results, are related only to analysis of investigated muscle cross-section,
- corrected surface which we have used in further calculation is not a physiological cross-section,
- calculated relations of muscular momentums are representing the condition of tension in particular cross--section and influence on bone segment in particular bone cross section,
- when the bone is intact, muscular forces are participating in dynamic modeling tension distribution of bone cross-section,
- the computerized tomography layers above and below the particular cross-section could enable more accurate analysis of muscle influence on bone,
- this study model is not related to muscle influence on joints because of need for serial CT analysis below and above muscle attachment, including the joints themselves,
- computerized tomography in this study model was performed with patient in supine position, with extremities in extension, foot in neutral position and relaxed muscles.

TABLE 7
MUSCULAR MOMENTUMS ON THE RIGHT AND LEFT FIBULA

	Momentum on right fibula (nm)			Momentum on left fibula (nm)		
Muscle	30	60	90	30	60	90
M. tibialis anterior	5.49	10.99	16.49	5.80	11.61	17.42
M. extensor digitorum longus	1.20	2.40	3.60	1.29	2.58	3.88
M. extensor hallucis longus	0.14	0.29	0.43	0.14	0.29	0.44
M. fibularis longus	1.95	3.90	5.85	1.61	3.22	4.83
M. soleus	16.91	33.82	50.73	17.77	35.54	53.31
M. tibialis posterior	1.89	3.78	5.67	1.73	3.46	5.19
M. gastrocnemius (c.mediale)	20.82	41.64	62.46	22.04	44.08	66.12
M. gastrocnemius (c.laterale)	5.28	10.56	15.84	5.33	10.66	15.99
M. plantaris	0.54	1.08	1.62	0.55	1.10	1.66

Serial computerized tomography of extremities could give us more accurate biomechanical analysis, especially with muscles in relaxed and contracted condition[17,18]. But for such analysis it will be required to perform additional CT scans what will cause high amount of x-ray emission which is ethically not acceptable[17,18].

Nevertheless, additional important data can be given by use of ultrasound like Ikai and Fukunaga in their investigation[19].

Ultrasound echography in some transverse and longitudinal cross-sections can define surface relation and volume of particular muscles as well as their direction.

Ultrasound echography can present real time situation which means muscles in relaxed and contracted condition an moment of contraction[19–21].

Furthermore, it enables serial cross-section analysis of muscle through longitudinal axis like on CT but without negative effects of irradiation[19–21].

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MODEL KVANTIFIKACIJE MIŠIĆNIH SILA I MOMENATA U DONJIM EKSTREMITETIMA ČOVJEKA

S A Ž E T A K

U ovom istraživanju je evaluirana mogućnost za izračun rasporeda sila i momenata djelovanja mišića na skelet u živa čovjeka primjenom kompjutorizirane tomografije. Na ovaj način, određena je za svaki mišić površina i korigirana površina kako bi se dobila realna vrijednost površine, zatim udaljenost od centroida mišića do neutralne osi kosti, snage mišića te moment djelovanja na kost. Moment djelovanja mišića dobiven je množenjem sile mišića i udaljenosti centroida od neutralne osi kosti. Upotreba kompjutorizirane tomografije, kao metode za stvaranje modela za kvantifikaciju mišićnih sila i momenata, se pokazala pouzdanom zbog preciznosti u evaluaciji svih struktura donjih ekstremiteta u čovjeka, što je osnova za izračun biomehaničkih karakteristika.

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A New Model of Selection in Women's Handball

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A B S T R A C T

The aim of the study was to assess the basic motor abilities that determine top performance in women's handball, and to identify test panel for primary selection at handball school. The study included 155 female attendants of the Split Handball School, mean age 12.5 years. Differences in the basic motor abilities between the subjects that developed into elite handball players after 7-year training process and those that abandoned handball for being unable to meet the competition criteria were evaluated by use of discriminative analysis. The former were found to have also been superior initially in all variables analyzed, and in arm coordination, overall body coordination, throw and jump explosive strength, arm movement frequency and repetitive trunk strength in particular. Motor superiority based on the abilities of coordination, explosive strength and speed determines performance in women's handball, qualifying these abilities as reliable selection criteria. Based on this study results, a new model of selection in women's handball, with fine arm coordination as the major limiting factor of performance, has been proposed.

Key words: *women's handball, selection, motor abilities*

Introduction

Recognizing true talents for a particular sport is a very complex process because definitive conditions that determine performance in this sport have to be reliably predicted on the basis of initial anthropologic characteristics at the youngest age possible. The process requires good knowledge of the developmental pattern of anthropologic characteristics that are relevant for top performance in this particular sport, in this case women's handball. These requirements include knowledge of the congenital and acquired developmental components of these anthropologic characteristics as well as the sequence and magnitude of this development. As the performance in any sport, including handball, cannot be explained simply as the sum of individual abilities and characteristics but implies their active relationships, the monitoring should address development of the functions describing the anthropologic system of female handball players of a particular age group, i.e. according to a particular stage of performance development.

There are two methodological procedures and/or models to obtain relevant information on the factors determining particular sport performance: regression model and discriminative model. The use of either model depends on the data available. Regression correlation analysis is generally employed in case of results expressed in variables assessing the anthropologic status of an individual and this individual's achievement in a particular sport[1–6]. Regression analysis yields an equation of the respective sport specification, containing three crucial sets of information: which factors influence the performance and to what extent, and what is the correlation between the factors influencing the result in that particular sport. However, when results are expressed in variables assessing the anthropologic status of an individual but without data on the performance achieved by this individual in the respective sport, the overall athlete population in this sport can be divided into groups achieving top results and average results; in this case, discriminative analysis is employed[7–10], however, the respective sport specification equation can then be less precisely determined. When both regression and discriminative models are applicable, then prediction of the study phenomenon is almost complete.

In the present study, discriminative approach was used to obtain information relevant for orientation and selection in women's handball. The model was set in an

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original fashion to enable prediction of definitive status of performance at age 19 and 20, based on the initial motor status at age 13. The aim of the study was to identify motor abilities that differed between the group of players that initially attended handball training program to discontinue it during the 7-year selection procedure and those that continued their handball training program to grow into elite handball players, all this based on the measurements recorded during their initial handball school attendance. The question to answer was whether the present elite handball players (some of them national team members) and those that had to give up handball for being unable to meet the competition criteria had differed significantly according to their motor status at the mean age of 12.5 years. In this way, the motor abilities yielding significant between-group differences at baseline would be identified as crucial for handball performance, thus also serving as reliable criteria for efficient player selection.

Subjects and Methods

A battery of 13 motor variables were used in a sample of 155 female handball players, mean age 12.5 years, after three years of work at Split Handball School[2]:

- for psychomotor speed assessment (movement frequency) – hand tapping, foot tapping, hand rounds and foot rounds;
- for (explosive and repetitive) strength assessment: standing long jump, medicine ball throw from supine position, front support and support pushups; and
- for (arm, leg and whole body) coordination assessment – ball bouncing with tennis racket, juggling with matchboxes, foot slalom around stands with two balls, agility on the ground, and polygon backward.

Seven years later, the study sample was divided into two subgroups: subgroup of 136 subjects who had given up handball in the meantime due to their inability to meet the competitive selection criteria, and subgroup of 19 subjects who had remained active in handball and become elite handball players.

Canonical discriminative analysis was used to solve the set problem, i.e. to determine between-group differences according to motor variables.

Results

Table 1 presents the basic descriptive parameters of motor variables, results of univariate ANOVA analysis of variance, and results of canonical discriminative analysis of motor variables between the study subjects that abandoned handball training and those that continued handball training to turn to elite handball players. The data presented clearly indicate the players having become elite players to have initially achieved considerably better results than those who abandoned handball training in the meantime. This means that the elite handball players were superior according to their motor characteristics already at the beginning of the training process, suggesting the higher level of motor abilities to have significantly contributed to their superior handball performance.

TABLE 1
BASIC DESCRIPTIVE PARAMETERS OF MOTOR VARIABLES IN TOTAL STUDY SAMPLE (T) AND RESULTS OF CANONICAL DISCRIMINATIVE ANALYSIS BETWEEN ELITE HANDBALL PLAYERS (E) AND OTHERS (O)

Variable	T(n=155) X±SD	E(n=19) X^1	O(n=136) X^2	DF	F^A	p^A
Hand tapping	28.46±2.79	29.76	28.28	0.43	4.76	0.03
Foot tapping	39.00±3.67	40.00	38.87	0.25	1.57	0.21
Hand rounds	29.31±3.96	29.67	29.26	0.09	0.18	0.67
Foot rounds	19.06±2.46	19.51	19.00	0.17	0.72	0.60
Standing long jump	169.65±17.16	177.17	168.59	0.41	4.23	0.04
Medicine ball throw	3.71±0.63	4.08	3.66	0.55	7.76	0.01
Front support	50.74±26.30	63.11	49.01	0.44	4.88	0.03
Support pushups	17.74±8.39	20.21	17.39	0.27	1.88	0.17
Ball bouncing	2.57±1.62	2.74	2.54	0.10	0.24	0.63
Matchbox juggling	18.57±6.69	23.13	17.83	0.66	11.07	0.00
Slalom with 2 balls#	46.76±7.87	44.70	47.04	–0.24	1.47	0.22
Ground agility#	18.72±3.36	16.68	19.00	–0.56	8.26	0.00
Backward polygon#	21.45±4.75	19.23	21.76	–0.44	4.82	0.03
Centroids			0.09	–0.09		
Delta					0.40*	

#variable with opposite metric orientation, *$p<0.01$

X^1, X^2 – means for groups 1 and 2, DF – discriminant function, FA – F-test for ANOVA, pA – probability for ANOVA, Delta – canonical discrimination

The highest between-group difference was recorded in the variable of matchbox juggling to assess arm coordination, in handball manifesting as manipulative ability of ball handling. Between-group differences were also found in the variables assessing the ability of solving complex motor problems, the variable of agility on the ground in particular, which represents whole body coordination. In handball, this ability manifests in the performance of complex technical elements of attack and defense such as feinting, falls, blockade, etc.

Analysis of variance yielded considerable between--group differences in explosive strength of throw and jump type. The difference was especially pronounced in the explosive strength of throw type (throwing medicine ball from supine position), in handball manifesting in the pass force. Situation performance is predominantly determined by explosive strength because elite handball imposes the need of maximal utilization of the jump, throw or sprint. Numerous studies have confirmed the prognostic value of explosive strength tests to predict situation efficiency[1,2,7–9].

Significant between-group differences were also obtained in the variables of the trunk repetitive strength and movement frequency (hand tapping). These motor abilities significantly determine the quality of performance because the trunk strength and speed are necessary for efficient performance of structural movement entities, especially in the conditions of situation confrontation with the opponents.

Discriminative function confirmed the results obtained by the analysis of variance and clearly discriminated the two subgroups, i.e. those who continued their handball training with pronounced abilities of upper extremity and whole body coordination, explosive strength of throw type, hand movement frequency and trunk repetitive strength, and those that discontinued their handball training with a lower level of development of these specific abilities.

Based on the results of the present and previous studies, handball could be characterized as a sport of high complexity, where successful performance depends on a number of basic motor abilities, predominantly on the ability of cortical regulation of movement, explosive strength, throwing in particular, basic trunk strength, and psychomotor speed. Obviously, top results in handball cannot be achieved without the above-average levels of motor abilities. Therefore, the selection process should include the use of measuring instruments intended specifically for assessment of those motor abilities that predominantly determine the quality of performance.

Discussion

In 1982, Pavlin *et al.*[11] isolated five factors or five situation motor abilities that exist in senior handball players, ranking them according to their importance as follows: situation precision, skill of ball handling, speed of movement with ball, speed of movement without ball, and explosive strength of ball throw. However, in particular stages of performance development in female handball, the predominance of the mentioned specific motor abilities follow an inverse pattern, in parallel with the quality of acquiring specific motor skills. In stage 1, age 9–11 years, the performance in handball mostly depends on the speed of movement without ball and speed of movement with ball; in stage 2, age 12–14 years, it predominantly depends on the explosive strength of ball throw; and at age 15–17 years on situation precision and skill of ball handling. The timing of particular stages in the process of developing performance quality in women's handball should be taken as approximate figures, while the main concept is that the performance of specific motor skills is closely associated with the development of specific and basic motor abilities, which definitely results in the integration of specific and basic motor abilities into the locomotor system. This is consistent with the results reported from the studies performed in female elementary school fifth- to eighth-graders training handball[2,7], elite female handball players[1,8,9], and studies investigating motor development in general[12–17].

Based on the results obtained in the present study and studies carried out in subjects of various age and level of performance, a model of selection in women's handball can be established, which should be conducted step-wise, in stages (as it has been done in women's volleyball[6]):

- stage 1: after age 9, selection should be done on the basis of psychomotor speed and psychomotor coordination. These motor abilities will eventually limit top performance quality; psychomotor speed through facilitated technique performance, and coordination through faster motor learning and efficient situation solutions. The abilities of movement frequency (hand tapping test), repetitive strength of the trunk that is considerably saturated by movement frequency (test of front support to sitting position), and explosive strength of jump type (standing long jump test) should primarily be developed to the level that enables integration of these basic motor abilities into specific abilities of movement speed without and with ball. This stage of the performance quality development is predominated by acute, i.e. rapid and simple action which limits handball performance, so-called »catch and run« pattern; it can be objectively and reliably assessed by 20-m standing-start run;

- stage 2: after age 11, upon selection based on the running speed (sprint) performed in the preceding stage, this motor ability will not predominantly influence handball performance anymore but will be substituted by explosive strength of throw type (medicine ball throw from supine position), manifesting in the explosive strength of ball throw, i.e. pass force. This stage is characterized by marked development of arm and shoulder girdle strength and musculature, thus enabling active performance in defense or attack;

- stage 3: upon selection based on explosive strength of throw type, i.e. explosive strength of ball pass, performed in the previous stage, the players will not vary significantly in this ability anymore; now, after age 13, their performance will be predominantly influenced by whole body coordination (agility on the ground test), ensuring integration of the mentioned basic and specific motor abilities in the general motor efficiency to solve all play situations. This will manifest in high speed of technique performance and speed of movement direction exchange in handball game. The formation of performance quality is gradually transferred to a higher level;
- stage 4: after age 15, having achieved a satisfactory level of pass force and its integration with specific agility, selection should be based on the pass precision and skill of ball handling because these specific motor abilities now take the leading role in the determination of performance quality; and
- stage 5: after age 17, selection should be done by evaluation of all specific motor abilities, especially the ability of ball handling, for which hand coordination in terms of object manipulation (matchbox juggling test) is responsible. This facilitates the performance of catching and throwing the ball, which eventually mostly determines the level of performance in women's handball by significantly reducing the number of lost balls.

Accordingly, in the presented model of selection in women's handball the skill of ball handling is the specific motor ability that limits the achievement of top performance to the greatest extent. The skill of ball handling requires finely coordinated regulation of arm movement, from the upper arm and forearm through the wrist and hand, which depends on functional coordination of primary motor abilities that is highly genetically determined. Therefore the skill of ball handling in the play can fully manifest only when other basic and specific motor abilities have reached a satisfactory level. Thus, the following sequence should be followed: speed of movement without ball and with ball from selection stage 1 through stage 3 (determined by basic motor abilities of speed, explosive strength of jump type, and coordination); explosive strength of ball pass and specific agility in selection stages 2 and 3 (determined by basic explosive strength and basic coordination of the whole body); pass precision in selection stage 4 (determined by basic precision and basic explosive strength of throw type); and skill of ball handling in selection stages 4 and 5 (determined by arm coordination).

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NOVI MODEL SELEKCIJE U ŽENSKOM RUKOMETU

SAŽETAK

Cilj rada je bio utvrditi bazične motoričke sposobnosti koje determiniraju vrhunsku igračku kvalitetu u ženskom rukometu, te izvršiti izbor testova za primarnu selekciju u rukometnoj školi. U tu svrhu, na uzorku od 155 polaznica rukometne škole grada Splita, prosječne starosne dobi od 12.5 godina, putem diskriminativne analize utvrđene su razlike u bazičnim motoričkim sposobnostima između ispitanica koje su kasnije nakon sedmogodišnjeg trenažnog procesa

postale kvalitetne rukometašice i onih koje su napustile rukomet jer nisu zadovoljile natjecateljski kriterij. Utvrđeno je da su ispitanice koje su ostale u rukometu bile superiornije i na početku u svim analiziranim varijablama, a osobito u koordinaciji ruku, koordinaciji cijelog tijela, eksplozivnoj snazi bacanja i skoka, frekvenciji pokreta rukom i repetitivnoj snazi trupa. Motorička superiornost temeljena na sposobnostima koordinacije, eksplozivne snage i brzine uslovljava igračku kvalitetu u ženskom rukometu. Zato te sposobnosti možemo smatrati pouzdanim selekcijskim kriterijem. Temeljem rezultata ovog istraživanja predložen je novi model selekcije u ženskom rukometu u kojem fina koordinacija ruku u najvećoj mjeri limitira igračku kvalitetu.

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Modeling the Influence of Body Size on Weightlifting and Powerlifting Performance

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A B S T R A C T

The purpose of this study was to examine 1) if lifting performance in both the weightlifting (WL) and powerlifting (PL) scale with body mass (M) in line with theory of geometric similarity, and 2) whether there are any gender differences in the allometric relationship between lifting performance and body size. This was performed by analyzing ten best WL and PL total results for each weight class, except for super heavyweight, achieved during 2000–2003. Data were analysed with the allometric and second-order polynomial model, and detailed regression diagnostics was applied in order to examine appropriateness of the models used. Results of the data analyses indicate that 1) women's WL and men's PL scale for M in line with theory of geometric similarity, 2) both WL and PL mass exponents are gender-specific, probably due to gender differences in body composition, 3) WL and PL results scale differently for M possibly due to their structural and functional differences. However, the obtained mass exponents does not provide size-independent indices of lifting performances since the allometric model exhibit a favourable bias toward middleweight lifters in most lifting data analyzed. Due to possible deviations from presumption of geometric similarity among lifters, future studies on scaling lifting performance should use fat-free mass and height as indices of body size.

Key words: *allometric scaling, body size, muscle strength, polynomials, powerlifting, weightlifting*

Introduction

Muscle strength has been defined as the maximum force or torque developed during maximal voluntary contraction under a given set of conditions[1]. Although performance in almost every sport depends on certain amount of muscle strength[2], this is particularly emphasized in two sporting activities: weightlifting[3] and powerlifting. In both sports, the objective is to perform maximum lifts using different lifting techniques. Three events make up the sport of powerlifting (PL): bench press, squat, and dead lift. Weightlifting (WL) consists of two events: snatch, and clean-and-jerk. By summing the results in each lifting event, a total result can be calculated, which is usually used as a criterion of overall lifting (or strength) performance in both WL and PL.

It is well known that body size represents an important factor that affects muscle strength[1,4,5]. In particular, there exists significant positive relationship between muscle strength and body size[1]. In order to eliminate the effects of body size on lifting performance, athletes in both sports compete in different weight classes. However, in weightlifting competitions comparisons are often made among lifters who win the different weight classes so that the best lifter in the competition can be identified[6]. In order to be able to compare results of lifters from different weight classes, the results should be appropriately normalized for body mass (*M*).

The simplest method of normalizing muscle strength (or any other physiological or performance variable) is to divide the result (in this case mass lifted) with subjects' *M*, i.e. ratio standard[7–9]. However, for this ratio standard to be valid the true relationship between two variables should be linear and the regression should pass through the origin. Unfortunately, in WL and PL neither of these two conditions is satisfied, so this scaling method is inappropriate[8–11]. In particular, many studies (see Jaric[1] for review) have shown that muscle strength (and lifting performance) appears to increase at a lower rate than *M*. Therefore, ratio scaling have been shown to penalize heavier individuals because too much an adjustment would be made for *M* thus disproportionately »deflating« the overall result of heavier individuals[10,11].

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The theoretical explanation for these results is based on the presumption of geometric similarity[10], by which muscle strength should be proportional to muscle physiological cross-sectional area – which is proportional to $M^{2/3}$ – rather than M[7,13]. Therefore, other scaling models, the allometric model in particular, have been proposed and used frequently[6,7,14–16]. Allometric modelling is based on the assumption that the true relationship between depended variable (in this case, muscle strength) and an independent anthropometric variable (in this case, body mass) is curvilinear and passes through the origin (for review see Nevill and Holder[9]). Specifically, it presumes an allometric relationship:

$$S = a \cdot M^b, \quad \text{(eq. 1)}$$

where S is muscle strength, M is body mass, b is the allometric exponent and a is constant multiplier. When allometric exponent b is determined, muscle strength (in this case mass lifted) could be expressed independent of M:

$$S_n = S / M^b, \quad \text{(eq. 2)}$$

where S_n represents normalized muscle strength. Note that S_n corresponds to constant multiplier a (for details see Jaric[1] and Jaric et al.[4]).

This approach has been frequently applied in normalizing WL and PL performance[6,7,10,11,17–21]. However, while several studies have determined that the overall WL and PL results should scale with a theoretical mass exponent $b = 0.67$[6,11,20], some studies showed that the allometric exponent b in the WL lifting is considerably lower than 0.67, predicted by the presumption of geometric similarity[10,17]. In contrast, some researchers that modelled the total PL performance (e.g., Vanderburgh and Dooman[21]) reported mass exponent b significantly higher than 0.67.

Batterham and George[10] have also shown that body composition represents an important factor in determining the relationship between muscle strength and body mass. By excluding the subjects heavier than 91kg from their analysis, the authors obtained allometric exponent $b = 0.68$, instead of $b = 0.48$, when all weight categories were considered in analysis. It must also be noted that the majority of studies analyzed men's lifting performance[6,7,17,20] and used a rather small samples (≤ 30[6,7,10,11,17,19]). Jensen et al.[22] have elegantly shown that sample size represents an important factor in determining a true relationship between physiological variables and body size. Recently, Kauhanen et al.[8] analysed WL performance – body mass relationship on large data sets of elite WL results ($n = 1572$) and demonstrated that the overall lifting performance does not scale with theoretically predicted exponent 0.67 ($b = 0.55$; 95% confidence interval 0.53–0.56). When heaviest weight class was excluded from their analysis, mass exponent b was 0.60 (95% confidence interval 0.53–0.66), still significantly lower ($p < 0.05$) than 0.67. However, one possible confounding factor might be included in the study of Kauhanen et al.[8]. Namely, the authors included in an analysis men's lifting results from 1973 to 1999. Due to increased use of anabolic-androgenic steroids and other anabolic stimulus among strength athletes in the mid 1970s and early 1980s[23], possible increase in upper limits of lean body mass could significantly influence to the relationship between lifting performance and body mass[10].

This brief literature review suggests that different results obtained in previous studies could be the result of: a) differences in body compositions among lifters (lifters in heaviest category may be heavier because of excess body fat); and b) small sample size studied. In addition to these divergences, recent findings have indicated that second-order polynomial model provides a superior fit to elite WL and PL results than allometric model[7,10,24,25]. For this reason, Batterham and George[10] suggested that the allometric modelling should be applied only when all underlying model assumptions (i.e., regression diagnostics) have been rigorously evaluated and satisfied. Finally, there is an obvious lack of empirical data in scientific literature about the relationship between lifting performance and body size in female athletes. To further examine if lifting performance scale with the theoretically predicted exponent $b = 0.67$, and whether there are any differences in scaling lifting performance for body size between genders, we analyzed lifting performance on a relatively large sample of elite men and women weightlifters and powerlifters using both allometric and second-order polynomial model.

Materials and Methods

Performance and body size data

In this study the WL and PL data from 2000 to 2003 were analyzed. The data for WL were selected from results made in the World Championships and Olympic Games during 2000–2003 with the respective body mass measured at the official weigh-in before each competition (official web-site of the International Weightlifting Federation; www.iwf.com). The data for PL were selected form PL results made in the World Championships during 2000–2003 with the respective body mass (M) measured at the official weigh-in before each competition (official web-site of the International Powerlifting Federation; www.powerlifting-ipf.com). Although both the WL and PL consists of several lifting disciplines (see Introduction), we focused only on the total results (the sum of results in each lifting discipline) as a criterion of overall lifting performance.

Subjects

For WL, best 10 two-event total results (sum of snatch and clean-and-jerk) for each men and women weight category, except for super heavyweight (men's +105 kg and women's +75 kg), achieved during 2000–2003 were used in this study. If the same lifter had more than one result in top 10 results, only his/her best result was used in further analysis. For PL, best 10 three-event total results (sum of squat, bench press, and dead lift) achieved during 2000–2003 for men's categories up to 110 kg, and women up to 75 kg were used in this study. As for WL, if

the same lifter had more than one result in top 10 results, only his/her best result was used in further analysis. The greatest weight category in WL, and categories over 110 kg for men and over 75 kg for women in PL were omitted in order to avoid possible confounding effect of body composition common in super heavyweight categories[10,11].

Data analyses

To examine if lifting performances scale with theoretically predicted mass exponent 0.67, an allometric or power function model was applied (see eq. 1). A regression technique applied on the log-transformed data provided the values of the allometric exponent b for each lifting result (see Batterham and George[10] for details of the method). In short, a log-transformation of the presumed allometric relationship (see eq. 1) between lifting performance S and body mass M gives:

$$\ln S = \ln a + b \ln M + \ln \varepsilon, \qquad \text{(eq. 3)}$$

where $\ln a$ and b, respectively, correspond to the intercept and slope of the regression line fitted through the logarithmic values of the experimentally recorded lifting results and body mass, and ε is an error term. Normality of log-transformed variables was confirmed using a Kolmogorov-Smirnov test ($p > 0.3$). Commonality of slopes of the lifting result-body mass relationships between men and women were tested by including gender and gender-by-ln M interaction term in a multiple log-linear model, as described by Batterham and George[10]:

$$\ln S = \ln a + d(\mathrm{G} \ln M) + c\mathrm{G} + b \ln M + \ln \varepsilon, \text{ (eq. 4)}$$

A significant gender-by-ln M interaction term ($p < 0.05$) for both WL and PL results was found. Therefore, separate allometric exponents for men and women in both lifting disciplines were calculated. Allometric exponents were reported as mean ± standard error (mean ± SE), allowing construction of 95% confidence intervals (95% CI).

In addition, a second-order polynomial model was also applied to the same data, as recommended by Batterham and George[10]:

$$S = a + bM + cM^2, \qquad \text{(eq. 5)}$$

The differences in the lifting performance-body size relationship between allometric and second-order polynomial model were computed using two-tailed t-test for testing differences between two dependent correlation coefficients. A detailed regression diagnostics recommended by Nevill and Holder[9] and Batterham and George[10] was used to examine model fit. In brief, normality of the distribution of the residuals in both analyses was ascertained using the Kolmogorov-Smirnov test. The homoscedasticity of data (constant error variance) was tested by calculating a correlation between the raw residuals and ln M. Appropriateness of log-linear regression model was checked via detailed inspection of the scatter plot of residuals and ln M.

Results

Figure 1 depicts the scatter plots of M *vs*. lifting performances by gender in both WL (Figure 1a) and PL (Figure 1b). As expected, absolute indices of lifting performance and M suggest a strong positive relationship. The plotted relationships are curvilinear, similar to those reported by Batterham and George[10] and Vanderburgh and Batterham[11] for the WL and PL results, respectively.

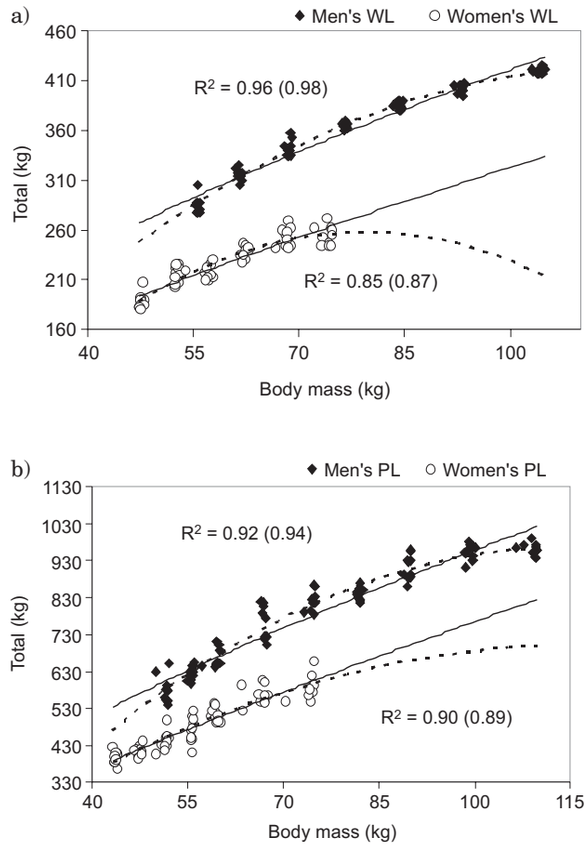

Fig. 1. Scatter plot of total lifted in weightlifting (a) and powerlifting (b) vs. body mass for men and women. Both allometic (solid line) and second-order polynomial curve (dashed line) fits are displayed together with their corresponding coefficients of determination R^2 (R^2 for second-order polynomial models in parenthesis).

The results of the allometric and the second-order polynomial model applied to the lifting performances are shown in Tables 1 and 2. The resulting solutions explained between $R^2 = 85\%$ and $R^2 = 96\%$ of the variation in lifting performances analyzed in this study. Although the second-order polynomial model explained ~ 2% more variance than allometric model for most lifting performances, this improvement was not significant ($p > 0.05$).

The obtained slopes of the regression lines correspond to allometric exponents b needed to assess body mass in-

TABLE 1
ALLOMETRIC MODEL RELATIONSHIPS FOR WEIGHTLIFTING (WL) AND POWERLIFTING (PL) PERFORMANCE (S) TO BODY MASS (*M*)

	N	b	95% CI	Equation
Men's WL	70	0.61±0.02	0.58 – 0.64	$S=25.56M^{0.61}$
Women's WL	70	0.68±0.04	0.62 – 0.76	$S=13.85M^{0.68}$
Men's PL	90	0.69±0.02	0.65 – 0.74	$S=36.31M^{0.69}$
Women's PL	80	0.80±0.03	0.74 – 0.87	$S=19.11M^{0.80}$

N – number of subjects, 95% CI – 95% confidence interval, *b* – allometric parameter (X±standard error)

TABLE 2
SECOND ORDER POLYNOMIAL MODEL RELATIONSHIPS FOR WEIGHTLIFTING (WL) AND POWERLIFTING (PL) PERFORMANCE TO BODY MASS (*M*)

	Equation
Men's WL	$S= -0.037M^2 + 8.68M - 71.77$
Women's WL	$S= -0.068M^2 + 10.76M - 169.11$
Men's PL	$S= -0.081M^2 + 20.04M - 232.37$
Women's PL	$S= -0.070M^2 + 15.59M - 151.16$

S – normalized performance

dependent indices of lifting performance (see parameter *b* in eq. 3). These allometric exponents *b* (Table 1) represent the main result of the study. The exponents of 0.68 and 0.69 for women's WL and men's PL data are similar to the theoretical prediction of 0.67. In contrast, 95% confidence intervals of the mass exponents *b* for men's WL and women's PL performance do no not include the value of 0.67, suggesting that these lifting performance scale with negative and positive allometry, respectively. It must also been pointed out that women's mass exponents *b* were significantly higher ($p<0.05$) than men's exponents in both lifting sports (see Methods section for details).

All residual errors from fitting both models were found to be acceptably normal when using Kolmogorov Smirnov test ($p>0.4$). In addition, no linear relationship was found between residual scores and body mass for allometric modelling (correlation coefficients *r* ranged between –0.11 and 0.09, $p>0.05$), suggesting that the model errors displayed homoscedasticity.

The presented data suggest that both models demonstrated excellent fit to the lifting performances analyzed.

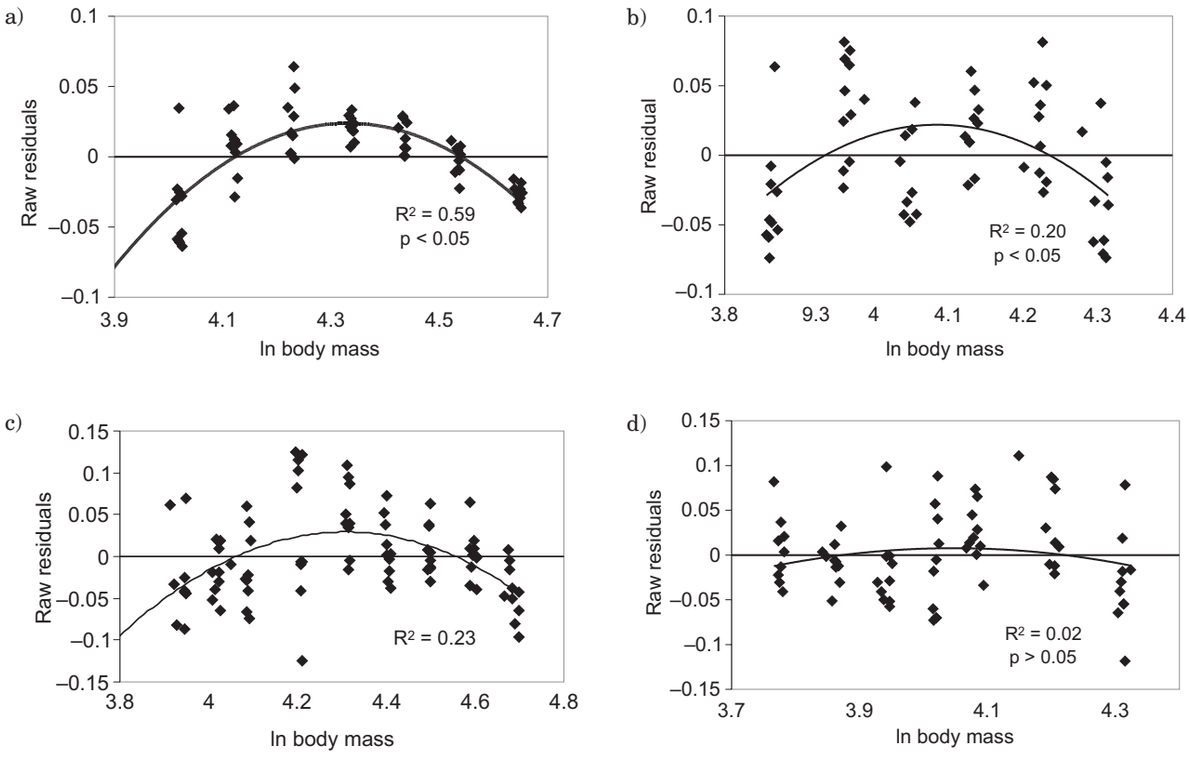

Fig. 2. Scatter plot of raw residuals vs. ln body mass for allometric model applied to: (a) men's weightlifting results, (b) women's weightlifting results, (c) men's powerlifting results, and (d) women's powerlifting results. Second-order polynomial curve fits are displayed (solid line) together with associated coefficient of determination (R^2).

However, a detailed inspection of scatter plots of residuals *vs*. ln *M* (Figure 2) shows a curvilinear relationship for all allometric models, except for women's PL results. In particular, a moderate to strong non-linear relationship was found between raw residuals and ln *M* (R^2 =0.20–0.59, $p<0.05$) for men's and women' WL and men's PL performances. These results clearly indicate that the allometric model applied to all lifting performance, except in women's PL, exhibit a favourable bias toward lifters in middleweight classes.

Discussion

The present study examined the allometric and second-order polynomial relationship between lifting performances in both WL and PL on the one hand, and *M* on the other. Performance in WL and PL certainly represent the maximum of achievable physical strength, so that it seems justified to call these performances 'maximal strength'[25]. The theory of geometric similarity suggests that maximum muscle strength (*S*) should scale to *M* to the 2/3 power (i.e., $S \propto M^{0.67}$), also known as isometric scaling[1,6]. In our study, mass exponents *b* for the men's and women's WL performances were 0.61 and 0.68. For PL performances, gender-specific mass exponents *b* were 0.69 for men and 0.80 for women. While women's WL and men's PL performance appeared to scale in line with theory of the geometric similarity, men's WL performance scale with negative allometry, and women's PL performance scale with positive allometry, respectively.

Modelling both WL and PL results in this study did not include men over 105 kg (over 110 kg for PL) and women over 75 kg. Therefore, possible confounding factor of disproportionate increase in body fat, common in the overweight subjects [26,27] (i.e., super heavyweight categories), was excluded from the analyses. In addition, a relatively similar number of lifting performances (between 70 and 90) was analyzed in both WL and PL. Still, the obtained mass exponents varied from 0.61 to 0.80, suggesting that other factors than body composition and sample size might have had significant influence on the lifting performance – body mass relationship. Comparisons of the allometric exponents *b* obtained in this study also raised two important questions: a) *Why do women have significantly higher mass exponents in both WL and PL than men,* and b) *Why do PL performances have significantly higher mass exponents than WL performances?*

Regarding the first question, significantly higher mass exponents obtained in the women's WL and PL results can be attributed to several factors. First, it must be underlined that WL and PL for women is a relatively new sport event, when compared to men's WL and PL. For example, 72 men's and only 15 women's World Championships in WL were held until the present date. It is, therefore, feasible to expect that men's lifting performance exhibit more homogeneity within body weight classes. Figure 1a confirms this assumption, showing greater dispersion in WL results of women than of men. Thus, defined heterogeneity in strength across body weight classes in women can decrease the possibility to define a true relationship between lifting performance and *M*. The second explanation is based on the fact that women generally tend to have less active muscle mass[28] and more fat tissue (i.e., different body composition) than men[29–31] regardless of the body-weight class analyzed. Moreover, it has been demonstrated that women generally have more fat-free mass in their lower body than men do. It is therefore, likely that the observed gender-specific relationship between muscle strength and *M* could be the result of gender differences in body composition and regional distribution of contractile tissue. Finally, one cannot exclude possible use of illegal but non-detectable drugs of a higher prevalence among one gender over another (e.g., growth hormones[11]).

Regarding the second question, the authors recognize two possible explanations for PL performances having significantly higher mass exponents than WL performances. The first explanation is related to technical and biomechanical differences between WL and PL events. Namely, WL events (snatch and clean-and-jerk) are far more complex movement patterns than PL events (bench press, squat and dead lift). Therefore, besides raw muscle strength, specific lifting skill has a profound influence on the performance in WL. It is not uncommon that a weightlifter misses the maximum lift due to a small technical error at the end of the lift (e.g., loss of balance). In that case, athlete's overall lifting performance would not represent his/her true maximum strength capabilities. Since characteristic motor-skill influences WL performance more than PL performance, the stated differences in the relations between *M* and performances are easy to follow.

It must also be pointed out that the WL performance depends on several other motor abilities besides strength, like power, speed, flexibility and coordination, which do not scale with *M* similarly as muscle strength. Several authors[7,10,32] have pointed out that the WL performances should be recognised as a combined measure of strength, power and skill, while PL performance is a pure measure of strength. Thus, lower mass exponent *b* for the WL performance when compared to the PL performance could be the result of the abovementioned structural and functional differences between these two lifting techniques.

The second explanation is related to grip strength. Namely, in both WL disciplines (snatch and clean-and-jerk) an athlete pulls the barbell up from the floor by holding it with his/her palms. It can be, therefore, expected that grip strength may be one of the limiting factors for success in WL. In PL, only one lifting discipline is related to grip strength – it is dead lift. Thus, the overall performance in PL is probably to a smaller degree influenced by the grip strength than the performance in WL. Vanderburgh and Batterham[11] also reported similar finding. Since grip strength scales to *M* with negative allometry in both men and women[15,16], differences in mass exponent *b* between WL and PL could be also the result of the specific influence of grip strength on lifting performance in WL and PL.

Another important finding of this study is related to regression diagnostics of the allometric and second-order polynomial model applied to the WL and PL data. Analysis of residuals showed that allometric modeling for *M* does not provide an appropriate fit for men's and women's WL and men's PL results. In particular, the results have shown that the allometric modeling exhibit a favourable bias toward athletes in middleweight classes. Results of the present study also confirmed previous findings[10,21] that the second-order polynomial model provides statistically superior solution when modeling lifting performances for differences in *M*. However, in agreement to the results of Vanderburgh and Dooman[21], the authors have found no satisfactory explanation for the superiority of the second-order polynomial model other than its better statistical fit. Several research studies[10,11,21] have demonstrated that allometric modeling is statistically incorrect when used to scale the WL and PL performances for *M*. However, as far as we could ascertain, most researchers did not provide a possible biological explanation for the results obtained. Vanderburgh and Batterham[11] revealed that half as many powerlifters are found in the lightest and heaviest weight classes as in the intermediate classes and that intermediate classes' top lifters are perhaps more likely to achieve at a higher body mass adjusted level than those powerlifters in lower and higher classes. However, this explanation cannot be applied to the WL results.

