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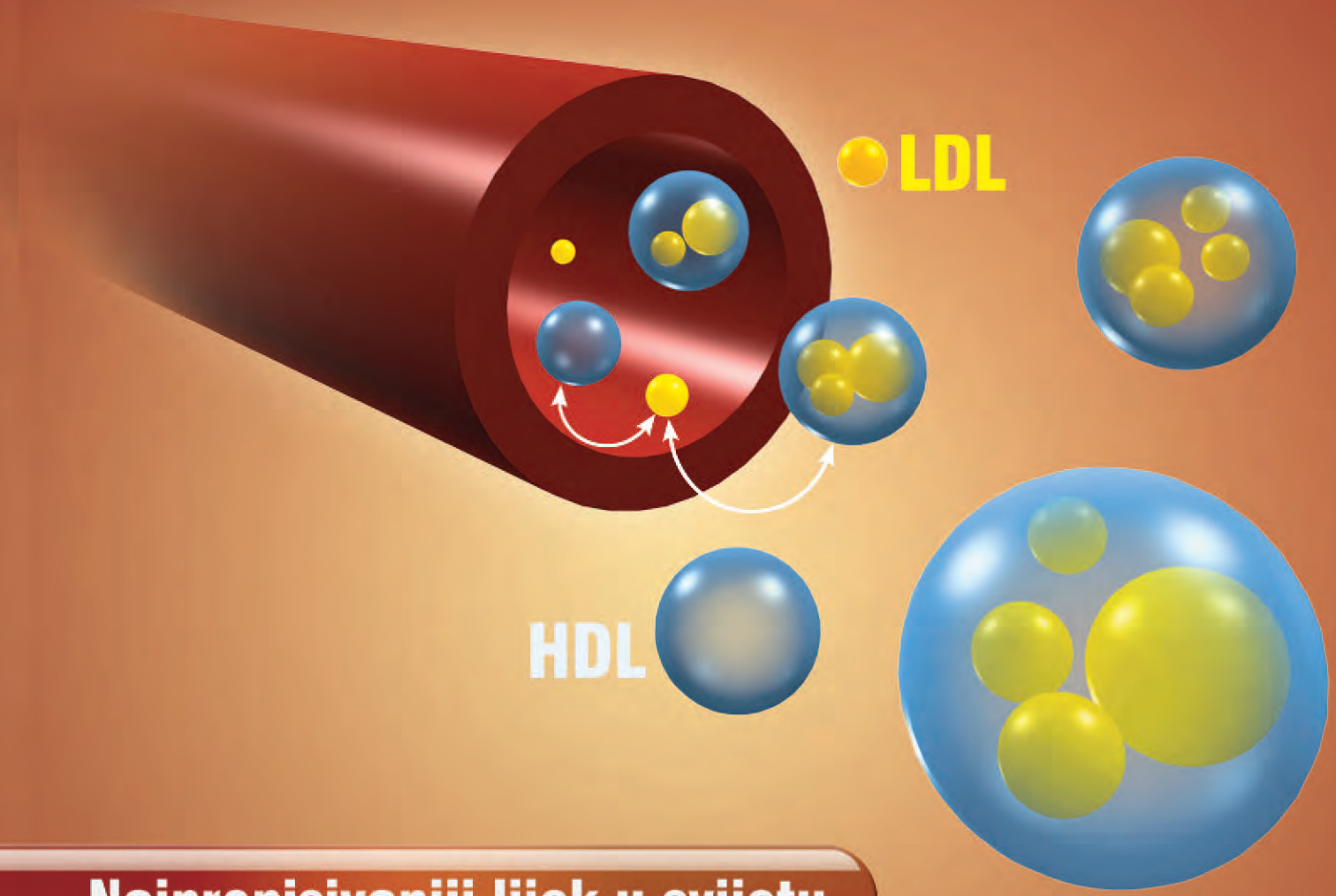

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Gender differences in lifestyle components among patients with coronary heart disease

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Numerous studies had shown that lifestyle modifications can reduce the risk for subsequent coronary events or death in patients with pre-exiting coronary heart disease (CHD). Stopping smoking, regular physical activity and making healthy food choices are an integral part of total risk management in patients with CHD. We evaluated gender differences in lifestyle components of secondary prevention for CHD (smoking status, physical activity and dietary fat intake) in patients with established CHD. This prospective trial included 130 randomly selected patients from Family Medicine Teaching Center Tuzla (66 men and 64 women), aged 40-80 years, with established CHD. We determined smoking status in all participants and assessed dietary fat intake by using modified Dietary Intake Nutrition Evaluation method (DINE). We assessed intensity of physical activity in all participants by using Borg scale for perceived exertion. Mean age of participants was 64.9 ± 7.8 years; 28/130 patients were daily smokers (22%), while 60/130 patients were ex-smokers (46%). More than one third of patients had never smoked (32%), with significantly more women than men ($p = 0.003$). Mean dietary fat intake was 35.4 ± 6.0 g/day; 59/130 patients self-reported regular physical activity (45%). Mean intensity of physical activity was 9.3 ± 1.6 and significantly higher in men than in women ($p = 0.002$). Results of this study showed unhealthy lifestyles in patients with coronary heart disease that indicates the need for more effective intervention by primary care teams to change behavior and modify lifestyles in order to reduce risk for recurrent coronary events.

Key words: Lifestyle, Components, Coronary heart disease.

Introduction

Cardiovascular disease is the leading cause of death worldwide and contributes to nearly one third of all global deaths. By 2020 approximately 25 millions deaths annually are expected from cardiovascular disease, and almost half of those deaths will be related to coronary heart disease (1). According to the limited statistical data, we can conclude that

cardiovascular disease is a leading cause of morbidity and mortality in our country, for men, as well for women, causing 50% deaths of the total mortality. In addition, because of inadequate health culture in the community (high prevalence of smoking, alcohol consumption, obesity, physical inactivity, unhealthy diet and obesity) we can expect a further trend of increasing cardiovascular morbidity and mortality (2).

Numerous studies have shown that lifestyle modifications can reduce the risk for subsequent coronary events or death in patients with pre-existing coronary heart disease (3). Causal relationship between cigarette smoking and increased risk for cardiovascular disease is strong, continuing, linear and independent. Clinical trial evidence suggests that reduction or modification of dietary fat intake may be sufficient to reduce cardiovascular events in certain patients (4,5). The Lion Diet Heart Study found that among survivors of the first myocardial infarction, those who followed a Mediterranean diet (rich in monounsaturated fatty acids) for a mean of 46 months, had a significantly lower risk of cardiac death or recurrent myocardial infarction than those who followed a Western-type diet (14 vs. 44 events; $p < 0.001$) (5). Physical inactivity is one of a major independent risk factors for coronary heart disease. Inactive people have twice the risk of dying from coronary heart disease as active people. The epidemiologic evidence indicates that physical activity can reduce risk for recurrent cardiovascular events in patients with coronary heart disease. An exercise program will significantly increase patient survival and reduce all-cause mortality (6, 7). Physical activity also has a positive effect on other risk factors for coronary heart disease including reducing blood pressure in people with hypertension, improving blood lipid profiles and improving insulin sensitivity (8).

An integral part of the work of primary care physicians for patient's health is prevention of disease and health promotion. Most people with coronary heart disease come to the primary care surgery thinking that primary healthcare professionals are persons who will suggest and give advice regarding regular use of antihypertensive medications, anti-platelet agents, lipid lowering therapy, as well as lifestyle modifications. Optimal secondary prevention includes control of medical components of secondary prevention for coronary heart disease (blood pressure < 140/90 mmHg, serum total cholesterol level < 4,5 mmol/l, prophylactic use of secondary preventive therapies), as well as promotion of healthy lifestyles (smoking cessation, regular physical activity, moderate alcohol consumption, healthy diet and weight reduction if overweight or obese). Interventions to change behaviour and modify lifestyles are an integral part of the primary care physician's tasks for the patient's health. However, many investigations have shown a high prevalence of unhealthy lifestyles in patients with coronary heart disease (9, 10).

Little is known about current lifestyle components of secondary prevention for coronary heart disease among patients in primary health care. This is why we studied lifestyles among patients with coronary heart disease in primary health care to assess lifestyle components of secondary prevention for coronary heart disease and to investigate possible gender differences in lifestyles of patients with coronary heart disease.

Material and methods

This trial was conducted as a prospective, randomized controlled study that enrolled 130 randomly selected patients from the Family Medicine Teaching Center, Tuzla, aged 40-80 years: 66 men (50.8%) and 64 women (49.2%). Every consecutive patient with established coronary heart disease,

who came to the family physician's office for examination during the period March-July 2006, was included in this study. No patient refused involvement in this study. Notes from medical records were reviewed to ensure that patients were documented by hospital or cardiologist letters as having coronary heart disease. We placed a limit of 130 patients for data collection. All patients gave informed consent to the study before attending the clinical assessment.

The main outcome measures were lifestyle components of secondary prevention for coronary heart disease: smoking status of all participants, dietary fat intake, and physical activity. According to the Third Joint European Societies Recommendations on Prevention of Coronary Heart Disease in Clinical Practice criteria used to define appropriate lifestyle, the components of secondary prevention for coronary heart disease were low fat diet, moderate physical activity and not currently smoking (11).

Patients were asked about their smoking habits at base line. Smoking status was analyzed by a questionnaire according to the Standard Questions on the Use of Tobacco among Adults (12). Smokers were defined as persons who smoke daily or occasionally. Ex-smokers were defined as persons who do not smoke at all now, but smoked at least 100 cigarettes or a similar amount of other tobacco products in their lifetime. Never smokers are persons who do not smoke now and have smoked fewer than 100 cigarettes or similar amount of other tobacco products in their lifetime.

Dietary fat intake was assessed by using a modified Dietary Intake Nutrition Evaluation (DINE) questionnaire which provides a quick assessment of an individual's diet by adding the scores relevant to the frequency of consumption of the groups of foods to give a total fat score. For fat, three categories were then derived grouping the scores: low intake (less than 30), medium intake (30-40) and high intake (more than 40). A total fat score of 30 or less on the DINE is estimated to represent a fat intake of 83 grammes per day (g/day) or less, which corresponds to about 35% of the energy recommended dietary allowance for adults. A score of 40 or more indicates a fat intake greater than 122 g/day, or about 40% of energy (13).

Physical activity more than three times a week, at least 30 minutes, was considered as regular physical activity. We assessed the intensity of physical activity in all participants by using a Borg scale for rating perceived exertion (RPE). This is a scale of how a person is feeling while exercising, which ranges from 6 to 20. RPE is a psychophysical scale developed to have a high correlation with heart rates and other metabolic parameters. The RPE scale subjectively measures exercise intensity by using other metabolic parameters. The RPE scale subjectively measures exercise intensity by using verbal expressions to evaluate the perception of effort during walking or running. The RPE scale 6 corresponds to sedentary physical activity, number 7 to 10 to light physical activity, number 11 to 14 to moderate physical activity, and number 15 and more corresponds to hard physical activity (14).

We used Arcus Quickstat Biomedical to manage data and standard statistical methods for statistical analysis. The chi-square test with significance of $P < 0.05$ and independent samples t test respectively were used for comparing proportions and means between groups. We expressed effect size as the difference between groups with a 95% confidence interval.

