The Town of Čabar, Croatia, a High Risk Area for Multiple Sclerosis – Analytic Epidemiology of Dietary Factors

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A B S T R A C T

Multiple sclerosis (MS) is demyelization disease of central nervous system of unidentified causes. Analytic epidemiological research of 19 patients, clinically approved cases of MS and 25 controls, autochthonic inhabitants of town of Čabar, Croatia, the high-risk zone for the disease, was made. The research plan included case-control investigation – the »door to door« questionnaire – about nutrition habits. An odds ratio (OR) was calculated for all the factors which were more frequently found in the patients than in the controls, and vice versa. The variables that were connected with significant risk for MS in the town of Čabar included: alcohol consumption (p=0.05), animal fats/dried meat products consumption (p=0.007), nitrate salting (p=0.03), strong spices (p=0.007), mixed bread (p=0.002), oat and oat products consumption (p=0.0075). No connection was found with regular consumption of vegetables and fruit (p=0.009), blue fresh fish (p=0.028), other fresh fish (p=0.03), freshwater fish (p=0.002), canned fish (p=0.004), dormouse meat (p=0.007), air-dried meat products (p=0.004) and using the water from water supply (p=0.011). In the town of Čabar nutritional customs, primarily food rich in animal fats, alcohol-abuse, and oat consumption could have an influence on MS pathogenesis in genetically inclined individuals.

Key words: multiple sclerosis, analytic epidemiology, nutritional effects, Croatia

Introduction

Neuroepidemiological research of multiple sclerosis (MS) in Croatia, rank the town of Čabar (TČ) among the high-risk area for this disease in Europe[1,2]. Regions with high risk for MS support the theory that the inclination to the disease is under the influence of multiple interactions of environmental and genetic factors[3–5]. The aim of this study was to evaluate the influence of dietary factors on MS etiopathogenesis.

Patients and Methods

Geographical position of the town of Čabar is a special micro-region on the far north-west part of Croatia, with about 54 km long frontier with Slovenia. Territory, of approximately 800 m heights, is rich with water flows. The climate is severe mountain one, with the number of sunny hours 1973. TČ, is the rainiest and well the most wooded Croatian region (215 km² of territory under the coniferous and beech forest). On January 1st, 2002, on the 280.18 m² territory in 41 settlements and 42 villages, 4387 inhabitants lived: 2200 (50.1%) male, 2187 (41.9%) female. Average settlement density was 16 inhabitants/km² 6–10. The most important settlements of the TČ are Čabar, Prezid, placed in the valley of Čabranka river, Gerovo and Tršće.

Traditional TČ cuisine

The diet of the inhabitants of TČ depends on traditional habits, partly on environment and working especialties. There are no recipes, cooking books, nor any written evidences about the nutrition in the past. There are only some inherited recipes from the end of XIX and XX centuries; originated from Austro-Hungary, and they are very similar to the present ones, still in use. Diet re-

gime did not suffer important changes during last decades. Daily menu consists of three meals; daily calorie input is, in average, higher than organism needs. Fat/ meat diet (pig fat and butter), milk (pure, non-skimmed, nonpasterized) and milk products dominate. Of meat, the most frequent is pork, mostly dried (mainly home- made or industrial sausages – headcheese, roast), then beef, venison, rabbits, mainly roasted and fried. Smoked meat reigns at least 6 months during the year on their tables, especially from the autumn to late spring. Eggs are also frequent on the menu.

Of vegetables potatoes and cabbage is consumed, then beet; of grains – barley, wheat, and oat, then beans (cooked with barley). The potato, basic Goran's food is on daily menu and almost in every meal. Most often they are consumed baked in their jackets, covered with piece of bacon. The food is prepared mainly with lard; herbal oil is used usual only for salad dressing. Green leafy vegetables and fresh fruits are rarely – once or twice a week – on the menu. They pickle the cabbage by salting it firstly, then adding popper, chaperones and fresh horseradish. Once, mushrooms were often used. Traditionally they use home-made syrups of romanac, mint, elder, carrot, beet and apple. The most often used teas are chamomile, elder, linden, mint, dog rose, forest strawberries, melisa, blackberries, and dried apples. They make brandy from fruits (brinjevac, medica). Forest berry fruits are used for home-made liquors, juices, and cakes as well. Herbs and fruits they collect and dry by themselves. The specialties of the cuisine are salmonidae in corn flour, the cake called »zlevanke« and season (autumn) consumption of dormouse meat (Myoxus glis v. Postus) commonly prepared as a stew or roasted[6–10].