A possible biological explanation for an inappropriate fit of the allometric model in the WL and PL performances could be related to a violation of the presumption of geometric similarity among lifters. For example, it is common that lifters in both sport events move to a higher weight class by increasing his/her muscle mass, especially in the middleweight classes. In this case, lifters with similar heights and other linear body dimensions would have disproportionately greater limb girths and body masses. It would be, therefore, advisable to include more anthropometric measures (e.g., height) in the analysis when modeling lifting performance (or any other physiological or performance variable) for differences in body size as suggested by Nevill[33]. Recently, Ford et al.[34] have shown that muscle mass, but not *M*, varies almost exactly with the cube of height over the entire range of body size of weightlifters, so that strength varies almost exactly with height squared or with muscle mass to the two-thirds power. Ford et al.[34] have also showed that the fraction of *M* devoted to non-contractile tissue increases abruptly in heavier lifters. This abrupt transition produces corners in the curves relating performance variables to *M*, and these corners preclude a good fit by any continuous allometric function relating a power of *M* to measures of strength[34]. More recently, Nevill et al.[35,36] provided evidence that athletes' physiques could substantially deviate from geometric similarity and concluded that this deviations have serious implications for the allometric scaling of physiological and performance variables. In particular, Nevill et al.[35,36] proposed the use of corrected limb girths (e.g., thigh, calf) instead of *M* in the allometric scaling of human biological functions. These data suggest that other indices of body size, like height, fat-free mass or muscle mass should be used when modeling muscle strength for body size.

Conclusion

To summarize, our result together with some previous data[8,10,11,21] indicate that the allometric modeling of WL and PL results for *M* does not provide size-independent index of lifting performance. Instead, it exhibits a favourable bias toward middleweight lifters in most lifting data analyzed. The possible explanations for this inappropriate statistical fit of the allometric model applied to the WL and PL data are: 1) lifters have disproportionately greater limb girths than bone lengths, thus violating the presumption of geometric similarity, and 2) muscle mass does not represent a constant proportion of body mass within the sample of elite lifters. Moreover, we demonstrated that WL and PL results scale differently for *M* possibly due to their structural and functional differences (i.e. PL is a pure strength sport, while WL is strength-speed sport with strong emphasis on specific and rather complex lifting skill). Finally, we showed that the allometric relationship between lifting performance in both WL and PL is gender-specific, probably due to gender differences in body composition and regional distribution of contractile tissue. Based on these results we suggest using muscle mass (or fat-free mass) and height as indices of body size when modeling WL and PL performances. This would allow comparisons of size-independent lifting performance (separately for WL and PL) of all lifters, regardless of weight category (i.e. super heavyweight athletes included) and gender. These findings together recent observations obtained on other human populations[35,38,39] highlight the importance of using more suitable indices of body size than M when comparing physiological performance of humans of different body size.

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MODELIRANJE UTJECAJA VELIČINE TIJELA NA NATJECATELJSKE REZULTATE U OLIMPIJSKOM DIZANJU UTEGA I »POWERLIFTINGU«

SAŽETAK

Glavni cilj ovog istraživanja bio je utvrditi 1) da li natjecateljski rezultati u olimpijskom dizanju utega (WL) i »powerliftingu» (PL) skaliraju sa tjelesnom masom (*M*) u skladu s teorijom geometrijske sličnosti, i 2) da li postoje razlike među spolovima u alometrijskoj povezanosti između dizačkih rezultata i veličine tijela. U tu svrhu analizirano je deset najboljih rezultata u WL i PL u svakoj težinskoj kategoriji osim super-teške, postignutih u razdoblju 2000–2003. Podaci su analizirani primjenom alometrijskog modela te polinomijalnog modela drugog stupnja, dok je provjera o primjerenosti primijenjenih modela provjerena detaljnom regresijskom dijagnostikom. Rezultati istraživanja pokazuju kako 1) ženski rezultati u WL i muški rezultati u PL skaliraju s *M* sukladno teoriji o geometrijskoj sličnosti, 2) postoje značajne spolne razlike u alometrijskoj povezanosti između dizačkih rezultata u oba sporta i *M*; te su razlike vjerojatno rezultat spolnih razlika u kompoziciji tijela, 3) rezultati u WL i PL skaliraju s *M* različito vjerojatno zbog strukturalnih i funkcionalnih razlika koje postoje među njima. Međutim, alometrijski eksponenti dobiveni u ovom istraživanju ne omogućuju definiranje indeksa rezultata u WL i PL koji su nezavisni od veličine tijela iz razloga što alometrijski model favorizira rezultate kod dizača u srednjim težinskim kategorijama kod većine analiziranih podataka. Zbog mogućih devijacija u geometrijskoj sličnosti dizača utega, buduća istraživanja o skaliranju rezultata u WL i PL trebala bi koristiti nemasnu masu tijela i tjelesnu visinu kao pokazatelje veličine tijela.

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Original scientific paper

Influence of Passive Smoking on Basic Anthropometric Characteristics and Respiratory Function in Young Athletes

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A B S T R A C T

The primary objective of this study is to investigate the maintenance difference in basic anthropometric characteristics and to outline the dynamics of respiratory function change in youngsters athletes exposed to passive smoking (PS) and athletes not exposed to passive smoking in their families (NPS). High and weight were determined as basis anthropometric characteristics. Measured parameters for respiratory function were vital capacity (VC), forced expiratory volume in the first second (FEV1), maximum expiratory flow (PEF), forced expiratory flow at 50% forced vital capacity (MEF 50) and forced expiratory flow at 25% forced vital capacity (MEF 25). Significant statistical differences in separate spirometric variable were found in three variables (FEV1, MEF50, and MEF25) for group older youngsters. Analysis of variance showed statistical differences between athletes unexposed to passive smoking (NPS) and athletes exposed to passive smoking (PS) in even four spirometric variables (VC, FEV1, MEF50 and MEF25).

Key words: *passive smoking, respiratory function, athletes*

Introduction

Smoking is a major public health issue due to its direct and indirect effects on health outcomes[1–3]. Previous studies have suggested that passive smoking (involuntary inhalation of tobacco smoke by nonsmokers) reduces small airways function[1,3]. Passive smoking may affect children directly, by decreasing pulmonary function, or indirectly [4,5] by increasing their exposure to infectious diseases, since smokers have a higher incidence of respiratory infections[1–3]. Maternal smoking during pregnancy is a major risk factor for preterm delivery, low birth weight, intrauterine growth retardation, and intrauterine death[6].

According to World Health Organization (WHO), almost half of the world children are exposed to tobacco smoke of adult smokers. WHO states that passive smoking is thru cause of bronchitis, pneumonia, coughing and troubled breathing, and fits of asthma, middle ear infections, and cardiovascular damages in children and adults[7–9].

Previous studies in this field have mostly been based on the follow-up of forced expiratory volume in the first second (FEV 1) and forced expiratory flow at 25–75% FCV (MEF 25–75)[10,11]. Respiratory function tests provide objective, quantitative data on the functioning of the respiratory system. They are used for both diagnostic and prognostic purposes and can also be used as a method of diagnosis and prognosis of functional abilities of young athletes.

We evaluated the exposure to passive smoking and its effects on pulmonary function in five spirometric indicators and two anthropometric parameters in a group of 8 to 15 year-old youngster's athletes.

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Materials and Methods

Study design and patients

Basic anthropometric characteristics and respiratory functional indicators have been measured on 100 youngster's athletes (swimmers, handball players, and soccer players) with mean age of the test subjects 12.98 year (8–15 years old). As basics anthropometric characteristics high and weight were determined. The examiners train on average 1.5–4 hours, 4–5 times a week. All of the subjects have been training from the age of six. All of the subjects included in the study have been training constantly for at least the past 6 months.

None of the examiners is an active smoker. The group of subjects exposed to passive smoking (PS) consisted of those examinees whose one or both parents smoke at least 10 cigarettes in family surrounding, and time of exposure to passive smoking amounted to at least 2 hours a day. They have been exposed to passive smoking for at least five years, almost on a daily basis. The number of people smoking in their surrounding has been limited to two, generally their parents. The exposure to passive smoking in age 8 to15 years is possible almost in family surrounding, because they have very little free time outside the family home.

Six subjects have been excluded from the study. One of them has been excluded because of training pause longer than month. One test subject has been excluded because of several years' history of asthma. Two subjects have not been included due to virosis during the course of the study and still two because their exposure to smoking was lower than the prescribed criteria. None of the subjects included in the study has been diagnosed with acute or chronic diseases. Both the parents and the leaders of the soccer, handball and swimming teams have given written consent to the participation of test subjects in the study. The parents also have given data about exposure to passive smoking their children.

The examiners were divided into two groups according the age. In fist group (Group A) were 24 athletes from 8 to 11 years old with mean age 10.13 year (12 PS and 12 non passive smokers – NPS). In the second group (Group B) were 70 youngsters from 12 to 15 years old with mean age 13.80 year (31 PS and 39 NPS).

Sample of variables

As basis anthropometric characteristics, high and weight were determined according International biological programme (IBP) recommendation. Respiratory functional indicators have been measured in resting by means of the pulmonary function measuring device »Master Lab« produced by Jaeger. The pulmonary function measuring device is completely automatic. It consists of three functional units: »Master Lab Pneumo«, »Master Lab Transfer« and »Master Lab Body«, which runs on a computer system. The computer is running CAP (Computer Aided Pulmonary Diagnostic software) consisting of programs for examinations pulmonary function: spirometry flow-volume curve**,** diffusion by means of the techniques of one inhalation and body pletismography. The programs contain all technical criteria, technical indicators and reference values in Civil Engineering Contractors Association (CECA) and American Thoracic Society (ATS) standards. Spirometry is the method for measuring the volume of air in the lungs during breathing. Before the measurement itself, the spirometer is gauged several times and 2 l of air is introduced into it, while the computer monitor records the air inspired/expired flow-volume plot. In spirometric measurements, the examinee, using a mouth piece, first exhales maximally from normal breathing regime, and then inhales maximally. The computer program uses the data on the age, height and mass of the examinee to determine the values of parameters expected according to standard defined, and also report their actual values. The difference between the values expected and actual is expressed in percentages. The following parameters have been measured: lung vital capacity (VC), forced expiratory volume in the first second (FEV1), maximum expiratory flow (PEF), forced expiratory flow at 50% forced vital capacity (MEF50), forced expiratory flow at 25% forced vital capacity (MEF25), height (H), and weight (W). Ventilatory measurement results were expressed automatically as percentage of referent values (ESC norms), depending of age, sex, high and weight.

Data processing methods

By using the software package »STATISTICA«, i.e. the program Statistic for »Windows Ver5.5«, basic statistical parameters have been obtained. All differences in which the probability of the null hypothesis was $p<0.05$ were considered statistically significant. In line with objective of the study, the multivariate analysis of variance has been performed in order to determine the relevance of differences between the obtained statuses of all tests simultaneously. Univariate analysis of variance has been performed to determine differences at each variable between groups of examinees.

Results

From 100 youngsters, 6 subjects (6%) have not been included in the study because they have not fitted in testing criteria. Between 94 youngster's athletes were 52 swimmers, 30 handball players and 12 soccer players. Basic anthropometrics characteristic were showed in Table 1. Data are pointing that athletes in group A unexposed to passive smoking (NPS) are higher 0.84 (0.56%) cm and heavier about 2.17 kg (5.62%) than athletes exposed to passive smoking (PS). The youngsters unexposed to passive smoking (NPS) from the group B were higher 1.62 cm (0.93%) and heavier 0.8 kg (1.35%) from exposed athletes (PS). Altogether, data are pointing that athletes unexposed to passive smoking (NPS) are higher 2.44 cm (1.45%) and heavier 2.09 kg (4.88%) than athletes exposed to passive smoking (PS). Multivariate analysis of variance (Table 2) for anthropometric characteristics and age showed that statistical differences between

TABLE 1
CHARACTERISTICS OF CURRENTLY EXPOSED AND UNEXPOSED ATHLETES

	Group A		Group B		Total	
Variable	PS (N=12)	NPS (N=12)	PS (N=31)	NPS (N=39)	PS (N=43)	NPS (N=51)
Age	10.00 (0.6)	10.25 (0.97)	13.77 (1.12)	13.82 (1.14)	12.72 (1.98)	12.98 (1.88)
Height (cm)	148.83 (9.23)	149.67 (8.80)	171.84 (9.78)	173.46 (8.13)	165.42 (14.13)	167.86 (13.08)
Weight (kg)	36.50 (4.62)	38.67 (5.73)	58.61 (10.69)	59.41 (10.25)	52.44 (13.71)	54.53 (12.88)

Data are mean % (SD %), Group A – 8–11 years, Group B – 12–15 years, PS – passive smokers, NPS – non passive smokers

TABLE 2
MANOVA BETWEEN EXPOSED AND UNEXPOSED ATHLETES FOR ANTHROPOMETRIC VARIABLES

	Wilks' λ	Rao R	p
Group A	0.91	0.68	0.57
Group B	0.99	0.27	0.85
Total	0.99	0.25	0.86

MANOVA – Multivariate Analysis of Variance, Wilks´ λ, Rao R – multivarijate measure of group differences over several variables, p – significance level of the difference between two groups

athletes unexposed to passive smoking (NPS) and athletes exposed to passive smoking (PS) in high and weight were not statistically significant ($p>0.05$). That means that the differences between arithmetic means of NPS and PS athletes are not statistically significant in applied system of variables for these two groups of athletes.

Multivariate analysis of variance for spirometric variables is showed in Table 4. Wilks' Lambda and Rao R (which tests value of lambda) are statistically significant for group B and altogether athletes. That means statistically significant differences between arithmetic means of NPS and PS athletes in these two groups in applied system of variables.

TABLE 4
MANOVA BETWEEN EXPOSED AND UNEXPOSED ATHLETES FOR SPYROMETRIC VARIABLES

	Wilks' λ	Rao R	p
Group A	0.68	1.71	0.18
Group B	0.82	2.80	0.02
Total	0.85	3.14	0.01

MANOVA – Multivariate Analysis of Variance, Wilks´ λ, Rao R – multivariate measure of group differences over several variables, p – significance level of the difference between two groups

Differences at each observed spirometric variable between unexposed (NPS) and exposed (PS) athletes to passive smoking are presented in Table 3.

Results analyses (ANOVA-Table 3) clearly show significant statistical differences in whole system of spirometric variables between NPS and PS athletes for group B and altogether athletes. Significant statistical differences ($p<0.05$) in separate spirometric variable (ANOVA – Table 3) were found in three variables (FEV1, MEF50, and MEF25) for group B. Altogether athletes exposed to passive smoking (PS) have significant statistical differences in four variables (VC, FEV1, MEF 50, and MEF 25) in opposite of youngsters unexposed to passive smoking (NPS).

Discussion

The data we have obtained confirm some results of the previous studies based on influence exposure to passive smoking young athletes[11,12]. The results noted nega-

TABLE 3
ANOVA BETWEEN EXPOSED AND UNEXPOSED ATHLETES FOR SPYROMETRIC VARIABLES

	Group A		Group B		Total	
Variable	PS (N=12)	NPS (N=12)	PS (N=31)	NPS (N=39)	PS (N=43)	NPS (N=51)
VC	98.28 (10.56)	104.28 (18.00)	93.95 (11.50)	99.46 (13.33)	94.48 (12.34)	100.15 (14.25)*
FEV 1	111.20 (9.50)	119.72 (17.89)	102.52 (8.61)	111.86 (13.29)**	104.94 (9.59)	113.71 (14.63)**
PEF	97.85 (9.50)	93.53 (20.21)	101.19 (13.37)	107.27 (20.31)	100.26 (12.39)	104.04 (20.93)
MEF50	104.88 (8.04)	117.94 (24.76)	101.01 (18.09)	115.28 (22.81)**	102.07 (15.93)	115.91 (23.05)**
MEF25	110.65 (7.46)	130.45 (37.28)	113.48 (31.38)	128.23 (33.27)*	112.63 (26.83)	128.75 (33.88)*

Data are mean % (SD %), ANOVA – Analysis of Variance, *$p<0.05$, **$p<0.01$, PS – passive smokers, NPS – non passive smokers, VC – vital capacity, FEV 1 – forced expiratory volume in first second, PEF – maximum expiratory flow, MEF 50 – forced expiratory flow at 50% forced vital capacity, MEF 25 – forced expiratory flow at 25% forced vital capacity

tive influence of passive smoking to respiratory function and anthropometric characteristics included in the study. Although no statistically relevant differences in height and weight have been found, considerable differences in high and weight are evident. NPS athletes are on average higher (2.44 cm) and heavier (2.09 kg) than PS athletes, which are in correlation with French study[12] who explored influence of smoking, at young athletes, on increasing body weight and proven statistically significant less body weight in PS athletes.

Unexposed athletes (NPS) have at average higher results in all observed static and dynamic respiratory parameters in respect to exposed athletes (PS). Previous studies explored static or dynamic parameters separately. Even 4 from 5 spirometric parameters showed statistically significant difference between athletes exposed and unexposed to passive smoking: VC, FEV1, MEF50 and MEF25 (Table 3). Unexposed athletes have average higher results in MEF25 for 16.12%, MEF50 for 13.84%, FEV1 for 8.77%, and VC for 5.67% in respect to passive smoking athletes. Significant statistical differences in separate spirometric variable in three variables (FEV1, MEF50, and MEF25) for group older youngsters (group B) could be consequence of longer exposure to passive smoking in relation to younger group (group A).

The difference in vital capacity (VC) is significant on level $p<0.05$ which emphasize negative effects of passive smoking not only on dynamic but also on static pulmonary capacities. In variables, which represent dynamic in small airways, the levels of significant are $p<0.01$ (MEF50) and $p<0.05$ (MEF25). Negative impact of passive smoking on small airways is obviously inflammatory reaction caused by irritants in cigarettes and decrease in speed current of air in lungs as a result of enhanced resistance. Our results acknowledge published investigations where authors have found a four times higher increase of reduced FEF25–75 (these parameters appropriate the values of MEF 25–75 and depend of manufacturer terminology) and/or the occurrence of coughing in sport players exposed to passive smoking in respect to sport players who have not been exposed[11].

In variable forced expiratory volume in the first second (FEV1) the difference is significant on level $p<0.01$ which statements conclusion that passive smokers athletes have developed great disorders of ventilation obstructive type.

All harvested data indicate the conclusion of negative influence of passive smoking on, in this investigation measured, anthropometric and spirometric variables. The results of our investigation indicate effects of passive smoking on growth and development young athletes and their pulmonary function as well as cause bad performances and insufficiently physical condition young athletes. It is considerably observation that NPS athletes are better in all measured ventilatory parameters then PS athletes.

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UTJECAJ PASIVNOG PUŠENJA NA OSNOVNE ANTROPOMETRIJSKE KARAKTERISTIKE I RESPIRACIJSKU FUNKCIJU MLADIH SPORTAŠA

SAŽETAK

Cilj studije je istražiti postojanje razlike u osnovnim antropometrijskim parametrima i promjene u respiracijskim funkcijama kod mladih sportaša izloženih pasivnom pušenju (PS) unutar obitelji u odnosu na mlade sportaše koji nisu izloženi pasivnom pušenju (NPS) unutar obitelji. Kao osnovni antropometrijski parametri mjereni su visina i težina mladih sportaša. Respiracijska funkcija je istražena mjerenjem vitalnog kapaciteta (VC), forsiranim respiracijskim volumenom u prvoj sekundi (FEV1), vršnim ekspiracijskim protokom (PEF), forsiranim ekspiracijskim protokom pri 50%

forsiranog vitalnog kapaciteta (MEF 50) te forsiranim ekspiracijskim protokom pri 25% forsiranog vitalnog kapaciteta (MEF 25). Statističkom obradom rezultata uočena je značajna razlika u pojedinačnim varijablama kod grupe sportaša u dobi 12–15 godina (grupa B) izloženih pasivnom pušenju u tri varijable (FEV1, MEF50 i MEF25) u odnosu na mlade sportaše koji nisu izloženi pasivnom pušenju u dobi 8–11 godina (grupa A). Analiza svih respiracijskih parametara u grupi sportaša izloženih pasivnom pušenju (PS) pokazala je statistički značajne razlike u čak četiri varijable (VC, FEV1, MEF50 i MEF25) u odnosu na grupu sportaša neizloženu pasivnom pušenju.

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Relations of the Morphological Characteristic Latent Structure and Body Posture Indicators in Children Aged Seven to Nine Years

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A B S T R A C T

With the aim of determining the connection between the indicators of body posture and latent structure of morphological variables in children aged 7 and 8 years, first and second grade of primary school, a set of 17 morphological measures and 12 body posture indicators were longitudinally applied to a sample of 110 boys and 114 girls. The latent structure of morphological variables in both sexes was defined by three factors but at a different order of significance: in boys, the order was longitudinal dimensionality, voluminosity, mass and subcutaneous fat tissue and transverse dimensionality, whereas in girls the order was voluminosity, mass and subcutaneous fat tissue, longitudinal dimensionality and transverse dimensionality. The latent structure of torax body posture indicator was defined by two factors, the status of body posture of the rear part of the thorax, and status of the body posture of the front part of the thorax. The results obtained by canonical correlation analysis between predictive variables, morphological latent structure and criterion variables, latent structure of thorax body posture indicators with two posture indicators of the chest and one of the foot status, showed two important pairs of canonical roots on each measurement, suggesting a significant association between these two sets of parameters.

Key words: *body posture, scoliosometer, morphological status*

Introduction

Many factor studies have determined the structure of latent dimensions of a group of manifest morphological variables. The majority of these studies were performed in older children, children in the end stage of development, or children whose growth and development had been completed. Relatively reliable indicators of the final morphological structure and dimension relations that can be considered final or permanent were thus obtained (Szirovicza et al., 1980; Hofman and Hošek, 1985)[1–2]. These results show that four morphological dimensions can generally be identified in adult individuals: longitudinal dimensionality of the skeleton, transverse dimensionality of the skeleton, voluminosity and body mass, and subcutaneous fat tissue. A two-dimensional model is found in children (Bala, 1977; Katić et al., 1994; Katić, 2003)[3–5].

A study by Bala (1977)[3] is best comparable in terms of specific age. In this study, 11 anthropometric variables were measured in a specimen of 1,750 female subjects aged 6–10, and demonstrated that there were only two morphological dimensions both in boys and girls: dimensionality of the skeleton, voluminosity of the body with subcutaneous fat tissue. Isolated dimensions were similar in both sexes, although the structure obtained was much better expressed in boys.

The general developmental tendencies reflect on all other body subsystems, which are inter-related and require a multisegment and multidisciplinary approach whenever possible (Katić et al., 1994; Katić, 2003; Katić et al., 2004)[4–6]. Developmental processes lead to the formation of a general morphological factor defined as ectomesomorph and two general mechanisms responsible for motor efficiency in the form of strength regulation and speed regulation[6].

There are studies in the area of anthropometric characteristics and development of certain deformities, in which the relations between the growth and development of the spine and its deformities, mostly scoliosis, were assessed. Lončar-Dušek et al.[7] observed subjects

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over a 3-year period in their longitudinal study including 698 children aged 9–12, measuring their body height and recording the development of secondary sex characteristics every six months. The data obtained showed faster growth and secondary sex characteristic development in children with idiopathic scoliosis. Furthermore, in the second part of the study[8], an important association was established between the development of scoliosis in puberty and growth, recorded in 8.9% of the study subjects, i.e. in those that developed scoliosis during puberty, while initially exhibiting normal body posture. Other researchers report on a connection between the evolution of idiopathic scoliosis and spinal growth, i.e. the process of growth[9].

The specificity of the age of the study subjects, 7–9 years, was not taken into consideration in the area of morphologic characteristics and their relation to some body posture indicators. Therefore, it is important to start with some studies that are not directly connected with this paper, in order to establish the basic morphological characteristics and relations to body posture in a specimen of this age. Children aged 7–9 are in an important and very specific period of growth and development. Any abnormal external influence may impair proper growth and development of a child and prevent the child from growing and developing properly later in life[10]. It is therefore important to determine all possible relations mentioned above to be able to act quickly and appropriately to prevent possible deviations.

The aim of this study was to determine the association between the indicators of body posture and latent structure of morphologic space in children aged 7 (first grade of primary school) and 8 (second grade of primary school).

Material and Methods

Sample

Study sample included 110 boys and 114 girls, pupils of three Split primary schools (Pojišan, Dobri and Skalice). The longitudinal study was carried out at two points: at age 7 years (±3 months) and at age 8 years (±3 months). Inclusion criteria were freedom from malformation and regular class attendance.

Variables

A set of 17 morphological variables were chosen, according to the International Biological Protocol (IBP) standards. All measures were taken on the left side of the body. The latent morphological structure with four different latent dimensions was taken from a previous study[1,2]: longitudinal dimensionality, transverse dimensionality, voluminosity and body weight, and subcutaneous fat tissue; body height (BH), sitting height (SH), leg length (LL), hand length (HL), knee diameter (KD), hand diameter (HD), bicristal diameter (BCD), bisacromial diameter (BAD), chest diameter (CD), chest depth (CDE), body weight (BW), chest circumference (CC), forearm circumference (FC), calf circumference (CAC), subscapular skinfold (SS), abdominal skinfold (AS), and triceps skinfold (TS). All measures were taken three times on the left side of the body. Final results were obtained by Burt method of summation.

A set of 12 body posture indicators were used. Eleven parameters were obtained by the method of symmetric body parts using a scoliosometer[11–13] (a measuring instrument with a plexiglass board with a centimeter grid). The indicators of foot status were estimated by the four-grade plantography method (0, normal foot; 1, pes valgus; 2, pes planovalgus; and 3, pes planus).

Body posture indicators were: SCDIFF1 – difference in distance from the scapular upper edge to the spine (cm); SCALT1 – difference between the scapular upper edge highs (cm); SCDIFF2 – difference in distance from the scapular lower edge to the spine (cm); SCALT2 – difference between the scapular lower edge highs (cm); SHOALT – difference between shoulder highs (cm); PAPDIF – difference in distance between the nipples (cm); PAPALT – difference between nipple highs (cm); SIDIFF – difference in crista suprailiaca superior anterior (cm); SIALT – difference between highs of crista suprailiaca superior anterior (cm); FOST – foot status; PECEXC – pectus excavatum (0 – normal, 1 – deformity 1°, 2 – deformity 2°); and PECCAR – pectus carinatum (0 – normal, 1 – deformity 1°, 2 – deformity 2°).

Data processing methods

Latent structure of the morphological variables and thorax body posture indicators (9 variables) were calculated by factor analysis, with the principle component method and varimax rotation. For the extraction criterion of significant components, Guttman-Kaiser criterion[14] was chosen: factor correlations, λ – eigenvalues.

Determinants of relations between predictive variables, morphological latent structure and criterion variables, latent structure of thorax body posture indicators with two posture indicators of the chest and one of the foot status were calculated by canonical correlation analysis: correlations with canonical roots, Can R – canonical correlation, Can R^2 – canonical determination, χ^2 – Chi square, df – degrees of freedom.

Results and Discussion

General examination of body posture in first-grade children showed incorrect thorax posture in 41.1% and incorrect foot status in 47.3% (Figure 1). On second examination (second grade), posture examination revealed an increase in the percentage of incorrect thorax posture (42.2%) and incorrect foot status (60.7%) (Figure 1). Ostojić et al.[15] report on incorrect thorax posture in 28.3% of first-graders and 28.9% of third-graders. Thus, both this and the present study showed the rate of incorrect posture to be on an increase.

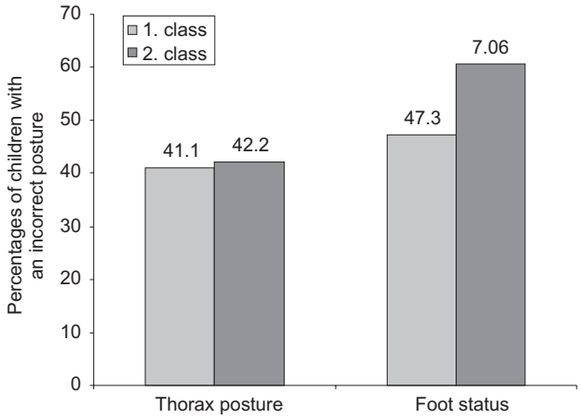

Fig. 1. Prevalence of incorrect thorax posture and foot status in first- and second-graders.

The latent structure of the morphological space in children aged 7 (first-graders) and 8 (second-graders) is explained by three significant latent dimensions.

The significant sex differences in anthropometric characteristics pointed to the need to separately estimate the latent structure of anthropometric variables. By use of factor analysis, Guttman-Kesier criterion of extraction of different components and Varimax normalized rotation, three important factors were distinguished in girls and boys. An almost identical latent structure of anthropometric space of 17 variables was obtained in all measurements. The factors obtained were named as follows (Table 1): voluminosity, mass and subcutaneous fat tissue (VSFT); longitudinal dimensionality (LD); and transverse dimensionality (TD). The factor of voluminosity, mass and subcutaneous fat tissue became apparent as most important in both sexes, and most variables were associated with this factor (8). Four parameters were connected with the second factor of longitudinal dimensionality, and three to five parameters with the third factor of transverse dimensionality.

The latent structure of anthropometric variables in girls and boys aged 7 was defined by two factors (Table 1): voluminosity, mass and subcutaneous fat tissue (VSFT), longitudinal dimensionality (LD). In girls aged 8 was defined by three factors (Table 1): voluminosity, mass and subcutaneous fat tissue (VSFT), longitudinal dimensionality (LD), and transverse dimensionality (TD). The latent structure of anthropometric variables in boys aged 8 was well defined by three factors but in different order of significance: longitudinal dimensionality, voluminosity, mass and subcutaneous fat tissue, and transverse dimensionality.

The estimate of the latent structure of body posture indicators pointed to a conclusion that body posture as the main study objective could be divided into two components (Table 2). It was decided to measure the body

TABLE 1
LATENT STRUCTURE OF MORPHOLOGICAL VARIABLES (F)

	Girls 7 years			Girls 8 years			Boys 7 years		Boys 8 years		
	F1	F2	F3	F1	F2	F3	F1	F2	F1	F2	F3
BH	0.17	0.92	0.05	0.25	0.93	0.07	0.60	0.76	0.88	0.22	0.30
SH	0.37	0.77	–0.21	0.28	0.80	0.22	0.48	0.65	0.84	0.22	0.12
LL	0.59	0.58	–0.22	0.39	0.79	0.00	0.55	0.61	0.70	0.33	0.40
HL	–0.45	0.72	0.11	–0.14	0.70	–0.10	0.41	0.57	0.85	0.09	0.03
KD	0.19	0.00	0.84	0.73	0.27	0.19	0.69	0.39	0.55	0.66	–0.09
HD	0.62	0.56	0.28	0.67	0.42	–0.14	0.55	0.39	0.66	0.46	0.07
BCD	0.33	0.24	0.81	0.60	0.35	–0.66	0.71	0.55	0.22	0.57	0.76
BAD	0.07	0.39	–0.76	0.13	0.27	0.91	–0.29	0.75	0.17	0.08	0.95
CDE	0.83	0.10	–0.08	0.78	–0.04	–0.16	0.69	0.21	0.53	0.55	0.01
CD	0.43	0.28	0.42	0.75	0.27	–0.48	0.70	0.29	–0.17	0.17	0.83
BW	0.44	0.52	0.09	0.83	0.44	0.11	0.78	0.53	0.69	0.70	0.17
CC	0.66	0.17	0.30	0.92	0.23	–0.06	0.84	0.32	0.69	0.56	0.28
FC	0.75	0.34	0.04	0.76	0.17	0.06	0.80	0.18	0.65	0.68	0.13
CAC	0.24	0.60	0.13	0.74	0.45	0.07	0.81	0.35	0.44	0.77	0.10
SS	0.83	0.11	0.16	0.86	–0.04	0.07	0.95	0.08	0.55	0.71	0.05
AS	0.80	0.06	0.08	0.88	0.07	–0.03	0.92	0.16	0.49	0.74	0.18
TS	0.81	0.11	0.29	0.69	0.19	–0.20	0.80	–0.10	0.45	0.78	0.04
λ	5.35	3.71	1.85	7.49	3.65	1.70	8.00	3.51	6.13	5.04	2.62

BH – body height, SH – sitting height, LL – leg length, HL – arm length, KD – knee diameter, HD – hand diameter, BCD – bicristal diameter, BAD – bisacromial diameter, CDE – chest depth, CD – chest diameter, BW – body weight, CC – chest circumference, FC – forearm circumference, CAC – calf circumference, SS – subscapular skinfold, AS – abdominal skinfold, TS – triceps skinfold, λ – eigenvalues

TABLE 2
LATENT STRUCTURE OF BODY POSTURE INDICATORS (F)

	Girls 7 years		Girls 8 years		Boys 7 years		Boys 8 years	
	F 1	F 2	F 1	F 2	F 1	F 2	F 1	F 2
SCDIFF1	0.87	0.11	0.88	0.08	0.88	0.07	0.87	0.05
SCALT1	0.89	0.22	0.89	0.27	0.89	0.28	0.89	0.26
SCDIFF2	0.93	0.09	0.89	0.03	0.91	0.03	0.93	0.02
SCALT2	0.86	0.38	0.88	0.27	0.85	0.36	0.86	0.37
SHOALT	0.62	0.29	0.63	0.24	0.71	0.36	0.62	0.37
PAPDIF	0.04	0.79	0.14	0.72	0.05	0.78	0.03	0.79
PAPALT	0.13	0.73	0.25	0.60	0.10	0.72	0.11	0.73
SIDIFF	0.22	0.70	0.02	0.76	0.25	0.69	0.12	0.61
SIALT	0.45	0.61	0.54	0.51	0.35	0.61	0.34	0.51
λ	3.77	2.13	4.55	1.31	3.55	2.01	3.46	2.11

SCDIFF1 – difference between distance from scapular upper edge to the spine (cm), SCALT1 – difference between highs of scapular upper edge (cm), SCDIFF2 – difference between distance from scapular lower edge to spine (cm), SCALT2 – difference between highs of scapular lower edge (cm), SHOALT – difference between highs of shoulders (cm), PAPDIF – difference between distance of nipples (cm), PAPALT – difference between highs of nipples (cm), SIDIFF – diference between crista suprailiaca superior anterior (cm), SIALT – difference between highs of crista suprailiaca superior anterior (cm), λ – eigenvalues

posture status of the rear part of the thorax (REARTHORAX) and body posture status of the front part of the thorax (FRONTTHORAX) in separate by use of a scoliosometer. Of the nine segments measured by the scoliosometer, the segments estimating the shoulder--blade and shoulder position were joined into a single component, whereas the segments estimating the papilla and crista suprailiaca anterior position were joined into another component.

The canonical correlative analysis (Table 3) yielded two different significant pairs of canonical roots that explained the connection of the two sets of parameters in first-grade girls, and three important pairs of canonical roots that explained the connection of the two sets of parameters in second-grade girls. In 7-year-old girls, the first pair of canonical roots explained 31% of total variability. In the anthropometric space it was negatively defined by the factor of voluminosity, mass and subcutaneous fat tissue, and by the factor of transverse dimensionality. In the space of body posture parameters, this pair of canonical roots was positively defined by chest deformity (pectus excavatum). Such a structure of the first pair of

TABLE 3
RESULTS OF CANONICAL CORRELATION ANALYSIS IN GIRLS

Variable	Girls 7 years (n=114)		Variable	Girls 8 years (n=114)		
	CAN1	CAN2		CAN1	CAN2	CAN3
VSFT	–0.74	–0.42	VSFT	0.97	–0.06	0.37
LD	–0.29	0.91	LD	–0.13	–0.94	–0.84
TD	–0.60	0.07	TD	0.20	–0.33	0.39
REARTHORAX	–0.09	–0.12	REARTHORAX	0.12	0.09	–0.65
FRONTTHORAX	0.05	0.54	FRONTTHORAX	–0.33	–0.31	0.14
FOST	–0.24	0.94	FOST	0.85	–0.94	–0.33
PECEXC	0.99	–0.06	PECEXC	–0.24	0.41	–0.79
PECCAR	–0.01	0.26	PECCAR	–0.21	0.07	0.15
Can R	0.55[a]	0.36[a]	Can R	0.70[b]	0.36[b]	0.28[a]
Can R^2	0.31[a]	0.13[a]	Can R^2	0.49[b]	0.13[b]	0.08[a]
χ^2	61.8	22.1	χ^2	93.0	23.5	0.08
df	15	8	df	15	8	3

[a]$p<0.05$, [b]$p<0.01$, CAN – canonical variable, Can R – canonical correlation, Can R^2 – canonical determination, χ^2 – Chi square, df – degree of freedom, VSFT – voluminosity, mass and subcutaneous fat tissue, LD – longitudinal dimensionality, TD – transverse dimensionality, REARTHORAX – status of body posture of the rear part of the thorax, FRONTTHORAX – status of body posture of the front part of the thorax, FOST – foot status, PECEXC – pectus excavatum (0 – normal, 1 – deformity 1°, 2 – deformity 2°), PECCAR – pectus carinatum (0 – normal, 1 – deformity 1°, 2 – deformity 2°)

canonical roots indicated that chest deformity (pectus excavatum) was present in children who had a less pronounced transverse and voluminous-fat component. The second pair of canonical roots (13% of total variability) could explain the association of longitudinal dimensionality mostly with flat feet, and then with the factor of body posture of the front part of the thorax. The presence of flat feet was explained by this pair, and asymmetry of the body posture parameters of the front thorax part by the emphasized longitudinal component.

In 8-year-old girls, the first important pair of canonical roots explained 49% of total variability. It could explain the connection of the voluminous-fat component with foot status. Their relation was expressed by positive sign and very high correlations. Accordingly, this pair of canonical roots showed that there was a significant association with a higher flat foot severity in children that had a greater voluminous-fat component. The second pair of canonical roots explained 13% of total variability. In this pair there was a high negative correlation with the longitudinal component and parallel correlation with foot status. The parallel connection of these two parameters was obtained in first-graders of both sexes. There was a significant connection between body height and the length of the levers with a higher flat foot grade. The third pair of canonical roots explained 8% of total variability and showed connection of a higher transverse component with a lower rate of pectus excavatum deformity and asymmetry of body posture parameters of the rear thorax part. This connection was also recorded in first-graders.

Canonical correlative analysis (Table 4) yielded two important pairs of canonical roots that explained the connection of the two sets of parameters in first-grade and second-grade boys.

In 7-year-old boys, the first pair of canonical roots explained the connection of longitudinal and transverse dimensionality factors with most of all chest deformities (pectus excavatum), body posture factor of the rear thorax part, and foot status. This pair explained 40% of total variability. This pair explained a similar connection as in girls. In boys who had a pronounced longitudinal and transverse component, chest deformities were less present (negative correlation of the parameter with this root), therefore there was a higher rate of foot deformities in the children thus morphologically defined. The second pair of canonical roots explained 27% of total variability and connected the factors of dimensionality with the body posture factor of the front thorax part and foot status. In this pair of canonical roots, there was positive connection of body dimensionality with a higher rate of flat foot as well. This pair of canonical roots explained the positive connection of dimensionality factors with more emphasized asymmetry of the body posture parameters of the front thorax part.

In boys, the connection of longitudinal dimensionality with foot status was best explained (it was present in both pairs of canonical roots). This connection was positively defined, which could be explained by the greater longitudinal dimensionality of the skeleton being connected with a higher rate of flat foot in boys, which was also observed in first-grade girls. The second important connection that was observed in both sexes was the connection of the less expressed transverse component with the presence of pectus excavatum deformity. One of the important parameters explaining the transverse component was the width of the chest cavity, which influenced

TABLE 4
RESULTS OF CANONICAL CORRELATION ANALYSIS IN BOYS

	Boys 7 years (n=110)			Boys 8 years (n=110)	
Variable	CAN1	CAN2	Variable	CAN1	CAN2
VSFT	0.64	0.70	LD	0.85	–0.38
LD	0.21	0.23	VSFT	0.14	–0.52
			TD	–0.51	–0.77
REARTHORAX	–0.52	0.18	REARTHORAX	0.09	–0.44
FRONTTHORAX	–0.16	0.65	FRONTTHORAX	–0.10	–0.57
FOST	0.57	0.48	FOST	0.57	–0.75
PECEXC	–0.72	–0.22	PECEXC	–0.40	0.30
PECCAR	–0.02	0.20	PECCAR	–0.19	0.42
Can R	0.63[a]	0.52[a]	Can R	0.46[b]	0.36[a]
Can R^2	0.40[a]	0.27[a]	Can R^2	0.21[b]	0.13[a]
χ^2	89.8	36.1	χ^2	15.5	10.8
df	15	8	df	15	8

[a]$p<0.05$, [b]$p<0.01$, CAN – canonical variable, Can R – canonical correlation, Can R^2 – canonical determination, χ^2 – Chi square, df – degree of freedom, VSFT – voluminosity, mass and subcutaneous fat tissue, LD – longitudinal dimensionality, TD – transverse dimensionality, REARTHORAX – status of body posture of the rear part of the thorax, FRONTTHORAX – status of body posture of the front part of the thorax, FOST – foot status, PECEXC – pectus excavatum (0 – normal, 1 – deformity 1°, 2 – deformity 2°), PECCAR – pectus carinatum (0 – normal, 1 – deformity 1°, 2 – deformity 2°)

this connection. The depth of the chest cavity was mostly related to the factor of voluminosity, mass and subcutaneous fat tissue. In girls, this factor was closely connected with the deformity of pectus excavatum. Lower values of the chest cavity depth explained the higher rate of pectus excavatum, as indicated by the signs observed. Two significant pairs of canonical roots were obtained 8-year-old boys, together explaining 34% of total variability. The structure of these canonical roots was more vaguely defined. The first pair of canonical roots (21% of total variability) explained the parallel connection of the voluminous-fat component with the pectus excavatum deformity. The second pair of canonical roots ($CanR^2$= 13%) explained the connection of the dimensionality factor with foot status and asymmetry of the body posture parameters of the front and rear thorax parts. This connection was defined by the same direction, indicating that deformities such as flat foot and asymmetry of body posture parameters would occur at a higher rate in children with a more pronounced body dimensionality.