Results

This trial included 130 patients from the Family Medicine Teaching Center, Tuzla with established coronary heart disease: 66 men (50.8%) and 64 women (49.2%). There were no significant differences in propor-

tions of participants related to gender (p = 0.8041) and age (p = 0.4501). Significantly more men had diagnosis of myocardial infarction than women (p = 0.0002), while significantly more women had diagnosis of angina than men (p = 0.0002). Characteristics of patients with coronary heart disease are shown in Table 1.

Table 1 Characteristics of patients with coronary heart disease

Characteristics	Patients with coronary heart disease		
	Men	Women	Total
Number of participants (n; %)	66; 50.8	64; 49.2	130; 100.0
Age (years; $\bar{x} \pm SD$)	65.8 ± 8.0	64.2 ± 7.5	64.9 ± 7.8
Diagnosis			
Myocardial infarction (n; %)	34; 51.5	13; 20.3	47; 36.2
Angina (n; %)	32; 48.5	51; 79.7	83; 63.9
Complications			
CABG (n; %)	11; 16.7	3; 4.7	14; 10.8
PTCA (n; %)	2; 3.0	1; 1,6	3; 2.3
CHF (n; %)	32; 48.5	24; 37.5	56; 43.1

CABG = coronary artery bypass grafting; PTCA = percutaneous transluminal coronary angioplasty; CHF = congestive heart failure.

Analysis of smoking status of patients with coronary heart disease indicated that 28/130 patients were daily smokers (22%), while 60/130 patients were ex-smokers (46%). Approximately one third of patients never smoked (32%). Significantly more women than men had never smoked (p = 0.003). According to the DINE questionnaire about fat intake we found that 23/130 patients had a low fat diet (17%), 79/130 patient had moderate fat intake (61%), while 28/130 patients had high fat intake (22%). We did not find significant differences for any category of fat intake related to gender. Fifty nine patients self-reported regular physical activity (45%), while more than half of patients had no regular physical activity (55%). Significantly more men had regular physical activity compared with women (p = 0.0017). According to the Borg scale for rating perceived exertion, sedentary physical activity was reported in 3/130 patients (2%), while 110/130 patients had light physical activity (85%). Only 17/130 patients had moderate physical activity (13%), and no one had hard physical activity. We did not find any significant differences in level of physical activity

Table 2 Lifestyle components in patients with coronary heart disease

Lifestyle components	Men		Women		Total	
	n	%	n	%	n	%
Smoking status						
Smokers	17	25.8	11	17.2	28	21.5
Ex-smokers	35	53.0	25	39.1	60	46.2
Never smokers	14	21.2	28	43.8	42	32.3
Fat intake						
≤ 83 g/day	14	21.2	9	14.1	23	17.7
84-122 g/day	41	62.1	38	59.4	79	60.8
> 122 g/day	11	16.7	17	26.6	28	21.5
Physical activity						
Regular	38	57.6	21	32.8	59	45.4
No regular	28	42.4	43	67.2	71	54.6
Intensity						
Sedentary	–	–	3	4.7	3	2.3
Light	57	86.4	55	85.9	110	84.6
Moderate	9	13.6	6	9.4	17	13.1
Hard	–	–	–	–	–	–

related to gender, except that significantly more women had sedentary physical activity than men (p = 0.0376). Data are shown in Table 2.

Mean dietary fat intake was 35.4 ± 6.0 per day with no significant difference related to gender (35.1 ± 6.5 vs. 35.7 ± 5.5; p = 0.554). Generally, all patients had moderate fat intake and light physical activity. Mean level of physical activity was 9.3 ± 1.6, and it was significantly higher in men than women (9.7 ± 1.4 vs. 8.9 ± 1.6; p = 0.002). Patients smoked average 18.5 ± 15.2 cigarettes daily. The mean number of cigarettes smoked daily was significantly higher in men compared with women (22.7 ± 17.2 vs. 10.8 ± 5.8; p = 0.037). Mean values of lifestyle components in patients with coronary heart disease are shown in Table 3.

Table 3 Mean values of lifestyle components in patients with coronary heart disease

Lifestyle components	Men ($\bar{x}$ ±SD)	Women ($\bar{x}$ ±SD)	Total ($\bar{x}$ ±SD)
Fat intake	35.1 ± 6.5	35.7 ± 5.5	35.4 ± 6.0
Physical activity	9.7 ± 1.4	8.9 ± 1.6	9.3 ± 1.6
Number of cigarettes	22.7 ± 17.2	10.8 ± 5.8	18.5 ± 15.2

Discussion

The overall objective of coronary heart disease prevention is to reduce the risks for subsequent coronary events, and thereby reduce premature disability, mortality and to prolong survival. Opportunities for family physicians and general practitioners to undertake preventive activities for coronary heart disease and other cardiovascular diseases in clinical practice are possible, but are not optimally realized.

Lifestyle changes can modify coronary heart disease and significantly contribute to reduction in cardiovascular mortality in established coronary heart disease. Evidence is available that stopping smoking, taking more exercise, losing weight, drinking less alcohol, and eating a more favourable diet can all play a part in secondary prevention of CHD (3). General practitioners have been encouraged to target patients with coronary heart disease for secondary prevention, but putting this into practice has proven challenging.

Our results showed unhealthy lifestyles among patients with coronary heart disease in family practice. Despite the benefits of smoking cessation in order to reduce cardiovascular risk and recurrent coronary events, 22% of our patients continue to smoke. Generally, all patients in our trial had moderate fat intake and light physical activity and more than half of them had no regular physical activity.

Results of EUROASPIRE II study showed a high prevalence of unhealthy lifestyles, modifiable risk factors (21% of patients smoked cigarettes) and inadequate use of drug therapies to achieve blood pressure and lipid goals. There is considerable potential throughout Europe to raise the standard of preventive cardiology through more effective lifestyle intervention, control of other risk factors and optimal use of prophylactic drug therapies in order to reduce coronary morbidity and mortality (8).

Numerous prospective investigations demonstrated a substantial decrease in coronary heart disease mortality for former smokers compared with continuing smokers. Persons with diagnosis of coronary heart disease experience as much as a 50% reduction in risk of myocardial re-infarction, sudden cardiac death, and total mortality if they quit smoking after the initial heart infarction (15). Our results showed that 22% patients with coronary heart disease smoked, which is similar to other studies that analyzed secondary prevention in primary care (8, 9). We did not find any difference in the proportion of patients who smoked related to gender, but men smoked significantly more cigarettes than women (p = 0.037).

Many trials indicate that incorporating moderate-intensity activities into the lifestyle may have benefits for coronary risk factors comparable to those derived from structured exercise programs (6, 7). More than half of patients in our trial had no regular physical activity (55%) which was significantly more in women than men (67.2% vs. 42.4%; p = 0.0118).

The effects of dietary modification in secondary prevention were investigated more than 30 years ago by the Oslo Diet-Heart Study, in which survivors from myocardial infarction were given a low fat diet. Results of this study showed reduction in fatal and nonfatal myocardial infarction by 37% at 5 years, while the rate of coronary mortality was decreased by 44% (16). Most patients in our study, more than half of them (61%) had moderate fat intake and they took 84-122 g/day of fat in their diet with no significantly differences in mean fat intake between men and women (35.1 ± 6.5 vs. 35.7 ± 5.5; p = 0.554).

The American Heart Association Guidelines for Preventing Cardiovascular Disease in Women emphasize focusing on the lifetime heart disease risk for women 20 years and older. The new American Heart Association Guidelines for women's heart disease prevention strategy has three main components. These include: living a healthy lifestyle, addressing heart risk factors, and using medications appropriately. Recommendations are included on physical activity, nutrition, and smoking cessation, as well as detailed recommendations on blood pressure treatment, cholesterol, hormone and aspirin therapy, and use of supplements based on new data (17).

Results of our trial showed that all the lifestyle components of secondary prevention for coronary heart disease were being managed suboptimally. Management was worse in the women, but not significantly. Male patients showed a higher mean level of physical activity than women (9.7 ± 1.4 vs. 8.9 ± 1.6; p = 0.002), but generally a gender-equal level of physical activity and dietary fat intake was established for patients in secondary prevention of coronary heart disease in our primary health care. The exception was sedentary physical activity, which was significantly higher in women than men (p = 0.0376).

Conclusion

Results of this study showed unhealthy lifestyles in patients with coronary heart disease, especially in women. Integrating comprehensive lifestyle changes into standard cardiovascular risk modification programs may be the necessary first step towards improving care for patients with coronary heart disease. It indicates more effective primary care teams intervention to change behaviour and modify lifestyles in order to reduce risk for recurrent coronary events. It will require more effective public health messages and changes in healthcare systems that promote a healthy lifestyle.

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Analysis of alfa-fetoprotein concentrations in maternal serum of Romany and non-Romany women in the Prešov Region of Eastern Slovakia

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Aim. The aim of the study was to estimate concentrations of alpha-fetoprotein in the maternal serum of Romany and non-Romany women in the Prešov region (Slovakia) in the period from 2003-2006 and their comparision with attention to ethnic origin. AFP testing involved 1983 women. **Methods.** The study includes 265 Romany (14-44 years; mean age 23.64±0.57 year) and 1718 non-Romany women (17-45 years; mean age 28.22±0.24 year). To show the statistical difference between the ethnic groups, we employed multiple regression analysis. **Results.** The median values of MSAFP at 15-18 weeks gestation in Romany women were lower in comparision with non-Romany women of majority population in the Prešov region. **Conclusion.** The obtained results suggest that a correction for AFP concentrations or the use of group-specific medians for various ethnic populations would be appropriate.

Key words: Romanies, Ethnic differences, Ethnicity, Alpha-fetoprotein.

Introduction

Alpha-fetoprotein (AFP) is an embryo-specific serum alpha-glogulin protein that is a major component of early embryonal serum in mammals. The biological function of AFP is still not known. AFP produced by the fetal liver appears in the maternal serum in a measurable quantity by the 15th completed week and increases in concentration until term.

Neural tube defects (NTD) are the most common congenital malformation of the central nervous system. Screening of all pregnancies for neural tube and other birth defects using the triple test which combines the AFP (alpha-fetoprotein), hCG (human chorionic gonadotropin) and uE3 (estriol) is now a routine practice. Screening methods improve screening by addition of new markers and combination of serum and ultrasound markers.

Romanies (Gypsies) in Eastern Slovakia constitute a considerable part of the popula-

tion. According to the classic classification the Romanies belong to the White race also called Caucasian, Euro Asiatic or Indo-European. The exact number of Gypsies is not known, it is estimated to be about 10 million in the world, about 5 million of Gypsies live in Europe (12). In the Slovak republic live approximately 400 000 Romanies. The highest concentration of the Romany population lives in Eastern Slovakia.

Results of population genetic structure have revealed high consanguinity and coefficients of inbreeding in the Romany population in Slovakia (2). In literature ethnical differences in the levels of biochemical screening markers were described (4, 11, 1, 8). Benn et al. (1997) concluded that a correction for maternal weight and race should be applied when values for AFP in maternal serum are being interpreted (1).