Preparing of smoked meat

After slaughtering, the intestines are removed, and after washing meat are cut to pieces. After 24 hours, salting process commences: for one kg of meat, 100 g of salt mixed with popper and garlic is used. Usually in a wide oak vessel hams are placed on the bottom followed with other pieces of meat. Bacon is salted separately, and after three or four days it is put into the drier, and the sausages are put in the drier the second day. The pieces of meat reside in brine 10 to 15 days; hams at least a month, the bigger ones even several days longer. The meat in brine is rotated every day and the lost liquid is added. When the meat is taken out it is wiped and left on the drier at least one day to extract the residual liquid. Smoking process starts the following day. The meat is mainly smoked in the attic, near the chimney, thus the meat is simultaneously exposed to the smoke and the air. Total duration of smoking depends on the weather conditions. Warmer weather asks for longer, and windy and cold weather shorter time. The process depends on the part and the shape of the meat. Hams are exposed to the smoke even to the period of three months. Dried and healthy beech from the forest is used for smoking; in addition dried beech sawdust is used – for more intense, but shorter smoking. Juniper and fir are very seldom used

for fire wood as the meat becomes sooty and sticky. Sometimes, to the beech an amount of »brinje« grapes is added for the smell and aroma.

Patients

The MS patients in TČ have been systematically explored from 1964. i.e. for 45 years. Medical records from Department of Neurology, KBC Rijeka and other Departments of Neurology in Croatia and Slovenia, and several neurological clinics in Germany were collected.

Diagnostic criteria

Patients with a definite diagnosis of MS according to the criteria of Poser[11–15,] and criteria of neurovisual diagnosis of MS using magnetic resonance imaging (MRI) were included[15–20]. On the day of the beginning of the questionnaire 19 MS patients fulfilled the criteria as the cases of approved MS, were united and accepted. The control group was of the same sex and approximately the same age (they could differ from the patients' utmost in 2 years). Controls that were in no birth connection with the patients could suffer from any illness except MS, or other diseases of know immunological or inherited degenerative causes and psychic diseases. Possible other diseases should not last more then 3 years. Parents and ancestors of the examinees were born and lived on the same area, at least for 4 or more generations. Attempts to reduce differences between the patients and the controls as much as possible resulted in small number of the controls; we succeeded in selecting 25 of them.

Questionnaire – leading the questionnaire

The questionnaire covered a long period – from the examinees' childhood to the day of questioning. We attempted to write down as much facts as possible about the number and ingredients of daily meals (once or several times a day, once or several times an week, once or several times a month, or not one time during the month), about nutritional habits of the parents, and to make evidences of the differences. There were 73 questions, partly connected and partly not. The language of the questionnaire was adapted to colloquial language of the examinees. A »door to door« type of questionnaire was made during 2 weeks period: from June 15th to June 30th, 2004. The attention was primarily paid on the ingredients of the examinees' food, which are traditionally present in their cuisine. The answers to the questions about the nutrition were »yes« or »no«, and questions dealt with the food rich with animal fats, on the consumption of butter instead of margarine, on using herbal oils in preparing the food, and the use of bread. The questions about the consumption of animal brain, sausages, and home-made meat products were categorized in three groups: »often«, »sometimes«, »never«. Special attention was given to the process of preparing and canning of meat products in the patients and controls in which the meat was treated home. To estimate the frequency of home-made meat products in the diet, there was a question about whether there were less or more than a half

meat products made at home, or were they bought in the store. The list of kinds of home-made sausages was adapted to researched area. For the sausages and dried meat products, it was questioned whether they were smoked, dried on the wind, or both.