In second-graders, the structure of the connection obtained in first-graders was confirmed. The most obvious connection was recorded between foot status and voluminous-fat component, and longitudinal component. In both cases the connection was parallel, as expected. The children greater body weight, and thus with greater body height were demonstrated to have a higher rate of flat foot. It is well known that the foot arch structure deteriorates under higher pressure, as in this case. The second important connection, which was not so well explained, was the connection of dimensionality components with the body posture indicators. The children with emphasized longitudinal and transverse component were more prone to weaker postural muscles that are in charge of correct body posture.

The results obtained in this study and those from the studies that served as a hypothesis show that there is a connection between these two sets of parameters. Bižaca and Kučić[16] report on the connection between body weight and flat foot in primary school first- to fourth-graders. Another study[17] performed in a specimen of primary school fifth- and sixth-graders demonstrated the chest deformity (pectus excavatum) to be largely connected with anthropometric characteristics, depth and width of the chest cavity. Lončar-Dušek et al.[7] correlated body growth with a higher rate of spine distortion. All these studies show that there is a connection of anthropometric characteristics with some indicators of body posture; however, none was carried out using the same methodological approach. It could be stated that the connection between body posture parameters and anthropometric latent structure has been successfully explained in primary school first- and second-graders. Rather high canonical coefficients of correlation (0.28 to 0.70) have been reported, suggesting a significant connection between the given parameters.

Conclusion

The aim was to establish the connection of anthropometric measures with the body posture indicators. Two important pairs of canonical roots were obtained on each measurement. Rather high canonical coefficients of correlation show that there are significant relations between anthropometric measures and body posture measures. The most important connection was established between a higher rate of flat foot and pronounced voluminous-fat component and longitudinal component. The foot arch structure is under a great strain in children that have bigger longitudinal and voluminous-fat component, so the arches of their feet deteriorate, entailing a higher rate of flat foot. The second obvious connection, which has been demonstrated in many measurements, was the connection of transverse dimensionality with pectus excavatum chest deformity. The parameter of chest cavity depth is connected with transverse dimensionality. The body posture factors are connected to longitudinal and transverse dimensionality. In children with pronounced body dimensionality factors, greater thorax asymmetries were observed. On all measurements, the voluminosity, mass and subcutaneous fat tissue factor had opposite value in relation to the thorax posture factors. Therefore, asymmetries of the thorax posture parameter were not as observable in children with the pronounced voluminous-fat component.

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ODNOSI LATENTNE STRUKTURE MORFOLOŠKIH ZNAČAJKA I POKAZATELJA TJELESNOG DRŽANJA U DJECE U DOBI OD 7 DO 9 GODINA

S A Ž E T A K

Na uzorku od 110 dječaka i 114 djevojčica u dobi od 7 i 8 godina primijenjeno je 17 morfoloških i 12 mjera za procjenu tjelesnog držanja kako bi se utvrdili odnosi između ovih dvaju nizova pokazatelja. Pokazatelji tjelesnog držanja sastavljeni su od devet pokazatelja držanja trupa, pokazatelja spuštenosti stopala i dva pokazatelja prsnih deformiteta. Latentna struktura morfoloških varijabla u oba spola definirana je trima čimbenicima, ali drukčijim redoslijedom važnosti čimbenika: longitudinalna dimenzionalnost, voluminoznost i potkožno mason tkivo, te transverzalna dimenzionalnost. Latentna struktura pokazatelja tjelesnog držanja trupa je definirana dvama čimbenicima, statusom tjelesnog držanja prednje strane trupa i statusom tjelesnog držanja stražnje strane trupa. Kanoničkom korelacijskom analizom između latentnih morfoloških varijabli kao prediktorskog skupa i kriterijskog skupa od: dvije latentne varijable tjelesnog držanja trupa, dviju varijabli prsnih deformiteta, i varijable spuštenosti stopala, dobivena su po dva para značajnih kanoničkih korijena u svakom mjerenju, te je glavni zaključak kako postoji statistički značajna povezanost između ovih dvaju nizova pokazatelja.

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The »Harsh Inhabitants of Hvar« in the Speech of Vinko Pribojević (A. D. 1525)

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A B S T R A C T

The aim of this paper is an analysis of the genesis and representation of local group identities of the island of Hvar, based on the information from the oldest historiographic and literary source dating to the 16th century. The speech by the Croatian Dominican monk Vinko Pribojević, entitled »De origine successibusque Slavorum« (»On the Origin and Glory of Slavs«), was held in the Latin language in the town of Hvar on the island of Hvar in 1525. It was published in 1532 in Venice, and represents one of the most famous works of Croatian literature in Latin language. The speech consists of three parts. Most locally specific information on Hvar and its inhabitants can be found in the last part of the speech. The island itself is divided into three geographical parts in Pribojević's speech (the eastern part, consisting of the high plain plateau, the western part, consisting of the Hvar plain, and the town of Hvar). The division of the island's inhabitants corresponds to this division. In the description of the islanders and their supposed characteristics we can recognize many of the stereotypes still ascribed to many inhabitants of islands even today. People from the eastern part of the island, mostly shepherds that came to the island as refuges before the Turkish army, are referred to as »the others«. They are described in quite negative context and stereotyped as being different from the rest of the island's population. In contrast, the inhabitants of the western and central part of the island are presented as ideal, homogenous, and harmonious community. Shepherds from the mountainous area in the east part of the island have been excluded from the collective representation of idealized indigenous population, the Mediterraneans that lived in the western part of Hvar. In Pribojević's speech we find the oldest form of the stereotype on the island's highlanders, the ever present »others« and »different« people of the island of Hvar, a view still present to this day.

Key words: *Vinko Pribojević, island of Hvar, Humanism, stereotypes, local communities, island highlanders*

Introduction

The island of Hvar is one of the most explored Croatian islands. Holistic anthropological research on the island of Hvar has been conducted since 1971[1]. During the past 35 years, detailed characterizations of migration, demography, isonymy, linguistic differences, anthropometric traits, physiological properties, quantitative and qualitative dermatoglyphic traits, radiogrammetric metacarpal bone dimensions and genetic traits were performed. Through the connection of cultural and bio-anthropological data with a holistic analytical methodology, we get more complete insight into the processes that formed the population structure of the present-day inhabitants of this Middle Dalmatian island. Migratory processes resulted in interaction of various populations on the island of Hvar, which influenced the construction and representation of the different island's group identities (stereotyping, emphasis of the exotic, allegorization). Literature and historiography are important for the study of images and stereotypes that are strongly connected with the formation of autoimages (images and presentation of »our space« and »our group«) and heteroimages (images of »others« and »their spaces«) of a particular group identity. The aim of this paper is to analyze the construction and representation of local group identities on the island of Hvar presented in the oldest literary and historiographic work from the 16th century.

Vinko Pribojević (Vincentus Priboevius)

One of the best known works of Croatian Latin literature, »*De origine successibusque Slavorum*« (»*On the Or-*

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igin and Glory of the Slavs«), was a speech given in Latin by the learned Dominican monk Vinko Pribojević in 1525 in Hvar, and printed in 1532 in Venice. This oration by Pribojević reflected humanist-intellectual trends, drawing its inspiration from the writers of classical antiquity, but also from the works of contemporaries (such as Juraj Šižgorić, »*De situ Illyriae et civitate Sibenici*« /*On the Position of Illyria and the Town of Šibenik*/ in 1487), and became a model and inspiration to later generations of writers and historians (Petar Zoranić, Ivan Gundulić, Mavro Orbini, Juraj Križanić, and others). With this speech, Pribojević passed beyond the limited ideological framework of the medieval communes, introducing pan-Slavic idea so as to grant a historical legitimacy to his native Hvar, Dalmatia, and the Slavs in general, interweaving historical and literary elements.

What little that is known about Pribojević's life is based more on hypotheses and considerably less on documented historical facts. The only autobiographical details about this humanist from Hvar are contained in his famous speech. His date of birth is unknown, as is his place of birth. Historians have found evidence in archival sources that the Pribojević family came from the central part of the island of Hvar, and two settlements from the plain of Hvar, Vrboska and Vrbanj, are noted (however, according to Niko Duboković Nadalini, Vrboska first arose as a settlement as late as the 15th century and was founded by settlers from inland Vrbanj). Data cited in support of Vrboska would be »the mention of his relative Petar (*consobrinus* = son of his maternal aunt), a hero in battles with pirates off the African coast, who sailed all the way to England. Pribojević spoke of him under the Latinized name »Blaseus« or »Blasius«. On Hvar, however, there are no old surnames derived from the name »Blaž« [Blaise], except Blašković, which is noted from the 15th century in Vrboska.«[2] Additionally, those bearing the surname Blašković »together with the surnames Pribojević and Stipišić, are mentioned among the earliest inhabitants of Vrboska«[3]. His basic humanistic education has also been suggested to have occurred at several possible institutions – the Dominican monastery in Hvar[4], or the educational institution of the Dominican Order in Zadar, the Universitas Jadertina[5]. Father Vicko of Hvar »in 1511 became a master of students in the Dominican monastery of Santa Maria Novella in Florence, and in August of the same year a lecturer of the Holy Testament (*biblicus*) in the same monastery, and ten years later, in May of 1521, he was delegated to solve some dispute related to the prior of the Dominican monastery in Senj«[2]. From the speech, we find that he had a brother Jerolim, also a Dominican, and that he dwelled in Poland for three years, where he came across the pan-Slavic ideas that were to become the theme of his speech in Hvar.

Given such a series of similar surmises about details of his life and work, perhaps it is both best and most secure to be content with how he introduced himself in his work, as »*Father Vinko Pribojević of Hvar, a Dalmatian, a professor of holy theology, a Dominican monk*«[6]. Pribojević first defined himself in local terms: »Hvaranin« – an inhabitant of the Hvar commune and the island of Hvar, then regionally: »Dalmatinac« – an inhabitant of Dalmatia, whose entire coastal region was under the control of the Republic of Venice in that time.

The Oration in Hvar

The speech »*De origine successibusque Slavorum*« (»*On the Origin and Glory of the Slavs*«) is divided into three parts. The title in fact only corresponds to the first section, which glorifies the Slavs and Slavism, the first time the pan-Slavic idea had been put forwards in Croatian history and literature. The second section discusses Dalmatia and the Dalmatians, where for the first time was presented »the history of Dalmatia from the earliest times, on the basis of sources«[7]. The last section of the speech was the most locally specific, as it was devoted to Hvar, both the town and the island. The Hvar segment, like the first two, is also unique, as it represents the only description of Hvar prior to the Turkish attack in 1571, with its looting and burning in which most of the original structures were destroyed, along with all the data preserved in the archives of Hvar and the library of the Dominican monastery. A similar tripartite structure can be found in another famous Croatian Latin text, »*De situ Illyriae et civitate Sibenici*« (»*On the Position of Illyria and the Town of Šibenik*«) (1487) by Juraj Šižgorić, which Pribojević evidently knew and took as a model. If we compare the thematic sections, a similarity can be seen »in the choice and arrangement of material:

Šižgorić: Illyria – Dalmatia – Šibenik and its vicinity,

Pribojević: Slavism – Dalmatia – Hvar and its vicinity«[8].

Slavism and the Slavs, which were announced as themes, were addressed by Pribojević in the smaller and undependable (because of overemphasized and distorted information) part of his speech, while he described Dalmatia and Hvar in more detail. »Of the 55 pages of Pribojević's Latin text in the Yugoslavian Academy edition only 23 refer to the subject as cited in the title, while 32 pages were devoted to the second and third sections, i.e. Dalmatia 14 and Hvar 18 pages«[9]. For Pribojević history was continuity, a duration, in which he followed the fundamental traits of European humanism. He presented a historical synthesis that through the past confirmed the present of his island, describing its genealogy from mythic ages, firmly placing it in space and time through emphasis of its lengthy continuity. As each family tree is recited before the members and on the property of the community to which the individual belongs, hence confirming his or her place within it, so did Pribojević, of Hvar, a Dalmatian, professor of holy theology and a Dominican priest, give his speech in Hvar and in front of the inhabitants of Hvar. The speech was in Latin, the international language of humanistically educated people of that time, and hence it was not intended for all Hvar residents, just those who shared the same classical education as the speaker – the educated citizens and nobles. It was also intended for those who did not belong to the Slavic people, who had no place in the genealogy, but

governed Hvar and Dalmatia, the Venetians and all non-Slavs that were present in the town or that were later to read the speech.

The most homegrown part of the speech was the last bit dedicated to the island of Hvar and its inhabitants. It began with the geographic location of the island, with Pribojević noting »like in a modern tourist guide, its exact dimensions, just expressed in ancient stadia«[9], and »describing his island in the terminology of the utopian literature of the time and geographic-travelogue tracts.«[10]. Pribojević described his home island from the east (the part closest to the mainland) to the west. Hvar was divided in the speech into three geographic regions:

1. The side of the island facing the mainland with 5 settlements on the highlands.
2. The plain that extends in the center of the island towards the north surrounded by 11 villages.
3. The southern coast of the island where the town of Hvar is located.

If we compare Pribojević's division to that of Ivo Rubić from the 20th century[11], adding only the names of towns and settlements that the famous Dominican monk did not cite in his speech, it is apparent that after half a millennium the island of Hvar is still described geographically in the same manner.

1. The western part of the island – the settlements from Stari Grad to Jelsa.
2. The southern foothills of the island – two settlements: Sveta Nedjelja and the town of Hvar.
3. The eastern part – Plame, the area southeast of Jelsa.

All of these sections of the island of Hvar were accompanied by Pribojević's descriptions of their inhabitants and their occupations. Pribojević declared himself a citizen of Hvar, a member of the community of the island of Hvar, despite the fact that he was highly educated in comparison to the rest of the population of the island, and in his description we can perceive local stereotypes related to individual inhabitants of the settlements of Hvar that he probably shared with the community to which he belonged. Accompanying the humanistic tone used to describe all the classes and residents of the various parts of the island of Hvar, it is possible to perceive from the text specific local animosities related to certain residents of Hvar. The division of the population in the speech corresponds to the geographical division of the island (Table I).

It is possible to note in Pribojević's description of the inhabitants of Hvar and the traits attributed to them and the manner in which they are discussed certain divisions that correspond to some widespread stereotypes even today present on the island.

The inhabitants of the eastern part of the island, shepherds, were described with a comparative adjective: *harsher than the other inhabitants of the island of Hvar* (pp. 199), with which they were placed in opposition to all the inhabitants of the island, clearly marked as the island »others«. They were reduced to only one, fairly negative characteristic – harsh, while the other island inhabitants were described with a series of positive and panegyric epithets (Table 2).

A comparison of the pronouns used in the descriptions of individual Hvar inhabitants is interesting.

TABLE 1
PRIBOJEVIĆ'S DIVISION OF THE ISLAND OF HVAR AND ITS INHABITANTS*

Eastern part of the island (Plame)	»*Now in these flatlands there are five villages, mostly settled by* ***shepherds, who are harsher than the other inhabitants of the island of Hvar.*** *Nonetheless, some of them cultivate fields for grain (as that part of the island is quite suitable for growing grain) and have vineyards.*« (p 198–199)[6]
Western part of the island (Plain of Hvar)	»*These are mountainous areas settled by numerous inhabitants,* ***who are endowed with physical strength and a lively spirit,*** *and* ***due to some inborn virtue they are sober****, and avoid excessive enjoyment of wine. Although there is a great abundance of wine on Hvar (the inhabitants truly do not even drink a tenth of their wine), they nonetheless consider it a great evil, and even a crime to drink wine undiluted with water. Hence, if a newcomer appears drunk on the street, everyone, and particularly the children (as you yourselves well know), insult him as a lunatic, making fun of him and laughing at him.*« (pp. 199)[6] »A true proof of the ***generosity*** *and* ***affluence*** *of the* ***village inhabitants*** *of this area is offered by the Monastery of St. Peter the Martyr…*« (pp. 200)[6] »*... as almost the entire plain is yours and they are only laborers*« (pp. 200)[6] »*... these areas are visited by many traders from all over the world to buy fish. In traffic with them,* ***our countrymen*** *have acquired not only abundant material goods, but also a* ***certain quick-witted guile****.*« (pp. 200)[6] »*This world is quite* ***hard-working****, despising slackness and laziness.*« (pp. 201)[6]
Town of Hvar	»*... the* ***refinement and good manners of the citizens of Hvar*** *because of the frequent contacts with people of various nationalities that arrive by boat in this town.*« (pp. 205)[6]

*The citations from Pribojević's speech are taken from Vinko Pribojević: »O podrijetlu i zgodama Slavena« (introduction and notes by Grga Novak, translation from Latin and name index by Veljko Gortan), JAZU, Zagreb, 1951.

TABLE 2
FEATURES OF CHARACTER ACCORDING TO GEOGRAPHICAL DISTRIBUTION IN PRIBOJEVIĆ'S SPEECH

INHABITANTS OF HVAR			
Eastern section (Plame)	Central Plain		Town of Hvar
shepherds	laborers (farmers)	fishermen	nobles, citizens
harsh	physical strength, lively spirits inborn virtue, sobriety generosity, affluence	quick-witted guile hardworking	refinement good manners

TABLE 3
USAGE OF PRONOUNS FOR DEFINING »WE – THEY« RELATION

shepherds	laborers	fishermen	nobles, citizens
»***who*** *are harsher«* (**they**) (pp. 199)[6] »*some of* ***them****«* (pp. 199)[6]	»***those*** *rural inhabitants«* (pp. 200)[6] »***they*** *are only laborers«* (pp. 200)[6]	»***their*** *swift bracera type ships«* (pp. 200)[6] »***our*** *countrymen«* (pp. 200)[6] »***that world****«* (pp. 200)[6]	»*as you well know«* (**you**) (pp. 199)[6] »*transported to this town, in which we live«* (**we**) (pp. 200)[6] »*that plain is* ***yours****«* (pp. 200)[6] »*except the main town where we live«* (**we**) (pp. 201)[6] »*I am not reporting this to* ***you*** *because* ***you*** *would not know that«* (pp. 201)[6]

Pribojević described the inhabitants of Hvar from the point of view of a citizen of the town of Hvar, as can be seen from the quotes »*in this town, in which we live*« (pp. 200)[6], and »*other than the main town where we live*« (pp. 201)[6], written in the first person plural (**we**). All the other islanders are »they« to the inhabitants of the town, with the exception of the fishermen, who through selling their fish communicate with traders from various parts of the world and who Pribojević called »*our* [*fellow*] *countrymen*« (pp. 200)[6]. The most positive epithets noting ideal physical, moral, and character qualities were used by Pribojević to describe the inhabitants of the central plain of Hvar. If this data is further related to the exaggeration in the number and architectural luxury of the houses in the settlements of the central part of the island[2], we have yet another confirmation that Vinko Pribojević probably came from the central plain of Hvar. Despite the description praising the virtues of these inhabitants, a caution and certain pandering to the citizens of the town of Hvar can also be noted, presumably because of the fact that the flash point of the peasant rebellion on Hvar and its main protagonists were related specifically to this part of the island (Matij Ivanić was born in Vrbanj). The most obvious section featuring this cited diplomatic care and tact is a sentence subsequently added to mitigate the impression left by the previously uttered rhetorical question »*what would the situation be if all the products of that plain were theirs?*« (pp. 200)[6], and it reads: »*as almost the entire plain is yours, and they are only laborers*« (pp. 200)[6]. This returns the original, revolutionary idea and aroused memories of the recent rebellion (directly referred to in one more place in the speech: »*Although in the attempt of individuals to acquire equal parts in the administration of our commune you have often fallen into various conflicts*« – pp. 211[6]) into the fixed and only acceptable framework to the Hvar commune under Venetian rule – they are in fact only laborers who work for you.

The Excluded »Others«

The Hvar commune with all its classes was described as a harmonious, unified, idealistic community. The shepherds and their five villages in the highland were excluded from this collective representation, from its autoperception. They were expelled from the Mediterranean framework of all the other Hvar inhabitants. According to Vladimir Vratović, Mediterranean culture on the eastern part of Adriatic Coast is (rather restrictively) determined by four factors: 1. extensive remains, both material and spiritual, of classical culture; 2. membership in the Catholic, Latin Church; 3. a classical-humanistic education with a broad spectrum of repercussions on the total cultural development; 4. a fertile closeness to Italy with various mutual ties and influences[12]. In the Hvar speech, Pribojević verified the historical and cultural identity of Dalmatia and his home island »along with the part played in the conception of Pribojević's work by contemporary historical circumstances, just such a perceived Mediterraneanism seems to us to be one of the fundamental guidelines of the speech.«[13]. He confirmed the identity of the region and the community with actual, and sometimes invented genealogies, citations, myths, and ideas about the civilized, cultural Mediterranean such as was shared by the Europe of his age.

Along with the Mediterraneanism based on the cited categories of values, in the Hvar speech we can find one more typical Mediterranean note – the earliest stereotypical conception of the shepherd and highlander as representing an eternally »different« culture and civilization of the Mediterranean. They do not enter into the urbanity, the brilliant culture, and ancient heritage of the Mediterranean cities, or into the recognized stratum of peasants-laborers on whose production those same towns depend. Pribojević did not place them directly into any of the glorified genealogies that he cited and verified through this speech: Slavic, Dalmatian, and of Hvar. On the contrary, he explicitly ejected them from the glorious genealogy of Hvar as different from all the other islanders, not citing any objective indicator of the emphasized differences, just the unambiguous characteristic of harshness. Speaking of recognized things before the community to which he belonged, the Dominican monk from Hvar also utilized its »known« collective imagery. As a member of the Hvar community in which and in front of which he was speaking, he knew its autoimages and heteroimages, skillfully including them into the perceptual inventory of humanistic Europe. In this manner, along with idealistic projections of Hvar and its population as cultured, civilized, educated, and hard-working inhabitants of an area settled and coveted even from ancient times, Mediterranean people who shared all the values and ideals of the contemporary cultured world, he also introduced into the speech stereotypes about a foreign, different, and alien Mediterranean and Hvar community.

The shepherds, settlers from the mainland, fleeing from the Turkish invasions, were strangers in the coastal world with their different culture and history. The period of danger from the Turks saw large migrations of populations from the continental hinterlands (of Bosnia, Herzegovina, and Montenegro) towards the Makarska coastline and the nearby islands. Settlers moving from the mainland coast to the Dalmatian islands were fairly numerous in the 13th and 14th centuries, while the flow greatly increased after the fall of Bosnia in 1463, after which Venice permitted the desperate population to seek shelter on the islands, and when Skender-Pasha captured the Krajina region from the Venetians in 1499, the inhabitants of Makarska had already requested permission from the local Venetian government to take refuge on Hvar. Documents and reports of Venetian legal counselors have been preserved about the migrations of new inhabitants and the demographic situation on the island of Hvar during the 16th, 17th, and 18th centuries. From these reports, we find that in 1525 there were 1000 people capable of bearing arms from the total population. In 1553, the syndic for Dalmatia, Giambatista Giustignan, reported that »the island has 7700 inhabitants, with 1400 capable of bearing arms«[4]. According to data from the other central Dalmatian islands, it appears that for each 2500 inhabitants there were 500 men suitable for the army. Hence we can calculate that the number of inhabitants of the island of Hvar increased from 5000 to 7100 in the period from 1525 to 1553[14].

The first migratory waves and settlement of the Christian population forced out by Turkish conquests were provisionally overseen by Venice, while the migrations after the Candian (1645 – 1669) and Morean (1684 – 1699) Wars were legally regulated and better organized. Hvar faced its first refugees from the mainland in Pribojević's time, new inhabitants who had found safety on the island from the Turkish conquests. They settled in the eastern, sparsely occupied, and less attractive part of the island. According to Pribojević's description of the eastern part of Hvar, the settlers occupied an area with remains of ancient monuments, where the land was suitable for cultivation and growing, fertile and offering rewards to all who knew how to exploit it. Some of »those shepherds« evidently knew enough to recognize the productive potential of the place where they had been settled, modifying their customs and adapting their economic lifestyle to their new surroundings (»*Some of them, it is true, cultivate the fields for grain (as that part of the island is quite suitable for raising grain) and have vineyards.*« pp. 199). However, in contrast to the rest of the population of the island, who raised Mediterranean plants such as grapes, figs, and olives, the new arrivals grew a typical continental crop – grain. Despite all their adjustments, the new immigrants were never to be accepted as »real« islanders and Mediterraneans, the indigenous Hvar population was to turn them into their permanent »others«, with whom they would compare themselves, creating boundaries and constructing stereotypes.

The Hvar community determined and defined itself in relation to this other and different community. The Hvar identity was validated spatially (Mediterranean island, sea), and through history and heritage. The new settlers were excluded from all categories, arriving on the island from the mainland, which to the islanders had always represented a place of otherness, as an anonymous collective without a written history and a tangible heritage. Pribojević defined his islanders as an ideal model with all the most desirable traits in comparison to which all the new settlers were reduced to harsh shepherds. In interaction with the immigrants, the Hvar islanders transformed themselves into a unified collective, and the classes integrated themselves into a perfect organism that functioned ideally, advancing the island of sun and security. The subject of the speech can be identified with various groups (nobles, commoners, peasants, fishermen) and identities (Slavic, Dalmatian, Hvarian) through positive stereotypes that confirm the well-established relations and values of its milieu. The Hvar island community established distinguishing frames of reference, *markers* of the group identity. They »belong to two types: the first depends on objective characteristics (attire, speech, religious affiliation, socio-historical differences, and so forth); the other lacks an objective foundation and is based on the desire of the group to be different and to distinguish themselves, a desire that seemingly is present even in relations with neighboring, culturally similar settlements between which numerous and varied contacts usually exist«[15].

Objective distinguishing markers between the Hvar islanders and the new arrivals would be language: Chakavian – Stokavian dialects[16], attire, customs: marriage customs, beliefs[17], occupations (laborers, fishermen – shepherds), oral poetry and prose (heroic decasyllabic epics – lyric songs, ballads, romances). Identity markers without objective foundations that are based on subjective projections and value judgments are stereotypes. These are constructed, standardized, and schematic conceptions, and in descriptions of groups they appear as generalizing attributes and characterizations that are often repeated and vary minimally. Stereotypes take over information from reality, reconstruct and properly place it, serving as a kind of filter for simplification that allow through only unambiguous symbols, annulling every multiple meaning and offering only one possible interpretation. The seductiveness of the stereotype lies in its capability of simplifying the complicated and incomprehensible into the straightforward and understandable. They can be both positive and negative.

Pribojević used positive stereotypes to represent the indigenous Hvar islanders in his speech, utilizing all available historical, linguistic, and cultural knowledge. He applied negative stereotyping to certain immigrants, designating them as harsh. Harshness was a trait that the citizens of Athens and Rome had already applied to barbarians, meaning the foreign and different peoples that they had come across. The Hvar inhabitants did not define themselves according to the Venetians, those who ruled them, rather they attempted to approach as closely as possible the customs and values of the inhabitants of Italy, so that the immigrants in fact represented an ideal opposition group. The older stereotypical image of the »Vlachs« (the name is pejoratively used for inhabitants of the continental hinterland), which the islanders applied, in general to every person from the mainland, now acquired a closer and actual context on the island itself. The indigenous Hvar inhabitants had acquired with these immigrants from the mainland their very own island highlanders. In the time of Pribojević, these new islanders were the first massive mainland refugee settlers that had found security and a new home on their island. Settled in a rocky, climatically unattractive part of the island with limited possibilities for existence, poorly connected with the developed Hvar commune, with its culture and heritage, in fact they represented unknown strangers to be talked about, some strange, foreign, perhaps even unfriendly mainlanders. The Hvar inhabitants from the »golden age« of the island, as yet untouched by the future Turkish attacks, the explosion of the arsenal, and the exterminations of the plague, the major waves of immigrants after the Venetian-Turkish wars, under the protection of the powerful *Serenissima*, fenced off the new island inhabitants, not giving them a name, and not showing any need for their integration or assimilation into island life. Several decades later, in the comedies of Martin Benetović (renaissance comedy writer from Hvar), the »harsh shepherds« acquired a name – Plamjani = those of Plame – and were incorporated into the life of Renaissance Hvar. It is true that they were the subject of caricature and ridicule, but laughter usually embraces what is known, what is no longer foreign, what is close through exposure. In contrast to Pribojević's harsh shepherds settled in the distant and rocky plains of Plame, Benetović's Plamjani are still the »others« of Hvar, but are already considered islanders, clearly separated by sea from the mainland »Vlachs«.

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»SUROVI HVARANI« U GOVORU VINKA PRIBOJEVIĆA (1525. GODINE)

S A Ž E T A K

Cilj ovoga rada je analiza stvaranja i reprezentacije lokalnih grupnih identiteta otoka Hvara iznijetih u najstarijem književnom i historiografskom djelu iz 16. st. Govor hvarskog dominikanca Vinka Pribojevića *De origine succesibusque Slavorum* (*O podrijetlu i zgodama Slavena*) održan 1525. na latinskom jeziku u gradu Hvaru na otoku Hvaru, a 1532. tiskan u Mlecima, jedno je od najpoznatijih djela hrvatske latinističke književnosti. Govor je podijeljen u tri dijela. Najzavičajniji je posljednji dio govora posvećen otoku Hvaru i njegovim stanovnicima. Otok je u Pribojevićevu govoru podijeljen na tri geografska područja (istočni dio otoka – visoravan, zapadni dio otoka – hvarsko polje i grad Hvar) prema kojima je napravljena i podjela hvarskog stanovništva. U opisu Hvarana i odlikama koje su im pripisane, mogu se uočiti podjele koje odgovaraju nekim i danas raširenim otočnim stereotipima. Stanovnici istočnog dijela otoka, pastiri, doseljenici s kopna, pobjegli pred turskim osvajanjima označeni su kao otočni »drugi«. Opisani su negativnim stereotipnim predodžbama kao surovi i drugačiji od ostalih Hvarana. Stanovnici zapadnog i središnjeg dijela otoka predstavljeni su nizom idealističkih odlika kao skladna, jedinstvena zajednica. Pastiri planinskog područja na istoku otoka Hvara, isključeni su iz kolektivne reprezentacije idealiziranih starosjedilaca, Mediteranaca nastanjenih na zapadu otoka. U Pribojevićevu govoru sačuvan je najstariji oblik i danas prisutnog stereotipa o otočnim gorštacima, vječnim »drugim« i »drugačijim« Hvaranima.

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Patterns of Sexual Dimorphism from Birth to Senescence

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A B S T R A C T

Sexual dimorphism is expressed as median of the female values in percent of the median of the male values, of 4 length measurements, 3 circumferences, and 5 measurements of corpulence respectively fat. Data were obtained from a cross-sectional sample of more than 41.000 German subjects, aged from birth to age 62. The pattern of sexual dimorphism is similar in the length measurements. Girls are shorter at birth, but they increase in length at higher rates than boys and even temporarily overgrow the boys up to age 12. Thereafter, males show an obvious growth advantage leading to some 6 to 9% more length in adult males. In contrast, female circumferences are always smaller, from birth to senescence. Though, the differences between the sexes are low in circumferences, up to age 13, sexual dimorphism increases to 17% in the thoracic circumference at adulthood. Sexual dimorphism in weight and BMI is comparably with that in length measurements while subcutaneous fat and total body fat content are always higher in females. The results highlight that sexual dimorphism develops at different pace in the various components of the body and that it associates with a sex specific growth tempo.

Key words: *sexual dimorphism, biological age, growth-age, adulthood*

Introduction

There is only little sexual dimorphism in humans compared to most non human primates, but it exists already at birth. Girls are born with less body length and body weight compared to boys, but newborn girls already have more subcutaneous fat than boys[1–3]. During childhood and adolescence, sexual dimorphism usually results from different growth velocity in both sexes. In comparison to boys, girls on an average grow and develop faster. They follow the typical human growth curve with a higher tempo and finish their length growth earlier. This leads to a first minor female growth advantage in some measurements around the age of 6 or 7 because already at this age girls may be biologically older compared to boys of the same chronological age and thus may undergo their mid-growth spurt earlier. It often leads, depending on the measurement, to an obvious overgrowth in girls between the age of 9 and 14. At this age, girls are biologically advanced by about 2 years, with an early adolescent growth spurt responding to their biological age. After the adolescent growth spurt the growth velocity decelerates and goes to zero in most, but not in all, dimensions. Now males show a clear growth advantage because of their later and longer lasting growth spurt. This leads to the typical morphological differences between adult females and males. The sexual dimorphism is most distinct at young adult age and decreases during later life.

Whereas these facts are well known for growth age from longitudinal studies[4–9], little is known about the sexual dimorphism within one population during the whole course of life. The present paper offers results of a cross-sectional study from birth to age 60. Cross-sectional studies are not able to highlight individual growth velocity, but they are able to present median anthropometric differences between females and males of the same age, and they are able to record yearly growth increments. By this, they may contribute to a better understanding of the correlations between sex-specific different median developmental velocities and sexual dimorphism at different ages.

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Subjects and Methods

Anthropometric data from a well stratified cross-sectional sample of 41.035 German subjects, aged from birth to age 62 and measured at the late 80th of the 20th century were reanalysed for sexual dimorphism. 12 measurements were included into the study according to Martin and Saller[10]: four length measurements (height, trunk length, leg length, total arm length), three circumferences (head circumference, neck circumference, thoracic circumference), and five measurements of corpulence respectively fat (weight, body mass index, triceps skinfold diameter, subscapular skinfold diameter, total body fat content). The total body fat content was calculated from the triceps and the subscapular skinfold diameter using the age-specific equations of Slaughter et al.[11] and Johnsen[12]. Age- and sex-specific medians of the variables investigated were calculated and smoothed by the LMS-method of Cole[13]. A disadvantage of this sophisticated method is the insufficient fitting of the first and the last values of a succession. Therefore, the raw data at birth were used and the curves were cut at age 60. The anthropometric differences between the two sexes were calculated as differences out of the smoothed sex-specific median curves. The proportional sexual dimorphism (PSD) was calculated as the female median in percent of the male median at the same age: $PSD = 100 \times p50_{female} / p50_{male}$.

Results

Sexual dimorphism of length is demonstrated in Figure 1. The medians of female measurements are expressed as percent of male values (PSD) are below 100% at birth in height, trunk length, leg length, and total arm length as well as. Girls are born shorter compared to boys, and they have a shorter trunk and shorter limbs. PSD of all 4 length measurements investigated increases annually up to the adolescent growth spurt. The same medians are reached first in leg length, followed by trunk length and height, while arm length remains shorter in girls compared to boys at all ages. Girls have higher medians in leg length between the ages of 2.5 and 12, in height between the ages of 8.5 and 13, and in trunk length between the ages of 8 and 14.5. Sexual dimorphism is first most pronounced in leg length at the age of 10, followed by arm length and height at 11 and at least in trunk length at the age of 12. Thereafter PSD decreases annually with minimum values at young adulthood, where the sex differences in body lengths are profoundly expressed. Sexual dimorphism gradually disappears again in the elderly.

Figure 2 shows sexual dimorphism in head circumference, neck circumference, and thoracic circumference. In contrast to length measurements, circumference measurements are smaller in females compared to males at all ages, yet the differences are low before the age of 13. Later on and up to age 40, a strong sexual dimorphism develops in neck and thoracic circumference. Women of 40 only reach 86% of the male neck circumference and 84% of the male thoracic circumference. Sexual dimorphism in head circumference however stays relatively small. As in length measurements, PSD increases again at older age, and the differences between the sexes gradually disappear in the elderly.

Sexual dimorphism in measurements of corpulence and body fat is shown in Figure 3. Girls are born with 96% of the male weight and 98% of the male body mass index (BMI). The curves of both variables approach 100% indicating absence of sexual dimorphism at the age of 9. Girls have higher medians of weight compared to boys between the ages of 10 and 13, and higher medians of BMI between the ages of 10 and 17. The lowest percent values are reached at the age of 30. At this age median weight of women is only 78% of that of men, and median

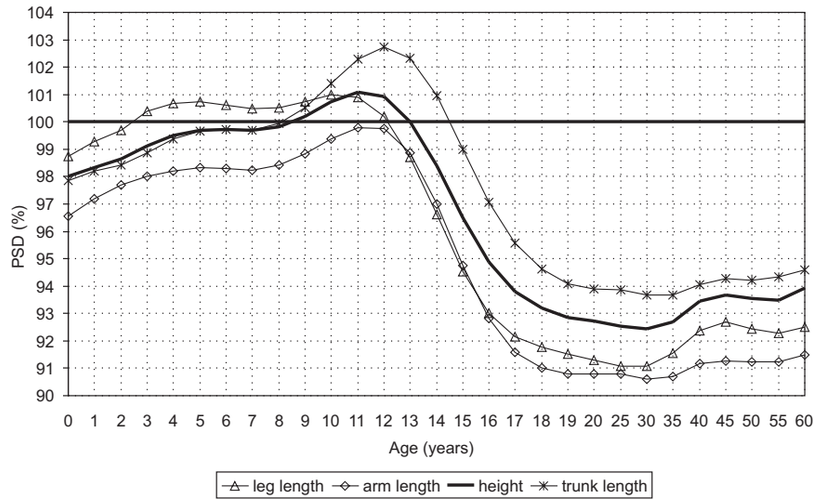

Fig. 1. Sexual dimorphism (PSD) for height, trunk length, leg length, and total arm length calculated as the female median in per cent of the male median at the same age.

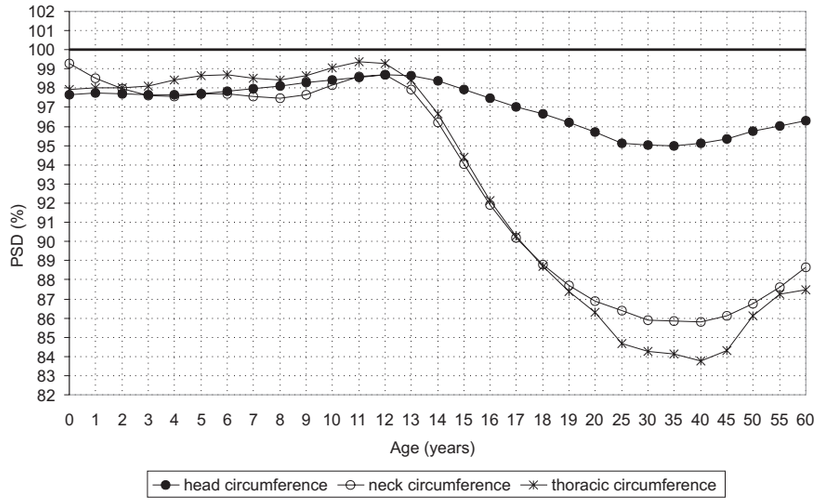

Fig. 2. Sexual dimorphism (PSD) for head circumference, neck circumference, and thoracic circumference calculated as the female median in per cent of the male median at the same age.

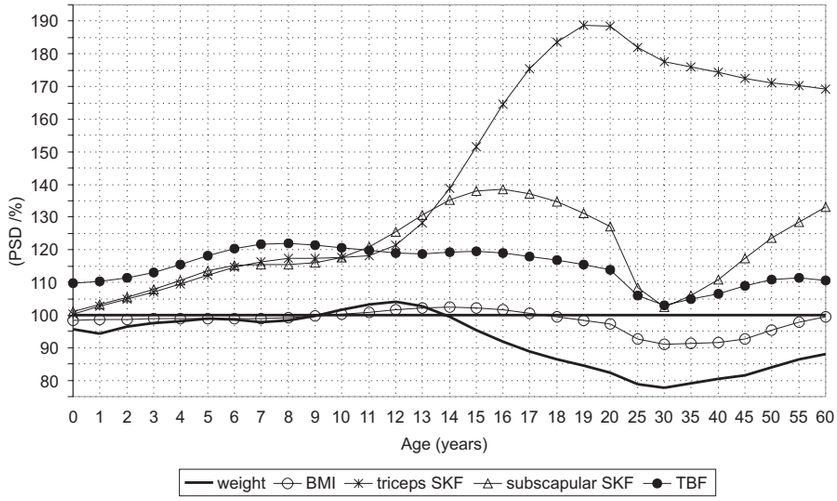

Fig. 3. Sexual dimorphism (PSD) for weight, body mass index (BMI), triceps skinfold diameter (triceps SKF), subscapular skinfold diameter (subscapular SKF) and total body fat content (TBF) calculated as the female median in per cent of the male median at the same age.

BMI is only 91 %. Sexual dimorphism of weight and BMI decreases in the elderly. Body fat differs from all other measurements. Females have more subcutaneous fat and higher total body fat content compared to males at all ages. Sexual dimorphism is more expressed in measurements of body fat than in the other physical signs investigated. High medians of subcutaneous fat are characteristic for the female sex. Young adult women nearly reach 190% of the triceps skinfold diameter of young adult men, and women of 60 still hold 170% of the male median. Compared to the triceps skinfold diameter, subscapular skinfold diameter and percentage of total body fat content do not differ so much between the sexes. Females only have some 10 to 20% more total body fat content than males with a minimum sexual dimorphism at the age of 30. At the same age the sexual dimorphism of the subcutaneous skinfold diameter is also low in contrast to the triceps skinfold diameter.