Patients and Methods

The aim of the study was to estimate the concentrations of AFP in the maternal serum of Romany and non-Romany women in the Prešov region (Slovakia) between 2003-2006 and their comparison with attention to ethnic origin. AFP testing involved 1983 women. Data were collected from AFP tests performed on singleton, non-diabetic pregnancies at 14. to 18. week of gestation. The study includes 265 Romany (14-44 years; mean age 23.64 ± 0.57 year) and 1718 non-Romany women (17-45 years; mean age 28.22 ± 0.24 year). AFP concentrations in maternal serum were measured by standard radioimmunoassay (RIA-test-AFP, Human Lab, CS) according Wald et al. (1987) in the Laboratory of Nuclear Medicine in the Faculty Hospital of J.A.Reiman Prešov (16). AFP values are expressed as multiples of the median (MoM) value established for each week of gestation.

To show the statistical difference between the ethnic groups, we employed the multiple regression analysis using gestational week and ethnicity as explanatory variables (SPSS test, version 15.0). Here, ethnicity was a dummy variable which expresses the two ethnics as „0“ and „1“. Maternal age was also included in the analysis.

Results

The concentrations of AFP in maternal serum in the 14th to the 18th week of gestation in Romany and non-Romany women were analysed. Table 1 summarizes the statistical significance of MSAFP concentrations in Romany and non-Romany women in separate gestational weeks. The median values of MSAFP at 15-18 weeks gestation in Romany women were lower in comparison with non-Romany women of the majority population in the Prešov region.

Results of maternal body weight and maternal age analysis, which are included in the analysis also are given in Tables 2 and 3.

Table 1. MSAFP levels (MoM) in Romany and non-Romany women in the Prešov region (2003-2006)

Gestational week	MSAFP value							
	Romany women				Non-Romany women			
	N	Median	Mean	Standard deviation	N	Median	Mean	Standard deviation
14.	4	0.9950	1.000	0.35954	69	0.960	0.9941	0.3611
15.	21	0.9000	0.9243	0.37633	463	1.010	1.0892	0.3934
16.	131	0.9000	0.9176	0.38832	870	1.060	1.1159	0.3983
17.	63	0.8000	0.9443	0.38864	219	1.000	1.1057	0.4437
18.	46	0.8000	0.9022	0.38583	97	1.000	1.0936	0.4482

Table 2 Maternal body weight (kg) of Romany and non-Romany women in the Prešov region (2003-2006)

Gestational week	Maternal body weight							
	Romany women				Non-Romany women			
	N	Median	Mean	Standard deviation	N	Median	Mean	Standard deviation
14	4	55.00	56.25	6.344	69	59.00	61.28	10.357
15	21	51.00	50.26	7.806	463	58.00	60.91	11.257
16	131	56.00	58.61	12.992	870	60.00	62.53	11.308
17	63	54.00	56.89	13.067	219	60.00	62.72	11.519
18	46	52.00	55.90	11.604	97	60.00	61.91	11.115

Table 3 Maternal age (years) of Romany and non-Romany women in the Prešov region (2003-2006)

Gestational week	Maternal age							
	Romany women				Non-Romany women			
	N	Median	Mean	Standard deviation	N	Median	Mean	Standard deviation
14	4	26.50	27.25	6.131	69	27.00	27.32	4.470
15	21	21.00	22.48	4.106	463	27.00	27.66	4.902
16	131	22.00	23.72	5.564	870	28.00	28.41	4.857
17	63	23.00	23.63	5.865	219	28.00	28.84	5.288
18	46	24.00	23.41	4.906	97	27.00	28.38	5.239

A multiple regression analysis (included MSAFP levels, maternal age and maternal body weight) revealed evidence of a significant trend (beta coefficient for ethnicity -0.194; t = -6.872) (Table 4).

Table 4 Statistical significance of obtained results

Control variables	Unstandardized coefficient Beta		Standardized coefficient Beta	95% confidence interval for B		t	Sig.
				Lower bound	Hoper bound		
Gestational week	0.017	0.009	0.043	– 0.001	– 0.035	1.836	0.066
Maternal weight	– 0.003	0.001	– 0.072	– 0.004	– 0.001	– 3.180	0.001
Maternal age	0.000	0.002	– 0.004	– 0.004	0.003	– 0.155	0.877
Ethnicity	– 0.194	0.028	– 0.170	– 0.249	– 0.138	– 6.872	0.000

Dependent Variable: AFP

Results of analyses confirmed the differences in MSAFP concentrations between Romany and non Romany women. Detected differences in MSAFP concentrations were independent of ethnic differences in body weight (partial correlation: coefficient of correlation: -0.15).

Discussion

Anthropological characterization of isolated ethnic groups takes an important place in the anthropological research. The specific development of Romany children starts in prenatal development influenced by factors which negatively impact the foetus. From

the results of their observations, Bernasovský and Bernasovská (1998) state that Romany newborns differ substantially from other populations. Statistically significant differences were found between the average birth weight, the length as well as head and breast circumferences in Romany and non-Romany newborns of the East Slovakia region. The question arose whether the World Health Organization limit for the low birth weight 2500g is correct for Romany full-term newborns (2). According to data from population genetic investigations amongst Romanies the gene pool of the present day Romany population in Europe is significantly different from the majority of the European population (3).

Selecting appropriate levels of screening tests is also affected by the prevalence of disorder in the population to be screened. The prevalence of neural tube defects varies. The highest incidence of NTD has been reported in Ireland and Wales (6.38-10.92 per 1000 births), whereas its incidence in other European countries has been only 0.1-0.6 per 1000 births (10). The prevalence of neural tube defects in Slovakia was estimated to 1-8/1000 births (7). The incidence of NTD in Romany ethnics in Slovakia has not yet been researched.

Knowledge about the distribution of blood groups, serum and isoenzyme variants, HLA systems and DNA polymorphisms suggests the similarity of the Romany ethnic group to Indian people (3). According to literature data the prevalence of NTD from different parts of India has been reported to vary from 0.5 to 1.1 per 1000 births (14, 15). Kulkari et al. (1989) have found a prevalence of NTD in consanguineous couples to be 16.3–20.6/1000 compared to 5.9–8.4/1000 in couples without consanguinity. The high prevalence of NTD has been attributed to consanguinity (9).

For AFP, the absolute value of a pregnant woman's AFP level is modified by other factors that affect the result. Biological factors and assay conditions are two variables that obviously affect the median serum AFP value; maternal weight is a third, less-obvious one, maternal weight and AFP concentration in serum apparently are inversely related. Race has been suggested as a fourth variable (4).

The median values of MSAFP at 15 to 18 weeks gestation in Romany women in the Prešov region (Slovakia) in 2003-2006 were lower in comparison with non-Romany women of the majority population. These findings are consistent with data from previous studies showing ethnic differences in MSAFP concentrations in screening for open neural tube defects (1, 5, 13). Dar et al. (1996) concluded that there is a predisposition for abnormal levels of serum markers influenced by genetic and/or environmental factors (6).

Many corrections were made regarding gestational age and multiple gestations, thus decreasing the number of abnormal results. Further studies are necessary, especially with regard to detection of neural tube defects and Down syndrome. The results of our study suggest that a correction for AFP concentrations or the use of group-specific medians for various ethnic populations would be appropriate.

Conclusion

Maternal serum screening programs have the potential to decrease fetal morbidity and mortality by providing access to earlier diagnosis, by enabling families to make more informed reproductive decisions, and may by designing appropriate delivery strategies.

There is a need for more studies on the value of MSAFP in monitoring pregnancies with attention to ethnic origin in MSAFP screening of neural tube defects. The aim for physicians and anthropologists remains henceforward to deal with this problem in numerous surveys.

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Lack of association between endothelial nitric oxide synthase glu298Asp variation, visceral obesity and insulin related phenotypes in Turkish type 2 diabetic patients

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Nitric oxide (NO) is an endothelium derived relaxing factor (EDRF) important in regulating heart-vessel physiology. The objective of this study was to investigate whether the eNOS gene Glu298Asp variation influenced the lipid parameters, visceral obesity, insulin related phenotypes and type 2 diabetes mellitus (T2DM) development, for the first time in a Turkish study group. We analyzed the the eNOS gene Glu298Asp genotype frequencies in 115 type 2 diabetic and 68 healthy control subjects. Serum lipids and insulin-related phenotypes were also analyzed. No significant difference for genotypic frequencies was observed for the Ban II (Eco241) restriction site in T2DM patients as compared to controls. eNOS Glu298Asp polymorphism was not found to affect visceral obesity and insulin related phenotypes. However, T2DM patients with Asp/Asp genotype were found to have lower hepatic insulin sensitivity (HIS) in comparison to Glu/Glu. In healthy controls, the insulin and HOMA levels were found to be lower in Glu/Asp genotype with respect to Glu/Glu genotype carriers ($p>0.05$). In T2DM patients, visceral obesity was observed in higher frequencies with Asp/Asp genotype, in comparison to Glu/Glu genotype. eNOS Glu298Asp polymorphism was not found to affect serum lipid levels in the T2DM group. However in the control group, lower serum apoB levels were observed in Asp/Asp genotype carriers in comparison to Glu/Glu genotype ($p \leq 0.05$). The eNOS gene Glu298Asp polymorphism was not found to be associated with T2DM in the present study group. Although not significant, since the eNOS Glu298Asp genotypes were found to be related to HIS, insulin, HOMA and visceral obesity in the present study, further studies on larger samples are needed to explore the exact role of eNOS Glu298Asp polymorphism in insulin related phenotypes and visceral obesity.

Key words: Glu298Asp polymorphism, Type 2 diabetes, eNOS, Hepatic insulin sensitivity, β-cell index.

Introduction

Type 2 diabetes mellitus (T2DM) is a heterogeneous disorder that develops in response to both genetic and environmental factors (1). The predisposition to T2DM is thought to be conferred by a number of different genes that in isolation may have minor effects, but in combination lead to the characteristic pathophysiological condition (2).

Nitric oxide (NO) is synthesized from L-Arginine by nitric oxide synthase (NOS). NOS has three isoforms; neuronal (nNOS), induced (iNOS), and endothelial (eNOS). eNOS is the only isoform constitutively synthesized both in vivo and in vitro (3). Arginine deficiency is a rare occurrence, however, there can be competitive inhibition by the endogenously produced asymmetrical dimethylarginine (ADMA) and nitroarginine. ADMA is emerging as an important cause of endothelial cell dysfunction. The relative deficiency of L-arginine due to elevation of ADMA levels contributes to oxidative stress and results in the atherosclerosis associated with insulin resistance, metabolic syndrome, prediabetes and overt T2DM (4).

Genetic polymorphisms of eNOS have been shown to have a significant effect on NO levels, plasma lipids and have been associated with T2DM (5), heart failure (6), coronary spasm (7), atherosclerosis (8), myocardial infarction (9) and hypertension (9) in some studies. Several studies have reported restriction fragment length polymorphisms (RFLP) of eNOS Glu298Asp to be associated with type 2 diabetes while others did not find such an association (5, 10, 11). Glu298Asp polymorphism of the eNOS gene is caused by a base substitution (G→T) in the position 894 of the exon 7, changing Glutamic acid to Aspartic acid (12).

Since the contribution of eNOS gene polymorphisms to the development of type 2 diabetes differ among populations, the aim of the present study was to evaluate the frequency distributions of eNOS Glu298Asp genotypes in Turkish patients with T2DM as compared to controls. Also the influence of Glu298Asp polymorphism over lipid parameters, visceral obesity, insulin related phenotypes together with their association with type 2 diabetes was evaluated.