Medical ethics

The recommendations of World medical association declaration of Helsinki[25] were followed in the research. All the subjects were acknowledged with the aims of the research and they signified their agreements for the questioning.

Statistical analysis

In the research the estimation of rate relation (odds ratio, OD) was calculated for all the factors more frequent in the patients than in controls, and vice versa. The calculation was made by using Compare226 module. The differences were tested by X^2, by fixing 95% interval of reliability for OR, and Fisher's exact frequency test – 2x2 tables. Statistically significant were considered all the values $P \leq 0.005$.

Results

Body mass index did not differ in MS patients and control group. Nutritional variables connected with significant risk for MS in TČ, include use of strong scents in the diet (p=0.007), salting the meat (p=0.03), daily consumption of the mixed bread (p=0.002), frequent oat and oat products consumption (p=0.006). MS patients, more frequently than control group, consumed alcohol, home-made or industrial (p=0.05), along with food rich in animal fats, and pork or beef meat (p=0.017) (Table 1). Consumption of liver sausages and blood sausages had no

statistical importance. Meat and meat products were obtained in home slaughter in more than 50% of all the subjects. Daily consumption of fresh fruit and vegetables (p=0.009) was observed in control group. Onion consumption, as well as carrots, leek, spinach, gourd, mangle and cauliflower covered only 8.3% of the subjects that got the MS late. Fruits consumption; plums, apples, grapes, and strawberries was reported in 33,3% of patients. None of the subjects mentioned blackberries and cherries. Sea fish, primarily blue fish (p=0.028) water fish (p=0.002), and industrially canned fish (p=0.01), was consumed by control group more than MS group. The dormouse proved to be protective (p=0.03). Meat products dried on the air proved to be healthier than smoking process. Controls in TČ consumed the most frequently meat dried on wind (p=0.004). Mother's milk consumption and duration of breast feeding, along with potatoes eating – the most frequent article on the Goran's table, tea and coffee drinking – were the nutritional variables whose frequency were not statistically significant.

Discussion

Present epidemiological research of high-risk areas for MS showed that the probability of finding specific environmental agents is small: there are numerous statistically significant variables, but only a small part of them is excluded as possible biological acceptance[27–34].

In the world, these environmental factors are primarily geographic width, the soil rich with peat, cold and wet climate, and the nutrition rich with the fat and meat, and poor with the fish. The less important influence presents living on the mountain area, coniferous forest, consumption of milk and dairy products, closeness to industrial

TABLE 1
THE COMPARISON OF STATISTICALLY SIGNIFFICANT DIFFERENCES IN CONSUMING DIETARY FACTORS BETWEEN MS PATIENTS AND CONTROLS AND VICE VERSA IN THE TOWN OF ČABAR, CROATIA

CHARACTERISTICS	PATIENTS (N = 19)	CONTROLS (N= 25)	OR	CI	p
Alcohol	31,6	8,0	5,31	0,76–58,8	0,05
Animal fat/meat	89,5	56,0	6,68	1,11–69,0	0,017
Strong spices	50,00	8,0	11,5	1,44–132,8	0,007
Nitrite salting	33,3	4,0	12,0	0,92–6146	0,03
Mixed bread	58,3	8,0	16,1	2,46–127,9	0,002
Oat and oat products	33,3	0,00	Inf.	1,61-inf.	0,0075
Leguminosae	8,3	75,0	0,03	0,001–1,04	0,02 7
Raw fruit and vegetables	58,3	96,0	0,06	0,001–0,70	0,009
Blue fish	75,0	100,0	0,0	0,00–1,06	0,028
Fresh Fish	66,7	96,0	0,08	0,002–1,07	0,03
Canned Fish	8,3	52,0	0,08	0,002–0,78	0,0011
Freshwater fish	58,3	100,0	0,0	0,00–0,40	0,002
Dormouse meat	50,0	92,0	0,09	0,008–0,60	0,007
Air-dried meat	33,33	84,00	0,10	0,01–0,59	0,004
Water from water supply	42,1	80,0	0,18	0,04–0,82	0,011