Discussion

Differences in human body size and body proportions within populations are well known for various age groups, but little is known about the whole age range from birth to senescence. In several animal species, sexual dimorphism may be viewed as a response to selection for fecundity. Guegan et al.[14] evaluated the hypothesis that the ex-

tent of sexual dimorphism in human populations, which is relatively small compared to non human primates, also results from the interaction between fertility and size-related obstetric complications. Observations about a markedly increase of sexual dimorphism of many anthropometric parameters at puberty in association with the development of sexual maturation support this hypothesis. There is a high correlation between sex hormones and the sex-specific development of the physique. Jaffe et al.[15] found already the regulatory mechanisms of growth hormone secretion to be sexually dimorphic. Different approaches have been undertaken to analyse sexual dimorphism. Some more or less sophisticated indices like the index of androgyny were developed to quantify anthropometric differences between the two sexes[10]. Loesch et al.[16] offered a general score between the poles »maleness« and »femaleness«. The present results support the opinion of al-Haboubi et al.[17] that sexual dimorphism develops at different pace in the various parts and compartments of the body and therefore, should be estimated separately for different body measurements. Lovich & Gibbons[18] published a review of techniques for quantifying sexual size dimorphism. They recommend an index based on the mean size of the larger sex divided by the mean size of the smaller sex resulting in positive values when females are larger and negative values in the opposite case. In this paper a similar approach is used. The median of the female sex is expressed as percentage of the median of the male sex.

Adult height is associated with the age at adolescent growth spurt[19]. A late growth spurt means high final height. The age at the onset of the adolescent growth spurt seems to be influenced also by socio-economic conditions in a sex-specific way. Valenzuela[20], Holden & Mace[21] and Spencer[22] found a larger sexual dimorphism in height in poor countries or in populations where women contributed less to food production. This corresponds to the results presented in this paper. The amount of sexual dimorphism in the German population signalises good living conditions. The pronounced sexual dimorphism in circumferences at adulthood is well known. Head circumference is an exception. Here the highest PSD is no more than 95%. Hajnis[23], Henneberke & Prahl-Andersen[24], and Joffe et al.[25] published similar results. They argue that the postnatally growth of the head is completed to about 95% at the age of 5. A small adolescent growth spurt was found only in boys, but not in girls. The higher total body fat content in females at all ages is well known. The results given here highlight especially the PSD of subcutaneous fat at triceps. Webster-Gandy et al.[26] found a significantly larger percentage body fat already in 5 to 7 years old children, but no different fat patterning in the two sexes. This agrees also with the results given above. The large sexual dimorphism in subcutaneous fat distribution with a high degree of fat accumulation at the upper arm at adult age is a result of a larger change in fat distribution in boys compared to girls at puberty, while girls hold better their prepubertal fat pattern. He et al.[27] found similar postpubertal sex-specific changes in fat distribution by age in different populations.

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OBRASCI SPOLNOG DIMORFIZMA OD ROĐENJA DO STAROSTI

S A Ž E T A K

Spolni dimorfizam izražava se u kao medijan vrijednosti za žene u postotku medijana vrijednosti za muškarce, za 4 mjerenja dužine, 3 mjerenja obujma i 5 mjerenja debljine, odnosno masti. Podaci su dobiveni iz presječnog uzorka od više od 41,000 njemačkih subjekata, od rođenja do 62. godine. Obrazac spolnog dimorfizma pokazuje sličnost u dužini mjerenja. Djevojčice su niže kod rođenja, ali rastu u dužinu u većoj mjeri nego dječaci te privremeno prerastaju dječake do 12. godine. Zatim muškarci pokazuju očitu prednost u rastu koja vodi povećanju dužine u odraslih muškaraca od 6 do 9%. Za razliku od toga, ženski obujam se smanjuje od rođenja do starosti. No, razlike među spolovima su smanjene u obujmu do 13. godine, a spolni dimorfizam se povećava do 17% u torakalnom obujmu u odrasloj dobi. Spolni dimorfizam težine i BMI može se usporediti s mjerama dužine, dok su potkožno masno tkivo i sadržaj ukupne tjelesne masnoće uvijek veći kod žena. Rezultati pokazuju da se spolni dimorfizam razvija različitom brzinom u različitim dijelovima tijela te je povezan sa spolno specifičnom brzinom rasta.

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Gonadal Cell Proliferation Dimorphism

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A B S T R A C T

Dimorphism between testis and ovary in germ cells proliferative behavior, shows remarkable differences in foetal and neonatal period [14.5 days post conception (dpc) – 7 days post partum (dpp)]. Immunostaining of the foetal testis, with the PCNA and Ki-67 antibodies [estimation of Labeling Index (LI)], reveals increasing germ cells population until birth. Afterwards, a sharp decline in the first 3 days of postnatal life and a transient increase, between 3 and 5 dpp, is observed. Then, the mitotic activity of germ cells ceases. In the foetal ovary, germ cells proliferation reaches a peak value before birth, decreasing thereafter. Somatic (Sertoli or follicular) cells behave similarly in both sexes. Increased mitotic activity is observed throughout the examined period. Thus, the gonadal dimorphism in proliferative behavior, concerns only germ cell lineage and is established during the foetal and neonatal period.

Key words: *dimorphism, testis, ovary, PCNA, Ki-67, germ cells, Sertoli cells, follicular cells*

Introduction

The establishment of the germ cell line in the embryo involves the segregation of the primordial germ cells (PGC's) from the somatic lineages, migration of PGC's to the gonad ridges, proliferation of PGC's and finally their differentiation within the gonads[1,2].

In the rat testis, at 13 dpc, germ cells become enclosed by somatic (Sertoli) cells in the emerging seminiferous cords[3,4]. Calculation of mitotic figures reveals that, the number of germ cells on 14.5 dpc is $20X10^3$ and increases until 17.5 dpc. At 18.5 dpc, $85X10^3$ germ cells are counted while, at foetal day 20.5, their number peaks reaching a maximum value of $140X10^3$. From postnatal day 1–4 onwards, germ cells differentiate in spermatogonia which remain in a quiescent stage, their cell cycle being blocked in G_1 phase and, their number is reduced at least by half[5,6].

The percentage of proliferating Sertoli cells increases during foetal life to reach a maximum on foetal day 20.5, resulting in a considerable increase in the diameter and length of the seminiferous cords[7,8]. Thereafter, the mitotic activity steadily decreases and a stable population of non-dividing Sertoli cells is established by post-natal day 15–20[9–11].

Counting of mitotic figures in previous studies revealed that, during ovarian morphogenesis, germ cells population varies considerably before and after birth. On foetal day 14.5 germ cells number reaches $11.5X10^3$. Thereafter, it increases sharply and reaches a peak value of $75X10^3$, at 18.5 dpc. At this point, mitotic activity ceases and germ cells enter the prophase of meiosis I, after one last round of DNA replication[12,13]. After birth, germ cells number drops significantly, resulting to a minimum level of $27X10^3$ cells on neonatal day 2[6,13].

According to Brambell, the proliferation of somatic cells continues without interruption, although slowly, in the mouse ovary, throughout the foetal period and after birth, until weaning time or even later. Thus, the number of follicular cells increases with age[14].

Most of the studies performed to date, besides counting of mitotic figures, used also measurement of the incorporation of tritiated thymidine and evaluation of 5-bromo-2'-deoxyuridine (BrdU) incorporation[15]. Now days, the S-phase related proteins, PCNA (Proliferating Cell Nuclear Antigen) and Ki-67 are detected by immunohistochemistry, as markers of cell proliferation, with excellent results, in most cases.

PCNA is an auxiliary protein to DNA polymerase-δ, being involved in nucleotide excision repair mechanisms. With a biological half-life of ~20h, PCNA is expressed in the nuclear matrix of cells during all phases of the cell cycle with a maximum in S- and G_2- phases. The proliferation-associated antigen Ki-67 is expressed in the nuclear

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matrix of cells during G_1-, S-, G_2- and M- phases of the cell cycle with a maximum in G_2- and early M-phases. Its biological half life is ~1 h. The Ki-67 protein is absent in resting cells (G_0-phase of the cell cycle) and in cells during early G_1-phase, nor is it detectable during DNA repair process[16–18].

The aim of the present study is to investigate differences in cell proliferative behavior between male and female gonadal cell populations, by immunohistochemical detection of PCNA and Ki-67 and to compare these with findings from previous studies. An attempt is made to establish a gonadal dimorphism in somatic and germinal element during foetal and neonatal period.

Material and Methods

Animals

Female and male Wistar rats, weighing 250–300g were purchased from Pasteur Institute (Athens, Greece). They were kept at 24°C on a 12L/12D cycle, fed with standard food for laboratory animals and drank tap water.

Five or six females were caged with a male for one night. The estimated time of ovulation and fertilization was 02.00 h and the following day was counted as day 0.5 post-conception (0.5 dpc)[19]. Pregnant rats from gestational days 14.5–21.5 were anaesthesized using Diethylether (Merck) and the foetuses were removed from the uterus. Sexing the embryos and gonadal explantation were done as previously described[4]. Foetal gonads from rats aged 14.5, 18.5, 20.5 days *post conception*, (dpc), were examined. Birth occurred between day 21.5 and 22.5. Testes and ovaries were surgically removed at 1, 3, 5 and 7 days *post partum* (dpp).

Histology and immunohistochemistry

Foetal and neonatal gonads were fixed in 4% paraformaldehyde in phosphate buffer 0.1 M (PB; 14,2 g/L Na_2HPO_4, 12 g/L NaH_2PO_4, pH=7.2) for 1 hr, dehydrated, embedded in paraffin, serially sectioned at 5 μm and mounted on poly-L-lysine-coated slides. Every 5–10 sections, one was stained with hematoxylin and eosin.

For immunohistochemistry, after deparaffinization and rehydration, quenching of the tissue in 1% H_2O_2 (commercial 30%) in methanol, was done in the dark for 15 min. For antigen retrieval, sections were placed in Coplin jars, filled with citrate buffer 10 mM (pH=6.0) and microwaved at 750 W for four 2.5-min periods. Sections were rinsed in tap water and subsequently PBS solution. The tissues were then incubated for 15 min in normal horse serum (NHS; Dako, Denmark) in PBS. Primary antibodies, i.e. Clone PC10 (Dako) and NCL-Ki-67 (Novocastra), diluted 1:200 and 1:100, respectively, in phosphate buffer saline 1 M (PBS; 120 mM/L NaCl, 11.5 mM/L NaH_2PO_4, 31,3 mM/L KH_2PO_4, pH=7.4) were applied on sections for a period of 1 hr, at 37 °C. After thorough rinsing with PBS, secondary biotinylated antibody diluted 1:100 in PBS was applied, for a period of 30 min at 37 °C, to all sections. The sections were then rinsed in PBS. Streptavidin-biotin peroxidase complex was applied to all sections, for 10 min at room temperature. Following rinses with PBS, sections were initially incubated for 1 min with a 0.1% Triton solution and then a solution of 3, 3' diaminobenzidine tetrahydrochloride (DAB) diluted in distilled water containing H_2O_2, was applied to all sections for 5–10 min, at room temperature. All sections were rinsed with tap water and counterstained for 45 sec in hematoxylin. Sections were then dehydrated in graded ethanols, dipped in xylene, mounted with DPX and coverslipped.

Image analysis

For the estimation of the Labeling Index (LI = percentage of labeled / total germ or somatic cells), i.e. the proliferation index (PI) in all age groups, 10 visual fields were examined with an average of 1000 cells. The processing of data was implemented with the aid of Image Pro Plus software and Zeiss Axiolab microscope.

Results

In rat testis, the ratio of reactive cells (both germ and Sertoli) to the PCNA and Ki-67 markers of cell proliferation is analytically presented in Tables 1 and 2, respectively.

Before birth, using the PCNA antibody, the percentage of labeled / total germ cells (Labeling Index, LI) increases from 29.68 at 14.5 dpc to 80.26 at 18.5 dpc. It then decreases to 73.93% at 20.5 dpc. After E20.5, the mitotic activity of germ cells resumes, resulting to a significant increase of the labeling index, at birth (peak value=88.06%). Immediately after birth, the LI drops, reaching 51.19% at 3 dpp. Two days later, a slight increase is observed (65.22% at 5 dpp) followed by a further decrease (63.74%) at 7 dpp.

The percentages of labeled / total germ cells, using Ki-67, are generally lower than those observed using PCNA antibody. Particularly, no significant changes of

TABLE 1
RATIO OF REACTIVE CELLS (GERM, SERTOLI) TO THE PCNA MARKER OF CELL PROLIFERATION

PCNA	14.5 *dpc*	18.5 *dpc*	20.5 *dpc*	22.5 birth	1 *dpp*	3 *dpp*	5 *dpp*	7 *dpp*
Germ cells	29.68	80.26	73.93	88.06	68.93	51.19	65.22	63.74
Sertoli cells	66.67	89.74	75.24	98.70	98.36	82.95	88.98	99.90

dpc – days post conception, dpp – days post partum

TABLE 2
RATIO OF REACTIVE CELLS (GERM, SERTOLI) TO THE KI-67 MARKER OF CELL PROLIFERATION

Ki-67	14.5 *dpc*	18.5 *dpc*	20.5 *dpc*	22.5 birth	1 *dpp*	3 *dpp*	5 *dpp*	7 *dpp*
Germ cells	43.95	36.66	43.74	45.77	54.43	45.82	55.02	35.13
Sertoli cells	60.37	67.96	77.40	71.62	77.37	70.74	71.24	95.76

dpc – days post conception, dpp – days post partum

the LI (43.95% at 14.5 dpc, 36.66% at 18.5 dpc) are observed until 20.5 dpc (43.74%). A notable increase is observed after 20.5 dpc until 1 dpp and, at this point, the LI peaks reaching a maximum of 54.43%. After 1 dpp, the mitotic activity ceases until 3 dpp (45.82%). It then resumes until 5 dpp, leading to a slight increase of the LI followed by a considerable drop (35.13%) at 7 dpp.

As far as Sertoli cells are concerned, using the PCNA antibody, the LI in the foetal testis, increases from 66.67 (at 14.5 dpc) to 89.74 (at 18.5 dpc) and then drops to 75.24 (at 20.5 dpc). At birth, the percentage of reactive Sertoli cells, reaches 98.70% and remains high thereafter, attaining a peak value of 99.90% at 7 dpp.

The foetal testis contains reactive to Ki-67, Sertoli cells from E14.5 (43.95%) to E20.5 (77.40%). The proliferation rate does not alter considerably, in the neonatal testis, until 5 dpp. At this point, a significant increase of the LI is observed and the peak value of 95.76%, is reached at 7 dpp.

In rat ovary, the ratio of reactive cells (both germ and follicular) to the PCNA and Ki-67 antibodies is analytically presented in tables 3, 4, respectively.

Before birth, using the PCNA antibody, the LI of germ cells increases from 71.19 at 14.5 dpc to 75.66 at 18.5 dpc. It then decreases to 73.26%, at 20.5 dpc. After E20.5, the mitotic activity ceases, resulting to a significant decrease of the labeling index, at birth (28.57%). In the neonatal ovary, mitotic activity resumes slightly after birth and the percentage of reactive cells increases, reaching 43.58%, at 1 dpp. Two days later, a slight drop is observed (42.17% at 5 dpp) followed by a further decrease in the LI (18.41%) at 7 dpp.

Values obtained with the Ki-67 antibody are lower than those observed using PCNA. This is also the case for the male gonad, as previously mentioned. In the foetal ovary, a significant drop of the LI is observed until E18.5 (43.67% at 14.5 dpc to 27.09% at 18.5 dpc), followed by a slight increase at 20.5 *dpc* (32.11%). At birth, the percentage of reactive cells is 28.62%. Postnatally, the mitotic activity ceases and the LI reaches a minimal value of 9.42 at 7 dpp.

As far as follicular cells are concerned, before birth, using PCNA antibody, immunostaining increases from 0.70% (at 14.5 dpc) to 28.90% (at 18.5 dpc) and then drops to 18.03%, at 20.5 dpc. At birth, the percentage of reactive follicular cells, reaches 27.66% and remains high thereafter, reaching a peak value of 49.22% at 7 dpp.

Prenatally, follicular cells immunoreaction to the Ki-67 antibody, increases from 0.87% at 14.5 dpc to 24.57% at 20.5 dpc. The proliferation rate does not alter considerably until birth. Thereafter, a striking increase of the LI is observed resulting to a peak level of 44.28% at 7 dpp.

Discussion

In this article, we report that germ cells mitotic activity differs significantly between male and female gonads, both prenatally and postnatally. We also present our re-

TABLE 3
RATIO OF REACTIVE CELLS (GERM, FOLLICULAR) TO THE PCNA MARKER OF CELL PROLIFERATION

PCNA	14.5 *dpc*	18.5 *dpc*	20.5 *dpc*	22.5 birth	1 *dpp*	3 *dpp*	5 *dpp*	7 *dpp*
Germ cells	71.19	75.66	73.26	28.57	43.58	42.17	41.71	18.41
Follicular cells	0.70	28.90	18.03	27.66	33,69	22.88	34.25	49.22

dpc – days post conception, dpp – days post partum

TABLE 4
RATIO OF REACTIVE CELLS (GERM, FOLLICULAR) TO THE KI-67 MARKER OF CELL PROLIFERATION

Ki-67	14.5 *dpc*	18.5 *dpc*	20.5 *dpc*	22.5 birth	1 *dpp*	3 *dpp*	5 *dpp*	7 *dpp*
Germ cells	43.67	27.09	32.11	28.62	26.83	25.99	11.88	9.42
Follicular cells	0.87	9.74	24.57	24.96	32.12	32.53	41.92	44.28

dpc – days post conception, dpp – days post partum

sults concerning gonadal somatic cells proliferation in both sexes.

The number of germ cells in the foetal and neonatal rat testis has been, previously, estimated in the work of Clermont and Perey[20]. In their study, they counted mitotic figures of germ cells in cross-sections of seminiferous cords and reported that, this cell lineage decreases sharply between late foetal life and 4 dpp.

In order to estimate germ cells absolute numbers, in the developing testis, Beaumont and Mandl, determined the testicular volume occupied by its 3 main cell lineages and substructed the volume of germ cells population. They calculated an approximate mitotic index by dividing the number of mitoses by the total number of germ cells. They concluded that, the number of germ cells in the rat testis increases from 14.5 dpc until 17–18 dpc to reach a peak value at 20 dpc. Mitotic activity then ceases and the population of germ cells remains static until 2–3 dpp. Between 2.5 and 4.5 dpp there seems to be a slight fall in the total population of germ cells, while afterwards (until 6.5 dpp), their number practically doubles[5].

J. Prepin, using the same technique (counting of mitotic figures), confirmed the latter results and revealed that the mitotic activity of germ cells is intense until 18 dpc. E20 represents the day in which a peak number is attained. This rise is followed by a transient decrease between 20.5 and 21.5 dpc and an arrest of cell proliferation at 22 dpc (just before birth). Postnatally, a sharp decrease of reactive germ cells is observed, associated with extensive degeneration. From 3 dpp, a further decline in the total number of germ cells is marked coincident with the formation of spermatogonia type-A[6].

More recently, Livera *et al.*, calculated the 5-bromo-2'-deoxyuridine (BrdU) incorporation index into the mitotically active germ cells, by immunocytochemistry and showed that, there are two periods of mitotic activity in the foetal and neonatal population of germ cells[8].

Comparative studies of BrdU and PCNA staining, through a dual immunohistochemical procedure, showed that, although both techniques may be effective for evaluating cell proliferation rates, the PCNA-labeled nuclei are significantly different from the population of cells marked by BrdU (BrdU is incorporated in cells only during S-phase of the cell cycle, i.e. during DNA synthesis)[21].

In the present study, we have examined the ratio of dividing germ cells (LI) by immunohistochemical detection of PCNA and Ki-67. These two antibodies were selected because they are expressed in almost the entire cell cycle, except Ki-67 protein which is absent in resting cells (G_0-phase of the cell cycle) and in cells during early G_1-phase.

Our findings, prenatally, agree partially with the above mentioned works. Before birth, using the PCNA antibody, we observed an intense mitotic activity between 14.5 and 18.5 dpc which coincides with the findings of Beaumont and Mandl's study. Until 20.5 dpc the percentage of reactive germ cells slightly decreases, followed by a significant increase of the LI, at birth (peak value). Using Ki-67 as marker of cell proliferation we observed that, the LI of germ cells increases until 20.5 dpc, which is in agreement with the latter results. Nevertheless, the fluctuation of germ cells LI is similar using both antibodies. The observed differences between our findings and those reported by Beaumont, Mandl and Prepin are, probably, due to the fact that, we examined the variation of the percentage of reactive germ cells and not the fluctuation of dividing germ cells numbers. Our results from neonatal male gonads are in accordance with their findings. In every case, significant decrease in germ cells number is observed after the 5th dpp.

It must be pointed out that, the overall percentage of PCNA positive germ cells exceeds considerably that of Ki-67 antiserum. This discrepancy is attributed to the differential expression of the two antigens during the cell cycle as well as to the significantly shorter biological half-life of Ki-67 and the involvement of PCNA in nucleotide excision repair mechanisms[16–18].

In the prenatal rat ovary, Beaumont *et al.* as well as Prepin *et al.*, showed that the increase in the germ cells number, between 14.5 dpc (11.5×10^3) and 18.5 dpc (75×10^3) is followed by a rapid decline, before birth. This was associated with the beginning of the prophase of meiosis I. After birth, a further reduction in germ cells number was observed leading to a minimum value of 27×10^3 at 2 dpp, which equals the one third of their peak value[6,13].

Results of the present study, using PCNA as cell proliferation marker, are in agreement with the latter studies but those, using Ki-67, differ as to the peak value of cell proliferation. The change of immunoreactive pattern obtained with both antibodies, reflects the differences associated with impairment of DNA[16–18].

Comparing germ cells proliferative behavior in both sexes, we observe that, in the male gonad, regardless the antiserum used germ cells exhibit proliferative behavior twice before birth and once during the first days of the postnatal life. On the other hand, female germ cells exhibit proliferative behavior only once before birth (Figures 1 and 2).

Sertoli cells mitotic activity in the seminiferous tubules of the rat varies according to the age of the animal. The Sertoli cell population increases from foetal day 13.5[9,22] until day 15–20 after birth[23]. According to Steinberger and Steinberger, no labeled Sertoli cells are found after postnatal day 15, using ^{3}H-thymidine and autoradiography, *in vitro* and *in vivo*[24]. Similarly, Clermont and Perey observed no dividing Sertoli cells in testes older than 15 dpp[20]. In 1982, Orth reported that, the percentage of Sertoli cells incorporating ^{3}H-thymidine, increased progressively from 16 dpc to a maximum on day 20. Thereafter, the percentage dropped steadily until, in 21 dpc, no labeled Sertoli cells were detected. These findings suggest that the foetal period is the time of greatest expansion of the Sertoli cell population and indicate that, at birth, proliferation of these cells is already in decline[25]. In a later study, in 1988, Orth *et al.*, developed a Sertoli cell-depleted rat model, estimated the percentage of cells

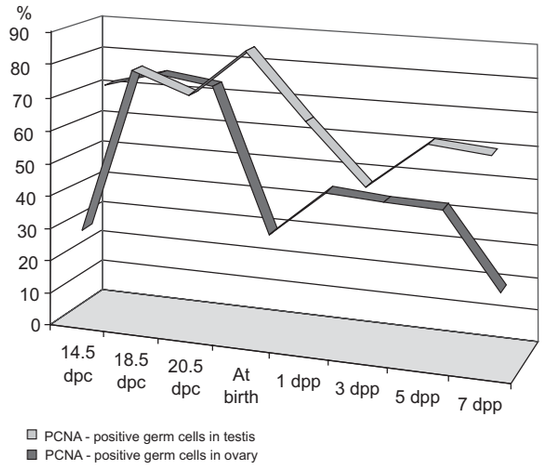

Fig. 1. Percentage of reactive to PCNA germ cells in both testis and ovary (dpc – days post concetion, dpp – days post partum).

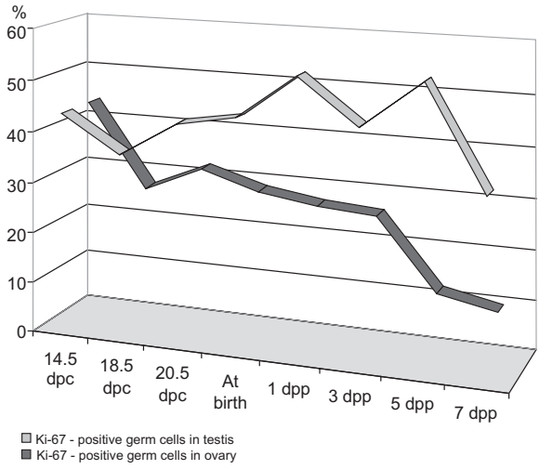

Fig. 2. Percentage of reactive to Ki-67 germ cells in both testis and ovary (dpc – days post concetion, dpp – days post partum).

labeled by ^{3}H thymidine and reported that, Sertoli cells division is observed during both foetal and early postnatal period[26]. Scientists estimating BrdU incorporation by immunocytochemistry showed that, the percentage of reactive Sertoli cells increases steadily in the prenatal testis, reaching a maximum value at 20.5 dpc. Thereafter, the mitotic activity decreases gradually and the stable population of non-dividing Sertoli cells is established by postnatal day 15–20[7].

Our results are in close agreement with the findings from the previously reported studies but disagree with Orth's first report in 1982, since the percentage of reactive Sertoli cells (estimated by PCNA and Ki-67 immunoreactivity) increases with age, reaching a peak value at 7 dpp. We showed that, the LI is increasing in the prenatal period, during which the Sertoli cells population is established. In fact, intense mitotic activity is detected in the forming seminiferous cords and Sertoli cells number reaches a high value before birth. In the neonatal testis, in spite of some fluctuations, Sertoli cells LI does not alter considerably, until 5 dpp. At this point, a significant increase of the LI is observed and the peak value is reached at 7 dpp. Additionally, the review article by Sharpe *et al.*, with the extensive literature on Sertoli cells proliferation, coincides with our results[27].

According to Jegou *et al.*, a critical cell number ratio between germ and Sertoli cells is necessary for normal spermatogenesis, implicating that somatic and germ cell lineage communicate and their numbers differentiate during the foetal and neonatal period, determining their numbers in the adult[28,29].

As far as follicular cells are concerned, our results concur with the observations of Brambell, who indicated that the proliferation of epithelial cells continues with-

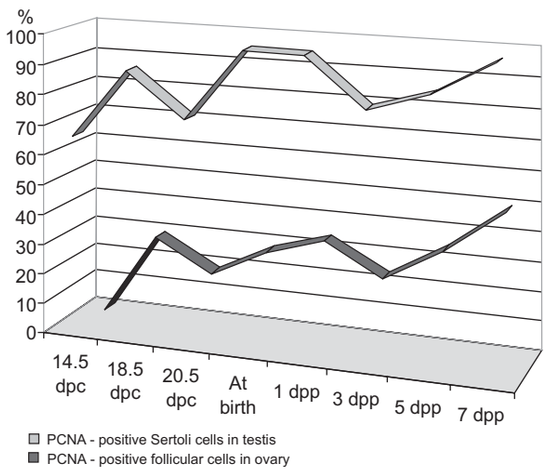

Fig. 3. Percentage of reactive to PCNA somatic cells in both testis and ovary (dpc – days post concetion, dpp – days post partum).

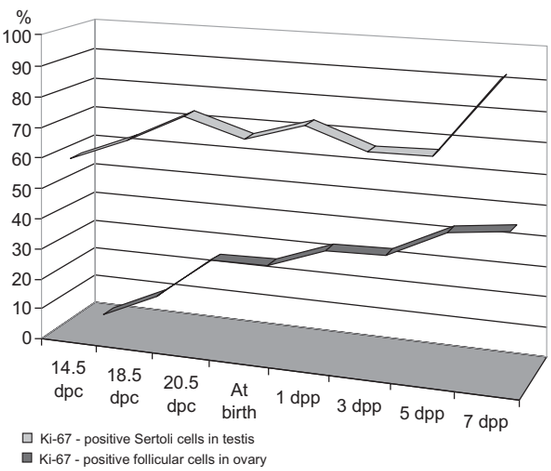

Fig. 4. Percentage of reactive to Ki-67 somatic cells in both testis and ovary (dpc – days post concetion, dpp – days post partum).

out interruption, although slowly, in the mouse ovary, throughout foetal period and after birth, until weaning time or even later[14]. Consequently, we observed that, the LI of somatic cells in the developing ovary is gradually increasing throughout the examined period, reaching a peak value at 7 dpp.

Our findings show a correlation between Sertoli and follicular cells proliferative behavior. Somatic (Sertoli or follicular) cells show no differences in the fluctuation of their population with age. In both sexes, regardless of the antiserum used, somatic cells exhibit an increasing proliferative behavior throughout the examined period (similar fluctuation[30,] Figure 3 and 4).

Overall, in this paper, we report that the gonadal dimorphism in proliferative behavior, concerns only germ cell lineage and is established during the foetal and neonatal period.

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DIMORFIZAM PROLIFERACIJE GONADNIH STANICA

SAŽETAK

Razlika između testisa i ovarija u proliferativnom ponašanju zametnih stanica pokazuje značajne razlike u fetalnom i neonatalnom periodu (14.5 dana nakon začeća – 7 dana nakon rođenja). Imunohistokemijskim metodama bojanja fetalnih testisa s PCNA I Ki-67 antitijelima (Labeling Index (LI)) otkriva populaciju zametnih stanica do rođenja. Nakon toga, opaža se oštar otklon u prva 3 dana postnatalnog života te kratkotrajan porast između 3. i 5. dana po rođenju. Tada prestaje mitotička aktivnost zametnih stanica. U fetalnim ovarijima proliferacija zametnih stanica doseže vrhunac prije rođenja, nakon toga se smanjuje. Somatske (Sertolijeve ili folikularne) stanice ponašaju se slično u oba spola. Primijećena je povećana mitotička aktivnost tijekom istraživanja. Usprkos tome, gonadalni dimorfizam tijekom proliferativnog procesa odnosi se samo na lozu zametnih stanica te je ustanovljen tijekom fetalnog i neonatalnog perioda.

Coll. Antropol. **30** (2006) 3: 649–652
Review

Sex Determinants in the Genome – Lessons from the Animal Kingdom

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A B S T R A C T

The immense value of sex differentiation as a means of enriching and evolving the genome has been proven by the vast variety of sex determining mechanisms to which organisms of all kinds resort. From single gene switching pathways found in lower level organisms to haplodiploid reproduction in hymenoptera, temperature-determined sex in reptiles and sex chromosomes in mammals and avians, nature and evolution have designated an impressive amount of effort to ensure that sex-specific variations remain under well-regulated control. Therefore enhancing our efforts to study some of the strategies recruited for the above may also lead to a better understanding of the inherent complexity of sexual dimorphism in general.

Key words: *differentiation, sex determining mechanisms, single gene switching pathways, temperature-determined sex, sex chromosomes, sexual dimorphism*

Introduction

Scientists throughout the ages have always been fascinated by the vast variety and ingenuity of reproduction mechanisms employed by various species. But even so, up to this day, we have yet to string the exact course of evolution that has led to the current wealth of sex determination mechanisms, a key element to the biological wonder also known as life.

However, despite the lack of a concise theory of how these mechanisms have come about, an organized system of studying them has been established. Sex determination mechanisms as a whole can be divided into chromosomal and non-chromosomal. Accordingly, non-chromosomal mechanisms can furthermore be distinguished into gene-related and environmental, with the latter generally considered as the evolutionally more ancient (Figure 1).

Environmental Sex Determination

Environmental sex determination, and especially when temperature related, is considered the forerunner of most contemporary mechanisms (Figure 2). It is mostly employed in several species of turtles, crocodiles, tuatara giant lizards and selected fish specimens.

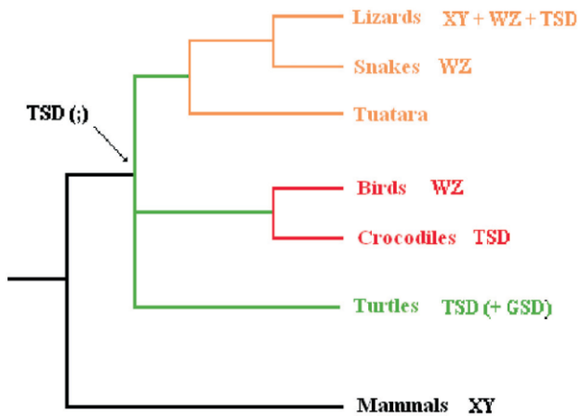

Fig. 1. A scheme that roughly indicates the evolutional pattern of sex determination mechanisms. XY – XY chromosomal sex determination, WZ – WZ chromosomal sex determination, TSD – temperature-dependent sex determination, GSD – gene-dependent sex determination.

For this assortment of mechanisms, the effect of several environmental factors, such as temperature, during

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Fig. 2. The basic principle of environmental sex determination is that environmental effects cause the affinity of several molecules for their receptors to increase or deteriorate.

fertilization and the primary stages of embryogenesis can alter the pattern of certain crucial proteins that effectively control the development of sex traits. For example, many species of reptiles, including most turtles and almost all crocodilians, have no discernible sex chromosomes, nor is their sex determined by the presence or absence of specific genes. In these organisms, it is the temperature of the environment in a specific period of incubation that can determine whether the animal in question will turn into a male or a female. This particular period, which usually coincides with the middle third of development, is also known as the thermosensitive period (TSP), and it has been mainly linked to the efficiency of aromatase, the key enzyme for the conversion of androgens into estrogens.

More specifically, in reptiles, while steroidogenesis begins very early, prior even to the thermosensitive period, aromatase activity tends to remain universally low. With the onset of the thermosensitive period however, aromatase activity seems to increase in certain temperatures, which vary for each species. For example, in marine and freshwater turtles, higher temperatures cause an exponential increase of aromatase activity, whereas in lower temperatures aromatase activity remains low. The different levels of aromatase activity then guide the differentiation of the indifferent gonad into an ovary or testis. Once the thermosensitive period is over and the fate of the gonad has been established, further changes in temperature seem to have no effects[1].

As mentioned above, the thermosensitivity of the gonads has also been demonstrated in several fish and some amphibians. These however tend to combine gene--dependent or chromosomal sex determination with the mechanism demonstrated here. As a result, the effects of temperature may go against the genotypic directions, allowing the existence of animals in genotypic and phenotypic sex discordance, a phenomenon known as sex reversal[2] (Table 1).

Gene Dependent Sex Determination

The next step in the evolutionary scale takes us to gene dependent sex determination. With these mechanisms, sex can be determined by focusing on a single gene.

Gene dependent sex determination can be differentiated by chromosomal from the fact that there is no specialized set of chromosomes (sex chromosomes), while the gene itself can often be found in various locations within the genome, due to transpositions. For example, in the *Megaselia scalaris* species the sex determining gene is actually a transposone that regularly alternates its home among the chromosomes.

For example, the haplodiploid genetic system we encounter in the insect order of Hymenoptera allows the laying of both unfertilized eggs that typically develop into uniparental haploid males and fertilized eggs that can give us biparental diploid females. The best understood strategy for this seems to be single-locus complementary sex determination (sl-CSD), in which sex is determined by multiple alleles at a single locus. Heterozygotes at that sex locus develop as females whereas hemizygotes and homozygous diploids develop as males, thus providing us with the pattern presented above[3].

Moving along the same lines, a single gene is also considered responsible for determining sex in *Drosophila melanogaster*, and more specifically the activation of the *sxl* gene (sex-lethal) in females during the early stages of development in response to the ratio of X chromosomes to autosomes (X:A ratio). The latter is communicated early in development through the delicate balance between the dose-sensitive X chromosome numerator elements, which include genes such as *sis-a*, *sis-b*, *runt* and less so *sis-c*, and the autosomal denominators, such as *dpn*, in conjunction with the maternally derived products of the *da* gene and the more recently studied *emc*, *groucho, her* and *snf*. An early form of the SXL protein is then produced that allows the correct reassembling of the later gene transcripts through sex-specific splicing of its mRNAs, unlike in males, where the delayed gene activation leads to the production of an inactive protein. Once the SXL active state has been established, it then goes on to regulate a series of other proteins that control female development, once again through the process of alternative splicing, leading finally to the two alternative products of the *doublesex* gene (*dsx*), DSX^F and DSX^M [4–6].

Similarly, the active state of the *xol-1* gene in males of the *Caenorhabditis elegans* species acts like a switch for

TABLE 1
SEX REVERSAL: THE COMBINATION OF GENOTYPIC AND TEMPERATURE-DEPENDENT SEX DETERMINATION ALLOWS A PHENOMENON KNOWN AS SEX REVERSAL, WHERE THE PHENOTYPIC SEX DOES NOT ALWAYS AGREE WITH THE GENOTYPIC DIRECTIONS

	Female-producing temperatures	Male-producing temperatures
XX (ZW)	Female (in accordance with genotype)	Female OR male (in discordance with genotype)
XY (ZZ)	Male OR female (in discordance with genotype)	Female (in accordance with genotype)

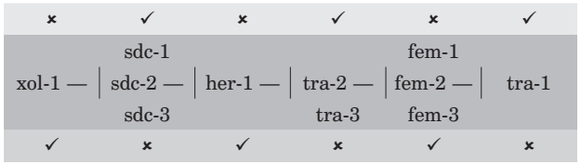

Fig. 3. Activating xol-1 acts like a switch in the sex determination pathway.

the pathway of genes that determine sex (Figure 3) and that, through a pathway of inhibitory genes, finally lead to the active or otherwise state of the TRA-1 protein, which acts as a transcription factor. As before, the X:A ratio is communicated with the help of several »X-signal elements«, such as the SEX-1 protein that acts on the level of transcription and the FOX-1 protein that acts post-trancrisptionally. These two, among others that have yet to be deciphered, manage to suppress the levels of the XOL-1 (XO lethal) key protein, setting off the mechanism that determines sex[7–9].

However, *C.elegans* worms are special in that the choice lies between males with one X chromosome and hermaphrodites with two. Indeed, the *C.elegans* hermaphrodites pose an interesting issue. These are specialized females which in the fourth and final larval stage (L4) produce around 300 sperm, to use for self-fertilization when there are no males available. This requires a careful regulation of switching between male and female differentiation of the same germ cells without the benefit of the usual sex determination gene pathway, since the »male« genes that normally regulate spermatogenesis are inherently inactive in hermaphrodites. Instead, a specific series of genes take over in a specific stage of development and act in place of the HER-1 protein to inhibit *tra-2* and allow spermatogenesis to take place for a specific period of time. Once this is over, a new series of genes take their place, *tra-2* is once again active, and the adult hermaphrodite is free to continue with oogenesis for the rest of its life[9,10].

Still, one should bear in mind that in both of these species the genes in question are permanently localized in a specialized set of chromosomes, despite the rest of the key factors in the sex determining pathways being scattered among the rest of the chromosomes. So it would be possible to consider these two an intermediate step between gene dependent and chromosomal sex determination.

Chromosomal Sex Determination

The next and final step in the evolutionary scale is what is commonly known as chromosomal sex determination. Along the passage of time, genes that were related to determining sex began to gather in specific chromosomes, which are now labeled as sex chromosomes since their presence or absence in an organism heralds the establishment of a particular sex.

Most species that adopt a chromosomal strategy of determining sex seem to follow a common pattern:

- There are two distinct sex chromosomes that differentiate in both size and content
- One sex requires a pair of sex chromosomes of one kind, while the other sex requires a pair of sex chromosomes of both kinds

Although the various sets of sex chromosomes that belong to contemporary organisms display a wide scale of differences and similarities, most of which can be traced to the existence of a common ancestral chromosome, we distinguish between two major varieties of sex determination mechanisms, depending on the sex that requires a unanimous set of said sex chromosomes:

Z/W sex determination

It can be attributed to species that require two sex chromosomes of the same kind for males (ZZ) and of two different kinds for females (ZW), such as birds, snakes, lizards and several fish.

The avian Z and W in particular seem to have no relation to the mammalian X and Y, but to have evolved from different pairs of autosomes. And this is part of the reason we are not yet certain which of the two carries the genetic trigger for sex determination. To this day, there are two major theories under investigation. Sex may depend on Z chromosome dosage, according to the example of *Drosophila melanogaster* and *C.elegans*. One candidate gene for this theory is the *DMRT1*, which is located on Z chromosomes, escapes dosage compensation and is expressed specifically in the gonads, and is thus capable of linking the number of Z chromosomes with gonadal differentiation. On the other hand, sex may be determined by the feminizing presence of the W chromosome, following the example of Y in eutherian mammals. There are two different mechanisms that are being studied and can support this theory. One includes the *FET1* gene, which is located on W, does not have a Z homologue and is expressed almost exclusively in the female urogenital system. The other includes the *ASW* gene, also known as *WPKCI*, and its Z homologue *ZPKCI*, since it has been proposed that the products of those two genes are capable of dimerisation, with a ZPKCI homodimer acting as a testis factor and a WPKCI/ZPKCI heterodimer preventing this effect.

One way to discern between the two theories would be to look into different combinations of Z and W chromosomes. Indeed, scientists have studied ZW aneuploidy in an effort to better understand how things work. It turns out that ZZZ animals develop testes but are infertile, ZWW animals die early in embryonic development, but ZZW combinations manifest as intersexual: the animals appear female on hatching, but slowly turn into males at sexual maturity. It is still possible, thus, that a combination of the above is in fact applied[11,12].

X/Y sex determination

It can be attributed to species that require two sex chromosomes of the same kind for females (XX) and of two different kinds for males (XY), such as mammals and several species of plants and insects.