Methods

Population sample

We studied 115 unrelated type 2 diabetic patients (67 men and 48 women; age: 58.24 ± 0.94 years). The patients were recruited from Caglayan Florence Nightingale Hospital (Istanbul, Turkey). Age at diabetes onset was 45.05 ± 11.85 years. Type 2 diabetic patients were selected according to WHO criteria (13). Of the 115 type 2 diabetic patients, 64 were treated with sulphonylurea drugs, 38 with metformin and 13 with sulphonylurea drugs in combination with metformin. The study protocol was approved by the Ethics Committee of the Kadir Has University, Faculty of Medicine, and informed consent was obtained from each participant. The control group consisted of 68 unrelated healthy individuals (47 men and 21 women; age: 55.31 ± 1.47 years) without medication, who attended a routine health check at a general practice in Caglayan Florence Nightingale Hospital (Istanbul, Turkey). The hepatic and endocrine functions of the patients were normal and all were relatively well controlled with glycosylated hemoglobin (HbA1c) ≤ 7% (normal range ≤ 8%). Patients with macro- and microangiopathic complications were excluded from the study. No member of the sample populations admitted to alcohol intake and none had a history of smoking.

Clinical and biochemical evaluation

Blood samples were collected after overnight (>12h) fasting. The biochemical analysis included determination of fasting plasma glucose, insulin, HbA1c, hepatic insulin sensi-

tivity (HIS), index of β-cell secretory force (HOMA), total cholesterol (T-Chol), triacylglycerol (TAG), apolipoprotein E (apo E), apolipoprotein A1 (apo A1) and apolipoprotein B (apo B). Serum TAG and T-Chol levels were measured using standard enzymatic methods (Merck, Darmstadt, Germany), automated on an AU5021 (Olympus, Merck). Serum apo E was determined by turbidimetry automated on a Cobas-Mira analyzer (Roche, Meylan, France); serum apo A1 and apo B were determined by immunonephelometry on a Behring Nephelometer analyzer with Behring reagents (Behringwerke, Marburg, Germany). Sera were analyzed without pretreament and diluted in double-distilled water when lipid or apolipoprotein levels exceeded reference values.

Anthropometric measurements
Body mass index (BMI)

The body mass index (BMI) was calculated and overweight (obese) was defined as a value ≥ 25 kg/m^2 (14).

Waist to hip circumference ratio (WHCR)

Waist circumference was measured at the level of the umbilicus while the subject was standing and breathing normally. Hip circumference was measured at the level of greatest hip girth. All participants were accepted as abdominal (visceral) obese, since their WHCR were greater than 0.95 and 1.0 for females and males, respectively.

Pancreatic β–cell secretory capacity

Pancreatic β–cell secretory capacity was estimated by β–index (index of β–cell secretory force; HOMA β–cell index) by the formula proposed by Hosker et al. (15), HOMA β–cell index= 20 × insulin^F / (glucose^F –3.5).

Hepatic insulin sensitivity

Hepatic insulin sensitivity was assessed by the following formulas realized by Matsuda and De Fronzo (16): Hepatic insulin sensitivity (HIS) = *k*/ (G^F x I^F) (k = 22.5x 18=405; G^F and I^F, fasting plasma glucose (mg/dl) and insulin (μU/ml), respectively).

Molecular analysis

Genomic DNA was extracted from leukocytes by a salting out procedure (17). The desired segments were amplified by PCR (18) using the eNOS Ban II (Eco241) protocol with primers (Integrated DNA Technologies, IDT, USA): 298F: 5'–GAC CCT GGA GAT GAA GGC AGG AGA–3' and 298R: 5'–ACC ACC AGG ATG TTG TAG CGG TGA–3'. The final 248 bp amplification products produce 163 bp and 85 bp products for the Glu298 allele, but fails to cleave the 248 bp fragment containing the Asp298 allele after digestion with Ban II. Restricted products were visualized on 2% agarose gel.

Statistical analysis

Statistical analyses were conducted using the Unistat 5.1 software program. Data were expressed as means ± SE. Baseline differences between patients and controls were examined by Student t-test. Hardy-Weinberg equilibrium for genotype frequencies was estimated by the Chi-square test. The variables across the various genotypes and groups were estimated by two way ANOVA with an interaction term to test the influence of eNOS Glu298Asp genotypes on analyzed parameters. The Bonferroni correction for multiple testing was applied to T2DM and control groups separately as required. P values less than 0.05 were considered significant.

Results

The genotype frequency distributions of the 115 type 2 diabetic and 68 control subjects with respect to Glu298Asp polymorphism were compared (Table 1).

Table 1 eNOS Glu298Asp genotype frequencies in type 2 diabetic patients and control subjects

	eNOS Glu298Asp Genotype frequencies		
	Glu/Glu; n (%)	Glu/Asp; n (%)	Asp/Asp; n (%)
Diabetic	15 (13.0)	43 (37.4)	57 (49.6)
Control	11 (16.2)	29 (42.6)	28 (41.2)

Results are expressed as numbers (percentage). The eNOS genotype frequencies of the control and diabetic groups were compared with Chi-square test and no significance was found ($\chi^2 = 0.243$, $p = 0.537$).

The eNOS gene Glu298Asp polymorphism frequencies for Glu/Glu, Glu/Asp and Asp/Asp genotypes were respectively 13%, 37.4%, 49.6% in subjects with type 2 diabetes and 16.2%, 42.6%, 41.2% in the control group. No significant difference was observed in genotype frequencies between the type 2 diabetic and control groups ($\chi^2 = 1.243$, $p = 0.537$). When the demographic characteristics of the study subjects, together with the eNOS Glu298Asp genotype effect and group genotype interaction were examined no significant difference was observed for any analyzed characteristic when the diabetic and control groups were compared (data not included). Also no significant effect of eNOS genotypes was found over the demographic parameters. The clinical characteristics of the study subjects are compared as a function of groups and eNOS genotypes. In detail, insulin ($p \leq 0.001$), HbA1c ($p \leq 0.001$), fasting glucose ($p \leq 0.001$) and β-cell index ($p \leq 0.001$) were significantly higher in diabetic patients compared to controls, whereas HIS ($p \leq 0.001$) was higher in controls. No significant difference was observed ($p \geq 0.05$) for the T-Chol, TAG, apo E, apo A1 and apo B levels when the diabetic and control groups were compared (data not included). The effects of the eNOS polymorphism on clinical or biochemical characteristics were analyzed and not found to be significantly effective (data not included). The demographic and clinical parameters were compared between eNOS Glu298Asp genotypes separately for type 2 diabetic and control groups (Table 2).

Although no significant difference was observed, T2DM patients with Asp/Asp genotype were found to have lower HIS levels in comparison to Glu/Glu. In healthy controls, the insulin and HOMA levels were found to be lower in Glu/Asp genotype with respect to Glu/Glu genotype carriers ($p > 0.05$). Also, in T2DM patients with Asp/Asp genotype, visceral obesity was found to be higher in comparison to the Glu/Glu genotype. None of the analyzed serum lipids were found to differ between the eNOS Glu298Asp genotypes in patients with T2DM (Table 2). In the control group, the demographic, clinical and biochemical parameters were not found to be different among eNOS Glu298Asp genotypes except for apo B levels. In detail, lower serum apo B levels were observed in the Asp/Asp genotype when compared to Glu/Glu genotype carriers ($p \leq 0.05$).

Discussion

Multiple studies provide evidence that genetic factors are important contributors to the inter-individual variation in diabetes susceptibility (19-21). The eNOS gene Glu298Asp polymorphism has been reported to decrease the basal NO production in healthy subjects (22). eNOS Glu298Asp polymorphism may interact with other gene polymorphisms of other endogenous antioxidant enzymes and especially environmental conditions such as smoking, obesity, toxicities of insulin resistance, metabolic syndrome and T2DM as we know their antioxidant reserve is compromised (23–24). eNOS Glu298Asp polymorphism causes endothelial dysfunction, and thus oxidative stress, may be responsible for insulin resistance and T2DM (4). For this reason we thought that

Table 2 Clinical characteristics of type 2 diabetic and control subjects with respect to eNOS gene Glu298Asp genotypes

Parameter	eNOS gene Glu298Asp Genotype					
	T2DM			Control		
	Glu/Glu (n = 15)	Glu/Asp (n = 43)	Asp/Asp (n = 57)	Glu/Glu (n = 11)	Glu/Asp (n = 29)	Asp/Asp (n = 28)
Weight (kg)	69.00 ± 2.38	76.90 ± 2.12	72.35 ± 1.57	72.72 ± 4.82	71.78 ± 2.08	73.14 ± 2.47
Height (m)	1.61 ± 0.02	1.65 ± 0.01	1.64 ± 0.01	1.63 ± 0.03	1.63 ± 0.01	1.63 ± 0.01
BMI (kg/m^2)	26.48 ± 1.02	28.22 ± 0.71	26.99 ± 0.56	26.94 ± 1.21	27.01 ± 0.75	27.47 ± 0.84
Waist (cm)	88.13 ± 5.94	98.62 ± 2.80	97.78 ± 2.47	90.81 ± 7.78	96.82 ± 3.89	96.92 ± 3.93
Hip (cm)	101.20 ± 2.53	104.97 ± 1.32	103.37 ± 1.14	100.45 ± 2.95	104.39 ± 1.43	104.71 ± 1.40
Waist to hip ratio (cm)	0.86 ± 0.04	0.93 ± 0.02	0.94 ± 0.02	0.90 ± 0.06	0.92 ± 0.03	0.92 ± 0.03
Fasting glucose (mmol/l)	9.43 ± 1.36	9.66 ± 0.56	9.61 ± 0.50	3.52 ± 0.17	3.74 ± 0.16	3.51 ± 0.13
HbA1c (%)	7.43 ± 0.65	7.63 ± 0.35	7.83 ± 0.30	4.46 ± 0.14	4.66 ± 0.09	4.54 ± 0.08
Insulin (μU/ml)	11.64 ± 1.62	15.43 ± 0.94	13.88 ± 0.79	4.00 ± 0.61	3.78 ± 0.27	3.87 ± 0.33
Hepatic insulin sensitivity	0.37 ± 0.07	0.22 ± 0.03	0.27 ± 0.03	1.98 ± 0.29	1.93 ± 0.19	2.00 ± 0.19
β- cell index (HOMA)	1.54 ± 0.24	1.95 ± 0.13	1.78 ± 0.10	1.36 ± 0.22	1.22 ± 0.09	1.34 ± 0.13
Total-cholesterol (mmol/l)	5.64 ± 0.41	5.37 ± 0.16	5.39 ± 0.16	6.33 ± 0.37	5.65 ± 0.24	5.30 ± 0.27
Triacylglycerol (mg/dl)	1.40 ± 0.17	2.17 ± 0.27	2.03 ± 0.35	1.69 ± 0.16	2.02 ± 0.19	1.67 ± 0.20
Apolipoprotein E (mg/l)	41.29 ± 3.31	47.28 ± 3.65	45.01 ± 4.19	43.44 ± 4.29	58.04 ± 8.61	49.56 ± 4.87
Apolipoprotein A1 (g/l)	1.45 ± 0.09	1.39 ± 0.03	1.43 ± 0.03	1.41 ± 0.07	1.37 ± 0.04	1.43 ± 0.05
Apolipoprotein B (g/l)	1.14 ± 0.09	1.11 ± 0.03	1.19 ± 0.06	1.33 ± 0.08^A	1.16 ± 0.06	1.08 ± 0.04

Values are represented as mean ± SE. BMI: body mass index. HbA1c: glycosylated hemoglobin. HIS: hepatic insulin sensitivity (= *k* / fasting insulin × fasting plasma glucose (where k = 22.5 × 18= 405). HOMA: homeostasis model assessment (= 20 × fasting insulin / (fasting plasma glucose – 3.5). $^A p \leq 0.05$ Glu/Glu versus genotype in controls.