plants (especially celluloses, paper, textile industries, chemical products, plastics and metal), agricultural works and the contact with animals (cattle and zoonoses). Density of inhabitants, social determinates of simple characteristics (sex, age, profession, education, income, etc.), and the level of the health service, frame the picture of potential environmental influences[35–39]. Nutritional habits of MS patients are the subject of repetitional research, frequently with contradict results. In these researches four possible links of nutrition and demyelization are quoted: absence of redundancy, toxic effects of some ingredients of food, allergic reaction to nutritional agencies, and ingestion of infective substances[40]. Autotrophic and heterotrophic nutrition of herbs, animals and humans in connected with the characteristics of the soil. During the last several years, harmful substances in soil and waters of TČ exceed the borders ruled by the law. In Canada there were already rise charges caused by positive dependence between the content of heavy metals in the water and soil and MS[41].

There are numbers of quotations in the literature that consumption of animal fat (whole milk, butter) and meat (dried meat products) increase the incidence of MS[16,20,23,3]. Namely, animal fat can change myelin sheath and influence production of immunological mediators, prostaglandins or leukotriens[40].

However, there are opposite opinions: In Israel there was found no difference in the consumption if fats between MS patients and controls. In Italy, Casetta et al. negate harmful influence of saturated fatty acids in critical age for development of MS[30]. Epidemiologic researches suggest that non-saturated fatty acids may have positive influence on the development of MS. Thus, Dworkin et al. synthesize results of three double-bind clinical trials with linolein acid that included 87 MS patients and 85 controls, and concluded that cured MS patients with minimal or no invalidity at the beginning of the study show less development of inability and less severe and duration of aggravation, taking in account all the stages of the invalidity and duration of the illness at the including in the study[43]. Taking linoleic acid in the diet of MS patients, according to our experience, may be approved as the measure of help in therapeutic processes in some MS patients. However, up to date, there have been carried out only two analytic researches that give the data about the exact input of fats and fatty acids in the diet of MS patients.

The input of D vitamin is connected with lower incidence of MS[45]. The strongest D vitamin metabolite is 1.25 dihydroxy D3 vitamin. Receptors for this unit are found in monocytes of peripheral blood and activated by T-lymphocytes, which proves the importance of the D3 in the inflammatory process. D3 vitamin in vivo inhibits IL-2 cytokine that stimulates growth of the cells[46–48]. The importance of other vitamins, minerals, and oligoelements is not yet clear. TČ is the most wooded region of Croatia; the woods are prevalently coniferous[6]. In the MS ethnology, Laučer suggests possible influence of harmful agencies (conjugates of hapten carrier) from the smoke of the coniferous wood during the meat drying[49]. In some European states, the essential connection is proved among MS and traditional methods of conserving the meat[49]. In smoking the meat, almost all the examinees of TČ, used beech tree, smaller part of them used fir, sometimes industrially treated by chemicals. Nitrogen oxide, various nitrophenoles and nitrites in the smoke of the wood, and their uniting with proteins in meat, may be biological arguments in the autoimmune processes pathogenesis[28]. Namely, nitrogen oxide may cause severe oxidative damages on biological tissues[50–51]. In previous ecologic research in Gorski kotar, no positive correlation was found in MS and oat consuming[16]. Our repeated research in TČ clearly suggests harmful correlation of oat and oat products and the diet of potential MS patients in the researched area. Significant correlation of this ingredient in MS nutrition is found in some European states (Denmark, Sweden, and Swiss) and in some parts of Germany. Harmful effect of the oat is connected with pathogenic effect of the viruses related to this cereal on myelin[52].