One interesting prospect studied in the marsupial X and Y chromosomes is that they need not exclusively control all aspects of sex. The basic marsupial Y chromosome is the smallest of any mammal but retains its ability to turn the undifferentiated gonads into testes. However, the formation of the mammary glands and scrotum develops before gonadal differentiation takes place and is independent of gonadal hormones. In fact, it appears to be under the control of genes located on the X chromosome. So it happens that XXY animals have testes, but a pouch with mammary glands has replaced their scrotum, whereas XO animals have no testes, but an empty scrotum in place of a pouch[13].

Conclusion

It becomes apparent that the mechanisms devised to determine sex exhibit an extraordinary variety and ingenuity when examined as a whole. The purpose however is both to comprehend the molecular strategies employed by other organisms and to find ways of applying that knowledge as regards sex determination and differentiation on the species that interests us most, man[14].

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SPOLNE DETERMINANTE U GENOMU – LEKCIJE IZ ŽIVOTINJSKOG CARSTVA

SAŽETAK

Golema vrijednost spolne diferencijacije, kao sredstvo za obogaćivanje i razvoj genoma, dokazala se kao nepregledno šarenilo spolno određenih mehanizama kojima pribjegavaju sve vrste organizama. Od promjenjivih putova jednoga gena nađenog u organizmima nižih vrsta do haplodiploidne reprodukcije kod opnokrilaca, temperaturno određenog spola kod reptila te spolnih kromosoma kod sisavaca i ptica, priroda i evolucija uložile su impresivan trud kako bi osigurale da spolno specifične promjene ostanu pod dobro reguliranom kontrolom. Zbog toga, pojačavanje naših napora za proučavanje nekih strategija potrebnih za gore navedeno, može također voditi boljem razumijevanju prirođene kompleksnosti spolnog dimorfizma uopće.

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Review

Establishing Sexual Dimorphism in Humans

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A B S T R A C T

Sexual dimorphism, i.e. the distinct recognition of only two sexes per species, is the phenotypic expression of a multistage procedure at chromosomal, gonadal, hormonal and behavioral level. Chromosomal – genetic sexual dimorphism refers to the presence of two identical (XX) or two different (XY) gonosomes in females and males, respectively. This is due to the distinct content of the X and Y-chromosomes in both genes and regulatory sequences, SRY being the key regulator. Hormones (AMH, testosterone, Insl3) secreted by the foetal testis (gonadal sexual dimorphism), impede Müller duct development, masculinize Wolff duct derivatives and are involved in testicular descent (hormonal sexual dimorphism). Steroid hormone receptors detected in the nervous system, link androgens with behavioral sexual dimorphism. Furthermore, sex chromosome genes directly affect brain sexual dimorphism and this may precede gonadal differentiation.

Key words: *SRY, Insl3, testis differentiation, gonads, androgens, AMH, Müller / Wolff ducts, aromatase, brain, behavioral sex*

Introduction

Sex is a set model of anatomy and behavior, characterized by the ability to contribute to the process of reproduction. Although the latter is possible in the absence of sex or in its multiple presences, the most typical pattern and the one corresponding to humans is that of sexual dimorphism. The term sexual dimorphism has been used to describe morphological differences between the sexes, but can be extended to any biologically-related process that varies between males and females[1].

This quality achieves to offer the necessary variability in phenotype features, in gametogenesis and parental chromosome fusion in fertilization, while at the same time it ensures the maintenance of androgens and estrogens within an acceptable proportional ratio. Thus, sexual dimorphism is the phenotypic expression of a multistage procedure at chromosomal, gonadal, hormonal and behavioral level.

Chromosomal – Genetic Sexual Dimorphism

In humans, the typical male usually has a diploid karyotype of 46 chromosomes, including 22 autosomal pairs and an XY pair of gonosomes (46, XY). Alternatively, a standard female karyotype would be 46, XX, the latter referring to the two identical gonosomes in each diploid cell.

The basis of sexual dimorphism in mammals derives from the evolution of the sex chromosomes[2]. According to recent findings, both X and Y chromosomes have evolved from autosomal ancestors about 300 million years ago[3,4]. At the time, a failure in homologous recombination resulted in the formation of a small area that wasn't identical in the two chromosomes. The presence or absence of this region coincided with a different pattern of development that altered androgen activity, resulting in a sex – determining role. In all mammalian organisms surviving today, this area appears to retain the regulatory function and is therefore described as the sex-determining region of the Y chromosome (*SRY* gene)[5]. However, it must be pointed out, that the genetic basis of sexual dimorphism is not limited to a single gene. In fact, sequence analysis has suggested a gradual structural conversion process. According to this theory, the X and Y chromosomes have experienced repetitive recombination failures throughout time, leading to the accumulation of micro- and macroscopic specializations, which finally lead to the extensive differentiation in the current structure of the two gonosomes[4,6–8].

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The particularly small Y chromosome, in comparison to its X partner, cannot be simply attributed to chromosomal recombination. Therefore, one must assume that every failed recombination was followed by some level of genomic instability, which caused partial deletions of the Y chromosome-ancestor. Today, males carry a Y chromosome that almost entirely (95%) consists of non-recombinant sequences, allowing recombination with the X chromosome only in polar regions, the so-called pseudoautosomal regions, PAR1 and PAR2[9]. The non-recombinant region (NRY) seems to have developed around the *SRY* gene to include a variety of genes and regulatory elements that cooperate to produce the main features of the male phenotype. Owing to this observation, it has recently been proposed to rename this area as the male-specific region of the Y chromosome, or MSY. Moreover, it has been suggested that the MSY has the ability to exchange DNA between its own different units, allowing some level of variability. This unique quality is based on the presence of palindromes, which increase its stability and determine the positions of Y-Y recombination[9–12]. According to this, one should expect that, the Y chromosome retains its current length, after millions of years of gradual deterioration, a concept that remains to be proven[9,13,14].

For females, the double presence of the X chromosome seems to be related to sexual features. However, in all higher mammals that have developed an MSY region, extending the sex determining abilities of the *SRY* gene, some model of X chromosome inactivation has been employed[15]. This mechanism leads to dosage compensation between the two sexes for the large majority of X-linked genes[6]. The remaining genes escape inactivation, which predisposes for their differentiated role in females, owing to their double expression from the two X chromosomes, as opposed to a single copy in XY males. However, not all of these genes have been found to control sex-related functions. Therefore, one may assume that certain autosomal genes must also play an important role in the establishment of the female sex pattern.

The crucial role of the sex chromosomes in dimorphism is particularly stressed by the phenotypic disorders associated with cytogenetic alterations. Despite differences in their clinical manifestations, gonosome aneuploidies, such as Kleinefelter and Turner syndromes, are always characterized by gonadal dysgenesis and infertility.

Gonadal Sexual Dimorphism

The expression of the *SRY* gene is the key parameter in gonadal differentiation. In males, *SRY* is expressed in differentiating epithelial cells of the gonadal anlagen, i.e. Sertoli cells that encompass germ cells forming the seminiferous cords[16,17]. *SRY* is expressed only briefly, and therefore, one may assume that it acts as a form of molecular "switch", triggering a gene cascade that promotes male phenotype (Table 1, Figure 1). Indeed, of the numerous downstream genes, current research mainly focuses on two major gene products, both deriving from Sertoli cells. The first is the SOX9 protein, which appears to coordinate the formation of seminiferous cords within the developing testis. The action of SOX is necessary for

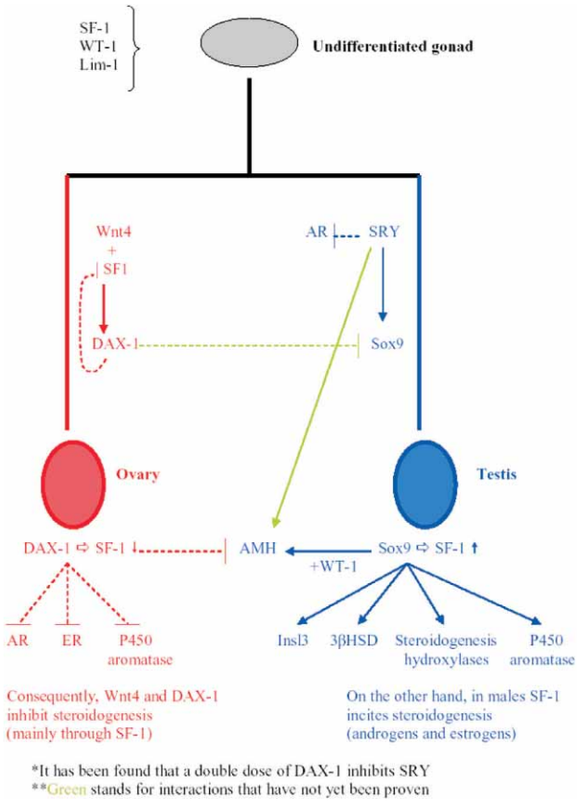

Fig. 1. Genes implicated in gonadal sex differentiation.

TABLE 1
MOLECULAR MARKERS IN THE EARLY STAGES OF GONADAL DIFFERENTIATION IN THE RAT FOETUS

Molecular markers	Testis (XY)	Ovary (XX)	Molecular markers	Testis (XY)	Ovary (XX)
11.5 days *post conception* (*dpc*)			12.5 days *post conception* (*dpc*)		
Sry	+	–	*Sry*	–	–
SOX9	++	–	*SOX9*	++	–
DAX1	++	++	*DAX1*	+/–	++
AMH	–	–	*AMH*	++	–

normal testis development, since its absence totally inhibits it, regardless of *SRY* expression, as experiments with mutant SOX deficient mice have shown. This involves both Sertoli and Leydig cells, since the latter are induced by SOX9 to express the *FtzF1* gene, whose product is the main regulator of androgen production, i.e. steroidogenic factor 1 (SF1)[18,19].

Although testis formation is largely androgen-independent, it seems that subsequent development of the organ depends on continuous trophic exposure to androgens. The latter are produced by Leydig cells and accumulated by the androgen binding protein produced by Sertoli cells. Experimental data in adult rats show that a stable androgen to estrogen ratio is vital to retain both ovary and testis histological organization.

Testosterone promotes Wolffian duct differentiation into the male reproductive tract through the formation of the epididymides, vas deferentia and seminal vesicles. On the other hand, Sertoli cells produce the Anti Müllerian Hormone (AMH), shortly following *SRY* stimulation. This hormone inhibits the development of the Müllerian ducts in the male embryo[20–22].

In females, absence of the *SRY* gene allows the formation of an ovary. Among the major regulators of this process, one may refer to the DAX1 protein, which is associated with follicular cell function and the formation of the primordial follicles. The importance of this product is understood by the result of its duplication in male rats. In this case, regardless of *SRY* or *SOX* action, DSS-AHC critical region on the X chromosome gene 1 (*DAX1*, also known as *NrOb1*) achieves the feminization of the genital structures, a process described as dosage-sensitive sex reversal[23]. In addition, experimental data suggest that its presence, in small quantities, in males, may actually be required for the initial organization of the epithelium forming seminiferous cords. As far as ovaries are concerned, steroid producing cells are represented by the inner and outer theca. The SF1 protein is the key regulator of androgen production in these cells as well, but it also helps to limit P450 aromatase activity, thus achieving an optimal androgen to estrogen ratio.

In the absence of male hormones, the Wolffian ducts degenerate, whereas the Müllerian ducts persist and differentiate into the female reproductive tract, including the oviduct (fallopian tube), uterus, cervix and upper portion of the vagina. Homeobox A (*Hoxa*) genes are expressed along the craniocaudal axis of the Müllerian ducts and specify the identities of the developing structures. The expression of a *lin-11*, *Isl1* and *mec-3* homologue (*Lim1*, also known as *Lhx1*) which encodes a LIM class homeodomain protein, in the epithelium of the Wolffian and Müllerian ducts highlights the initial sexual duality of the forming reproductive systems[24,25].

Hormonal Sexual Dimorphism

The external genitalia and the secondary sex features, such as osteology, muscular strength, voice depth, hair length, lipid distribution pattern, facial characteristics and breast development, are controlled by androgens and estrogens[26–29]. Both types of hormones may act in androgen and estrogen receptors, thus altering the outcome at a cellular and tissue level. It is important to note that, while estrogens mainly act in the form of estradiol, i.e. the final product in the aromatase chain of reactions, androgens act as both, testosterone, and the most enhanced form, dihydrotestosterone (DHT). The latter is produced from testosterone by the action of the enzyme 5a-reductase. Testosterone and DHT bind to a specific high-affinity intracellular receptor and, ultimately, this hormone-receptor complex enters the nucleus and modulates transcription of tissue-specific genes and their protein products. Testosterone-receptor complex mediates development of the Wolffian derivatives whereas DHT-receptor complex modulates differentiation of the urogenital sinus and male external genitalia. The response of target tissues to testicular hormones lasts for a particular developmental period, which constitutes the sensitive period for hormone action. Hormonal treatment of females in adulthood has negligible effects on genital morphology[27]. The differentiation of external genitalia into labia majora, labia minora, clitoris and part of the vagina is stimulated by estrogens. Androgens together with AMH and insulin-like growth factor 3 (Insl3) are involved in testicular descent to the scrotum, via activation of the Lim1 transcriptional factor. In the female embryo, the absence of androgen holds the ovary by the suspensory ligament inside the abdomen and, as Insl3 is not present, the gubernaculums fades away before it has a chance to yank the ovary outside[30].

Sexual Dimorphism of the Brain

Until recently, scientists believed that the way in which each individual chooses to determine his/her sex constitutes a final, "behavioral" level in sexual dimorphism, attributed to psychological rather than organic factors. However, it has been suggested that sex hormones might be involved in processes within the central nervous system (CNS) which cannot explain sexual preferences, but they may constitute some kind of predisposition to homo- or hetero-sexuality. This concept has given rise to extensive research on this field of Neuroscience, leading to reviews and original papers on the issue of the so-called "sexual brain"[31,32].

Of the various functional regions of the CNS, those that seem to be closely associated with the sexual behavior are the hypothalamus, the amygdala and the bulbocavernosus nucleus in the spinal cord. The hypothalamus represents a central area in the regulation of the autonomous nervous system and the function of vital organs. Regions related to sexual dimorphism, perinatally, include the preoptic area and the anteroventral periventricular nucleus, both bearing estrogen receptors. On the other hand, the septal AVP, the spinal bulbocavernosus nucleus (SBN) and the nucleus robustus archistriatum seem to retain a role in sexual differentiation throughout life. With the exception of the SBN, which

only contains androgen receptors, the others seem to be regulated by both androgen and estrogen receptors (glutamate secreting neurons). This is true for the posterodorsal medial amygdala in adults. Progesterone, on the other hand, is known to bind to the subunit of GABAnergic neurons.

Although the exact target for sex hormone activity in the CNS is not clear, research has provided some probable candidates. These include, for example, the ciliary neurotrophic factor (CNTF) receptor, which regulates neuronal development. Moreover, PGE_2 may promote "masculinization" of the preoptic area in the hypothalamus, but it cannot justify the differences in volume observed between the two sexes. Granulin is an androgen-induced modulator of epithelial growth, highly expressed in the ventromedial and arcuate nucleus of the hypothalamus[33]. Prenatal exposure to high androgen concentrations is often found in the history of homosexual women, while androgen insensitivity is detected in some men submitted to surgical sex reversal.

Differences between male and female brains are thought to arise largely through the actions of gonadal secretions during a critical period of brain development[34]. In humans (as in rats) circulating testosterone displays 2 peaks. The 1st peak, in male human embryo, occurs in the 2nd trimester of gestation and the 2nd peak in the 1st year of post-natal life (Figure 2). Thus, higher levels of testosterone during foetal and neonatal life cause the masculinization of the brain[31,32].

Due to the fact that, specific central nervous system regions and behavior can be fully sex-reversed by treating females with testosterone or preventing the action of testicular hormones in males, no other factor need to be invoked in order to explain the sexual differentiation process in those cases[35].

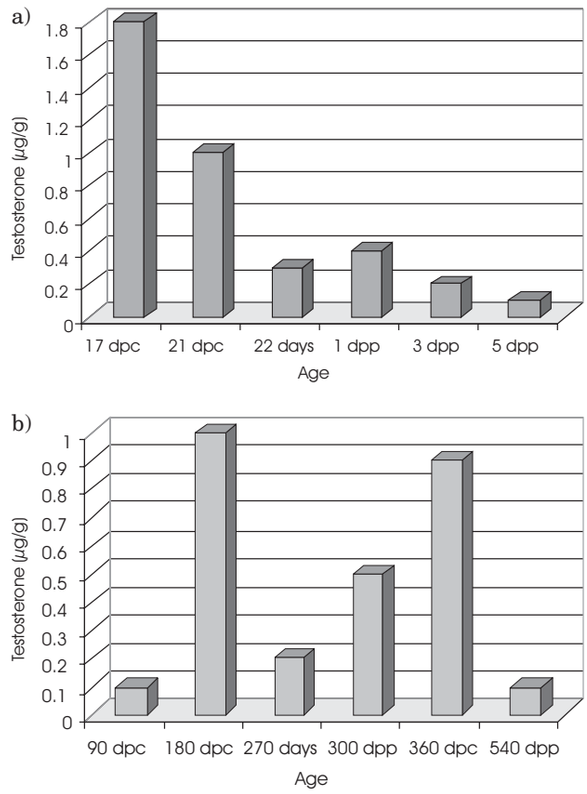

Fig. 2. Levels of testosterone in foetal and neonatal a) rats and b) humans: dpc – days post conception, dpp – days post partum.

Testis-Dependent Sexual Dimorphism

Several masculinizing effects of androgens in the brain result from aromatization of testosterone to estrogen, catalyzed by aromatase, an enzyme abundant in the hypothalamus[36]. Only aromatizable androgens, such as testosterone, exert a masculinizing effect in female rats, not non-aromatizable androgens, such as 5µ-dihydrotestosterone (5µ-DHT). But, in addition to aromatase, the brain contains 5µ-reductase. The type 2 isoform of 5µ-reductase (5µ-R2) is expressed in cerebral neurons and its maximal expression occurs a few days after birth, in males than in females. This enzyme (5µ-DHT) seems to be a morphogenetic signal for the development of aromatase-expressing neurons of the hypothalamus[32]. However, the importance of 5µ-DHT in brain masculinization is limited because men with homozygous inactivation of the 5µ-R2 gene and their animal model (5µ-R2 knock-out mice) display a proper gender identity and behavior[37,38].

Lesions of the entire preoptic area (POA) in the anterior hypothalamus eliminate virtually all male copulatory behaviors, whereas lesions restricted to the sexually dimorphic nucleus of the POA (SDN-POA) have more modest effects. Treating female rats with testosterone, just before and after birth, causes the SDN-POA in adulthood to be as large as in normal males, whereas castrating male rats at birth results in a smaller feminine SDN-POA in adulthood. Thus, sexual differentiation of this nucleus resembles that of the genitalia, i.e. androgen early in life permanently masculinize this brain region[39–41].

However, a divergence has been observed on these neural sexual dimorphisms. Some rely on perinatal actions of testosterone (SDN-POA, AVPV) and some require both perinatal and adult testosterone (septal vasopressin, BNST, SNB, RA)[34]. Yet, others require testosterone only in adulthood (MePD – Prostero Dorsal Medial Amygdala)[41, 42]. In some cases, testosterone acts only on estrogen receptors (SDN-POA, AVPV) or activates both androgen and estrogen receptors (septal vasopressin, MePD, RA)[43, 44]. In other cases, only androgen receptors act perinatally (SNB).

Testis-Independent Sexual Dimorphism

Recent studies have shown that, sex-specific differences observed in both mammals and birds can be associ-

ated with X and Y chromosome-linked genes acting directly on the brain cells[45]. In several species of songbirds, males sing more than females, a functional difference that matches a structural difference: the forebrain regions controlling song, including the Higher Vocal Center (HVC) and the nucleus Robustus Archistriatum (RA). These nuclei are much larger in males and contain larger neurons, than their female counterparts. The forebrain song circuit shows marked sexual differentiation, which does not seem to be due solely to gonadal hormones, but rather might result from differences in neuronal sex chromosome genotype[46].

Manipulating males with gonadal estrogen do not prevent masculine development, so it seems that brain cells may produce *de novo* estrogen (neurosteroids) to induce HVC ingrowth and masculine development. Neuronal transplantation studies in quails also support a role for genetic sex in controlling sexual differentiation in the brain. This means that, an important contribution to the process of brain sexual dimorphism is given by the action of sex chromosome genes, acting locally, within the brain (somatic) cells and steroid hormones are produced *in situ*, to virilize the bird song system[47].

Microarray screening of genes that were expressed differentially in the brain of male and female mice, before gonadal hormone secretion, allowed the identification of 57 female enhanced genes and 24 male enhanced genes, at embryonic day 10.5 (E10.5). This means that sexual differences in gene expression in neuronal cells, before gonadal hormone secretion, play an important role in sexual dimorphism in the brain[48,49].

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SPOLNI DIMORFIZMA KOD LJUDI

S A Ž E T A K

Spolni dimorfizam, odnosno raspoznavanje dvaju spolova unutar vrste, fenotipska je ekspresija višefaznog postupka na kromosomskoj, gonadnoj, hormonalnoj i bihevioralnoj razini. Kromosomsko-genetski spolni dimorfizam odnosi se na postojanje dvaju identičnih (XX) ili dvaju različitih (XY) gonosoma kod žena i muškaraca. To je posljedica različitog sadržaja X i Y kromosoma na oba gena te regulatorskih sekvenci, a SRY je ključni regulator. Hormoni (AMH, testosteron, Insl3) koje izlučuju fetalni testisi (gonadni spolni dimorfizam) priječe razvoj Müllerovog duktusa, maskuliniziraju derivate Wolffovog duktusa te su uključeni u testikularno propadanje (hormonalni spolni dimorfizam). Receptori steroidnih hormona, nađeni u živčanom sustavu, vežu androgene uz bihevioralni spolni dimorfizam. Nadalje, geni na spolnim kromosomima direktno utječu na moždani bihevioralni dimorfizam, što može prethoditi gonadnoj diferencijaciji.

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Review

Hormonal and Meta-Hormonal Determinants of Sexual Dimorphism

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A B S T R A C T

The role of hormones in the determination of sexual characteristics has been known for several decades. It has been shown, for example, that several products, including sex steroids, may influence the body development pattern, metabolic pathways, fat and muscle distribution and vocal cord anatomy, thus producing an overall outcome consistent with a masculine or feminine phenotypic pattern. These qualities are usually described as secondary sexual traits, so as to be distinguished from primary sex traits, usually referring to the gonads and external genitalia. However, it must be noted that hormonal regulation may not explain the full range of the sexual phenotype, since the central nervous system retains a significant role in the establishment of sexual identity, thus giving rise to a higher sex determination stage exclusively described in humans, namely behavioral or psychological sex. Recently, it has been suggested that differences among the sexes are not limited to brain function but they may also refer to anatomical differences and different biochemical profiles, including a distinct pattern of AR and ER distribution. This new aspect of sexual dimorphism suggests a whole system of meta-hormonal regulation, recently described as the sexual brain model. The role of local androgen and/or estrogen concentrations in the initial establishment of brain sexual dimorphism is still under evaluation, since the first results are relatively inconclusive and no direct cause and effect relationship has been proven so far. On the other hand, sex hormones have recently been found to participate in processes well beyond their initially suggested spectrum of action. For instance, ER interacts with EGFR in a number of ways, affecting development of a number of epithelial structures. Estrogen receptors have also been detected in a number of non-classic targets of steroids, such as the brain and the lungs. This observation may imply that sexual dimorphism goes a lot deeper than previously estimated, affecting virtually every organic system, suggesting, in essence, the existence of two different functional models for the whole human body, formulated and conserved throughout the evolutionary progress.

Key words: *sex steroids, steroid receptors, sexual brain, EGF receptor, sex traits*

Introduction

The determination of sex is a complicated, multistage process involving a number of genomic and biochemical determinants. Although largely dependent on inherent qualities, i.e. DNA content, environmental parameters may also influence the final outcome in terms of phenotypic variation. Since a) major environmental phenomena affecting local temperature at the hatching site have been shown to directly determine sex differentiation in a number of species, mostly reptiles, leading to the term »environmental-temperature sensitive sex determination« and b) a number of external interventions in the human fetus at the time of gestation, including drug administration and exposure to radiation, smoke or alcohol, have been associated with various distortions of sexual dimorphism observed in postnatal life, it is more than evident that sex is only the final product of a network of interactions occurring at any time throughout a large period of time, spanning from gestation to the acquisition of sexual maturity/puberty[1].

In terms of terminology, researchers in the field of developmental biology, experimental embryology and reproductive endocrinology use different terms to refer to the various phenomena associated with the acquisition of sexual traits in an individual. The term sex determination usually refers to phenomena *in utero*, based on the

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genomic determinants of sex and leading to the formation of the gonads and sex-specific genetic tracts. On the other hand, the term sex differentiation is larger, referring to the gradual appearance of all characteristics that are considered consistent with male or female sex, regardless of the developmental stage at which they are initially observed, i.e. during gestation, in perinatal life or even later in puberty. Finally, the term sexual dimorphism has attracted much attention recently, owing to the observation that sex-specific differences in macroscopic and microscopic anatomy and physiology exist in all organic systems, thus suggesting a completely distinct functional model for each sex, expanding in all reactions of the body, rather than simply the few qualities, previously considered exclusively as the sex traits[2,3].

Sex hormone-mediated reactions have been the focus of attention for many decades. Recently, the detection of hormone receptors in different organs than those traditionally viewed as the target tissues has opened the question for the possible presence of further regulatory actions also related to sexual identity, thus formulating the hypothesis of a meta-hormonal level in sex differentiation[4–6].

Regulatory Stages of Sex Differentiation

Since the time of Professor Alfred Jost's innovative work in gonadal development, sex is no longer considered as the result of a single genetic effect, but rather as a phenotypic variation regulated via a long, complicated process. Although the latter appears to be a continuum, where every previous step preconditions the available options for the next regulatory phenomenon, didactic purposes justify an attempt to facilitate relevant scientific discussion and research, achieved via the recognition of a multistage regulatory pattern. Each stage is distinct in the sense that its determinants do not seem to regulate events at a later stage (or at least not as a major contributor anymore) and the outcome is observed at a different morphological or functional level each time[2,3].

Initial sexual dimorphism is formed at a chromosomal level, since normal males will have a 46, XY and females a 46, XX DNA content. The role of X and Y as determinants of sex fate has long been recognized, leading to the distinction between autosomes (all the other chromosomes existing in homologous pairs in the nuclei of diploid cells) and gonosomes or sex chromosomes, i.e. the X and Y. This difference at chromosomal level is already installed at zygote formation, based on the content of the male contributing gamete, i.e. the spermatozoon, whereas the oocyte always bears an X sex chromosome.

The presence of a Y chromosome signifies development towards the male path. This may first be observed at a gonadal level. In the human fetus, the gonad is formed as a bipotential primordium or gonadal anlagen, dependent on the expression of major developmental genes also affecting adrenal and kidney development. This is followed by the differentiation towards an ovary or testis, i.e. gonadal dimorphism. The major determinant in this process is the *SRY* gene of the Y chromosome in males.

Downstream actions of SRY affect the subsequent development of most sex-specific structures. For instance, the *SOX9* gene family regulates local epithelial organization (spermatic cords, Seroli cells) while the *FtzF1* gene is responsible for the differentiation of steroid hormone producing cell populations (adrenals and gonads) and the *AMH* gene regulates the fate of the Muller duct products. A similar process occurs in females, with the absence of SRY action and the regulatory cooperation between *DAX1*, *FtzF1*/*SF1*, *SOX9* and *AMH*. It is interesting to note that, excluding *SRY* itself, no other major sex-related regulatory gene is actually unique to the male or female sex, the difference thus found at the time and level of gene expression[7].

Hormonal Actions in Sex Differentiation: The »Classics«

Sex-related hormones belong to two different categories. One of them includes a protein produced by the epithelial cells of the gonads, the Antimullerian Hormone or AMH (formerly known as the Muller duct inhibiting substance, or MIS). This molecule has long been studied in males as a determinant of internal genitalia. Indeed, it has been proven that AMH is produced by Sertoli cells following their differentiation in the testis via *SOX9* function. AMH may directly interact with the SRY and Sox family proteins due to the presence of a homeobox-related region. However, its receptors have also been recognized and thoroughly studied in terms of mutations that may be related to the various syndromes of resistance to AMH action. All these syndromes share the major common clinical manifestation of persistence of Muller duct products in the adult male. Indeed, the hormone is named after its most prominent quality, namely, the inhibition of further development of the Muller duct in male fetuses (i.e. 46, XY fetuses with an activated *SRY-SOX-AMH* pathway). Interestingly, AMH is also located in the female genitourinary system and experimental data suggest that its presence is necessary for normal ovarian development. The exact nature of AMH-SOX--DAX protein interactions and their role in the evolution and maintenance of the female sexual phenotype remains a debatable issue[8].

Other sex-associated hormones belong to the biochemical category of steroids. They are molecules of lipophilic nature, showing limited solubility in water--based solutions such as blood or the fluids of the extracellular space. Their circulation is based on the concomitant presence of binding agents-ligands, which form complexes with increased solubility in hydrophilic environment. For sex hormones, the major carrier protein is the sex hormone binding globulin (SHBG), a product particularly sensitive to the metabolic and hormonal equilibrium of the organism, with major differentiations of its value in extreme physiological states such as obesity and pregnancy.

The signaling pathway by which sex steroids, i.e. androgens, progesterone and estrogens mostly mediate their actions is based on intracellular receptors, named androgen, progesterone and estrogen receptors, or AR, PR and ER, respectively. It should be noted that the names suggest only the major sensitivity of each receptor and not a complete specialization. In other words, sex steroids and their receptors show some level of structural homology that results in the phenomenon of cross reaction, by which it is possible to observe actions of a sex steroid circulating in high concentrations (e.g. androgens) mediated through the receptors of a seemingly non-compatible receptor (e.g. ER or PR). In the case of androgens, it is also useful to note that a considerable number of their actions is also dependent upon their prior conversion to more active hormonal products. For testosterone, this is achieved via the activation of 5a reductase, leading to the formation of dehydrotestosterone or DHT, the most important contributor in the process of genital tract masculinization, including phenomena such as prostate development (morphological and functional maturity)[4,7,9].

It is also true that estrogens are also products of androgen metabolism, through the action of the P450 aromatase enzyme complex. This reaction is crucial for normal ovarian development and it requires the combined action of both of the gonad's major cell populations, i.e. the thecal cells, responsible for initial androgen production and the granulosa cells, sites of local aromatization and estrogen production. Modern data has shown that aromatization is maintained to some extend throughout female life and its limitation may result in masculinization phenomena even after menopause. In this case, the major site of aromatization is shifted from the aging ovary to the peripheral tissues. In particular, the adipose tissue has been shown to have extensive synthetic properties, some of which refer to tissue-specific hormones and paracrine messengers (adipokines) and others being homologous to hormones and cytokins also produced in other systems (e.g. resistin, TGFb, TNFa). In the case of sex steroids, the adipose tissue is considered a major site of production following menopause and ovarian follicle atresia. Aromatase activity is particularly increased locally, thus maintaing an adequate estrogen flow for the normal maintanance of a female phenotype (constant androgen/estrogen ratio). A recent finding of great potential implications refers to the possible existence of aromatization and sex steroid production sites in the central nervous system. If this is indeed so, then research will be faced with the challenge of the study of intracranial sex steroid metabolism and its potential in the establishment of sexual dimorphism throughout life[8].

Regardless of the target tissue, binding of a sex steroid to its receptor results in the formation of a complex, which is then shifted to the nucleus. Genomic actions of sex steroids are mediated via specific compatible regions in the DNA sequence named »hormone response elements or HREs« (e.g. androgen response element).

New Aspects in Sex Steroid Physiology

Until recently, genomic action was the main concept used to explain all sex steroid-mediated actions. Following the steroid: receptor complex binding to its special HRE, subsequent DNA manipulations was considered responsible for all sex steroid-associated actions affecting sex differentiation. These include formation of external genitalia, adipose tissue distribution, hair, bone and muscle development and distribution, testis descend in males and breast maturation in females[10–15].

Interestingly, recent studies revealed possible alternatives to this regulatory pathway. It has been suggested that estrogen actions are not necessarily mediated via the nucleus but also in an extragenomic sequence of events, which might involve a membrane receptor.

In addition, it has been shown that estrogens and epidermal growth factor (EGF) may share common messengers in their signaling pathway. This observation has been particularly useful to explain cases of cross-reaction in breast cancer patients treated with selective estrogen receptor modulators (SERMs). In this case, the expected/desired outcome would be a decrease in tissue growth, since estrogens, as previously mentioned, are a major contributor in breast development, bearing qualities similar to those expected of a growth factor. However, most patients show a gradual resistance to SERMs. It seems that this may partially be due to an increase in EGF and EGF receptor (EGFR) expression in tumor cells. The latter process implicates several molecules beyond the initial tyrosine kinase, leading to a different equilibrium in intracellular reactions that may actually eventually promote instead of inhibit tumor progress, in the presence of SERMs[16].

Moreover, significant conclusions can be reached via studies of androgen and estrogen receptor distributions in men and women of various age groups. Localization data have proven a much vaster presence and expression of AR and ER than previously assumed. Among the newly detected target-tissues for sex steroid action, one may distinguish, the lung, the liver and the central and peripheral nervous system (CNS and PNS, respectively), to name but a few characteristic examples[17].

In the case of the lung, ER type a presence has been described several years ago. In 1996, ER type 2 were also found in the tissue. Despite suggestions for a possible secondary effect and thus, limited importance for the organ's integrity, relevant research bloomed. More recent experimental data in this field from Erb knockout mice showed that absence of ER is directly responsible for abnormal lung development, with a large-scale distortion of alveolar micro-architecture. An analysis of local gene expression suggests that this phenomenon must be attributed to a generalized deregulation of several genes' action normally activated downstream the estrogen-ER-HRE pathway. These observations may also be useful in future advances in lung cancer treatment. In this case, EGF has already been studied as a potential target for oncological targeted treatment and some products are already avail-

able or under evaluation as therapeutic options. However, these recent findings for ER expression in the normal lung raise discussion for possible implications, such as the ability to attempt a combined chemotherapy strategy[18].

In the case of the liver, macroscopic and microscopic differences between the sexes have been known for decades. Recently, researchers attempted to explain these differences studying regional differences in the expression of hormones and growth factors. It seems that differences may include several target genes and receptors, including somatostatin (SS), a major inhibitory regulator already detected in the hypothalamus, the pancreas and the gastrointestinal tract. A local antagonist of SS action is the hepatocyte growth factor or HGF. It might also be possible to include other products in this multifactorial analysis of sex-associated differences in liver development. These might include insulin-like growth factors (IGFs) and ER expression. The exact manner by which all these different signals cooperate together and with external metabolic factors to achieve the dynamic equilibrium of liver function remains a challenge for current research in the field of Endocrinology[19].

Dimorphism in brain function has been a controversy for Neurosciences since the 19th century. Many observations have attempted to characterize specific modes of behavior as typically masculine or feminine, respectively, however, most of them were limited in few patient observations, largely biased by personal beliefs and mostly of subjective nature, thus considered inadequate for a modern scientific discussion. However, recent series have filled this gap, offering trustworthy data that prove statistically significant differences among the sexes in a behavioral level. These differences in cognitive functions are numerous, referring to arithmetic, positional and lexical skills, oral or written expression, synthetic and analytic procedures, determination and positioning in time and space and general state of memory. Overall assessment of this dimorphism in brain function leads to the conclusion of a general sex-specific organization of its function, described by the term »the sexual brain«.

Sexual Dimorphism in the Neural System: »Behavioral Sex« Revisited

Until recently, reproductive biology recognized a behavioral level in sex determination as a meta-hormonal regulatory phenomenon. By definition, scientists referred to the way in which each and every individual chooses to determine his/her sex and accepted the fact that it constitutes a final, distinct level in sexual dimorphism. This process was attributed to psychological, rather than organic factors. Recent data challenge this belief, claiming that at least some of the differences observed may also be explained via sex hormone activity in the CNS[20].

Furthermore, hormonal differences may constitute some kind of predisposition to homo- or heterosexuality, although there is no data suggesting that this is ultimately predetermined and emphasis is still placed on environmental influences and personal choice. Among the different parts of the CNS showing anatomical and functional dimorphism the most characteristic ones are the hypothalamus, the amygdala and the bulbocavernosus nucleus.

In terms of AR and ER expression in the CNS, immunohistochemical and *in situ* hybridization techniques have detected their presence in different parts of the system, depending on both the sex of the individual and the age at the time of study. Perinatally, estrogen receptors have been detected in the preoptic area and the anteroventral periventricular nuclei of the hypothalamus. On the other hand, throughout life, the septal AVP is positive for both androgen and estrogen receptors. The same is true in animal models for the nucleus robustus archistriatum. Contrary to these examples, the spinal bulbocavernosus nucleus appears to exclusively include androgen receptors. In adults, the posterodorsal medial amygdala is another sex-hormone sensitive region, with the expression of both ARs and ERs.

The role of progesterone in CNS has also been studied. It seems that PRs are not expressed locally. Nevertheless, progesterone may still mediate reactions in the area. This is attributed to its capacity to bind directly to a subunit of GABAnergic neurons, which are amply distributed in the CNS. Thus, progesterone may actually be a major player in sexual dimorphism, affecting both peripheral organ function and maturation in the female (e.g. breast, endometrium) and central regulation of dimorphic behavior in both sexes[21].

The sex brain model becomes even more attractive by observations of local steroid metabolism in the CNS. Indeed, aromatization has been detected within the brain. Several masculinizing actions of androgens in the brain seem to result from aromatization of testosterone to estrogen, further enhancing the theory of steroid hormone cross-reactions as the basis of sex-specific variation in humans. An interesting biochemical observation refers to the interaction between estradiol and a-fetoprotein (AFP). The two molecules form a complex, which, in the female, may inhibit estrogen access to the CNS and brain masculinization. To which extend such a process may be important for the pathophysiology of syndromes with increased AFP production remains a question. In any case, such an inhibitory effect would limit estrogen activity in the female considerably, allowing only reactions that mediated via local aromatization within the CNS. Ectopic androgen action would also be possible, through the previously presented cross-reaction model for steroid hormones.

It has been suggested that the maintenance of an optimal androgen: estrogen and AR: ER activity ratio in the brain is crucial for micro-environmental stability and prolonged neuronal survival. Distortions in this equilibrium may promote local cytotoxic effects, leading to apoptotic phenomena. Such a procedure may also justify differences between the gross anatomy of the CNS in the two sexes, as well as between hetero- and homosexual individuals. Although limited in few, very specific nuclei and regions of the brain, these differences must be an

adaptive developmental phenomenon, since they are not related to any obvious functional disadvantage and they are not extended to any other parts of the CNS or the rest of the body. Among the theories suggested for these cases of anatomical dimorphism, it is interesting to note the suggestion for a possible history of *in utero* exposure to high concentrations of sex steroids[21].

Naturally, all homosexual individuals do not have a history of exposure to androgens or estrogens, respectively. Additionally, differences in behavior and practice between the sexes, as well as between homosexual and heterosexual individuals extend beyond those affected by the few brain regions discussed above.

In effect, this implies that hormonal regulation of sex behavior may not be the only factor responsible for all the differences observed[22]. Further research will be necessary to clarify the exact role of hormones in sex determination and differentiation. Understanding the complexity of the regulatory patterns involved in this process is a necessary tool for the explanation of various pathophysiological phenomena affecting sex physiology as part of their clinical manifestations. This goal promises to allow the development of new therapeutic agents for sex-related diseases and the targeted, aetiological treatment of all affected individuals in the near future.

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HORMONSKE I METAHORMONSKE DETERMINANTE SPOLNOG DIMORFIZMA

SAŽETAK

Uloga hormona u utvrđivanju spolnih karakteristika poznata je već nekoliko desetljeća. Dokazano je, na primjer, da pojedini produkti, uključujući spolne steroide, mogu utjecati na obrazac tjelesnog razvoja, metaboličke putove, distribuciju mišićnog i masnog tkiva te anatomiju glasnica, stvarajući sveobuhvatni ishod u skladu s muškim ili ženskim fenotipskim obrascem. Takve odlike obično se opisuju kao sekundarne spolne osobine, a kako bi se razlikovale od primarnih spolnih osobina, obično se odnose na gonade i vanjske genitalije. No, mora se spomenuti da hormonska regulacija ne može objasniti cjelokupni opseg spolnog fenotipa jer središnji živčani sustav zadržava značajnu ulogu u uspostavljanju spolnog identiteta, dajući povoda višem stupnju spolne determinacije, posebno opisane kod ljudi kao bihevioralni ili psihološki spol. U novije vrijeme predloženo je da razlike među spolovima nisu ograničene na moždanu funkciju, nego se mogu odnositi i na anatomske razlike te biokemijske profile, uključujući različite obrasce AR i ER distribucije. To novo stajalište o spolnom dimorfizmu predlaže čitav sustav metahormonske regulacije, u novije vrijeme opisane kao model spolnog mozga (engl. the sexual brain model). Uloga koncentracije lokalnog androgena i/ili estrogena u početnoj uspostavi spolnog dimorfizma još se proučava jer su prvi rezultati relativno neuvjerljivi te do danas nije dokazan direktna poveznica. S druge strane, nađeno je da spolni hormoni sudjeluju u procesu koji je daleko od njihovog početno predloženog spektra djelovanja. Na primjer, ER međusobno djeluje s EGFR-om na puno načina, utječući na razvoj određenoga broja epitelnih struktura. Estrogenski receptori također su detektirani u organima kao što su mozak i pluća. Ovo istraživanje može implicirati na to da spolni dimorfizam ide još dublje nego što je prethodno utvrđeno, utječući praktično na sve organske sustave, predlažući, u osnovi, postojanje dvaju različitih funkcionalnih modela za čitavo ljudsko tijelo, izraženih i očuvanih kroz proces evolucije.