Glu298Asp variation may be responsible for T2DM.

A limited number of studies have examined eNOS gene Glu298Asp polymorphism (5, 10, 11, 25) in patients with T2DM. According to our knowledge, only one study (5) has found an association between eNOS Glu298Asp polymorphism and T2DM. In detail, Monti et al. (5) evaluated Glu298Asp polymorphism in exon 7 in 159 type 2 diabetic patients without macrovascular complications and in 207 healthy control subjects and described a significant association between eNOS gene Glu298Asp polymorphism and T2DM. Monti et al. (5) suggested eNOS Glu298Asp polymorphism as a new genetic susceptibility factor for hyperinsulinemia, insulin resistance and T2DM. In a study

from the United Kingdom (10), 152 SNPs in 71 candidate genes were examined in 2134 Caucasians, where no association was found between eNOS Glu298Asp polymorphism and T2DM. Thameem et al. (25) investigated whether the T-786C, Glu298Asp and 27bp-VNTR variants of the eNOS gene are associated with T2DM and its related traits in Mexican Americans and did not find Glu298Asp polymorphism as a significant contributor to disease. Similar to Barroso et al. (10) and Thameem (25), we were not able to demonstrate any association between eNOS Glu298Asp polymorphism and T2DM in the present study. A possible explanation for the lack of relationship of polymorphism with disease in the present study may be that patients with gene polymorphism of the eNOS enzyme may be capable of withstanding many years of redox stress before the defect in eNOS becomes evident.

The Glu298Asp allelic variation of the eNOS gene shows variations in different ethnic groups. Ukkola et al. (11) evaluated the presence of the Glu298Asp polymorphism in 239 Caucasian patients with T2DM with a high prevalence of macroangiopathy and 245 control subjects, but did not find any significant difference in the allelic frequency between the T2DM and the control groups. In 159 Caucasian T2DM patients without macrovascular complications Monti et al. (5) reported Glu298Asp Glu/Glu, Glu/Asp and Asp/Asp genotype frequencies to be 32.7%, 39.6% and 27.7%; whereas in 207 healthy control subjects they were 46.4%, 39.6% and 14%. Monti et al. (5) found Glu298Asp genotype frequencies significantly different among their study groups ($\chi^2 = 1$, $p = 0.0005$). In detail, Asp/Asp genotype frequency was higher in type 2 diabetic patients in comparison to controls. Thadeem et al. (25) evaluated 670 low-income Mexican Americans with T2DM, and all first-, second- and third degree relatives. They reported Glu298Asp Glu/Glu, Glu/Asp and Asp/Asp genotype frequencies to be 65%, 30% and 5% (25). In another study, Srivastava et al. (26) reported Glu298Asp Glu/Glu, Glu/Asp, Asp/Asp genotype frequencies respectively as, 71.22%, 28.06%, 0.72% in 139 healthy Indians, and did not find any significant difference between the groups with respect to Glu298Asp genotypes. In our study the Glu/Glu, Glu/Asp and Asp/Asp genotype frequencies were respectively as 13.0%, 37.4% 49.6 %for the diabetic group; and 16.2%, 42.6% and 41.2% for the control group. We did not find any significant difference between the T2DM and control groups, when the frequency of eNOS genotypes were compared ($\chi^2 = 1.243$, $p = 0.537$).

Monti et al. (5) were not able to find any difference in metabolic parameters (plasma glucose, BMI, TAG, systolic and diastolic blood pressure) except visceral obesity (waist to hip ratio). They found visceral obesity much higher in Asp/Asp genotype carriers in comparison to Glu/Glu and Glu/Asp (5). In agreement with the results of Monti et al. (5), although not statistically significant, visceral obesity was found to be higher in type 2 diabetic patients with Asp/Asp genotype when compared to Glu/Glu in our study. Furthermore, Yoshimura et al. (27) showed that Asp/Asp genotype lowered HIS levels in coronary artery disease. Interestingly, in our study we observed that in type 2 diabetic patients the Asp/Asp genotype carriers had lower HIS levels when compared to Glu/Glu genotype carriers. Exercise induced skeletal muscle glucose transport (GLUT4) is eNOS dependent. If the production of eNO were defective due to gene polymorphism and environmental interaction there would be increasing peripheral insulin resistance (28-30). Monti et al. (5) showed that in healthy controls, Asp/Asp genotype carriers had higher insulin, C-peptide, NO, and HOMA levels compared to Glu/Glu genotype. In the present study, in healthy controls, the insulin and HOMA levels were found to be lower in

Glu/Asp genotype with respect to Glu/Glu genotype carriers.

Paradossi et al. (31) evaluated Glu298Asp polymorphism in 118 healthy control subjects and found no influence on lipid parameters. In the present study the Glu298Asp variant of the eNOS gene was not found to be associated with the lipid parameters in the T2DM group. However, Ukkola et al. (11) showed that male diabetic patients with Asp/Asp genotype had higher plasma very-low density lipoprotein (VLDL) cholesterol and VLDL-triacylglycerol concentrations than those with the genotypes Glu/Glu or Glu/Asp. In the present study we found an association between the presence of the Glu-298Asp polymorphism of the eNOS gene and apo B levels in the control group. But we did not find any association between Glu298Asp variation and the clinical parameters in the T2DM group.

Conclusion

There was no significant difference in genotypic frequencies of the Glu298Asp polymorphism of the eNOS gene between the T2DM and control groups. In the present study, the Glu298Asp polymorphism of the eNOS gene is not associated either with visceral obesity or with insulin related phenotypes in Turkish samples with T2DM, but is related to apo B levels in the control group. Since the limitation of this study was the relatively small sample size, the study should be replicated with a larger sample. Increasing the sample size would improve the statistical power of the study to detect significant changes.

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Thyroid calcification: radiographic patterns and histological significance

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Background. Calcification of the thyroid gland may occur in both benign and malignant thyroid diseases. **Aim.** To determine the incidence, significance and plain radiographic patterns in goiters with calcification. **Materials and Methods.** Radiographs (chests, thoracic inlets and neck) and clinical records of 160 goiterous patients were reviewed retrospectively, and classified into two groups; those with calcification and those without calcification. Data analysis was by SPSS 11.0. **Results.** The majority of our patients (64.9 %) were in their 4th to 6th decade of life with modal age of 30 years. The ratio of males to females was 3: 17. Radiographic calcification was demonstrable in 17.0% (male 22.2% and female 77.8%) and this increased steadily by about three–fold per decade of life from 3rd–5th decade. All patients with calcification had tracheal narrowing, higher occurrence of cervical degeneration and 3–fold incidence of retrosternal extension of goitres. However, only four out of thirteen patients (14.8%) with malignant histology had calcification, while the remaining nine patients had no calcification. Subtotal thyroidectomy was offered in 89.3% of patients with calcification. **Conclusion.** Calcification of goitre increases steadily with advancing age and is more common in multinodular than solitary thyroid nodules. However, it does not suggest benignity or malignancy of the thyroid mass. There is high propensity of calcification in goitres having retrosternal extension and a strong tendency for concentric tracheal narrowing in calcified goitres. Hence, this may make the need for early thyroidectomy imperative.

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Keywords: Cervicothoracic radiograph, Goiters, Thyroid calcification, Thyroidectomy, Thyroid histology.

Introduction

Calcification within the thyroid gland may occur in both benign and malignant thyroid diseases. In 1958 Holtz and Powers (1) presented their work on thyroid calcification in papillary carcinoma of the thyroid gland. Thereafter, several authors have written on the significance of thyroid calcification, a common finding on thyroid imaging (1–4).

More recently, sonographically detected thyroid calcifications were related to the risk of cancer (5–10), but there is no agreement yet on the distinct ultrasonographic features that could distinguish benign from malignant thyroid nodules (9). Calcifications, often described in sonographic or radiographic reports, can be detected in both benign and malignant thyroid nodules. Peripheral calcification in thyroid nodules according to Kim et al (8), often presents a sonographic diagnostic dilemma to radiologists because its acoustic shadowing may hinder the visualization of the nodule internal architecture. In addition, the characteristics of thyroid nodules on ultrasound (US) can vary widely between observers. These two ultrasonography limitations are unlikely with the plain radiographs explored in this study.

Neck, thoracic inlet and chest radiographs are part of the preoperative work-up in patients with goitre at our centre. Apart from soft tissue swelling, evaluation of the trachea, oesophagus and cervical vertebrae, calcification in the thyroid swelling can be significant. This is an observational study aimed at determining the incidence of calcification in the various types of goiters and evaluating the importance of the various types of calcification.

Materials and Methods

All patients who had neck, thoracic inlet and chest radiographs for goiters were retrospectively reviewed. The patients' records were reviewed for clinical findings, preoperative radiological investigations, type of surgical operation and histological diagnosis. Only patients who had surgical operations and histological diagnosis were included in the study. The radiographs of this group of patients were reviewed for the extent of the goiter, calcifications, retrosternal extension, cervical vertebral configuration and bony changes. We grouped our patients into two groups; 1) patients with calcification in goiter (CIG) and 2) no calcification in goiter (NCIG) for the purpose of analysis. The data was analysed using SPSS 11.0.

Results

The 160 goiterous patients composing of 136 (85.0%) females and 24 (15.0%) males, who had neck, thoracic inlet and chest radiographs were evaluated. The majority (25.2%) of patients were in the 5th decade of life, followed by patients presenting in the 3rd decade (20.8%) [Fig 1]. The modal age at hospital presentation was 30 years. Calcification in goiter (CIG) was radiographicaly demonstrable in 27 (17.0%) patients whose ages ranged from 23 years to 70 years with modal occurrence at 45–years. There was a steadily increasing CIG of about three folds with each decade of life from the 3rd–5th decade and there was no occurrence before 23 years of life. Patients below 23 years accounted for 6.3% of our total patients and all belong to the NCIG group (Table 1). Amongst the twenty–seven (17.0%) patients who had their enlarged thyroid gland calcified there were six male (22.2%) and 21 female (77.8%). Amorphous and mixed types of calcification constituted about 63% with flat and curvilinear variants very rare [Table 1]. Sampled radiographs illustrating calcifications are shown in figures 2–4.