We conclude that fatty diet with lot of butter, pork, particularly dried meat, and fried sausages is connected with higher risk for the disease. Consumption of sea (blue) fish, fresh or canned, has an obvious protective effect on TČ inhabitants. It's consuming, increases input of higher concentration of omega-3 fatty acids from the fish (and fish oil). Similar observation was recorded in the coast region of Norway but not in Sweden and Finland[53,54]. In Croatia, Primorsko-goranska County has the highest rate of positive nutritional habits; firstly by frequent consumption of fruit, salad, leafy vegetables and no additional salting of the food[55]. In its' mountain part, especially in TČ, however, it is opposite; vegetables consuming is far more rarely, once or twice a week, except leguminosae, especially seedcase. Protective effect of vegetables and fruit is probably related to strong antioxidant effects, oligoelements and vitamines[56]. Pekmezović et al. stressed protective effects of cherries in MS patients in Belgrade (OR 0,5; 95% CI=0,3–0,9)[57]. In our research cherries were not mentioned.

Of unusual meals, control group consumed more frequently dormouse meat, and MS patients consumed more frequently headcheese sausage. The recipe for the headcheese is similar to the recipe for cooked pork head and the brain – a specialty consumed by inhabitants, mainly those who develop MS in Shetland that is also, a high-risk zone for the disease in Europe[58].

MS patients are much more dependant to alcohol, especially home-made. It is well known that alcoholism along with malnutrition leads to demyelinisation. An example is central pontine myelinosis[59].

Conclusion

Demographic, economic, historical and cultural characteristics of the Čabar region inhabitants were basic colors on the palette of the case-control research of nutritional components of life style MS patients and their controls in TČ. The diet rich with saturated fatty acids;

meat, especially smoked, oat consumption, and insufficient consumption of fish, fruit and vegetables – factors characterizing the diet of TČ inhabitants represent bio-

logically convincing factors that can interfere in a complex pathogenesis of demyelization in MS.

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GRAD ČABAR, HRVATSKA, PODRUČJE VISOKOG RIZIKA ZA MULTIPLU SKLEROZU – ANALITIČKA EPIDEMIOLOGIJA PREHRAMBENIH ČIMBENIKA

S A Ž E T A K

Multipla skleroza demijelinizacijska je bolest središnjeg živčanog sustava, neidentificiranoga uzroka. Analitičko epidemiološko istraživanje 19 bolesnika, kliničkih sigurnih slučajeva multiple skleroze i 25 kontrolnih ispitanika, autohtonih staniovnika Grada Čabra, Hrvatska, područja visokog rizika za tu bolest, provedeno je sredinom 2004. godine. Plan istraživanja uključivao je case-control ispitivanje – anketu »od vrata do vrata« – o prehrambenim navikama. Izračunat je omjer odnosa (OR) i 95%-tni interval pouzdanosti za sve pokazatelje koji su bili učestaliji u bolesnika nego li u kontrola, i obrnuto. Varijable povezane sa zamjetnim rizikom za multiplui sklerozu u Gradu Čabru obuhvaćale su: pijenje alkohola (p=0,05), životinjske masti/suhomesnati proizvode (p=0,017), soljenje nitritima (p=0,03), jake začine (p=0,007), miješani kruh (p=0,002), potrošnju zobi i poroizvoda od zobi (p=0,0075). Nije ustanovljena povezanost sa redovotom portošnjom povrća i voća (p=0,009) plave svježe ribe (p=0,028), ostale svježe ribe (p=0,03), slatkovodne ribe (p=0,002) konzervirane ribe (p=0,011), mesa puha (p=0,007) suhomesnatih proizvoda sušenih na vjetru (p=0,04), te korištenejm vode iz vodovoda (p=0,011). U Gradu Čabru prehrambene navike u prvome redu masno-mesna prehrana, alkoholizam, korištenje nitrita u hrani i potrošnja zobi utječu na patogenezu multiple skleroze u genski sklonih jedinki. Potrošnja voća i povrća, riba, suhomesnatik poroizvoda sušenih na zraku te bolji higijensko-sanitarni standard djeluju zaštitnički.