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Review

DNA Methylation as a Regulatory Mechanism for Gene Expression in Mammals

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A B S T R A C T

Epigenetics refers to the study of heritable changes in gene expression that occur without a change in DNA sequence. In the last decade, it has been shown that epigenetic mechanisms provide an »extra« layer of transcriptional control that regulates genes expression. Three distinct mechanisms appear intricately related in initiating and sustaining epigenetic modifications: RNA-associated silencing, DNA methylation and histone modification. These mechanisms are critical components in the normal development and cell growth. DNA methylation is involved in transcriptional silencing of genes, regulation of expression of imprinted genes, a number of tumour suppressor genes in cancer and silencing of genes located on the inactive X chromosome. In this review, we are focused on the basic principles of DNA methylation as the main epigenetic mechanism for normal embryonic development and epigenetic alterations that contribute to carcinogenesis.

***Key words**: DNA methylation, genomic imprinting, cancer*

Introduction

Genomic DNA represents the base of cellular genetic information contained in a sequence of four nitrogen bases: adenine, guanine, thymine and cytosine[1]. The sequence of these four bases determines the primary structure of DNA, but it still does not mean that the existing sequence is really going to be transcribed and translated, i.e. the sequence would undergo expression[2]. Despite the fact that every single cell of our body contains the identical set of chromosomes, there are big differences among these cells both in their shape and in their functions. So, the cells of a multicellular organism are genetically homogeneous, but structurally and functionally they are heterogeneous due to differential gene expression[3]. It implies that these cells activate only those genes which are indispensable for their functioning and survival, while the rest of genes remain inactive[4]. Such a pattern of differential gene activation has been established very early during the period of embryonic development, and further on it is stably inherited from one cell generation to the next[5]. It is clear therefore, that there must be an additional level of control over the observed genetic activity, totally independent from the primary DNA structure[6]. Such variability of gene expression which is not the consequence of a change in DNA sequence is the topic of interest for epigenetics, the term used for the first time in the fifties by Conrad Waddington[7,8]. Epigenetic inheritance may be described as the transmission of information from a cell or multicellular organism to its descendants without that information being encoded in the nucleotide sequence of the gene. All of our cells contain the same number of genes; however, in a given tissue and at a given state, owing to an epigenetic code, only some of these genes are expressed, giving rise to the phenotype. Three systems, including DNA methylation, histone modification and RNA-mediated action, are considered today of having roles in regulation of gene expresion[9]. Upsetting any single factor of this interactive system could bring about inadequate gene expression and consequently present epigenetic diseases[10].

Here we are going to concentrate primarily on DNA methylation as one among the essential mechanisms for regulation of gene expression in mammals, and the other stakeholders in this story will be mentioned only for sake of better understanding of the mechanisms of action of methylation on gene expression.

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The Process of DNA Methylation

Methylation process is one among the key mechanisms for modification of DNA molecule, and hence the epigenetic control of gene expression in vertebrates11. Such a regulatory mechanism allows a cell to stop transcription, ensures inactivity of the majority of genes of one X chromosome of the female organism, and enables the process of genomic imprinting, as well as protection of the endogenous genome from eventual intrusion of a parasitic genome[12,13]. The very mechanism of methylation refers to the binding of a methyl group to the 5th carbon atom of the cytosine ring, and is carried out with the help of the enzyme DNA methyltransferase (Dnmt). The result is the formation of a new base, 5-methylcytosine (5mC)[14,15]. Addition of the methyl group is a process which takes place immediately after replication, and is completed within 1 minute after completion of replication[16,17]. The result of such modification changes the affinity for particular transcription factors towards DNA molecule which prevents the formation of the transcriptional initiation complex, or elongation of those already initiated, that is gene silencing[18].

DNA methylation typically occurs in a CpG dinucleotide regions of DNA[19]. Assuming random distribution of nucleotides the probability of a cytosine and guanine lying next to each other is very high. However, there are actually very few CpG sites in eukaryotic genomes[20]. This is due to the action of DNA methyltransferase, which recognizes these CpG sites and methylates the cytosine, turning it into 5-methylcytosine. Following spontaneous deamination, the 5-methylcytosine converts into thymine. If this has no effect (as in most cases), the error is not recognized by the repair machinery, thus resulting in the loss of the CpG site. CpG sites thus tend to be eliminated from the genomes of eukaryotes[21].

In mammals there are so far discovered 5 members of the family of DNA (cytosin-5) methyltransferases, enzymes involved in the methylation of CpG sequences: Dnmt 1, Dnmt 2, Dnmt3a, Dnmt 3b and Dnmt3L[22,23]. Dnmt1 is the proposed maintenance methyltransferase that is responsible for copying of the already established methylation pattern by recognizing the hemimethylated sites in DNA helix, and it is present always in replication forks of the cells undergoing division[24,25]. Inactivation of this enzyme in mice brings about the global loss of methylation and abnormal biallelic expression of imprinted genes[26,27]. In contrast to Dnmt1, the biological activity of Dnmt2 does not demonstrate affinity towards CpG sequences, and knock-out mice for this gene do not show recognizable abnormalities[28]. It is assumed that this enzyme plays some role in DNA methylation, but it appears not to have any DNA methyltransferase activity[29]. Dnmt3a and Dnmt3b participate in processes of *de novo* methylation that sets up DNA methylation patterns early in development.. Kaneda et collaborators reported the disruption of Dnmt3a and Dnmt3b in germ cells, with their preservation in somatic cells, by conditional knockout technology. Offspring from Dnmt3a conditional mutant females died in utero and lacked methylation and allele-specific expression at all maternally imprinted loci examined. Dnmt3a conditional mutant males showed impaired spermatogenesis and lacked methylation at 2 of 3 paternally imprinted loci examined in spermatogonia. By contrast, Dnmt3b conditional mutants and their offspring showed no apparent phenotype. The phenotype of Dnmt3a conditional mutants is indistinguishable from that of Dnmt3L knockout mice, except for the discrepancy in methylation at 1 locus (IG-DMR). The conclusion was drawn that both Dnmt3a and Dnmt3L are required for methylation of most imprinted loci in germ cells, but other factors are probably involved[30].

Beside these randomly distributed CpG sites there are regions of the genome which contain extremely high concentration of CpG sites. The concentration of these dinucleotides is more than ten times higher than in the rest of the genome[31]. These regions, known as CpG islands, are found at the promoters of 50–60% of all human genes[32,33]. Surprisingly, these CpG sites are unmethylated, and therefore any spontaneous deaminations of cytosine to uracil are recognized by the repair machinery and the CpG site is restored.

The question is being raised on how does the DNA methylation influence gene expression, i.e. how is the gene repression acquired by the mechanism of DNA methylation? It is considered that the repression proceeds in two ways: (1) directly – the methylation of CpG sequences changes the recognition site for a particular transcriptional factor, so that it does not recognise it any more and does not bind to it, and (2) indirectly – by binding of specific proteins which have affinity for methylated CpG sequences[34]. These are the proteins which bind to methylated CpG groups (methyl-CpG binding proteins, MeCPs), via a domain responsible for binding to CpG sequences (methyl-CpG binding domain, MBD), and are consequently called MBD1, MBD2, MBD3, MeCP1 and MeCP2[35,36]. MeCP2 is a transcription factor that recognizes and binds to a symmetrically methylated CpG dinucleotide[37]. It is also a component of the histone deacetylase complex[38]. MBD1 is included in histone deacetylation, too, while MBD2 is a part of MeCP1 protein complex which mediates the methylation-dependent repression of transcription[39,40]. The indirect repression of transcription which includes the mentioned proteins is undoubtedly connected with the degree of histone acetylation[41]. Every histone contains a domain which is responsible for mutual histone interactions and winding of DNA around them, and an aminoterminal end which protrudes out of the nucleosome, by which histones communicate with other regulatory proteins[42]. The aminoterminal domain is rich in lysine, which are the most frequent targets for acetylation, which in turn markedly decrease the affinity of histone H4 for DNA[43]. It is being considered that the histone acetylation is the molecular mechanism by which DNA becomes generally available for trans-regulatory factors, and at the same time retaining further the nucleosomal architecture[44]. DNA methylation together with such chromatin organisation contributes to repression of transcription by stimulating

binding of MeCP2 and the recruiting complex of histone deacetylation[45,46]. This suggests that specific behaviour of chromatin containing methylated DNA region forms molecular key-lock which might permanently silence down the transcriptional process[47]. Capacity of DNA methylation to increase the repression of transcription by reorganizing chromatin, largely contributes to genome division into hetero- and euchromatin in differentiated cells[48]. Covalent modifications of nucleosomal histones, like acetylation, methylation, phosphorylation and ubiquitinalization, comprise unique and sufficient gene configuration, promoting the idea which subordinates the genetic code to superior epigenetic regulatory system known as the »histone code«[49,50]. »Histone code« is represented by histone modifications like methylation of lysine 9 in H3 histone (H3mK9) and absence of acetylation of histones H3 and H4 in heterochromatin, and the other way around methylation of lysine 4 in histone H3 (H3mK4) and acetylation of histones H3 and H4 in euchromatin[51,52].

DNA Methylation and Embryonic Development

Embryonic development in mammals demonstrates the bimodal reprogramming of DNA methylation, which takes place in primordial germ cells (PGC), where the loss of methylation is connected with the creation of new methylation pattern specific for male or female gamete. After fertilization and during the preimplantation period the loss of methylation enables establishment of the totipotency of zygote[53]. Upon entrance into germinal ridge, particularly methylated PGC, undergo rapid demethylation followed by *de novo* methylation, and so the mature gametes display a high degree of methylation[54,55].

Surprisingly, recent data showed the ability of an environmental factor (for example, endocrine disruptor) to reprogram the germ line and to promote even a transgenerational disease. Namely, transient exposure of a gestating female rat during the period of gonadal sex determination to the endocrine disruptors induced an adult phenotype in the F1 generation of decreased spermatogenic capacity and increased incidence of male infertility. These effects are transferred through the male germ line to nearly all males of subsequent generations up to F4. The effects on reproduction correlate with altered DNA methylation patterns in the germ line[56].

Not only environmental factors, but even nutritional intervention is connected to altered DNA methylation patterns with transgenerational effects[57]. The finding is remarkable because it suggests that a pregnant mother's diet can affect her health in such a way that not only her children but her grandchildren and possibly great-grandchildren inherit the same health problems.

In another study in northern Sweden grandparent's prepubertal access to food was correlated with diabetes and heart disease. If food was not readily available during the father's slow growth period, then cardiovascular disease mortality of the proband was low. Diabetes mortality increased if the paternal grandfather was exposed to a surfeit of food during his slow growth period[58].

Nevertheless, there are still strong evidences that after fertilization the loss in DNA methylation takes place in both pronuclei. This methylation decline is stronger in paternal DNA which becomes demethylated several hours after fertilization, so it is considered to be the consequence of active demethylation, despite the fact that enzymes responsible for this active demethylation are not identified up to now[59]. Parallel with this process, the exchange of protamines with histones is taking place in paternal pronucleus[60]. Maternal pronucleus also undergoes demethylation, but it is more gradual and diminishes after each replication cycle for the lack of Dnmt1 responsible for the maintenance of methylation pattern[61]. Such a replication-dependent decline in methylation is called passive demethylation[62]. The second wave of methylation reprogramming takes place during the period between fertilization and appearance of blastocyst[63].

The crucial role of proper DNA methylation pattern changes during development was showed by our work with 5-azacytidine, a demethylating agent which was administered to pregnant rats in different stages of gestation. After application on day 12 and day 13 of pregnancy survival of foetuses was drastically reduced and limb malformations were present[64]. Moreover, the placentas were influenced as well when the methylation pattern was disturbed, in a way that not only the placental structure, but even the expression of different glycoprotein's was disturbed (own unpublished results).

Imprinted Genes and Human Disorders

Despite such a global loss in methylation, certain genomic sequences are excepted from this process in the preimplantation embryo. It is primarily about the imprinted genes whose expression depends on the fact whether they are inherited from mother or from father e.g. they are monoallelically expressed in contrast to most genes where two alleles contribute equally to the production of gene product[65]. The first experiments which have indicated the existence of imprinted genes in the mammalian genome were carried out by McGrath and Solter, by establishing the method for transplantation of nuclei into enucleated mammalian oocytes[66]. These experiments have shown that the prerequisite for the normal embryonic development of mammals is the genetic information contained in gametes of both parents. Gynogenetic embryos obtained by combining two female pronuclei were small and morphologically normal, but without extraembryonic membranes and were spontaneously aborted[67]. On the other hand, androgenetic embryos obtained by combining two male pronuclei developed normal extraembryonic membranes, but the embryonic part was reduced, a phenomenon almost identical to that occurring occasionally in abnormal human pregnancy, when instead of normal embryo one finds formation called mola hydatidosa[68]. Mola is aggregate of

extraembryonic membranes without presence of foetal tissue, and its chromosomal composition is mostly that of the father[69,70].

The mentioned data speak in favour that the expression of each of the parental genomes is different, and it is crucial that they mutually complement each other if we want to have the normal development[71]. The question is raised whether mammalian zygotes for their normal development require the presence of total paternal and maternal genome, or only of particular chromosomes, or even some critical genes. Experiments with mouse embryos which contained rearranged parts of some chromosomes, showed that particular genes or groups of genes are active when inherited from one parent, and inactive when inherited from from parent of opposite sex[72].

The first discovered imprinted genes were insulin-like growth factor 2 receptor (*Igf2r*) gene[73] discovered in the year 1991 and a few months later *Igf2*. At the time DeChiara and collaborators have observed that targeted mutation in this gene results in dwarfed growth in heterozygotes, but only in the case when the paternal gene is involved (and not the maternal gene). The reduced growth in the newborns was identical in heterozygotes with mutant paternal gene and in recessive homozygotes, indicating that only father influences the activity of *Igf2* gene[74]. Discoveries of new imprinted genes followed, particularly after establishing the method for restriction landmark genome scanning (RLGS) which was based on the fact that the „imprinting« is connected with methylation of CpG dinucleotides identifiable using this method[75].

The imprinted genes display certain, for themselves specific rules like the fact that so far identified genes are not randomly dispersed in the genome as singular genes, but display tendency for grouping[76]. One among the largest groups is located on the distal end of the mouse chromosome 7 as well as on the proximal end of the human chromosome 11[77]. In the majority of such groups one finds interwoven maternally and paternally imprinted genes[78]. To date over 70 human imprinted genes have been identified of a total of 100–200 expected in the whole genome. These imprinted domains are regulated co-ordinately, via long-range mechanisms such as anitsense RNA interference and methylation-sensitive boundary elements. The largest group of imprinted genes is located on the X chromosome[79,80]. It is known that in the female mammals, the dose compensation for genes on X chromosome is acquired by inactivation of one X chromosome in all somatic cells[81]. Although this inactivation is random, it appears that in the extraembryonic tissues the paternal X is turned off more frequently, with the only exception of *Xist* gene, which represent the inactivation centre and is the only active gene on the inactive chromosome, and *vice versa* the only inactive one on the active chromosome[82,83]. In any case inactivation of X chromosome as well as genomic imprinting of autosomal genes generates functional hemizygotes[84].

It seems that reason for grouping of imprinted genes in the genome is that the control of their expression is executed from the single cis-regulatory sites called imprinting control regions (ICRs) or differentially methylated domains (DMDs). Their distinctive characteristic is that they are always methylated on one of the two parental alleles[85,86]. The deletion of these sites eliminates imprinting and consequently both alleles are expressed[87]. Particularly interesting and well studied is the ICR between H19 and Igf2 genes on the human chromosome 11, methylation of which may be responsible either for activation or inactivation of the above genes depending on the choice of whether it is methylated on the maternal or paternal chromosome[88]. The behaviour of these two genes illustrates how differently they may react depending on the state of methylation of the control region which is located between them[89]. This region is methylated on the paternal chromosome and consequently *H19* is inactive, while *Igf2* is expressed and active[90]. On the contrary, on the maternal chromosome the same region is not methylated, so now *H19* becomes active, and *Igf2* inactive[91]. In this case the control region behaves as an insulator. The insulators are regulatory elements which prevent the activation or inactivation of genes, and can be found between promoters and enhancers, or even on the border between transcriptionally active euchromatine and inactive heterochromatine[92,93]. When the ICR is unmethylated, it is the binding site for CTCF which belongs to the group of transcriptional regulatory proteins with the Zink finger motif, by which it prevents activation action of enhancer upon the promoter of *Igf2* gene[94]. Here we see an unusual way of regulation of gene expression, where methylation indirectly activates the gene by blocking the insulator[95]. Regulation of *H19* expression in much more common because its methylation creates inactive, i.e. imprinted site, so the methylation directly affects the activity of the promoter[96].

The second significant characteristic of imprinted genes is their temporarily uncoordinated (asynchronous) replication in relation to the active alleles on the homologous chromosome[97]. It has been observed that *Igf2* gene and gene for its receptor (*Igf2r*), as well as *H19* and SNRPN are replicating earlier in S-phase of the cell cycle if they are of paternal origin[98]. Even though the time of replication within the cell cycle very frequently correlates with the degree of gene expression, so that active genes are mostly replicated at the beginning of the S-phase, this is not the general rule, inasmuch as *H19* and *Igf2r* normally transcribed from maternal chromosome are replicated earlier on paternal chromosome[99]. In reality, it appears that, within the so far studied imprinted genes, and irrespective of the degree of gene expression, the paternal allele always replicates first, which has been demonstrated by the method of fluorescent *in situ* hybridization (FISH) suitable for visualization of particular genes during S-phase of the cell cycle[100,101].

It is surprising that almost half of the so far discovered imprinted genes does not code for proteins but for untraslated RNA. So the RNA molecule of the *H19* gene expressed only from mother is indispensable during embryonic development and required for imprinting of the

other two genes which are transcribed from paternal chromosome[102]. Imamura and collaborators have proved that RNA which is not translated may induce demethylation of the tissue specific differentially methylated domain, which might serve as a potential new tool in epigenetic manipulation of mammalian cells[103].

Importance of methylation and consequently of genomic imprinting is unquestionable for normal functioning of an organism, which is documented by numerous diseases associated with inadequate methylation or with mutation of enzymes responsible for methylation and by them mediated repression of transcription[104]. Classical examples associated with the phenomenon of genomic imprinting are two rare diseases which are a mirror image of each other[105]. Both include a disturbance in growth and behaviour, and are called Angelman and Prader-Willi syndromes[106]. They are both caused by the identical chromosomal deletion of chromosome15[107]. When the deletion is inherited from the mother it results in the phenotype of Angelman syndrome, while the same deletion inherited from father gives rise to Prader-Willi syndrome[108,109].

The loss of genomic imprinting of the chromosome 11p15.5 presents the Beckwith-Wiedmann syndrome which is characterized by embryonic tumours, excessive growth of abdominal organs, macroglossia and omphalocoela[110]. Mutation of the gene MeCP2 and enzyme included in methylation-mediated repression of transcription, brings about the neurological disease known as Rett syndrome which affects one out of 10.000 newborn girls, though up to now the effect of sex on development of this disease has not been discovered[111]. It is considered that the mutation of this enzyme brings about disturbance in differentiation of primary neurones[112]. Consequently microcephalia, ataxia, and tonic clonic seizures are developed[113].

Epigenetic reprogramming is considered today as one among the most significant barriers to cloning, because when a differentiated somatic cell nucleus is put into oocyte, its genome-wide epigenetic pattern must be reprogrammed in order to restore totipotency. The difficulties associated with reprogramming in chromatin, histones, and methylation patterns along the entire length of the DNA sequence may explain why so many cloned embryos have so many developmental failures. And even after assisted reproduction there is elevated incidence of diseases associated with imprinting[114,115]. The reason for this may be because these methods, and particularly *in vitro* fertilisation (IVF) and intracytoplasmyc sperm injection (ICSI), include isolation, handling and cultivation of both gametes and embryos in early embryonic phase, when the genome methylation pattern changes dynamically, so the above mentioned cells are found themselves in a particularly vulnerable period during which this pattern might be disturbed[116,117]. The number of the up to now reported cases connected with these issues is too small to enable making any generalized conclusions, but in any case requests further scientific engagement in order to eventually eliminate the problems connected with genomic imprinting and the methods of assisted reproduction[118].

DNA Methylation and Carcinogenesis

During the past several years the interest of many scientists who are engaged in epigenetics has broadened into the field of carcinogenesis, because the status of DNA methylation changes during life, hence affecting the expression of genes associated with development of cancer[119,120]. Epigenetic silencing as the consequence of aberrant methylation of promoter regions, and CpG islands results in the loss of function of tumour suppressor genes[121,122]. Tumour cells frequently show increased activity of DNA methyltransferases, which are responsible for hypermethylation of promoters of these genes[123,124]. But there are no genetic proofs that would indicate the ectopic *de novo* methylation in cancer, neither for DNA methyltransferase genes mutations. Instead it is hypothesized that the cause is somewhere in the transcriptional machinery or in a signal transduction pathway genes[125]. So Butcher et al. consider that inactivation or disruption of these insulators may facilitate an epigenetic »hit«, in this case DNA methylation, leading to down regulation of tumor-supressor gene (e.g. BRCA1) contributing to tumorigenesis[126]. It follows from the above mentioned, that the epigenetic changes, i.e. the silencing of tumor-supressor gene promoters by methylation would trigger carcinogenesis. However, it is known that DNA methyltransferase gene mutation does not change the frequency of tumour appearance[127]. In some cell lines of colorectal carcinoma one mutant allele of *p16* gene has been found, which is therefore non-functional, but expressed, while its homologue was hypermethylated and totally silenced[128]. As the methylation can be detected very precisely with contemporary techniques, and the extent of methylation established in any sort of cells, the connection of methylation with appearance of cancer gives hope for discovery of new potential ways for prevention and treatment of these diseases[129].

Recent evidence indicates that epigenetic changes might 'addict' cancer cells to altered signal-transduction pathways during the early stages of tumour development. Dependence on these pathways for cell proliferation or survival allows them to acquire genetic mutations in the same pathways, providing the cell with selective advantages that promote tumour progression. Strategies to reverse epigenetic gene silencing might therefore be useful in cancer prevention and therapy[130]. Moreover, DNA demethylating agent 5-azacytidine has been currently used in human myelodisplastic disorders therapy[131]. Being aware that this agent has a detrimental effect upon mammalian embryonic development *in vitro*[132] and *in vivo*[133,134] one must be careful while using it on humans because of its teratogenic side effects.

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REGULACIJA EKSPRESIJE GENA U SISAVACA

S A Ž E T A K

Nasljedne promjene u ekspresiji gena koje se nasljeđuju neovisno o promjenama slijeda baza u molekuli DNA definiraju pojam epigenetike. Zadnja dekada, pokazala je da epigenetski mehanizmi omogućavaju dodatnu regulaciju ekspresije gena na razini transkripcije. Tri sistema, uključujući metilaciju DNA, modifikaciju histona i RNA-posredovano djelovanje, danas se povezuju s regulacijom ekspresije gena. Oni su kritični regulatori kako embrionalnog razvoja tako i samog rasta stanica. Proces metilacije molekule DNA predstavlja epigenetski mehanizam uključen u inaktivaciju transkripcije gena, genomski imprinting, osigurava neaktivnost većine gena na inaktivnom X kromosomu u stanicama ženskog organizma kao i tumor supresor gena u raku. Ovim pregledom iznjeli smo osnovne principe metilacije DNA molekule kao glavnog epigenetskog mehanizma regulacije genske ekspresije, s težištem na njezinoj ulozi u normalnom embrionalnom razvoju te poremećajima koji dovode do razvoja raka.

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Review

Nutritional Studies in Croatia – A Century of Research

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A B S T R A C T

This paper is dedicated to the pioneers of nutritional research in Croatia: to Professor Edvin Ferber, Professor Hubert Maver and Professor Ratko Buzina, to whom we owe exceptional contribution in the development of science of nutrition, as well as for many scientific publications from the fifties to the eighties in the 20th century, leaving us great information about nutritional state in Croatian population. The paper brings a review of nuitritional research in Croatia with an emphasis on history of research and papers published in Collegium Antropologicum. Since first publications on the subject, a number of institutions and scholars participated in numerous research projects which resulted in a vast number of published papers, depicting a multidisciplinary approach to the subject. In addition, the results of 44 analyses that have been a part of doctoral (18) and master's research (26) are discussed.

Key words: *nutritional research, anthropological history, Croatia, literature review*

History of Nutritional Research

Through civilizational history, man has been experiencing many processes, in which he has been creating nutritional habits in a constant fight against hunger, social and civilizational differences, so today we can regard nutrition as a part of the culture of each nation and individual, as well as a fundamental factor in watching health and fighting against diseases. The history of nutritional research has started in old Greece with Hippocrates (460–370 B.C.) (Figure 1), who was the first one who realized the importance of nutrition for good health and against diseases.

Erasistratus (330?–250? B.C.) was the first one who experienced with metabolism, and only after a long period, Galen (129–200 AD) (Figure 2) showed scientific interest for nutrition science. Many centuries have passed until the physicist Santorius Koperski (1561–1636) improved nutrition science. Subsequent development of nutrition science was continued by John Mayow (1641–1679), an English physicist, whose research was based on the investigation of the work of muscles and their connection with burning out of some substances that were unknown to him. In 1753, James Lind (Figure 3) made a famous experiment on twelve sailors suffering from scurvy, and he found out that orange and lemon had positive effects on curing that disease.

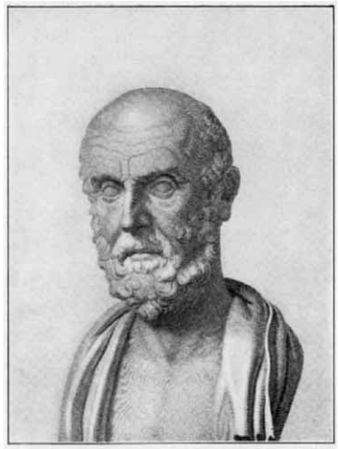

Fig. 1. Hippocras (460–370 BC).

Antoine L. Lavoisier (1743–1804) (Figure 4), a French chemist, was the first one who discovered the process of oxidation in metabolic decomposition of food, and was acknowledged as an establisher of modern nutrition sci-

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Fig. 2. Galen (129–199 BC).

Fig. 4. Antoine L. Lavoisier (1743–1804).

Fig. 3. James Lind.

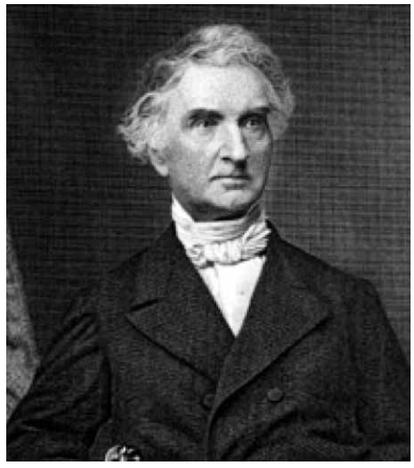

Fig. 5. Justus Liebig (1803–1873).

ence. William Prout (1785–1873) discovered hydrochloric acid in stomach, and found out that man is composed of proteins, hydrocarbons and fats, so he concluded that man must take a daily dose of these compounds.

Justus Liebig (1803–1873) (Figure 5) showed that human body consists of three types of organic substance liable to oxidizing processes. Much credit goes to Max Rubner (1853–1932) (Figure 6) for the development of nutrition science in the 19th century, as he was the first one who evaluated caloric values of proteins, hydrocarbons and fats, after the first calorimeter had been invented by an English scientist Atwater in 1832. Emil Fishere (1832–1919) explained the synthesis of proteins and hydrocarbons, and together with Frederik Hopkins (1861–1947), defined the primary role of amino acid as a component of proteins. The term »Vitamins« was introduced by Kazimir Funk, although Japanese officer Takaki and Christian Eijkman had been already engaged in the concept of vitamins.

Outstanding research on the development of digestive physiology was carried out by Claude Bernard (1813–1878) and Ivan Petrović Pavlov (1849–1936). Herman Helmholtz (1821–1894) confirmed transmission of energy during the work of muscles. Quick development of nutrition science in the 20th century is indebted to great scientific discoveries like X rays, radioisotopes, ultrasonography, magnetic resonance and modern biochemistry, up to genetics, molecular biology and immunology, on which principles was researched the nutritive influence on health and various diseases. Today we must point out the importance of multidiscipline, where we could have nutritionists, medical doctors, anthropologists, food technologists, veterinarians, agronomists, biologists etc, all of them in an ideal investigating team[1–4].

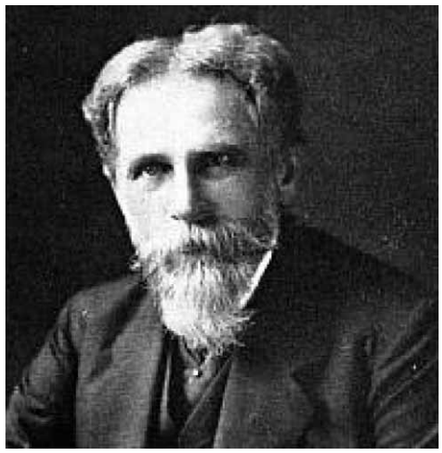

Fig. 6. Max Rubner (1853–1932).

Biomedical Nutritive Research

Biomedical nutritive research might be different: 1) directly connected with nutritive factors and the way they influence different aspects of biology or ecology of human population, or 2) they might be connected with man's nutritive adaptation.

Nutritive studies are defined through the research of various other studies, like nutritive, biochemical, anthropometric and clinical studies, with the aim of defining health state of an individual or the whole population. Apart from defining health state, nutritive studies can be very useful to anthropologists, even for research of human adaptability that is reaction on various physical, cultural and environmental stresses. The main questions imposed on anthropologists – nutritionists are: malnutrition, lack of specific nutriments, fatness related to different populations, socio-economic status, preservation of species, ecology of a disease and its connection with nutrition and nutritive status, sensitivity to infectious diseases, as well as to the children's growth and development. The methods accessible to nutritionist research can be divided into two main types: those concerned directly with food consumption and those concerned with nutritional status of an individual or a population. For the first type of research, the most important thing are figures about nutritional habits of various social sectors, the differences in nourishment during the season, age, sex or reproductive status. The methods of the first type of research are: 24 hours recall, estimated food record, weighed food record and food frequency questionnaires. Such researches are liable to a mistake, that's why they are mostly combined with the researches of the second type. The second type of research is based on anthropometry, defining composition of the body, biochemical, immunological and clinical methods. The most widespread researches are anthropometric, because of their simplicity and moderate expenses, and the most widespread measure is relative weight, which denotes the percentage of deviation from standard values for certain age, sex and height. The anthropometry is enough for measuring children, but there are two essential variables for classification of adults, both recommended by Ferro-Luzi and Waterlow, one for determining the mass of the body, BMI (body mass index, weight divided with height), and another one for determining the level of physical activity, PAL (total daily energy expenditure divided by basal metabolic rate). The measure of body composition is useful for evaluation of fatness and thinness, and measures of skin wrinkles are mostly used for this method. Biochemical tests are one of the essential indicators of nutritive state. Plasma albumin tests are mostly used from the wide range of such tests, and we use them to get results about nutritive state of proteins and hemoglobin, used for measuring the quantity of ferro-B-complex, ascorbic acid, copper and cobalt. Biochemical tests are also used for measuring nutrients in body fluids and tissues, which includes urine, hair, sperm, nails and skin. Immunological tests measure ability of strengthening immunological system on certain antigenetic stimuli, and weakening of immunological reaction is mostly combination of stress, lack of nutrients and infectious diseases. Clinical researches are used in detecting already serious nutritive deformations, so they are based on examining skin, eyes, hair, mouth, parathyroid and thyroid gland. Nutritive researches are the basis for making schemes, and the first one was made by »Food and Nutritional Board«, American academy of science in 1940, with recommendations that should be guidelines for regular planning of nourishment[5–8].

Socio-Culture of Nutritive Research

Socio-cultural researches include description and history of nutritive habits. Researches of socio-cultural type investigate nutritive habits in a cultural environment, and introduce us to the symbolic meaning of food in certain regions. Socio-cultural researches include history and description of nutritive habits, as well as nutritive behavior within a cultural group. These researches describe nutritive habits in certain cultural environment, and they give detailed description of preparing and serving traditional dishes. Croatia is definitely very interesting for such researches, because of great varieties of regional characteristics[9]. As an example of such researches we can point out investigations of traditional nourishment of the island Pašman[10], homesteads in Dalmatia[11], country nourishment in Baranja[12], Žumberk[13] and Cres[14], as well as cultural description of nourishment in different parts of Croatia[15]. In 1984 and 1989, Lazarević[16,17] introduced in her researches necessity for multidiscipline nutritive study, in other words, she introduced cooperation between cultural anthropologists and nutritionists, while Škreblin and Sujoldžić conducted researches of multidisciplinary type in 2002[18].

Nutrition Science in Croatia

In order to understand the possibilities and necessities of anthropological and nutritional researches in Cro-

atia, it is necessary to take into consideration its geo--strategic position, but we must not forget that a certain level of nourishment of one population is reflection of its economical and cultural status, which unfortunately was never at high level in Croatia. Specific position of Croatia, variety of its relief and climatic regions, as well as vegetarian varieties on a relatively small area, enabled wide and diverse choice of nourishment to its population[2].

When it comes to nutritional research in Croatian anthropology, we owe much to the medical doctors, who, in their daily practice, encountered various problems and diseases caused by malnutrition and dietary practices. First publications that deal with nutritional studies of Croatian populations are those of Mašek (1899), followed by studies of Mayerhofer (Figure 7), Lederer, Mikić and others at the beginning of the 20th century. Of great interest are books by Bogić (1927) in which he described the dietary practices of inhabitants of various regions of the former Yugoslavia, Grossmann's book (1934) in which we can find figures about necessary weights for certain age, as well as caloric and composing tables for certain nourishing food, and »The nutritional lexicon« by Mayerhofer (1944)[19–25].

The first institution in Croatia, which was dealing with nutrition science, was founded within Institute for Hygiene by Professor Edvin Ferber after the Second World War. Owing to Professor Ferber, Professor Buzina from Institute for Hygiene and Professor Hubert Maver from Institute for preventive medicine of former Yugoslav army, many important publications were made during the period from ž50s to ž80s. In 1984, the branch of nutrition science was founded on University of food and biotechnology in Zagreb, and even today it has great importance in educating young nutritionists.

The most important institutions that investigate anthropological-nutritive researches in Croatia today are: Croatian Institute for public health, that is department for physiology, observing and developing nutrition, headed by Zrinka Petrović, Institute for knowledge and control of raw materials and nutritive products, that is laboratory for the chemistry of food, belonging to University for food science and biotechnology in Zagreb, headed by Irena Colić-Barić, and Institute for testing food and control of quality of nutritive products, belonging to University for food science in Osijek, headed by Milena Mandić[2].

Fig. 7. Ernest Mayerhoher.

Researches in Croatia up to 1960

Although extensive nutritive researches in Croatia started only after the Second World War, as it has been already said, the first published writings of nutritive anthropological researches in Croatia date from 1899. That was the year when Mašek described two cases of pellagra. Not only the description of the history of disease, but also the description of the country nourishment of that time was very interesting. »The food of the patients was the simple country food like: bread, hardboiled corn mush, beans, corn mush fried with eggs and cheese. There were neither milk or alcohol in the house and they had meat only for Christmas, New Year, Epiphany and for Carnival«. In 1903 Lederer described the treatment of anemia. I will mention several interesting quotations from that article: »There are still doctors who oppose to the aim of necessity of blood letting« or »when a young doctor, after just graduating from college, starts his practice, he comes very often to the situation, especially in the country, to cut his veins. The same thing happened to me. Only after starting my practice, I had to let my blood very often, and to be quite honest, I was forced to do it«. Mayerhofer, who described food prices in Zagreb in 1924, recognized social sensibility, that is, the importance of the prices of food articles in the nourishment of the whole population. It is interesting to see the relationship of prices between some foodstuffs (the prices are expressed in the then dinars): 1 l of milk – 3.5 d, 1 kg of cheese 3.5 d, 1 egg – 1.37 d, cherries – 10.25 d, 1 kg pork meat – 29 d, 1 kg of peas – 9.35 d etc. Although the prices were said to be too high, the average income of that time population was not mentioned.

One of the most analyzed nutritive problems in ž30s, especially in the continental part of Croatia, was rickets. Mašek published the figure about great extend of rickets in the temperate climate, and he alleged that we could find in towns even 90% of »proletarian« children and 60% of children of »rich class« who suffer from rickets. In 1935, Dragišić discovered, by the means of clinical researches, great frequency of rickets, more than 90 % of country children, and he compared duration of lactation with the appearance of rickets. Dragišić brought out the figures of 1002 rickety children, 103 of them were artificially fed from the first day, 360 were artificially fed from 1 to 6 months, 492 were artificially fed from 6 to 48 months, while 47 children had unknown cause. After the Second World War, 7,654 cases of rickets were registered in 1950, and 9014 cases in 1951. Researches, made by

Buzina in 1952, discovered an extremely great appearance of rickets in Croatian villages around Zagreb 85.1%, Sisak 79.4%, Sinj 74.8%, Karlovac 72.1%, Slavonska Požega 68.3%, Split 64.3%, Rijeka 63.6%, Varaždin 62.6%, Ogulin 60.4% and Gospić 52.4%. The author attributes the great percentage of rickets not only to the lack of vitamin D, but also to very bad hygienic conditions in the villages. According to the lack of vitamin D, in 1954, Maver discovered the appearance of rickets in school children, 44.78 % of boys and 25.18 % of girls[19–21,26–30].

The great problem regarding health and nutrition, which according to some suppositions has existed in Croatia from the middle ages, is the lack of iodine, which causes goiter. Goiter was mostly widespread in the continental parts of Croatia, especially in some parts of Posavina and Podravina, the regions of endemic goiter, and the village of Rude, near Zagreb, where in the early ž50s; the frequency among school children was around 85% (Table 1). Goiter is determined by researches of school children and youth, because they are the best indicator of momentarily state in population, as the adults have goiter usually because the lack of iodine in the past. Daily man's necessities for iodine are 150–300μg, and reduced taking can cause troubles like reduced fertility, increased frequency of abortions, innate anomalies etc, and in the regions of striking lack of iodine, we can find even endemic cretinism. By the study from 1955, Buzina has analyzed iodine in Croatian waters, and conducted clinical researches of the population who have been using this water. The results about goiter in the villages in Savska Banovina (15446 goitred persons or 6.9%) were used in these investigations. As a conclusion, we can mention negative correlation between the quantity of iodine in drinking water and the mortality rate, caused by goiter. Researches carried out by Prebeg about extension of goiter and wide influence on psychophysical state, were realized on the sample of 19439 children in Zagreb and its environment at the end of 1953 and in 1954. From the total number of examined children, it was discovered that 9,033 children suffered from goiter, that is 46.5%, which was much more than 15.7%, evidenced by Ferber in 1951. For the sake of comparison, such frequency of goiter among school children was discovered in Lussan, Switzerland, in 1923. The results of investigations are also shown by the relationships between certain parameters, which discovered that the girls have more goiter than the boys in proportion to 50.9% – 42.2%. It was also found out that the illness doesn't depend on the age and the relationship between physical development and goiter, which showed that taller and overweight children are much more inclined to the illness. Researches of the influence of illness on learning showed that children with worse abilities are more liable to the illness. There are no differences in extension of goiter between country and town people, and neither are in altitude difference of dwelling[22,31–34].

According to clinical researches, Maver has conducted investigation about the state of well-fed persons on the sample of 2,443 school children in Zagreb in mid 1950s.

TABLE 1
FREQUENCY OF GOITER, MEASURED IN CROATIAN COUNTIES AT THE BEGINING OF ´90s (AFTER KUSIĆ[31])

Place	Number of examined children		% of goiter	
	7–11 years	12–15 years	7–11 years	12–15 years
Zagreb	379	361	20	19
Rude	88	112	26	43
Osiječko-Baranjska county				
Osijek	123	122	29	27
Vuka	102	101	23	37
Primorsko-Goranska county				
Rijeka	198	269	12	14
Delnice	100	101	18	33
Splitsko-Dalmatinska county				
Split	96	109	6	10
Lovreć	76	99	14	13

Although no lack of vitamins was discovered, there were some sub clinical signs of shortage, 14.54% of boys and 26.66% of girls showed the symptoms of lack of vitamin A, and 1.05% of boys and 4.43% of girls showed the signs of deficiency of riboflavin[30].

By clinical and laboratory examinations, Buzina determined the quantity and quality of nourishment of 42 pupils. The results showed that caloric value of food satisfied daily needs, as well as the quantity of daily taking of fat and proteins, while the quantity of taking of riboflavin was 31% less than recommended daily quantity. The results, achieved on the basis of index of height and body weight, showed good physical development and state of well-fed persons, without signs of malnutrition. Research of well-fed persons of the whole population in Croatia was carried out by Ferber and collaborators, as well as by Maver and Ferber at interval of several years. Nutritive survey of 250 homesteads was carried out by Ferber and collaborators in 1952 (Table 2), when they reached a conclusion about qualitative and quantitative scarce nourishment. In 1954, 64 villages with 1,200 homesteads in 12 different parts of the country and with 3,010 examined children, were tested and treated, and the results showed deficiency of vitamins A, D, C and riboflavin.