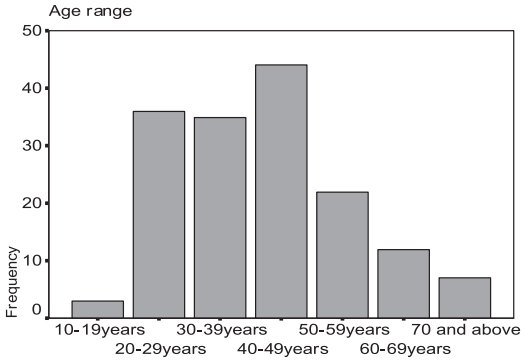

Figure 1 Age distribution of patients with goitres

Table 1 Cross Tabulation of Patient Age Groups with Calcifications and Types

Patients' Age Group	CIG group		NCIG group		Calcification types in CIG group					
	Frequency [n]	Percentage [%]	Frequency [n]	Percentage [%]	Nodular	Flat	Curvilinear	amorphous	Mixed	cloudy
20–29 years	1	3.7	3	2.3	–	–	–	–	1	–
30–39 years	3	11.1	32	24.1	–	–	–	2	1	–
40–49 years	8	29.6	28	21.1	–	–	–	2	2	4
50–59 years	6	22.2	32	24.1	2	–	–	2	1	1
60 years Above	8	29.6	15	11.3	–	–	1	2	4	1
Unsure	1	3.7	12	9.0	–	1	–	–	–	–
Total	27	100.0	133	100	2	1	1	8	9	6

Pre-operative diagnosis in these patients showed that 104 (65.4%) had simple multinodular goitre, 36 (22.6%) had toxic goitre, five (3.1%) had solitary thyroid nodule and 11 (6.9%) had malignant goitre. Histological diagnosis showed colloid 147 (91.9%) as the most common with thirteen (8.2%) having malignant goitre. Follicular carcinoma was the commonest type of thyroid malignancy (Table 2).

Various forms of tracheal narrowing were present in association with CIG. In 74.1% of CIG, there is circular tracheal narrowing in contrast to the 58.6% occurrence in the NCIG. Importantly, there was no patient with CIG without tracheal narrowing

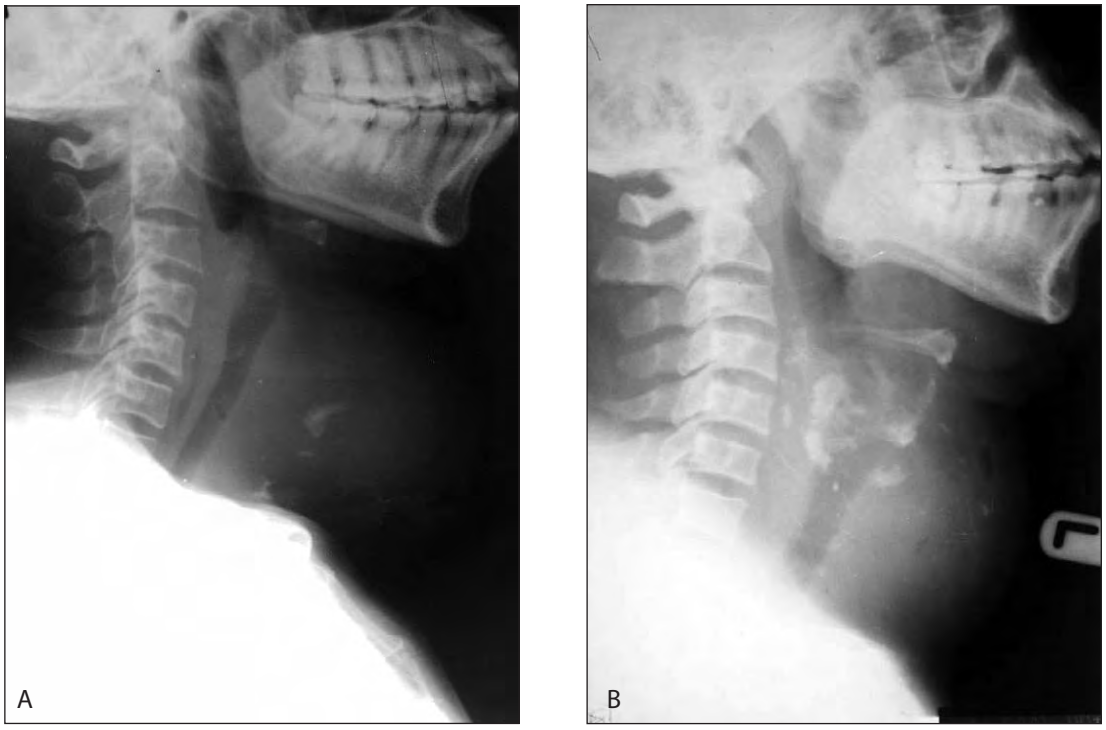

Figure 2 Lateral neck radiographs showing mixed calcifications (nodular and flat). Note tracheal narrowing and air oesophagogram in [A]

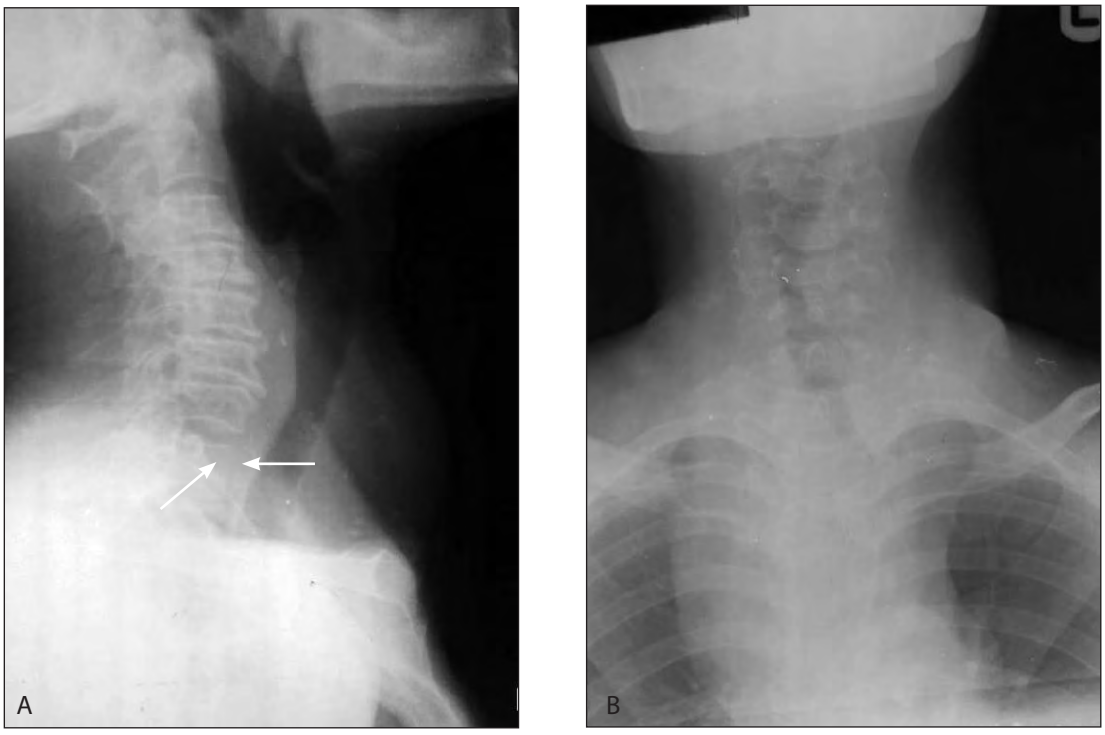

Figure 3 Lateral neck and frontal thoracocervical radiographs of a patient with malignant goitre having retrosternal extension. [A] Showing wide spread nodular calcifications in goitre, cervical osteophytes and erosion of the anterior body of T1 and [B] retrosternal extension with tracheal deviation

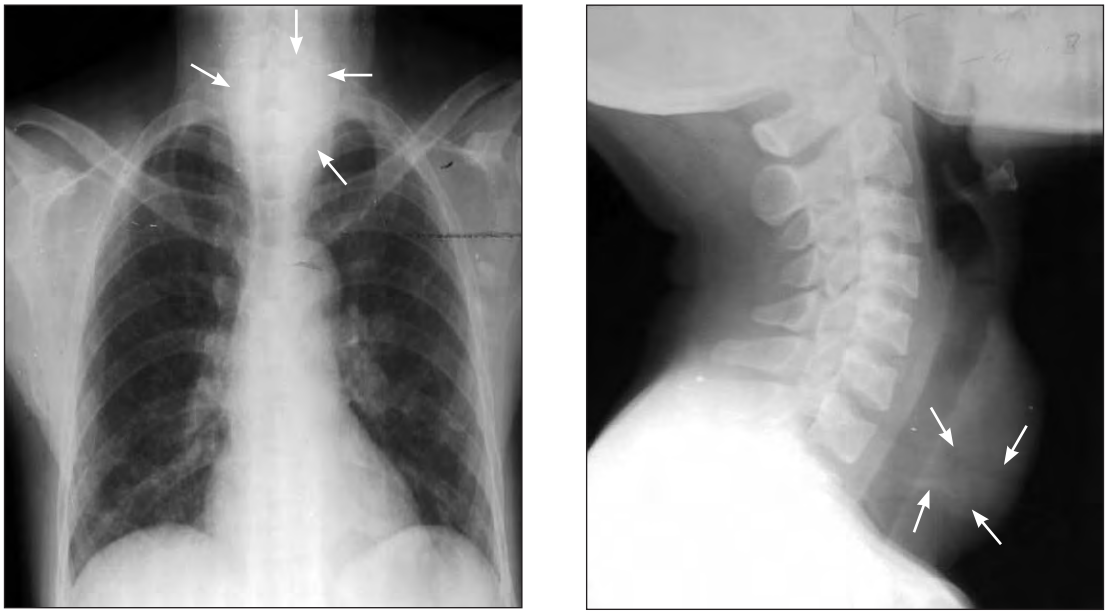

Figure 4 Frontal chest and cervical radiographs of a patient demonstrating cloudy calcification. Note the lobulated mass in the left hilum

Table 2 Histological Variance Cross Tabulation to Calcifications and Types

Histological types	CIG group		NCIG group		Types of calcifications					
	Frequency [n]	Percentage [%]	Frequency [n]	Percentage [%]	Nodular	Flat	Curvilinear	Amorphous	Mixed	Cloudy
Colloid goitre	23	85.2	124	93.2	2	1	1	8	7	4
Follicular carcinoma	3	11.1	5	3.8	–	–	–	–	2	1
Papillary carcinoma	1	3.7	2	1.5	–	–	–	–	–	1
Poorly differentiated	–	–	2	1.5	–	–	–	–	–	–
Total	27	100	133	100	2	1	1	8	9	6

Table 3 Types of Tracheal Narrowing Cross Tabulation to Calcifications and Types

Tracheal Narrowing	CIG group		NCIG group		Types of calcifications						Total
	Frequency [n]	Percentage [%]	Frequency [n]	Percentage [%]	Nodular	Flat	Curvilinear	Amorphous	Mixed	Cloudy	
No narrowing			13	9.8	–	–	–	–	–	–	–
Anteroposterior narrowing	3	3	17	12.8	–	–	–	–	2	1	3
Transverse narrowing	4	4	25	18.8	–	–	–	2		2	4
Concentric narrowing	20	20	78	58.6	2	1	1	6	7	3	20
Total	27	27	133	100	2	1	1	8	9	6	27

Table 4 Relates neck and chest findings to the thyroid groups [CIG and NCIG]

Thyroid group	Tracheal deviation			Retrosternal extension		Cervical degeneration		Air oesophagogram		Heart	
	Central	Left	Right	Yes	No	Yes	No	Yes	No	Normal	Enlarged
CIG	11 [40.7%]	4 [14.8%]	12 [44.4%]	17 [63.0%	10 [37.0%]	13 [48.1%]	14 [51.9%]	5 [18.5%]	22 [81.5%]	15 [55.6%]	12 [44.4%]
NCIG	57 [42.9%]	30 [22.6%]	46 [34.6%]	27 [20.3%]	106 [79.7%]	42 [31.6%]	91 [68.4%]	37 [27.8%]	96 [72.2%]	86 [64.7%]	47 [35.3%]

(Table 3). The sagital and transverse internal tracheal diameters were reduced to less than 1.2cm in 51.9% and 74.1%; and 43.6% and 54.4% in CIG and NCIG patients respectively.

The cervical spine shows predominant straightening in CIG, unlike the normal lordosis that predominated in NCIG (Table 3). The patterns of cervical degenerative changes were similar in both groups affecting c5 vertebra in 40.7% of CIG and 36.3% NCIG. However, the occurrence of cervical degeneration was much higher in CIG – 48.1% in comparison to the 31.6% in NCIG. There was retrosternal extension of goiters in 63.0% and 20.3% of patients with CIG and NCIG respectively. Other neck and chest findings are summarized in Table 4.

Of the patients with CIG, 23 (85.2%) had colloid goitre, three (11.1%) had follicular carcinoma and one (3.7%) had papillary carcinoma. Nine of the thirteen patients who had malignancy confirmed histologically had no calcification [Table 2].

The majority 25 (92.6%) of patients with CIG had subtotal thyroidectomy; only two (7.4%) had total thyroidectomy.

Discussion

Thyroid gland calcification is not uncommon and has been reported by various authors (1-4). There are three radiographic patterns of intrathyroid calcification: eggshell, dystrophic and fine stippled psammomatous calcification (6-11). Although this is not part of the aim of this study, several recent sonographic reports focused on thyroid calcifications have described similar patterns of calcification that include microcalcification, coarse and dense calcification, and rim-like peripheral calcification (7-10). However, the ultrasonic differentiation between malignant nodules and benign nodules based on calcification is still controversial. In a similar vein, studies correlating the histopathological and radiographic significance of thyroid calcifications are rare in the literature.

In this study, 17.0% patients with goitre had thyroid calcification. This is similar to Komolafe's finding in a similar environment (12). The radiological patterns of calcification were described as nodular, flat, curvilinear and cloudy (12). However, some patients present with a mixed pattern. Amorphous and mixed types of calcification constituted 66.7% of CIG in this study which is different from the findings of nodular variants in 72.6% in Komolafe's study (12). The probable explanation for this variation may be the patients' earlier presentation for medical intervention due to increasing awareness as evidenced by the modal age of 30 years at intervention in this study (Table 2).

We found out that CIG steadily increases by about three fold with each decade of life from the 3rd to the 5th decade (Table 1). Importantly, there was no occurrence of CIG amongst the 10 (6.3%) patients younger than 23 years. Again our findings of only four cases of CIG occurring before 42 years of age show that age is an important determining factor of calcification. This may not be unrelated to the length of time that may be required to have sufficient calcium deposition for a calcific focus to be sufficiently opaque on radiography.

The majority (65.4%) of the goiters in this study were simple multinodular goitres with only 6.9% presenting with a solitary nodule. Khoo et al (6) suggested that when calcification is noted within a solitary thyroid nodule, the risk of malignancy is very high. In this study, none of the patients with a solitary nodule had calcification. Of the patients with CIG, 85.2% had histological diagnosis of colloid goitre and 14.8% had malignant goitre. Calcification was found in 31% of patients with malignancy, with 69% of them not having any calcification. Amongst patients with CIG who had malignancy confirmed histologically, 75% of them had follicular carcinoma. Thus, follicular thyroid cancer is the commonest thyroid cancer to calcify with about 3-fold occurrence when compared to NCIG (see Table 2). Similar to other studies from Nigeria (13, 14), follicular carcinoma is the most common type of thyroid cancer in both CIG and NCIG. This study further confirms that calcification can occur in both benign and malignant goitres, though some studies have shown that calcifications were significantly more frequent in malignant goitres than simple multinodular goiters (15). The calcification in benign goitres can be explained from the point of view of haemorrhage, tissue necrosis and epithelial degeneration, which are factors that predispose to dystrophic calcification (12).

All patients with CIG had associated tracheal narrowing with predominance of concentric narrowing in 20 (74.1%). This is in contrast to the occurrence of 58.6% in the NCIG group. Again, in 63.0% of patients with CIG, there was retrosternal extension of goitres as against 20.3% in NCIG. Thus, we infer that CIG have about 3 times greater association with retrosternal goiters. Retrosternal extension may result in vascular compression leading to damping of blood or stasis and subsequent reduced perfusion, which in turn may promote ischaemia and necrosis, thereby playing a role in increasing the incidence of calcification. Cardiac enlargement, which is a recognized finding in thyrotoxic goitre, occurs with a higher frequency in CIG (44.4%) than NCIG (34.6%) as shown in Table 4.

The cervical spine curvature in CIG is essentially lordotic or straightened. The findings of no single case of CIG with kyphosis or scoliosis may show the protective mechanism to reduce the compressive effect of the calcified goiters, which becomes more rigid, on the trachea.

Calcification of goitre is more common in multinodular than solitary thyroid nodules and steadily increases with advancing age. However, the presence of calcification does not suggest benignity or malignancy of the thyroid mass. In malignant goitres, follicular variance is the most common to calcify with about a 3-fold chance of occurrence. There is high propensity of thyroid gland calcification in goitres having retrosternal extension. Similarly, CIG also has a strong tendency to produce concentric tracheal narrowing. Hence, this may create the need for early thyroidectomy imperative.

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Artificial neural network in prediction of the outcome of critically ill patients with perforative peritonitis

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Aim. The aim of the present paper is to compare the use of Artificial Neural Network (ANN) to APACHE II, MOF, TISS-28 and MPI scoring system in prediction of peritonitis-related death in patients with perforative peritonitis. **Patients and methods.** A prospective study was performed of 145 patients with perforative peritonitis, treated in the Surgical Intensive Care Unit. The main outcome of this study was peritonitis-related death. The Levenberg-Marquardt method was used for training, and 16 variables for entrance into the Artificial Neural Network. Sensitivity and specificity of scoring systems are graphically shown for the different values of cut-off points with the receiver-operating characteristic curve (ROC) curve. **Results.** We tested 92 cases in a network and found that the APACHE II system predicted the lowest number of wrong assessments with a score of 12, with all the other scoring systems predicting 19 wrong assessments. The area under the curve for the first postoperative day was 0.87 for TISS-28 score, 0.86 for APACHE II score, 0.83 for MOF and 0.72 for MPI score. The highest rate of correlation between the observed and the expected mortality rate was in the APACHE II system. This demonstrated that TISS-28 and APACHE II are significantly better than other systems ($P<0.01$). In addition, this discriminatory ability was also retained on the third and seventh postoperative days. **Conclusion.** APACHE II is superior in the prediction of patient outcome to the Artificial Neural Network and other tested scoring systems.

Key words: Artificial neural network, Perforative peritonitis, APACHE II, Surgical intensive care unit.

Introduction

Most scoring systems currently used in prediction of mortality in Surgical Intensive Care Units (SICU) are based on logistic regression. Although APACHE II (Acute Physiology and the severity of Chronic illness) system (1) was designed for severely ill medical patients, it has been validated in prediction of patient outcome in surgical pa-

tients with intra-abdominal infections and peritonitis (2, 3), but the usage of APACHE III system is not confirmed in these patients (4). There are scoring systems developed specifically for assessment of Multiple Organ Failure (MOF) (5), systems that reflect the amount of care and can provide useful information about severity of disease and prognosis (Therapeutic Intervention Scoring System – 28) (6) and a scoring system based on intra–operative data (Mannheim Peritonitis Index) (7). The Artificial Neural Network is an alternative technique in the prediction of mortality in SICU (8).

Artificial Neural Networks (ANN) are computer programs that simulate some of the higher level functions of the human brain. In the human brain, there are neurons and synapses, with various synaptic connection strengths, called weights, for each connected pair of neurons. There is a specific set of input and output neurons for each problem and each net corresponds to the inputs and outputs from a traditional computer program (9).

Despite the advancement of intensive care in medicine and the introduction of aggressive surgical techniques, the prognosis of peritonitis remains poor, especially if multiple organ failure has developed (10). About 80% of cases of secondary peritonitis in large hospitals are accounted for by perforative peritonitis, and 10 to 20% can be seen in patients after abdominal operations (11) Patients with peritonitis due to perforation of the hollow viscusa are among the most complex cases encountered in surgical practice (12).

The evaluation of the therapeutic approach requires a precise assesment of the risk to the patient, as mortality remains high, in some instances reaching ~60% (3) With this in mind, we performed a prospective evaluation of several prognostic models with the Artificial Neural Network, in prediction of peritonitis–related death in patients with perforative peritonitis.

Patients and methods

The prospective study involved 145 patients of both sexes with perforative peritonitis. Patients hospitalized in the SICU longer than 24 hours were included in the study. The inclusion criterion was perforative peritonitis, as determined by laparatomy. Exclusion criterion was post-traumatic peritonitis. Patients were tracked either to discharge or death.

The main outcome of this study was peritonitis-related death. APACHE II and Therapeutic Intervention Scoring System (TISS-28) scoring systems were calculated upon admission to the hospital (during the first 24 hours), and on the third and the seventh days after hospitalization. The MPI (Mannheim Peritonitis Index) scoring system was calculated during the first 24 hours after hospitalization or during laparatomy. Data were collected in a computer database made with the commercial program of Microsoft Access. Statistical analyses were performed using commercial software (SPSS 11.0).

Cut-off points were specified (26 points for APACHE II, 26 for MPI, 2 for MOF, 39 for TISS-28) and all values greater than the cut–off points were taken to predict death.

Sensitivity and specificity are graphically shown for the different values of cut-off points. They are presented by the ROC curve. The difference in the area under the ROC curve between scoring systems was tested statistically. The test of the difference between areas under the ROC curve was applied using the trapezoidal rule to approximate areas, conservative estimation for the standard deviation.

The Feed–Forward Artificial Neural Network had 4 hidden layers with 8 neurons in the layer. We used the Levenberg–Marquardt method for training, and 16 variables for the entrance into the network. One half of our data was taken by random selection for training, and the second half for testing

the neural network. The Artificial Neural Network was implemented in a Matlab software environment using the Neural Network Toolbox (Matlab 7.0).

Results

The prospective study involved 145 patients of both sexes with perforative peritonitis. The mean age of all patients was 58 ± 18; the ratio of men: women was 91/54, with no significant differences in the average age between the two genders. There were 92 patients that survived surgery (63.4%) while 53 patients died (36.6%).

Testing of scoring systems with Feed-Forward Artificial Neural Network (ANN)

By testing 92 cases in a network, with a cut off point for all the neural networks of 0.5, and all the other networks specified values of the cut off score, APACHE II system predicted the lowest number of wrong assessments with a score of 12, while TISS–28 and MOF system predicted 15 and 20 wrong assessments, respectively, with all the other systems predicting 19 wrong assessments.