In the analysis of nourishment in five towns in Croatia (Zagreb, Rijeka, Split, Osijek and Varaždin), anthropometric measurements, clinical controls and biochemical analysis were carried out on totally 11,294 citizens. Researches showed great qualitative and quantitative deficiency in nourishment, especially deficiency of vitamin A, riboflavin, ascorbic acid and calcium. Comparing researches from 1954 on country people with researches from 1956 on town people, it was discovered that the greatest differences in taking certain nutritive were in

TABLE 2
DAILY CONSUMPTION OF CALORIES, PROTEINS, FATS AND CARBOHYDRATES PER HEAD FROM 1952 (AFTER FERBER[36])

Region	Calories		Proteins		Fats		Carbohydrates	
	Town	Village	Town	Village	Town	Village	Town	Village
Rijeka	2518	2421	75.22	72.97	78.71	59.08	361.83	367
Split	2413	2263	72.87	68.66	77.34	62.34	381.49	315
Varaždin	2670	2264	83.96	74.9	96.38	59.27	358.63	334
Zagreb	2554	2338	74.60	77.26	88.46	58.93	355.70	346

TABLE 3
SLAVONIA / DALMATIA – FAMILY, AUTUMN 1958 – DAILY TAKING UP PER HEAD OF POPULATION, MEASURED IN 25 FAMILIES IN SLAVONIA AND 24 IN DALMATIA (AFTER BUZINA[40])

	Unit of measurement	Slavonia	Dalmatia	Δ	t
Calories		3,331±137	2,869±122	462	2.52
Proteins	g	104±4	82±3	22	3.79
Fats	g	93±7	97±7	4	0.39
Carbohydrates	g	445±27	353±14	92	3.02
Alcohol	g	42±2	36±2	6	2
Vitamin A	I.U.	3,152±479	5,180±1,057	2,028	1.78
Thiamine	mg	1.7±0.1	1±0.1	0.7	5.38
Riboflavin	mg	1.4±0.1	1.1±0.1	0.3	1.86
Niacin	mg	24±1	18±1	6	2.96
C vitamin	mg	135±9	122±18	13	0.83
Calcium	mg	626±45	524±63	102	1.32
Iron	mg	40±5	25±2	15	2.66

Split, which had the biggest value of vitamin A with 4,150 I.U., unlike the villages from the same region, which showed the lowest values with 930 I.U. Riboflavin and calcium were deficient in the village, while consuming of thiamine and niacin was within normal limits, both in the village and in the town[35–39].

Joining clinical, anthropometric and laboratory tests, Buzina and his collaborators (Table 3) initiated more intensive field researches in three regions of Croatia that differs in used fats. The chosen regions were Dalj in Slavonija, the region with mostly used animal fat, villages near Šibenik in Dalmatinska zagora – Boraje, Lepenica and Vrsno, the region in which people use both, animal fat and vegetable oil, and Sali on Dugi island, the region in which people use nearly only olive oil. Researches consisted of anthropometric measurements of body weight, skeleton, relative malnutrition and fatness, height, skin wrinkles and biochemical analysis. Statistic results of these investigations showed significant linear age trend of cholesterol in blood, and at a specific age (19–59) there wasn't much deviating from linearity. The value of cholesterol in blood for age set values was in proportion to 239mg/100ml (animal fat), 211mg/100ml (vegetable oil and animal fat), 201mg/100ml (olive oil)[40–42].

According to the great influence of economical situation on the nourishment of population in that time, we must mention Ferber's analysis carried out on 227 families in Zagreb, in which he compared the prices of food, personal income and energy taking up (Table 4). It was discovered that people were buying food of higher energy importance and that expenses for food were inadequate to incomes[43].

Researches in Croatia from 1960–1970

From the review of nutritive state from 1960s, represented by Ferber, we can see rather bad socio-economical situation of nourishment, like deficient dairy food, for example in Zagreb, around 100,000 l of milk was daily used, that is only 15g per head. Milk consumption of preschool and school children could be seen according to the figures about milk kitchens in schools, whose number was 3,200 in 1965, and they were used by 595,658 pupils, while significant decrease was evident in 1967, when we had only 660 kitchens with 226,908 pupils. Except of shortage of milk, meat consumption was also decreased because of high prices, and consumption of sea fish was around 0.5 kg per head yearly, what is below all world averages, which were around 17 kg per head at that time. That was the period with food too rich with carbohydrates and poor with proteins and vegetable oil, because of which there was deficiency of vitamins D, A, B2 and C[44].

TABLE 4
CONSUMPTION OF NOURISHING PRODUCTS, ACCORDING TO THE SURVEY OF POPULATION FROM 1958 (AFTER FERBER[43])

Food	Per head daily – grammas	Per head yearly – kilogrammas
Bread	312.8	114.172
Corn bread	0.08	0.029
Bread grains	115.85	42.285
Vegetables	108.46	39.587
Beans	26.18	9.555
Potatoes	142.65	52.067
Fruit	75.17	27.437
Jam	16.48	6.015
Honey	0.98	0.357
Fruit juice	0.61	0.222
Meat	86.73	31.656
Fish	10.69	3.901
Milk	224.77	89.341
Powdered milk	0.19	0.069
Dairy products	12.01	6.938
Eggs	0.35	0.127
Fat	58.89	21.494
Sugar	45.77	19.706
Chocolate, candy	1.45	0.529
Wine	19.49	7.113
Brandy	4.31	1.573

In 1958, Buzina and collaborators started a long – term and complex research in Slavonia and Dalmatia, which was continued in 1960, 1961, 1962 and 1963, in order to cover all seasons (Table 5). These researches were at the same time part of famous Keys's researches, which were carried out in seven countries (Greece, Yugoslavia, Italy, Finland, Netherlands, USA and Japan), and within Croatia, researches were carried out in Dalmatia and Slavonia.

The samples of 1,363 persons were taken in six places near Makarska; Puharići, Gornji Tučepi, Gornja Podgora, Donja Podgora, Drvenik and Gradac in Dalmatia, and in Dalj in Slavonija. Research methods of the state of nutrition were based on gathering nourishing figures in each family during seven days. Food, including drink, was measured and converted into caloric values by the means of nutritive tables. The annex to more precise calculating of caloric values of nutritive food was given by Brodarec, who by the means of chemical analysis of food, made local tables of caloric values. Researches of body composition consisted of measuring of six variables: height, relative weight, skin wrinkles, systolic and diastolic pressure and concentration of cholesterol in blood. Analysis showed that there was bigger taking of calories in Slavonija, while in Dalmatia there was bigger consumption of alcohol.

The biggest differences in nourishment were in the use of fat, according to which the ratio of unsaturated and saturated fatty acids was 1:1 in Slavonija and 3:1 in Dalmatia, while the content of mono-, di- and tri unsaturated fatty acids was bigger in Dalmatia. The results of these researches were connected with blood coagulability. The group in Slavonia showed shortened coagulation, although the total consumption of fats, converted into calories, didn't show great differences between these regions, they were relatively low in both populations. Although the blood pressure was nearly the same, bigger number of hypertension was found in Slavonia. Consumption of thiamine, niacin and iron was higher in Slavonija, while consumption of other nutrition was the same in Slavonija and Dalmatia. By investigating, it was proved that higher socio-economical status was connected with bigger weight, higher blood pressure and higher level of cholesterol in blood. Brodarec pointed out to a small difference between these two populations in variables important for etiology of heart diseases, like relative body weight, systolic and diastolic blood pressure, physical activity and cholesterol. The level of cholesterol in blood serum was higher in population in Slavonia, according to statistic figures, which showed higher mortality rate of heart diseases in Slavonija than in Dalmatia. The relationship in nourishment between the head of the family and other members of the family was also investigated, and we can say that before research it was believed that the head of the family had better quality and quantity of food. The results showed irrelevant difference in quality of food, while the quantity was a bit higher in the head of the family. For the purpose of nutritive seasonal research, individual testing of the head of the family was carried out, and it was concluded that in Dalmatia there was more constant nourishment during the whole year[45–51].

The comparison of supplies with nutriments in four Croatian regions: Dalmatia (1963), Slavonija (1963), Istria (1964) and Turopolje (1965), based on clinical, laboratory and anthropometric examinations, showed enough taking of 2500 calories in Istria, and enough taking of proteins, fats and carbohydrates in all tested regions. Deficiencies of riboflavin and thiamine are found in Dalmatia and vitamin A in Istria. Although it seemed satisfactory, Buzina mentioned existence of certain differences in distribution of food within tested groups, and he warned that one part of population was handicapped.

This period is also characterized by testing the state of well fed in certain categories of population. Discovery of anemia in 208 pregnant women, which was carried out by Buzina and his collaborators, by measuring hemoglobin, hematocrit and erythrocyte, showed that 17.8% of women had anemic values of hemoglobin, bellow 10g/100 ml in blood. Although the survey of nourishment, carried out in families of tested women, showed that the consumption of iron was adequate to the standards, it was concluded that the cause of inadequate supply of iron should be searched in distribution of food in families, or in difficult absorption of iron as a result of too much consumption of carbohydrates[52, 53].

TABLE 5
MIDDLE VALUES FOR MEN, CLASSIFIED ACCORDING TO THE AGE, IN DALMATIA AND SLAVONIA, AND SHOWN AS MEDIAN, % OF AVERAGE FOR ALL 18 SAMPLES FROM 1958 (AFTER BUZINA[45])

Region	Variables	Middle values				Median, % of average			
		40–44	45–49	50–54	55–59	40–44	45–49	50–54	54–59
Dalmatia	Height (cm)	175	173	173	172	103.1	102.3	102.8	102.6
	Rel.weight (%)	94	93	90	88	95.8	96.7	94.5	93.5
	Skin wrinkles	15	15	14	13	70.8	73.5	67.6	65.3
	Syst. B.P.	136	135	137	135	103.8	101.5	100.0	96.0
	Diast. B.P.	85	80	82	82	104.9	98.3	98.3	97.3
	Serum chol.	182	185	186	188	88.2	89.2	89.0	91.0
Slavonija	Height (cm)	170	168	166	168	100.1	99.4	98.6	100.2
	Rel. weight (%)	95	94	88	91	96.8	97.7	92.4	96.7
	Skin winkles	15	15	13	14	70.8	73.5	62.8	70.4
	Syst. B.P.	130	130	131	140	99.2	97.7	95.6	99.6
	Diast. B.P.	79	80	80	84	97.5	98.3	95.9	99.6
	Serum chol.	196	197	200	194	95.0	95.0	95.7	93.9

Ferber and his collaborators made analysis of nourishment of 114 persons of older Jewish population, what was the first analysis of that type in former Yugoslavia. The lack of protein was discovered by the analysis of daily menu, as well as by chemical analysis of menu, while the consumption of fat and carbohydrates was oversupplied. Biochemical tests of blood and urine were also made in investigations, and we couldn't see any connection between nourishment, health and long life[54].

Goiter in some parts of Croatia was still great problem, in spite of introduction of one prophylaxis from 1956. The region of endemic goiter in Croatia is Podravina, and the main reason for it was carried out by Maver and his collaborators. The lack of iodine is the main cause for goiter, but there are also many other factors that influence normal absorption and integration of iodine, like vinylthiooxazalidon and vitamin A, as well as economic factors that influence bad nourishment. Investigations in two villages, Legrad and Koprivnički Ivanec, discovered good supply of iodine in both villages, although there was great increase of goiter in Legrad, between 1960 and 1965, especially in women, from 42% to 64% and in men from 27% to 34%. The number of goitred persons was rather decreased in Ivanec after 1960, 5% in men and 23% in women. Prejac and his collaborators gave figures about investigations of serum lipids among population with great frequency of goiter, which included villages in Podravina – Legrad and Đelekovac, with frequency of goiter of 30% in men and 40% in women. The results of these investigations were compared with former quoted investigations, carried out by Buzina, about taking fat in regions of Dalj and Makarska. It was found out that the portion of fat in these villages was bigger than that in Dalj and Makarska, while the quantity of cholesterol in blood was less. Such results made the authors conclude about existence of endogen, or still unknown factors that caused deviation of present norms. Determination of the influence of iodine prophylaxis was carried out by Buzina and his collaborators, ten years after its regulation. 5000 children were tested from already tested regions (Osjek, Varaždin and Bjelovar region). The results showed the decrease of goiter, supposing that the places without decrease, had other strumogen factors in food that influence high level of goiter[45,55–57].

Buzina investigates attributing of body constitution to the ethnic characteristics and genes or nourishment, by anthropological measurements, carried out in Hrvatsko zagorje, on selected population of children and youth from Pregrad, Bednja, Krapina and Zabok. In that way, Buzina got genetically identical populations, influenced by different environmental factors. It was expected before researches that genetic factors influence the growth of bone tissue much more than the development of soft tissue-muscles and fats, which are liable to the influence of ecological factors. Comparing parameters between town inhabitants in Zagorje (Krapina, Zabok), taller population who had bigger values of relative body weight, better index weight/height and better muscle built, with country inhabitants (Pregrada, Bednja), and with inhabitants from Dalmatia, there were discovered obvious tendencies in the change of skeleton. It was concluded that the build-up of skeleton in town inhabitants in Zagorje approaches to tall inhabitants from Dalmatia, which helped Buzina to show that improvement in nourishment influences parameters of physical growth and development[58].

We get a better insight in energy needs, by knowing energy expenses of inhabitants, and in that way we can better plan nourishment and physical labor of individuals and the whole population. For this purpose many authors made tables of energy expenses for certain professions. The figures of energy expenses of various professions of this period were carried out by Maver, who included in his researches bakers, miners, textile workers and bus drivers.

The methods of investigation of energy expenses were based on chronometry during the whole working time, as well as on establishments of executed works. For each job, energy equivalent was determined by the analysis of sample of breathed out air gathered in respirometer, according to Franz-Muller, while the analysis was done according to Scholander. From the results of these researches, there were visible different expenses of calories at certain jobs within some professions, like at bakers, sifting the flour 981 calories, fuelling of the stove – 2,071 calories. It was also concluded that by introducing mechanization (miners), energy expenses are essentially reduced[59–64].

Researches in Croatia from 1970–1980

This period is characterized by the researches of state of well fed in certain age groups of population in Croatia, paying most attention to children.

Researches of nutritive status of 3,622 children at the age of 4 in whole Croatia, are carried out by Buzina and his collaborators, on the sample of population in Dalmatia (Šibenik, Makarska), Slavonija (Dalj, Đakovo, Slavonski Brod, Podravska Slatina), Zagorje (Zabok, Ivanec, Krapina), Zagreb counties (Velika Gorica, Samobor) and Rijeka (Table 6,7). Anthropometric measurements showed positive trend of well fed, according to the increase of relative bodyweight, although in some populations there were still some cases of malnutrition, even bellow 90% of relative weight, and in some regions (Rijeka, Zagreb) there was great increase of relative weight, above 120%. From the height measures it was obvious that the children from Dalmatia are on average taller than in Zagorje and Slavonija. Clinical researches discovered some symptoms of nutritive deficiency. Angular stomatitis, as a consequence of the lack of riboflavin was discovered in 13.9% of children in Zagorje and 6% in Slavonija, the bleeding of gums as unspecific, but in epidemiological researches good indicator of lack of vitamin C, appeared in 10% of children in Zagorje and 5% in Slavonija and Zagreb. The appearance of xerosis and follicular hyperkeratosis as a result of the lack of vitamin A, and atrophy of papilla as a result of the lack of vitamin B complex, were very low. The most important changes were decreases of enlargement of thyroid gland, probably as a consequence of introducing of iodine prophylaxis from 1956, since when 1kg of salt has iodized with 10mg KI. Biochemical researches showed decreased values of hemoglobin, from 3% to 16%, with the biggest aberration of 16.8% in Slavonija. Low values of hemoglobin are confirmed by the results of researches, carried out by Hibšer and his collaborators, on population of pupils in Vinkovci, which show figures of 11.7% of anemia in girls and 13% in boys. High appearance of sideropenic anemia in Croatia was pointed out by Sapunar and his collaborators in Dalmatinska zagora (25%), by Donadini in Split and its surroundings (36.35% and 20%) and also in Slavonia (22%). Biochemical researches discovered vitamin deficiency of riboflavin and vitamin C. The regions with the biggest deficiencies are Zagorje and Zagreb, with the lack of riboflavin of 54% and 30%, and Rijeka with 17% lack of vitamin C. Among other important deficiencies, there is pyridoxine of 9–20%, whose diagnostic criterion was investigated by Buzina and his collaborators, on a group of 70 children. The deficiency of thiamine can be found in Rijeka and Dalmatia, ranging from 14–19%. These results showed the relationship of vitamin deficiency between country and town inhabitants, and it was clear that there was bigger deficiency in country inhabitants. There were also researches about additions of vitamin deficiency (2.5 mg riboflavin and 50 mg C-vitamin per meal) in Hrvatsko zagorje, on a sample of two groups of school population with prevalence of angular stomatitis of 17–20%, and high percentage of gums bleeding. The results showed positive effects of vitaminised meals, and discovered that adding of riboflavin for only two months is enough to achieve optimal function of enzyme glutathione reeducates, and that in interval of six months; prevalence of angular stomatitis can be significantly reduced. It is supported by the figure that prevalence of angular stomatitis was reduced on 3.4%, while in a control group it grew to 29%. Adding of C vitamin was enough to keep optimal level of ascorbic acid in plasma, but it didn't influence on gums bleeding. The author mentions that deficiency of vitamin C is mostly of seasonal nature, so the problems with deficiency start in January and last until April and May, and in September the values of C vitamin in plasma are satisfactory, because of consumption of seasonal fruit[65–70].

There was an extensive study about the state of well fed of 3,744 school boys and 5,033 school girls at the age of 7 to 19 from Zagreb, as carried out by Prebeg and his collaborators. Researches discovered mild malnutrition in 10.6% of girls and 8.8% of boys, and high overweight in 11.15% of girls and 11.6% of boys. Such results discovered a bit higher body weight in children from Zagreb, compared with European average, which according to the author, could be the »consequence of quick acceleration of growth in the last decades«[71].

The analysis of nourishment of children in Split was carried out by Marušić, and it was concluded that daily meals were 13% caloric insufficient, although anthropometric measurements showed normal fed of relative weight from 90–110%. Apart from caloric deficiency, insufficient taking of vitamin B complex was discovered by the analysis of blood. The analysis of children in Split (1923) was carried out by Pasenti, who was comparing the relationship between the state of well-fed and plasmatic values of cholesterol. Higher values of cholesterol in blood was found by 3% of girls and 2.33% of boys, while the higher values of glucose were found by 1.69% of boys and 1.02% of girls. According to researches of relative weight, 12.3% of pupils suffer from malnutrition, while 8.7% of children are fat. Correlation between relative weight and cholesterol in blood was very low, as well as between relative weight and glucose. Ćurin spoke about insufficient fed of children in Split, according to the values of hemoglobin[72–75].

TABLE 6
RELATIVE BODY WEIGHTS (% OF STANDARD WEIGHT) OF A GIRL AT THE AGE OF 1 TO 14 IN REGIONS OF CROATIA TESTED IN 1954–56 AND 1973–76 (AFTER BUZINA[65])

Age (years)	Slavonija		Zagorje		Zagreb		Primorje		Dalmatia	
	1954–56 N=388	1973–76 N=380	1954–56 N=267	1973–76 N=443	1954–56 N=292	1973–76 N=379	1954–56 N=285	1973–76 N=213	1954–56 N=292	1973–76 N=379
1	98.0	98.5	94.1	97.8	97.0	102.4	98.0	106.5	94.9	101.9
2	100.1	98.9	93.3	99.5	97.4	103.5	102.1	106.3	99.2	98.6
3	99.7	99.9	95.3	100.6	98.3	99.4	100.9	107.2	98.6	100.3
4	98.3	99.5	96.7	99.8	98.1	106.5	99.5	109.4	96.5	100.6
5	98.5	99.7	99.0	99.5	97.4	108.6	99.1	108.1	97.8	98.9
6	99.3	100.7	97.9	102.0	98.0	103.3	98.7	105.5	97.6	102.6
7	99.5	100.3	102.5	100.4	97.4	105.4	100.8	108.3	98.1	100.8
8	102.6	100.9	103.5	101.7	95.7	104.0	103.7	106.0	99.4	97.8
9	100.0	99.8	100.7	100.0	103.2	110.3	101.0	108.4	95.5	99.7
10	97.9	100.0	99.8	100.3	99.2	107.6	98.2	104.7	95.2	98.4
11	97.8	99.7	100.7	97.6	98.9	104.1	98.6	101.7	95.2	94.7
12	94.2	102.4	105.1	98.3	97.1	103.7	98.6	94.4	94.0	98.6
13	98.2	99.5	96.7	98.1	104.7	108.3	101.7	101.4	91.8	97.6
14	94.0	99.8	96.6	99.7	94.8	105.6	94.2	102.6	97.0	100.0

TABLE 7
THE RESULTS OF CLINICAL RESEARCHES: PERCENTAGE OF CHILDREN WITH CLINICAL SYMPTOMS OF BAD NOURISHMENT IN CROATIA TESTED IN 1954–56 AND 1973–76 (AFTER BUZINA[65])

Symptoms	Slavonija		Zagorje		Zagreb		Primorje		Dalmatia	
	1954 N=767	1976 N=785	1954 N=550	1976 N=905	1954 N=504	1976 N=747	1954 N=556	1976 N=418	1954 N=572	1976 N=777
Skin										
Xerosis	0.4	0.6	6.2	2.1	3.8	1.2	0.8	0.4	0.4	0.4
Follicular hyperkeratosis	0.4	0.4	10.4	3.6	4.5	1.6	5.2	1.4	3.8	0.9
Lips										
Angular stomatitis	8.1	6.8	18.3	13.9	15.0	6.5	14.2	2.3	6.7	2.1
Gums										
Gums bleeding	9.5	5.4	18.8	9.8	8.2	5.1	18.6	2.1	5.0	0.8
Thyroid gland										
Goiter	66.4	1.7	70.1	1.4	57.1	1.8	23.8	0.9	27.3	1.1
Skeleton										
Caput quadratum	39.2	6.8	37.2	12.7	36.4	6.6	31.3	3.1	29.5	3.6
Thickening of epiphysis	43.1	5.6	44.2	2.4	39.9	3.7	82.7	3.8	62.3	4.8
Pectus carinatus	4.9	2.2	4.9	2.2	2.3	1.6	12.9	1.2	13.9	2.4
O legs	29.2	3.2	1.7	1.4	20.3	2.8	11.5	1.2	13.6	1.6
Tongue										
Atrophic papilla's	2.4	1.1	8.5	5.7	4.6	1.2	2.2	0.8	1.2	0.9

Kapetanović and his collaborators were researching 3,334 children at the age of 0 to 3, from three different parts of Croatia, Hrvatsko zagorje, Slavonski Brod and Knin (Table 8). Analysis showed that in two regions, 50% of children until 1 year started with artificial food, and insufficient food was found in children between 6 and 12 months, mostly in Hrvatsko zagorje and Knin. Malnutrition, discovered by anthropometric measurements, was found in 5% to 19.3% of children at the age of one year, and in 13% of children at the age of two and three years. Fatness was found in 8.8–19% of children at the age of 1 year, and 5–8% of children at the age of 2 and 3 years. By

biochemical measurements, lower values of proteins and albumin were found because of frequent infections and bad nourishment. Most children with lower values of proteins were in Hrvatsko zagorje, in the first year to 36.6%, and in the third year to 43.3%, while we can find lower values of albumin in the first year in 14–24.4% of children, and in the third year in 18.6–48% of children. From the specific nutritive deficiency, the most frequent are sideropenic anemia and rickets, which were found in the first year in 12.5–51%, and in the third year in 34–68.4% of children. These researches showed connection between mothers' education, caring out of prophylaxis of vitamin D and bone changes, and it was found out that the worst executing of prevention was among mothers with the low educational level[76,77].

Although widespread in nature and food, deficiency of calcium isn't rare, especially in women after menopause, when they often suffer from the loss of bone tissue that is osteoporosis. The research of influence of nourishment on the deficit of bone tissue was carried out by Matković on the population of Istria (town districts of Pazin, Buzet, Poreč) and Podravina (Virje, Novigrad, Gjurjevec, Molve, Novo virje, Ferdinandovec), when there were tested 1924 persons above the age of 30. The nourishment of these two populations differed in taking of proteins and it was found out that in Istria taking of proteins was more based on vegetable food, while taking of dairy products, as the source of proteins, was more characteristic for Podravina. By the analysis of nourishment, it was found out that people in Istria were taking 2–3 times less calcium, which didn't influence the loss of bone tissue because of vitamin D, which enables better absorption of calcium. The figures that resulted from work showed, according to the author, that we must influence prevention of osteoporosis with better nourishment in two periods of life, in the childhood and adolescence, in order to achieve bigger bone mass[78–82].

Researches of relative weight, as well as the influence of some widely used nutrients important for man's health, were carried out by Mimica and his collaborators. The sample contained 1,552 men and 1,667 women at the age of 37 to 58 in six town districts in Croatia (Zagreb-center and Črnomerec, Virovitica, Split-center, Omiš and Vis). The values of relative body weight of 96% to 110% had 42.6% of men and 32.1% of women. Less body weight, below 96% of relative body weight from the wished one, could be found in 10.7% of men and 5.1% of women, while the bigger weight from the wished one could be found (above 111% of relative body weight) in 23.9% of men and 25.2% of women. The fattiest people could be found in Split, Omiš, Vis and Zagreb–center, while the thinnest people were in Virovitica. By the analysis of the relationship of chronic diseases and the weight, it was discovered that fat people had more often cardiovascular diseases, diabetes and diseases of gall bladder, while thin people had very often chronic bronchitis. This study included researches of coffee influence and salty food on chronic diseases. It was discovered that 18.9% of men and 20.6% of women drink daily three or more cups of coffee, and we couldn't find the difference in weight between those who drink coffee and those who don't. Systolic and diastolic pressure showed bigger values in those who don't drink coffee, than in regular consumers of coffee. Besides, there were not found differences according to neurosis, bronchitis and gastrointestinal diseases. Testing of salting food discovered that out of 3,265 people, 62.8% »normally« salts the food, 17% salts more than necessary and 1,6% use too much salt. It's also discovered that country people salts food more than town people. The influence of salting food on systolic and diastolic pressure shows that the pressure is lower in those who use more salt than in those who normally salt the food. The only figure that shows that over salting causes hypertension is measurement in a group of 20 women who used more salt and had 8 cases of hypertension. There is connection between salting food and varicose veins, which are mostly diagnosed in people who use more salt in food[83–85].

The Period from 1980–1990

Keeping physical condition and work capability has always been for a man of vital importance, and it mostly depends on nourishment. That's why it's not difficult to conclude that the studies about the relationship between physical condition and nourishment have been always very interesting to the army. Physical activity has a strong influence on locomotor, digestive, respiratory and cardiovascular system, and there is no doubt that keeping good physical condition is used in the fight against overweight, as the cause of many diseases, like diabetes

TABLE 8
PERIOD OF BREAST-FEDDING, ACCORDING TO THE AGE OF CHILDREN FROM 1968–71 (AFTER KAPETANOVIĆ[76])

Period of breast feeding according to the age of children	Hrvatsko Zagorje		Slavonski Brod		Knin		Total	
	N	%	N	%	N	%	N	%
Number of breast feeding	57	4.37	83	7.79	38	4.82	178	5.64
1 month	275	21.1	220	20.66	68	8.63	563	17.84
2–3 months	377	28.93	353	33.14	138	17.51	868	27.51
4–6 months	237	18.19	196	18.4	168	21.32	601	19.04
7 and more	357	27.4	213	20	376	47.72	946	29.97
Sum of children	1303		1065		788		3156	

and coronary diseases. We can also conclude that we need optimal nourishment for a specific level of physical efforts, either in energy sense or in the sense of taking special nutrients. The source of energy for physical activity are first of all carbohydrates and fats, whose role is even bodily connected, while as for proteins, we must say that they lead to increased consumption of oxygen and consequently to decreased physical condition. According to Consolazi, physical condition in trained persons will be decreased for 50% in the period of 3 days, on a diet of high fats and low carbohydrates, and the best effect on condition and work ability is achieved in the period of 3 days on a diet of high carbohydrates and normal quantities of fats, as it is directly connected with the supplies of glycogen in the muscles.

The study of influence of nourishment on physical condition was carried out by Buzina and Subotičenec on the sample of 665 men at the age of 13 to 18. It was proved that under-weight persons with a relative weight under 90% of standard one had lower aerobic capacity, while the highest values of aerobic capacity were shown by the persons with a higher relative weight from 100% to 119%. Further increasing of relative weight above 120% of standard one was followed by decreasing of aerobic capacity. The persons under 90% of relative weight of standard one, had deficiencies of certain vitamins and minerals. According to the author, the development of soft tissues in the body can be considered as an indicator of well fed, and aerobic capacity depends mostly on development of muscle tissue, while the appearance of fat tissue will follow decrease of aerobic capacity. In regard of vitamin deficiencies on aerobic capacity, the most important parameters were iron (hemoglobin and hematocrit), the content o vitamins A and C in serum, as well as the content of riboflavin in erythrocytes. The proof for that is increasing of aerobic capacity after rehabilitation with vitamin deficiencies. It was also proved that receiving of exchangeable doses of pyridoxine and riboflavin, have no important influence on physical condition.

Subotičenec and his collaborators carried out a survey about the state of well fed of 112 older persons from pensioners' home in Zagreb. Analysis of the daily menus helped us to know more about energy sufficient nourishment, but the researches of the state of well-fed discovered great nutritive deficiencies, like lack of pyridoxine in 58% of persons, vitamin C from 21.4 –45.2% and riboflavin from 11.1–19.4%. The deficiency of iron was also diagnosed in tissues from 6.7–15.4%. For better investigating of functional meaning of present nutritive deficiencies, cell immunity was researched. It was proved by this research that around 20% of variability of cell immunity, in tested population of older persons, depends on supplies of vitamins and iron in nourishment. Scarce values of vitamin C, E, riboflavin and pyridoxine, as well as zinc and iron, are found in serum. The results suggest that nourishment is very important for immunological system of older persons[86,87].

The importance of daily nourishment of certain parts of population is to show certain lacks and deficiencies in nourishment, and by correcting them; we can improve our health and work abilities. In researches of nutritive and energetic needs of students, Colić discovered nutritive values of monthly menus of meals of students' social nourishment. The methods of these researches were analysis of daily menus according to nutritive tables and the results of chemical analysis, carried out by Public Health Institute in Zagreb. By analyzing meals, it was discovered that nutritive balanced quality and unevenness were very bad, and by the help of survey lists, nutritive and energy needs of this population were discovered. According to these researches, it was decided to make new standards for menus that would contain bigger portion of meat, vegetables and fruit, paste and rice and decreased portion of fat[88–93].

Breast-feeding nourishment and its influence on the later development of children at the age of 5 or 6 was investigated by Kapetanović. The state of well-fed was estimated on the sample of 648 children (338 boys and 310 girls) by percentage values of birth mass, body mass, length and height, at the age of 5–6 months and later at the age of 5–6 years. Birth masses were in 1/4 of children higher than in 90% of relative birth mass, and fatness was 2.5% in girls and 2.4% in boys. The number of fat boys until the age of 5 and 6 was doubled, and of girls quadrupled[94].

Kolaček was investigating the frequency of fatness in the group of persons from 14–19 years who were overfed in their childhood, as well as the roles of various factors in etiology of fatness. The methods of work demanded finding of population that was already measured in a unique way in their early childhood, so the figures by Kapetanović and collaborators from 1968 and 1969 were used for that purpose. There were 169 isolated children who were, according to the relative weight of that time, too fat, so they were examined again after 16 years. It was discovered that 137 persons out of 169 were overweight in the first 3 years of their life, and 32 were fat. After 16 years, it was found out that every second person has higher relative weight. Incidence of fat persons who were fat at the age of 3 was 43.8%, and in the group of former overweight children 20.5%, from which we can conclude that after 16 years more fat children come from the group of the overweight. It was also discovered that the most important predictor of the level of well-fed persons is relative weight of their mothers and fathers, what is seriously vivid in adolescent age. Such perceptions of these researches discovered hereditary characteristics of fatness, although social connection was also showed as relevant[76,95,96].

Socio-economic factors, important for breast-feeding nourishment, were investigated by Modrušan-Mozetič on 500 families, according to modified Graffar's social classification of families. The results of researches showed that social structure of families with mothers born in Rijeka is totally different from those whose mothers immigrated to Rijeka, which reflects on unpleasant situation of professional qualification and monthly income. The way of child's nourishment in the first 3 years of his

life differs according to the social class of his family, regardless of the same education of all mothers about the way of nourishment, from which we can conclude that mothers coming from families of lower social classes are more difficult in receiving advices about nourishment[97–99].

The Period from 1990 until Today

The 1990s were unfortunately war years in Croatia, the aggression of Yugoslav army left, among other things, consequences on nourishment of population, which can be felt even today. War events were accompanied with negative ecological changes, like devastations, negative socio-economical situation, great migrations, stress that causes the fall of immunity, radiological and chemical contaminations etc. It is necessary to have bigger quantities of qualitative food in the war because of many reasons like exposing to bigger physical efforts, and because of losing of energy, it is necessary to insure taking of caloric food. Man is exposed to various harmful biological agents, that's why organism needs protective nutrients in the form of various vitamins and minerals. The problem of bad quantitative and qualitative supply of food in such conditions is inevitable because of many reasons, like disarrangement of production and distribution of food. The way of nourishment often comes down to collective nourishment, and migrations of population cause quick growth of inhabitants in some regions, causing in that way deficiency of food. We also mustn't forget frequent devastations and contaminations of farmlands.

The result of such situation is often malnutrition, caused mostly by the lack of proteins, or as a partly or completely lack of vitamins in the form of hypo-or avitaminosis, of which the most often appearances are scurvy, beriberi, hemaralopia, pellagra etc. The consequences of acute and partly forms of hunger can turn to chronic forms, very dangerous for human health, children can suffer from deformations in growth and development, immunity is decreased, causing in that way appearance of infectious diseases, work ability is decreased, the wounded and sick recover very slowly. Because of great importance of nourishment during the war period in 1992 and 1993, there were held two important conferences 17th School of biological anthropology, named »Nourishment in war and postwar period« and 19th School of biological anthropology, named »Bread-Milk-Water« on which the lecturers were famous Croatian nutritionists and anthropologists: H. Maver, P. Rudan, S. Kolaček, T. Kapetanović, R. Živković, D. Matasović, A. Kaić-Rak, K. Antonić, J. Grgurić, and other respected scientists[100].

Kaić-Rak carried out figures about supply of energy and nutrients in Croatian population from 1991. According to these figures, average taking of energy was 2175 kcal, the portion of proteins was 71.7g, carbohydrates 281.4g, alcohol 7g, sodium 2.312mg, potassium 2.490mg, calcium 512mg, iron 9.1mg, vitamin A 343 R.E., vitamin B1 1mg, B2 1.3mg, B6 1.17mg and vitamin C 71mg. It is important to say that during that period Croatia entered DAFNE project (pan-European net of information about consumption of food got by surveys), in which it was important to ensure the control of daily taking of energy and nutrients, that is qualitative nourishment of population on a national level with indicators about healthy state of population and special reference to chronic diseases. According to the analysis of well fed of Croatian population from 1997, it was proved that there wasn't malnutrition in Croatia in the sense of energy deficiency. Although energy deficient food appeared only in 1.4% – 6.1% of inhabitants, the bigger problem was of qualitative nature. The main reasons for qualitative deficiency in nourishment were economical reasons, because of which people often buy cheaper and energy richer food instead of biologically more qualitative food. So we can mention the figures from 2003 about daily taking of cereals of 290g (of which 72% comes from bread), which was 30% of total daily taking of energy[101–103].

With the help of UNICEF, Zakanj and collaborators made a survey researches about the influence of war on breast-feeding in Croatia, which included 757 children under 2 years and 1,180 children between 2–5 years. The results showed that 94.6% of mothers have started breast-feeding, and the correlation between frequency of the beginning of breast-feeding and geographical position, or war events was not found. It was also discovered that breast-feeding was lasting longer in the parts of the country that didn't experience the war (Istria, Hrvatsko Primorje and Gorski Kotar) than in war-included regions (Slavonija). The results show that the war shortened the time of breast-feeding, and possible reasons for that are humanitarian donations with compensation of mother milk. Grgurić and collaborators were making researches on the sample of population from 3 regions in Croatia: in the free territory, from the first front line and on liberated territory. The respondents were households (1,563) who had children less than 5 years (1,937). Researches showed very low portion of breast feeding, while nourishment with cow milk was very high during the first six months (30%) and 1 year (60%) of child's life, which presents negative results in regard to high risk of sideropenic anemia in children fed with cow milk in their first year of life, as a result of lack of glycoprotein and lactoferrin, which can be found in mother's milk, and the lack of vitamin D was also noticed.

Researches, carried out by Berović on a sample of 500 mothers, showed that breast-feeding is more frequent in mothers who are older, more educated and non-smokers. It is also discovered that 30.7% of children are breast-fed after 3 months, only 11% after 6 months andμ 40% of children are fed with cow milk before their first year of life. Batinica investigated frequency of breast-feeding on 816 children (64% of totally born) in Međimurska county, 725 of children (51% from total number of born) in Sisačko-moslavačka county, and in Šibensko-kninska 716 children (61% from total number of born). The results showed that 61–85% of infants were breast fed until 2 months, 36–64% from 3–4 months and 14–53% from 6–7 months, from which we can conclude about positive trend of breast-feeding in Croatia. As for nourishment of

children, the subgroup for revision of National programme for children of State institute for family protection, maternity and youth, from domain of health and nourishment of children in 2002, defined the fundamental priorities for nourishment of children with three aims: promoting breast feeding, the programme of preschool children and the programme for improving nourishment of school children[104–107].

Unlike quantitive analysis, qualitive analysis of breast-feeding is carried out by Mandić and collaborators, by determining the quantity of copper and zinc in human milk on the samples taken in the clinical hospital in Osijek and in the refugee centre in Nabrde. The quantity of copper and zinc was tested depending on social status, age, number of breast feedings, day after breast feeding, smoking, information about child's taking of copper and zinc, which was varying, copper between 0.27–1.35mg/l, and zinc 0.62–15mg/l. Testing of daily taking of already mentioned chemical elements was carried out by Katalinić and collaborators. The choice of food and the way of preparing it corresponded to Dalmatian kitchen, while nutritive thickness of zinc, copper, manganese and iron was compared with recommendations of WHO/FAO, and it was concluded that the thickness of zinc was 30% less than recommended, while the thickness of copper responds to the recommendation[108–111].

The relationship between nourishment and etiology of various difficult and chronicle diseases is certainly the topic of many world researches of nourishment of this period. The researches of relationship between developing of stomach cancer and the way of nourishment, was carried out by Kaić-Rak. The sample in parallel investigations between 2 populations were the inhabitants of Vukovar and island Brač, and they included 80 persons at the age from 30–60 from each region. By comparing information about incidence of stomach cancer from 1982 and 1986 with incidence of stomach cancer in Vukovar, south regions and Croatia, there was great incidence of stomach cancer in Vukovar population in relation to Croatia, especially in relation to south regions. The researches showed that more qualitative nourishment is related to island population who has more satisfactory balance of saturated and unsaturated fats, because of use of vegetable oils, especially olive oil, while 90% of total taking of fat in Vukovar region are fats of animal origin. It was discovered that there was bigger consumption of complex carbohydrates, vegetables and fruit on Brač, that's why there is bigger taking of vitamins A and C as important elements in prevention of stomach cancer. There were also discovered statistically important differences in consumption of industrially prepared food, grilled food, sour ingredients, but also differences in frequent number of daily meals. Great differences are also in the structure of nourishment at the age of 25, when nourishment is poor with carotene, vitamin C and calcium, and consumption of alcohol is frequent, even at older age. Bad life habits and work conditions, low social status and unhealthy nourishment with less taking of carotene, vitamins C and B2 and calcium, as well as more taking of nitrates and nitrites from the environment, all that can initiate carcinogenesis[112,113].

Rudan and collaborators discovered differences in the rate of incidence of stomach cancer, colon cancer and pancreas, between two regions in Croatia, Koprivničko-križevačka county with continental way of nourishment, and islands in Dalmatia (Brač, Hvar, Korčula, Vis, Lastovo) with Mediterranean way of nourishment, for the period from 1986 to 1995. With great differences of nutritive habits, it was discovered that there was lower rate of incidence of stomach cancer and cancer of pancreas in both sexes in island population. Age standardized rates of cancer incidence of island population comparing with population in Koprivničko-križevačka county, was figured out on 100,000 inhabitants – 17.2 against 39.4 per mille, in men with stomach cancer – 9.1 against 16.5 per mille, and in women with stomach cancer – 34.5 against 31.4 per mille. Colon cancer shows more incidence in men of island population – 18.3 against 20.3 per mille, in women with colon cancer – 5.5 against 9.0 per mille, and in men with cancer of pancreas – 2.7 against 5 per mille. Standardized rates of incidence of stomach cancer and cancer of pancreas were much lower on these 5 Dalmatian islands. By comparing age standardized incidence for stomach cancer on our islands with corresponding age standardized rates in other European countries, similar values appeared in some parts of Italy, Spain, Germany, Czech Republic and Finland, while age standardized rates of incidence in Koprivničko-križevačka county are similar to those in Byelorussia, Estonia and Leetonia. Such results showed that Mediterranean way of nourishment decreases the risk of stomach cancer and cancer of pancreas, and according to researches of Car and collaborators, it is protective factor in the appearance of arteriosclerosis[114,115].

As many other diseases, cardiovascular diseases are very often connected with wrong nourishment, especially with too much taking of calories and saturated fat acids. In the purpose of early discovering of possible factors that might influence eventual cardiovascular diseases, additional researches were made by Kolaček and collaborators. After 18 years, 456 already tested persons were again invited to be examined, so anthropometric measurements were made, blood pressure was controlled, the puncture of veins was made and various questionnaires were carried out. By comparing these two researches, it could be concluded that the highest level of total cholesterol and LDL is most frequent in men who were thinnest in the first 3 years of life and fattest in adolescence. Taking in account results of anthropometric measurements, nourishment and social status of both life periods, the important factor was lower growth in adolescence, which was connected with malnutrition in the first 3 years of life. Systolic and diastolic pressures were also tested. The researches showed positive correlations between weight and blood pressure in children born with less body weight, and fattening in later years, which increased pressure, so it was proved that social situation influences higher values of systolic pressure[116–118].

The results of researches, carried out by Čubrilo-Turek and collaborators, showed that 10% of male popu-

lation has higher triglycerides above 6.01mmol/l, and female 3.13mmol/l, so it was proved that concentration of cholesterol in 10% of men was 7.6mmol/l, and in 10% of women 7.19%. Another important information is that 40% of energy in nourishment of Croatian population presents fat, and that higher cholesterol makes 50% risk of cardiovascular diseases according to Kaić-Rak. In favor of unbalanced taking of fats in east Croatia, we can mention researches about taking fats and fat acids, which was carried out by Primorac and collaborators, and according to which, daily taking of total fats was 33.4%, 9.9% saturated, 11.8% mono saturated, 9% poly saturated and 0.7% trans fats. Except of the influence of nourishment on already described diseases, the researches, carried out by Sepčić and collaborators in Gorski kotar, had the aim to show the connection of nourishment and multiple sclerosis, as Gorski kotar presents the zone of the greatest risk for this disease. 46 patients and 92 controls of autochthonous population were investigated, and it was proved that nutritive factors that might influence development of that serious disease in the zones of the highest risk, are unpasteurized milk, animal fat, smoked meat and potatoes[119–121].

At the beginning of 1990s, 40 years after iodine prophylaxis had been introduced to Croatia (10mg KI/kg of salt), there were carried out extensive researches on national level about frequency of goiter. Kusić was investigating 2,436 children of both sexes at the age of 7 to 15, as well as geographically and economically different regions in Croatia. The methods of discovering the goiter were palpation of the throat, according to the division of American health organization (PAHO) and world health organization (WHO), as well as ultrasound examination and measurement of iodine in urine. Analyzed regions were: Zagreb (740 pupils with 20% of goiter), Zagreb county (200 pupils from Rude district with 26% – 43% of goiter), Split (205 pupils with 6–10% of goiter), Lovreć (175 pupils with 14% of goiter), Rijeka (467 pupils with 12%–14% of goiter), Delnice (201 pupils with 18% – 35% of goiter), Osijek (245 pupils with 28% of goiter), Vuka (203 pupils with 23% – 37% of goiter). The results of researches showed that the decrease of goiter in Croatia isn't possible until obligatory content of iodine in salt increases from former level of 10mg KI/kg. In 2003, that is 6 years after regulation of iodizing of salt to 25 mg KI/kg, there were carried out new researches on national level, in order to confirm the effects of new regulations. Researches were carried out in 4 regions in Croatia: in the northwest part, Slavonia, north Adriatic and Dalmatia, on the sample of 927 pupils of both sexes at the age of 6–12. The values of sonographic volume of thyroid gland were in normal limits, according to the values WHO/ICCIDD, and the concentration of secreted iodine in urine of 14 μg/dl in average were within limits of normal values. Controls of domestic and imported salts that satisfied quantity of 25 mg KI/kg were made too. It was obvious from these researches that Croatia, after more than half of the century, reached normal values of iodine[122–125].

Researches on the sample of 1,048 pupils from Rijeka and 778 from Zagorje, Capak, show that in 40 years we achieved improvement of the state of well fed of school children at the age of 7 to 14. According to distribution of anthropometric parameters, population from Rijeka showed as taller and heavier for certain age, as well as heavier for certain height. We can find in both regions the values of anthropometric indexes 80%, which are interpreted as malnutrition, but we can also find the appearance of fatness, whose prevalence is higher in population from Rijeka. The study also showed that daily meal of examinees from Rijeka corresponds to the recommendations of RDA, by structure of carbohydrates, fats and proteins, but taking of vitamins A and C and calcium is lower than recommended, while total daily meal of examinees from Zagorje doesn't satisfy in energy sense, which resulted with the appearance of clinical symptoms of deficient nourishment, like follicular hyperkeratosis, angular stomatitis, atrophy of tongue papilla's etc, present in both populations. On the sample of 862 children from Prebukovje, Vrbno and Bednje, Lončar warned on being backward in growth and body, as well as in relative weight of children coming from poor villages, comparing with those from towns. The children from Prebukovje and Vrbno showed chronic nutritive deficiency, in favor of which we must mention, decreased development of soft tissues in relation to the size of skeleton. Nutritive deficient of country children were: proteins 5.3%, albumin 16%, hemoglobin 3.2%, hematocrite 5.2%, iron 12.3%, vitamin A 7.2%, vitamin C 21.6%, thiamine 1.5%, riboflavin 13.5% and pyridoxine 9.6% of examinees. For easy reference of the influence of nourishment on growth and well-fed of children, Antonić-Degač, in 1995, carried out researches in 2 ethnically homogeneous populations of school children in 2 geographically different regions, Bednja in Hrvatsko zagorje and Dugopolje in Dalmatinska zagora. Investigations were based on anthropometric measurements and interviews about 24 hours nourishment, and the results were compared with the same measurements from 1975. It was discovered that the boys in Bednja were taller in average for 3 cm, in Dugopolje for 4.7 cm, and the girls in Bednja for 3.5 cm, in Dugopolje for 6.4 cm. The most important difference in nourishment of children between 1975 and 1995 was increased taking of proteins of animal origin (Bednja 47%, Dugopolje 76%), which proves that there is positive correlation between taken quantities of proteins of animal origin and the growth of children[126–129].

The state of nourishment and well fed of school children in Split is investigated by Ćurin on the sample of 200 children in 1994, when it was discovered that there were 23.7% of malnutrition in boys and 27.1% in girls, and the values of hemoglobin under 120g/l was seen in 32.2% of boys and 19.6 % of girls. In 1999/2000, on the sample of 919 children registered in the first class of elementary school, there were made anthropological measurements and hematological researches (hemoglobin and hematocrite), and it was discovered that 64.8% of boys in all-day residence or 67.1% in shortened residence, as well

as 63.7% of girls in all-day residence and 68.2% in shortened residence were normally fed, while the signs of malnutrition were shown by 8.6% of boys in shortened and 4.9% in prolonged residence, and 6.9% of girls in all-day residence and 7.3% in shortened residence. Decreased values of hemoglobin were found in 13.3% of examinees with shortened residence and in 4.9% with prolonged residence, and the values above 120 g/l were shown by 55.6% of children with shortened residence and 63.4% with prolonged residence in the kindergarten. The investigation of children's nourishment in Bjelogorsko-bilogorska county on the sample of 1,399 children registered in the elementary school, discovers 14.9% of undernourished boys and 16.4% of girls, and 7.6% and 9.9% fat boys and girls. Jakovljević pointed out the frequent appearance of sideropenic anemia in children until 2 years, and the important factor for its development was bad socio-economical status with bad nourishment, composed mostly of flour and cow milk[130–134].

Researches of 575 school children and adolescents in Zagreb and Pazin were carried out by Colić-Barić and collaborators (Table 9). They discovered energy taking of 95.5 and 83.3% RDA in children and adolescents. Taking energy at breakfast was between 20–30% from the total taking, and there was also high taking of proteins of 253 and 139.6% RDA in children and adolescents. It was noticed that children had higher cholesterol, and adolescents daily taking of fibers, while consumption of fruit was 324 –204 g/per day. The differences in BMI index and nutritive parameters were not big. The researches in representative sample of 1190 children and adolescents showed the total taking of energy of 5% RDA for children and 23% RDA for adolescents, of which 26% of taking applies to breakfast, which has greatest share of milk and dairy products of 63.3%. The total taking of protein was 69%, 48.1% for children and adolescents, and like in former researches, cholesterol was higher in children – 24.3 mg and in adolescents – 19,6 mg, and taking of fibers was higher in adolescents. By comparing nourishment in rural and urban environments, it was noticed that in urban settlements there was trend inclining to healthier nourishment, while that trend was opposite in rural settlements. Energy daily taking was 27.5% RDA in urban and 23% RDA in rural regions, while divided on proteins, carbohydrates and fats, it was 15%, 32.6% and 52.4 % for both regions. Taking of cholesterol was higher in urban settlements, from 59.9% to 39.7%. Taking of micronutrients at breakfast (vitamins A, D, E, thiamine, riboflavin, niacin, vitamin B6, B12, calcium, phosphorus, magnesium, iron, zinc, iodine and selenium) was bigger in urban environment in %RDA and DRI, while taking of C-vitamin and folic acid was bigger in rural regions. Šatalić researches the state of nourishment of student population in Croatia in the school year 2002/03, in which he included 5 university centers (Zagreb, Split, Osijek, Rijeka and Zadar). By quantitative questionnaire (FFQ), 2,433 students were included (2.3% of total student population in Croatia). The results showed adequate taking of meals in both sexes, while men had in their nutrition more carbohydrates; women were taking food with bigger nutritive values. An average daily energy taking was relevant to recommendations, although taking of cholesterol was too high, and taking of nutritive fibers was too low. Adequate taking of micronutrients was discovered in 9.5 % of students, and it was also discovered that 50% of students didn't take enough quantities of iron, folacin and vitamin E. The quality of nourishment, according to the Mediterranean indicator of quality of nourishment (M-DQI), showed that the most number of students have bad nourishment (84.3%). The level of well-fed, regarding to BMI, showed that 80% of students are adequate fed and the biggest number of fat students were from Osijek and continental parts of Croatia, while the biggest number of under nourished students were from Zadar.

Investigation of differences in nourishment between fat adolescents and those with normal weight were carried out by Perl on the pupils of the 7th class of one school. The results showed that adolescents with normal weight prefer sweet, meat and cereals, which directed to psychological and social factors in fat adolescents. Antonić-Degač points to the great frequency of caries among school children in Croatia, about 52%. The main reason for it was the high percentage of consumption of refined sweets, sweet fruit syrup and carbonated drinks[135–141].

Celiac disease is chronical and it's caused by the deficiency of one enzyme, or by malfunction of metabolism after taking wheat, rye and barley flour, and it is demonstrated by insufficient absorption in intestines, because of gluten, which consists of glutenin and gliadin, causing gliadin shock in 10–20% of patients. Although the disease is genetically predetermined, immunological and envi-

TABLE 9
EVERYDAY TAKING OF ENERGY, PROTEINS, CHOLESTEROL AND FIBERS, INCLUDING RECOMMENDATIONS (% OF PERSONS (AFTER COLIĆ BARIĆ[136])

Parameters	Children	Adolescents
Taking in of energy (% RDA)		
<95	57.1	72.2
95–105	13.3	9.1
>105	29.6	18.7
Taking in of proteins (% RDA)		
<100	0.4	16.1
100–200	29.6	77.2
>200	70.0	6.7
Taking in of cholesterol(mg/day)		
<200	30.5	43.6
200–300	39.9	31.6
>300	29.6	24.9
Taking in of fibers (% of the age + 5 rule)		
<95	712	86.0
95–105	9.9	5.3
>105	18.9	8.8

ronmental factors are necessary for its appearance. The first signs of that chronic disease usually appear at the age of 5, and in Croatia, according to Matek, the number of sick persons is 1.9 per mille. In researches of Jadrešin, 71 patients were included in the way that 38 patients were on a strict nourishment without gluten, 23 were taking gluten occasionally, and 10 of them were constantly taking gluten, still there were not clinical, body or psychical symptoms of the disease: red blood test, smaller average mass, more frequent anemia and late sex. Kolaček points to the important decreasing of chromosome aberration in children who were on the diet without gluten.

The first evaluation of nutritive status of 284 gastroenterological patients (7% of pancreatitis, 10.9% of cirrhosis of the liver, 12.7% of gastritis, 10.9% of enteritis, 15.9% of chronical liver diseases, 17.2 of cancer and 25% without diagnosis) was carried out by Vranešić in Clinical Hospital in Zagreb. The nutritive status was evaluated by anthropometric measurements (body mass, height), dietetic estimate (qualitative questionnaire for estimate of food quality, way of living as well as self estimate of health and nutritive state) and biochemical analysis of parameters in blood (alkaline phosphatasis, albumin, total proteins, total cholesterol, triglycerides, potassium, sodium, chloride, calcium, phosphorus, magnesium, copper, iron, vitamin A, vitamin D, folic acid, vitamin B12, lymphocytes and thrombocytes). According to the estimate of BMI index 7.3% of patients were undernourished, 5.1% were exposed to that risk, while 37.2% were overweight and 20.8 were fat. Most of the patients were discovered to have inadequate taking of fruits, vegetables and dairy products. Frequency of malnutrition estimated by the method of subjective general appraisal (SGA) was 61.1% out of which 46.1% were only little undernourished and 15% were very undernourished persons. Examined persons were divided into groups (A – adequately well-fed, B – only little undernourished and C – very undernourished) by SGA method and statistically they differed widely according to BMI, the level of albumin, total proteins, calcium, iron, triglycerides, cholesterol, vitamin A and lymphocytes, so resulting with the lower values of these parameters in groups B and C. Vivid undernourishments (by methods BMI and SGA) were found out mostly among the patients who suffered from enteritis and there were also discovered radical departures of biochemical parameters from normal values.

Patients suffering from cirrhosis of the liver had very high values of body mass index (BMI) as a result of a specific pathophysiology (liquid retention), that's why the author points out that body mass and body mass index are unreliable parameters in evaluation of nutritive status in those patients, what directs to the methods of SGA and biochemical parameters. These patients had also lower serum level of vitamin A. The author also shows that an early nutritive intervention for the patients exposed to high risk can't evaluate efficacity unless evaluation of nutritive status was made while receiving them into hospital[142–146].

Apart from presenting the first and the only food for man in the first months of life, milk is admitted in the world as the fundamental nutritive product that contains not only energy value, but also vitamins and minerals. As most of people stands milk without problems, its consumption is pretty large in the world, so in some countries people consume 600 l of milk per person, while in our country, the consumption of milk is pretty low (around 100 l / per the member of the household). Important information is about everyday consumption of milk in only 66% of school children and 45% of adolescents. Colić Barić and collaborators test the influence of dairy products on certain population groups, as well as presence of milk and dairy products in the nourishment of socially handicapped older persons (Table 10). Milk and dairy producers presented the main products for breakfast of school children. We can also say that there was bigger taking of milk and dairy products in urban settlements, and as for sex, there was bigger taking in girls. Panjkota Krbavčić researches milk and dairy products in hospital children and the presence of milk and dairy products in hospital nourishment of pregnant women suffering from diabetes. The most consumed dairy products in pregnant women were milk and yogurt, which satisfied the needs of taking minerals (calcium and phosphorus), vitamins and riboflavin[147–153].

Recently, we have found in human nourishment many elements with harmful and toxic effects, which are the consequence of anthropogenic processes, mostly of industrialization of the last century. Many of these elements are pretty widespread, so we find them in the ground, water and air. In regard to the natural cycle of elements, such elements often finish in human milk, as well as in other animal milk and fats, but also in meat, vegetables and fruit. The research of daily taking of pretty widespread and toxic heavy metals, lead and cadmium, was carried out on Croatian population by Sapunar-Postružnik from 1988 to 1993. The results of researches showed daily taking of these elements: 701μg of lead and 4μg of cadmium per person, what would be according to tolerant table for 1 week (PTWI) 19.9% for lead and 24.4% for cadmium. Daily taking of arsenic per

TABLE 10
ANTHROPOMETRIC CHARACTERISTICS OF EXAMINEES
(AFTER COLIĆ BARIĆ[149])

Parameters	Urban environment	Rural environment
Body mass		
Boys	66.9±11.54	72.4±9.65
Girls	58.2±7.53	60.7±7.77
Body height		
Boys	177.4±5.8	178.8±5.07
Girls	167.2±6.15	162.3±7.22
Index of body mass BMI (kg/m^2)		
Boys	21.0±3.11	22.6±2.84
Girls	20.8±2.27	22.0±2.20

person was 7.8% from allowed quantity, according to PTWI table. Blanuša and collaborators, by measuring biological materials (fish, mussels, vegetables, bred animals), estimated taking of lead and cadmium on 6–40% from allowed taking, by PTWI table, and the most part of Croatian population consuming mercury was in Dalmatia. Opposite of researches of harmful elements, Klapec and collaborators, as well as Matek and collaborators, investigated the taking of selenium. Selenium, as essential element for man, has become very important recently, because of its strong antioxidant characteristics, what classifies it in preventive potential for many diseases (like Keshanov disease), harmful influences of heavy metal, and it has important role in etiology of cancer. Klapec researches supplying with selenium in regions with endemic nephropathy. With the analysis of food from endemic and controlled region and with determining its consumption, it was found out that endemic population takes more selenium than controlled, and some examinees even more than recommended daily dose. Considering the results, Klapec doesn't exclude the influence of selenium in etiology of nephropathy. Another important thing is taking of fibers that represent short chains of fat acids and gasses, and their positive effect is the reduction of glucose in serum, cholesterol and preventive importance in cardiovascular diseases. Perl researches taking of fibers on the sample of 54 persons from east Slavonia. Researches based on enzymatic-gravimetric methods showed sufficient daily taking of 30g. It is also pointed to 21% of higher taking of fibers during the summer period, what is caused by enlarged taking of fruit and vegetables, which after cereals, give the biggest contribution to the taking of fibers[154–161].

In Collegium Antropologicum

Collegium Antropologicum was edited for the first time in 1977 that was the journal with different fields of anthropology and similar scientific disciplines, certainly including nutrition. Concerning many important works about population researches in Croatia, it represents important part in totality of journals that edited works on mentioned theme. Collegium Antropologicum and nutrition are certainly connected by the fact that Professor Maver, a co-founder of this journal, was one of the pioneers of anthropological nutritive science in Croatia.

Concerning nutritionist themes, Collegium Antropologicum deals with nutritive status, nourishment of children, regional populations, patients and the influence of nourishment on certain diseases. We must emphasize the works that stressed necessity for multidiscipline approach to nutritive science, and which were led by Lazarević's work. The most important authors who edited their works on nutritive theme in Collegium Antropologicum were Z. Kusić, A. Kaić-Rak, J. Grgurić, N. Smolej-Narančić, A. Sujoldžić, L. Škreblin, S. Turek, M. Strand, E. Mesaroš-Kanjski, Ž. Prebeg, M. Čubrilo-Turek[16,162].

With the idea of multidiscipline approach, Lazarević researches the nourishment of two islands belonging to Zadar archipelago, Silba and Olib. In his work, Lazarević represents socio-cultural teaching of nourishment on these two islands, giving most attention to tradition and culture of the inhabitants´ nourishment. In order to determine biological status of these islands, Smolej and collaborators carried out researches a year earlier. There were measured 49 morphological and physiological variables, which discovered great difference in certain parameters on this specific island population[16,163].

Evaluation of nourishment state and nutritive status of adolescents were carried out with multidiscipline approach by Škreblin and Sujoldžić in researches on island Hvar, on a sample of 299 inhabitants at the age of 15 and 19 (40% of total number of that part of population). Multidiscipline approach consisted of biological, collecting anthropometric figures, weight, height with the aim of counting index of body mass ITM, social, standardized nutritive questionnaire and psychological questionnaire about demographic, psychological and social characteristics of examinees. It was shown that the greatest number of examinees belong to the category of normal or wished index of body mass, that nourishment mostly corresponds to recommendations of the world healthy organization, and that nourishment habits were different according to demographic differences of examinees, sex, place of birth, origin and socio-economical status of the family[18].

In order to define nutritive status of Dalmatian population, Smolej Narančić and collaborators tested 4,507 people at the age of 18–74. In these researches, there were used the results achieved by numerous anthropometric investigations in Dalmatia (Pag, Olib, Silba, Brač, Hvar, Korčula, Pelješac), in the period from 1978 to 1987. The results got by anthropometric measurements were compared with the results of US NHANES II documents, and there were noticed numerous differences, for which it was not clear whether they were the result of different arrangement of body fats or body construction. According to BMI, Dalmatians have more body fats, what may be the result of centralization of body fats or stronger body musculature. Researches, that had already been carried out by Prebeg and collaborators, and which had been comparing school population within Croatia, as well as with USA, showed that BMI of school children in Zagreb was higher than in other parts of Croatia, as well as it was higher than BMI of school children in USA[164–173].

The studies of evaluation of nutritive status of 181 pregnant women, investigated by Zekan and collaborators, showed that the average increase of body weight at the end of pregnancy was 4.4kg, of which 5.7kg was body fat. According to parameters of body mass index (BMI) and the thickness of skin wrinkle of triceps, the weight of children was estimated. The researches, carried out by Puljević and collaborators, show connection between body mass and appearance of rheumatic symptoms, which discovers that increased body weight is an additional factor in the appearance of mentioned symptoms. Puljević and collaborators also estimate the relationship between higher body mass and complex illnesses on the

sample from 1,583 workers of a railway company. The investigations showed less frequency of such illnesses in people with ideal body mass, while another study showed higher frequency of osteoporosis in fat women. This study proved that nourishment is absolutely one of the essential ecological factors that influence the development of these illnesses[174–176].

In conducted analysis of lipids, lipoproteins and markers of fibrinolitic activity, carried out by Čubrilo-Turek, it is showed that higher concentration of fibrinogen represents the most risky factor in myocardial infarction, and Vincelj and collaborators point out a higher total cholesterol in 75% of patients with acute myocardial infarction, as well as pretty higher LDL cholesterol. Sučić and collaborators investigate nourishment with pilchards in patients with hyperlipoproteinemia, and show that nourishment with fish, statistically, greatly decreased the level of total cholesterol for 10.7%, LDL cholesterol for 11.7%, VLDL cholesterol for 14.8% and triglycerides for 12%. These results help the author to conclude that nourishment with pilchards can help to decrease the development of arteriosclerosis[177,178].

After liberation of occupied territories in the period between 1995–1997, very important researches were carried out by Turek and collaborators, by the help of Ministry of health and Croatian health insurance. These researches were conducted with the aim of development and planning strategy of prevention and necessary help to the total population in Croatia. By evaluation of BMI values, 48.1% of men and 34.7% of women showed overweight, measurements of blood pressure showed that around 50% of men population has higher pressure, systolic above 83mmHg and diastolic above 13mmHg, 27.7% of population suffered from hypertension, that is systolic pressure above 140mmHg and diastolic above 90mmHg. Comparing men and women, percentage of hypertension was 31.9/23.6. By measuring triglycerides, it was discovered that men have higher values than women, consistently to the age, as well as values of HDL and LDL cholesterol according to nutritive questionnaires, it was discovered in 94.4% cases that cooked food is much healthier, as well as taking fish at least once a week[179].

Grgurić and collaborators research on a sample of population from 3 regions in Croatia, free territory, the first line of front and liberated parts. The questioned persons were from households (1,563) who had children under 5 (1,937). Researches discovered very low rate of breast feeding, and very high nourishment with cow milk during the first 6 months (30%) and 1 year (60%) of child's life, which represents negative results in regard to high risk of sideropenic anemia in children fed with cow milk in the first year of life. Apart from these results, it was noticed that children are badly supplied with iron, because of lack of glycoprotein and lactoferin, which can be found in mother's milk, and another discovered thing was lack of vitamin D[180].

The study by Strand and collaborators about geographical extension of cancer in Croatia with emphasis on nourishment, shows equal extension of colon cancer on the whole territory except Zadar and Lika, cancer of gullet is spread in Hrvatsko Zagorje, Varaždin, Osijek, Slavonski Brod and Istria, while liver cancer is mostly frequent in east Slavonia and south Damatia[181].

Identifying of goiter was carried out by Mesoraoš-Kanjski and collaborators, by researching on north Adriatic island Krk on the sample of 1975 school children between 7 and 19. Prevention of goiter in children was 29.8%, and average levels of vitamins A and E in plasma of children with enlarged thyroid gland were lower referential values. In 1996, Kusić, in his researches, connected with taking of iodine in organism, define that in continental parts of Croatia, 69–86% of children secret more than 5μg iodine per dl of urine, 17.34% secrets more than 10μg/dl, and in 5–13.2% of children in Zagreb, thyroid gland was above upper limit for their age. Kusić and Jukić, in 2005, also in Collegium Antropologicum, edited comprehensive and well laid out work about the history of goiter in Croatia[182–184].

Conclusion

In this well laid up paper, the intention was to include as many researches of nourishment as possible, which were carried out in Croatia, and which were characterized by varieties of its themes and methodology.

From the very beginning of nutritional research up today, certain number of institutions and scientists were included in many projects of nutritional researches and researching themes, and they gave wide and diverse opus of scientific works. Looking numerically, 44 qualifying works, of which 26 master's degrees and 18 doctoral dissertations (Table 11) were made on nutritional research of Croatian population. In the very development of nutritional researches, important contribution was given by Collegium Antropologicum – C.C. journal with publications of great number of works from that rich and wide scientific field. As this work pointed to the necessity of such researches on Croatian population, we can conclude that nutritional anthropology will go on with its development, including increasing number of scientists-researchers as well as amateurs.

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TABLE 11
QUALIFYING THESIS ABOUT RESEARCHES OF NUTRITION OF POPULATION IN REPUBLIC OF CROATIA

Type of qualifying thesis	Name of the author	Name of thesis	Year	Institution	Thesis advisor
Master's degree	Ivo Jelčić	Change of the attitude of school children to milk prepared from milk powder (use of technique of a small group): sociogrammes in experimental classes	1968	Faculty of Medicine, Zagreb	
Master's degree	Tomislav Horvat	Comparatively testing of knowledge and attitudes about nutrition of the 2nd and 8th graders of elementary school in town and village	1977	Faculty of Medicine, Zagreb	E. Ferber
Master's degree	Dagmar Loffler-Badžek	The choice of fundamental anthropometrics criteria for evaluation and observation of children's feeding up condition	1978	Faculty of Medicine, Zagreb	R. Buzina
Master's degree	Mirjana Smoljanović	The structure of nutrition in educational institutions is the essential factor in minors´ resocialization	1979	Faculty of Medicine, Zagreb	E. Ferber
Master's degree	Zlata Modrušan-Mozetić	The influence of socio-economic factors on the nutrition in infancy	1980	Faculty of Medicine, Zagreb	M. Juretić
Master's degree	Jadranka Marušić	Pupil's nutrition in Split	1980	Faculty of Medicine, Zagreb	R. Buzina
Master's degree	Mirjana Rumboldt	The relationship between arterial pressure and pupils´& youth's feeding up condition	1981	Faculty of Medicine, Zagreb	R. Buzina
Master's degree	Serafina Pasenti	The relationship between nutritive condition and level of cholesterol and glucose in pupils´ and youth's blood	1981	Faculty of Medicine, Zagreb	R. Buzina
Master's degree	Gorjana Gjurić	The activity of rennin and concentration of aldosteron in the plasma of infants with different taking of sodium and potassium into food	1981	Faculty of Medicine, Zagreb	D. Mardešić
Master's degree	Sanja Kolaček	Evaluation of factors that influence maintaining or change of fatness in overweight infants and little children in later development	1985	Faculty of Medicine, Zagreb	T. Kapetanović
Master's degree	Vesna Bosanac	The causes of early ablactation in contemporary conditions of socio-economical development of a family in one district	1985	Faculty of Medicine, Zagreb	I. Švel
Master's degree	Irena Colić	Nutritive values of the meals of social nourishment of students and confirming normative	1987	Faculty of food and biotechnology Zagreb	N. Jurković
Master's degree	Josip Lončar	Influence of the primary health protection in nutritive improvement of school children	1989	Faculty of Medicine, Zagreb	R. Buzina
Master's degree	Nevenka Jelić	The level of serum albumin and cholesterol in infants on a short lasting nourishment	1992	Faculty of Medicine, Zagreb	T. Kapetanović
Master's degree	Vesna Milas	The meaning of the way of nourishment on the beginning of infant diarrhea syndrome	1992	Faculty of Medicine, Zagreb	A. Votava
Master's degree	Krunoslav Capak	Nourishment and the state of well fed of the pupils In elementary schools in some parts of Croatia	1994	Faculty of Medicine, Zagreb	M. Sučić
Master's degree	Dobrivoje Godić	Distribution of pesticides and polychlorinated biphenyl in serum and mother's milk and in their infant's serum in the region of Prekomurje	1994	Faculty of Medicine, Zagreb	B. Štampar-Plasaj
Master's degree	Vesna Benjak	Clinical testing of preparation Bebimil 0 for infant's nourishment	1994	Faculty of Medicine, Zagreb	D. Mardešić
Master's degree	Ahmad Omar Awad Mustafa	The state of well fed of school children in refugee centers in Zagreb	1997	Faculty of Medicine, Zagreb	S. Kolaček
Master's degree	Tomislav Klapec	Evaluation of daily taking of selenium in food	1997	Faculty of food and biotechnology Zagreb	M. L. Mandić
Master's degree	Branka Rožić-Anđel	The influence of the factors of outer environment on children's well fed and the possibility of preventive activity of family doctor	1999	Faculty of Medicine, Zagreb	J. Grgurić
Master's degree	Katica Antonić-Degač	The influence of nourishment on growth and well fed in 2 ethnically homogeneous populations of school children	1999	Faculty of Medicine, Zagreb	A. Kaić-Rak

TABLE 11
CONTINUED

Type of qualifying thesis	Name of the author	Name of thesis	Year	Institution	Thesis advisor
Master's degree	Zora Zakanj	The influence of prenatal factors on growth and development of children during their first year of life	2001	Faculty of science, Zagreb	J. Grgurić
Master's degree	Lana Škreblin	Anthropological research of nutritive habits and biometric evaluation of nutritive status of adolescents	2003	Faculty of science, Zagreb	A. Sujoldžić
Master's degree	Olga Jadrešin	The influence of disregard of nourishment without gluten on health state of children with celiac disease	2003	Faculty of Medicine, Zagreb	S. Kolaček
Master's degree	Zvonimir Šatalić	Nutritive habits and the quality of nourishment of students´ population in Croatia	2004	Faculty of food and biotechnology Zagreb	I. Colić-Barić
Doctoral dissertation	Ratko Buzina	Coaguability of blood and its changes influenced by nourishment and some other factors	1964	Faculty of Medicine, Zagreb	E. Hauptman, B. Kesić
Doctoral dissertation	Ana Brodarec	Nutritive research of 2 populations connected with etiology of heart diseases in Croatia	1965	Faculty of Medicine, Zagreb	B. Kesić, R. Ivančić
Doctoral dissertation	Dinko Kello	The influence of age, sex and nourishment	1975	Faculty of Medicine, Zagreb	K. Kostial
Doctoral dissertation	Velimir Matković	The influence of age, sex and nourishment on the loss of bone tissue	1976	Faculty of Medicine, Zagreb	K. Kostial
Doctoral dissertation	Milivoj Kačić	The influence of age and nourishment on the level of lipids in the blood of older infant and little child	1976	Faculty of Medicine, Zagreb	B. Štampar-Plasaj
Doctoral dissertation	Tomislava Kapetanović	Clinical epidemic characteristics and the importance of nutritive disorders in the children in the first 3 years of life in 3 different regions in Croatia	1979	Faculty of Medicine, Zagreb	I. Švel
Doctoral dissertation	Ljiljana Audy-Kolarić	Comparative research of the influence of modality of chemical compensation on the balance of nitrogen in parental nourishment	1980	Faculty of Medicine, Zagreb	B. Štampar-Plasaj
Doctoral dissertation	Kornelija Subotičanec-Buzina	The influence of nourishment on physical condition of teenagers	1984	Faculty of Medicine, Zagreb	R. Buzina
Doctoral dissertation	Nada Panjatović	The influence of body weight and nourishment on tolerance of glucose in the examinees with damaged tolerance of glucose after 10 years of testing	1988	Faculty of Medicine, Zagreb	Z. Škrabalo
Doctoral dissertation	Irena Colić-Barić	Nutritive contribution of soya proteins to nutritive status	1996	Faculty of food and biotechnology Zagreb	N. Jurković
Doctoral dissertation	Antoinette Kaić-Rak	The influence of nourishment and some life habits in the beginning of intestinal of metaplasia precancerogenesis of stomach cancer	1996	Faculty of Medicine, Zagreb	I. Rotkvić
Doctoral dissertation	Ljiljana Primorac	Characteristics of part of population in east Slavonia concerning taking of fats and fat acids	1998	Faculty of food and technology, Osijek	M. L. Mandić
Doctoral dissertation	Elika Mesaroš Kanjski	Endemic goiter in school children on island Krk	1998	Faculty of Medicine, Rijeka	Z. Kusić
Doctoral dissertation	Ines Panjkota Krbavčić	Nutritive value and protein digestibility of milk food for infants	2000	Faculty of food science and biotechnology Zagreb	I. Colić-Barić
Doctoral dissertation	Tomislav Klapec	The role of taking of selenium with food in etiology of endemic nephropathy	2001	Faculty of food science and technology, Osijek	M. L. Mandić
Doctoral dissertation	Antonija Perl	Daily taking of nutritive fibers determined by 3 dieting methods	2002	Faculty of food and biotechnology Zagreb	LJ. Primorac
Doctoral dissertation	Kornelije Brkić	Biometric analysis of nutritive indicator of the state in Croatian army	2003	Faculty of Medicine, Zagreb	P. Rudan
Doctoral dissertation	Darija Vranešić	Assessment of nutrition status of the patients at department of gastroenterology	2005	Faculty of food and biotechnology Zagreb	Ž. Krznarić

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NUTRICIONISTIČKE STUDIJE U HRVATSKOJ – STOLJEĆE ISTRAŽIVANJA

S A Ž E T A K

Ovaj rad posvećen je pionirima nutricionističkih istraživanja u Hrvatskoj; profesoru Edvinu Ferberu, profesoru Hubertu Maveru i profesoru Ratku Buzini, kojima možemo zahvaliti za izvanredne doprinose u razvoju znanosti o prehrani, kao i za niz znanstvenih publikacija od 50-tih do 80 -tih godina 20. stoljeća, ostavljajući nam izuzetne informacije o prehrambenom stanju u Hrvatskoj populaciji. Rad donosi pregled rezultata dosadašnjih nutricionističkih istraživanja u Hrvatskoj. Posebna pažnja posvećena je povijesnom pregledu te radovima objavljenim u časopisu Collegium Antropologicum. Od početka nutricionističkih istraživanja do danas priličan broj institucija i znanstvenika bio je uključen u mnoge projekte, dajući širok i raznolik opus znanstvenih radova, što dovoljno dobro oslikava multidisciplinarni pristup ovoj temi. U radu se također osvrćem na rezultate istraživanja 44 kvalifikacijska rada od kojih je bilo 26 magistarskih radova i 18 doktorskih disertacija.

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The book Prehistoric Herders of Northern Istria (Croatia): The Archaeology of Pupićina Cave is a compilation of multidisciplinary studies on Neolithic and Bronze Age material from Pupićina Cave in Istria, Croatia. This is the first volume of several planned that will present detailed results from excavations at the Pupićina Cave, as well as from other prehistoric sites in Istria. After this volume additional two are planned. These will focus on Mesolithic and Upper Paleolithic remains from Pupićina. In preparing this book, editors had three goals: to introduce this prehistoric site, its setting and methods of excavation and analysis; to provide a complete account of the results of its post-Mesolithic deposits; and also to place the results from Pupićina Cave within the wider regional context of prehistoric Istria and the eastern Adriatic.

The book has 542 pages and is divided in 13 chapters. The text is accompanied with figures, tables and graphs, which provide aid in more comprehensive understanding of the subject. After the Introduction follows the main body of the book – specialist analyses in chapters 2–11. At the end of the volume there are two concluding chapters (chapters 12 and 13) in which the overall results of analyses are discussed. Every analysis focuses on different classes of excavated material using corresponding analytical techniques. Alongside standard analyses of pottery, lithics and stratigraphy of the site, the faunal remains, bone and antler artefacts, mollusks, charcoal and phytoliths, small vertebrates and pollen are subjected to specific analyses.

Book begins with a general introduction to Istrian landscape and archaeological research in Pupićina Cave. A chapter that examines regional evidence of vegetation change from the Ćićarija uplands follows. Third chapter gives the general information about the site and excavation methods used, as well as a detailed description of the stratigraphy and excavated contexts from the Neolithic period to modern times. Geological analyses focused on micromorphological evidence of stabling deposits are presented in chapter four. The discussion on the typology and technology of excavated ceramics is given in chapter five. Chapter six introduces us to analysis of flaked stone artifacts. The analysis of vertebrate remains is in chapter seven and worked antler and bone assemblage in chapter eight. In chapter nine, the molluskan assemblage is described. After the analyses of wood charcoal and phytoliths given in chapter ten, the last report in the book is about small vertebrate remains. At the end of each chapter there is relevant bibliography covering current issues in Croatian and European archaeology. Last two chapters of the book are concluding ones, bringing together the results of all the analyses. In chapter twelve editors combine all the categories of evidence from the Pupićina cave and its surrounding area to provide synthesis and interpretation of the site. In the last chapter they use the Pupićina Cave data to debate about the spread of agriculture in the eastern Adriatic.

Some chapters in the book offer raw data, completely or in a detailed summary form. This custom is rare, but the benefit from such practice is enormous, as it allows other scholars to evaluate given interpretations and use the data in their research. One of the singularities of this book is in the variety of the contributors. The editors gathered together an international team of specialists to analyze different classes of excavated material. In their analyses they used a wide range of modern scientific techniques that are not very common in Croatian archaeology. Majority of the authors were included in the fieldwork from its beginnings, which is one of the major reasons why they are so familiar with the material and the site.

Books of this length and thoroughness are rare in the Croatian scientific literature. The devoted editors and

multicomponent nature of the site made this possible. The cave has a long continuous prehistoric sequence and is relatively rich in different classes of excavated remains. Bilingual nature of the book makes it available both to Croatian and international archaeological audience to familiarize with the site and research. This will also enable the much needed development of Croatian archaeological technical terms.

Despite the fact that this is an archaeological book it is not exclusively devoted to stratigraphy, chronology and archaeological data summary, but also to the interpretations of archaeological materials in a wider anthropological context. We hope that the additional planned volumes will reach the high standards of this first volume in the series.

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IN MEMORIAM

Milan F. Pospíšil (1931–2006)

Emeritus Professor of Anthropology at the Department of Anthropology, Faculty of Natural Sciences, Comenius University in Bratislava, suddenly passed away on July 24th at the age of seventy-five.

Milan F. Pospíšil was born on 31st October 1931 at the village Pačlavice near Kroměříž (Czech Republic). Educated in Kroměříž, he graduated in zoology and anthropology from the Faculty of Natural Sciences in Brno. His pedagogical and research career began at the Anthropological Institute of the Faculty of Natural Sciences in Brno and later on continued at the Military Medical Academy J. E. Purkyne in Hradec Králové.

In 1957 he moved to Bratislava where he joined the former Department of Anthropology and Genetics of the Faculty of Natural Sciences, Comenius University. In 1971 he took over the function of Department's head and was in this position within full 20 years. After being retired from the employment in 2001, he did not cease his contacts with the University. He gave lectures not only for students of our faculty, but also for students of the Engineering Faculty of Slovak Technical University and of Academy of Fine Arts and Design – until his death.

His scientific interests and contributions were exceptionally wide, but his research activity was directed mainly to various aspects of dermatoglyphics. On this scientific discipline based his PhD dissertation entitled »*The Dermatoglyphics of Slovakia*«, habilitation dissertation »*Indian Remnants from the Province Oriente Cuba*«, dissertation for the scientific degree of DrSc. »*Variability of Human Chiridium and Factors Effected of it*«. In 1991 he was appointed Professor of Anthropology and in 2003 Emeritus Professor. The name of professor Pospíšil will always be connected with organizing international dermatoglyphic symposiums; from 1980 to 1990 entitled as the Valšíks's dermatoglyphic memorial, visited by foreigner specialists, too.

Besides the dermatoglyphics, he was interested in the fields of ethnic anthropology, human genetics, ergonomics, osteology, forensic anthropology, human ecology and ethology, and in the last years also of cultural and social anthropology. There was hardly an anthropological discipline, which was not a subject of his personal interest. Professor Pospíšil was engaged in all forms of pedagogical process. He wrote more than 120 original papers and reviews, eight textbooks, monograph and he was coauthor of publication written by Frank Spencer in two volumes under the title *History of Anthropology – An Encyclopedia*. Milan F. Pospíšil could have permanently inspired all people around him by his knowledge and experiences. His older age didn't negatively influence his activities or mental abilities. Many friends, colleagues, students and the members of the Slovak anthropological society, will sadly miss him.

Daniela Siváková and Milan Thurzo

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