The introduced error is related to the number of wrong assessments. If we compare the given results, we can conclude that the neural network gave better results than the MOF scoring system. Cases in which the neural network or the neural network and the APACHE II system, gave the right assessment, but not any of the other scoring systems, were recorded.

Discriminatory ability of prognostic systems

The APACHE II and TISS–28 scoring systems showed the highest sensitivity and specificity during the first and the third postoperative days, while the sensitivity and specificity during the seventh postoperative day was good for all the scoring systems (Table1).

The ROC curve (receiver-operating characteristic curve) for the prognostic scoring systems used (relationship between sensitivity and the false positive rates [1-specificity] for different cut-off points) was given for the first, third and seventh postoperative days (Figures 1, 2, 3, 4). The APACHE II and TISS–28 curve demonstrated that their discriminatory ability was better than that of the MPI curve. The area under the curve for the first postoperative day was 0.87 for TISS–28 score, 0.86 for APACHE II score, 0.83 for MOF, and 0.72 for MPI score. This demonstrated that TISS–28 and APACHE II are significantly better than other systems in predicting patient outcome ($P < 0.01$) (Figure 1 and 3). In addition, this discriminatory ability remained on the third and seventh postoperative day as well.

Table 1 Sensitivity and specificity of scoring systems.

Scoring systems	Sensitivity (%)			(Specificity %)			Positive predictive value (%)			Negative predictive value (%)		
	First day	Third day	Seventh day	First day	Third day	Seventh day	First day	Third day	Seventh day	First day	Third day	Seventh day
APACHE II	58.5	64.3	77.6	95.7	100.0	98.9	88.6	100.0	98.1	80.0	78.6	85.6
MOF	24.5	30.8	50.0	96.7	100.0	100.0	81.3	100.0	100.0	69.0	71.9	82.6
TISS-28	56.6	60.9	71.9	93.5	100.0	100.0	83.3	100.0	100.0	78.9	77.3	83.3
MPI	52.8	–	–	77.2	–	–	57.1	–	–	74.0	–	–

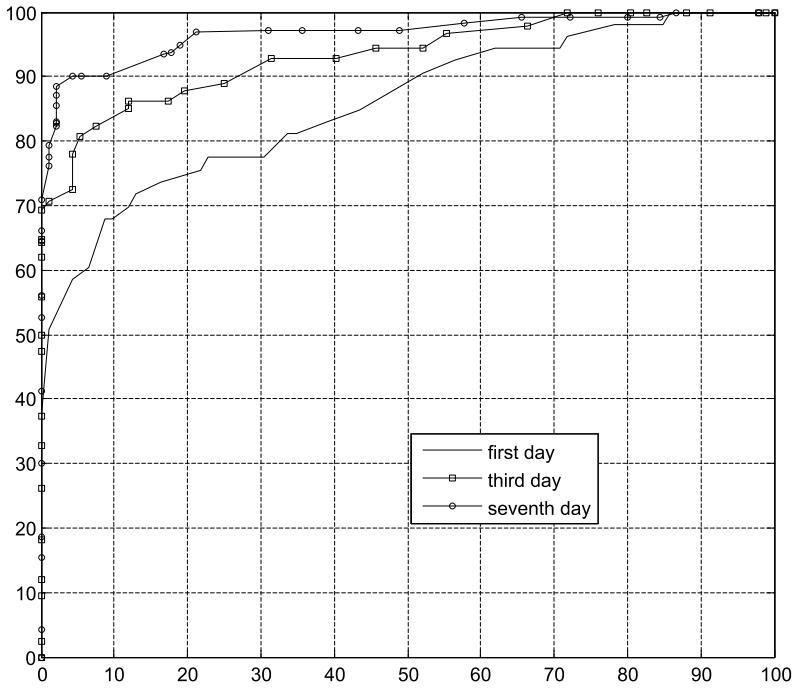

Figure 1 ROC curve (receiver–operating characteristic curve) for the APACHE II scoring system for the first, third and seventh postoperative days

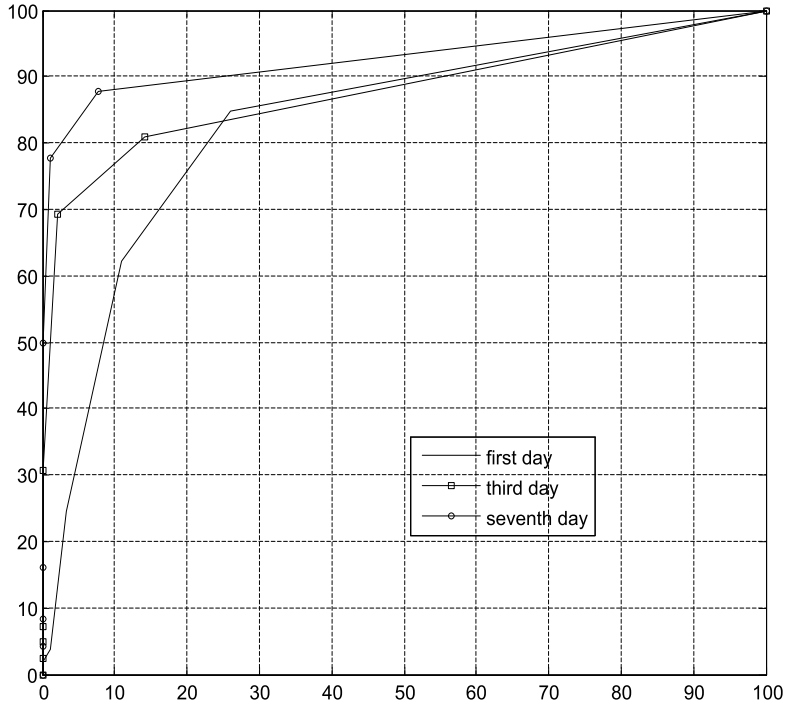

Figure 2 ROC curve (receiver–operating characteristic curve) for the MOF scoring system for the first, third and seventh postoperative days

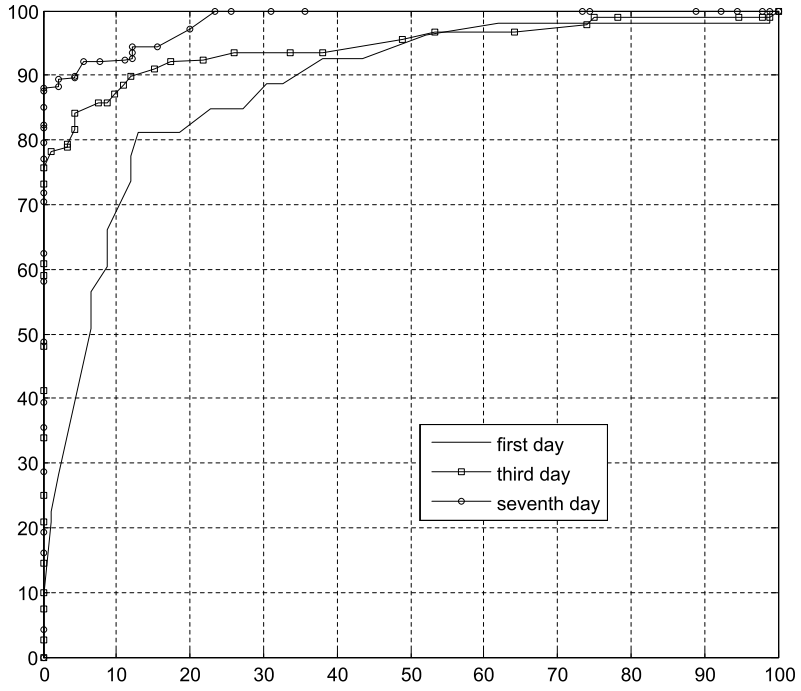

Figure 3 ROC curve (receiver–operating characteristic curve) for the TISS-28 scoring system for the first, third and seventh postoperative days

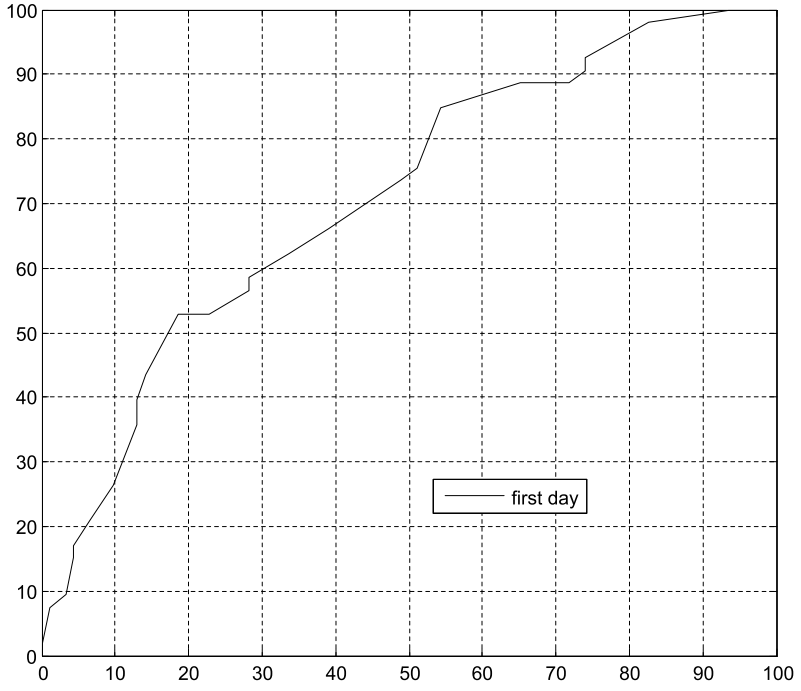

Figure 4 ROC curve (receiver–operating characteristic curve) for the MPI scoring system for the first day

Discussion

We demonstrate here that the APACHE II scoring system gives the best prognosis of patient outcome, and that the neural network itself gives better results than the MOF scoring system. Although some studies have shown that neural networks are superior to logistic regression models (8), or that there is no significant difference between regression models and neural network (13), our data suggest that the APACHE II system is more accurate than other scoring systems. In unvariant analysis, all tested scoring systems were relatively accurate in identification of patients with high risk of death from peritonitis. In multivariate analysis, only the APACHE II and TISS-28 systems independently contributed to prediction of outcome during all days of testing. Although we tested scoring systems on the first, third and seventh days, our study did not confirm the value of serial determination.

The APACHE II system is the best validated prognostic model which estimates general consequences of disease, taking into account age and previous diseases. It can be used in a defined population with the syndrome of systemic inflammatory response (2,3). All variables of the APACHE II system are a part of routine monitoring (14) which makes it easily applicable in everyday practice. APACHE II is extremely flexible, with good prediction capacity and without significant differences between elective and urgent surgery, in benign or malignant diseases, or in the prediction of complications (15).

Peritonitis generally responds promptly to surgical intervention and systemic antibiotics, but some patients continue to develop sepsis, organ failure and death. The seriousness of the disease and organ failure, but not recurrent infections, are the main reasons for lethal outcome in patients with peritonitis (16). Despite the advances in diagnostic techniques, the decision for re-operation of critically ill patients depends on a medical assessment and it can be the source of a variety of conflicts caused by a broad spectrum of pressure concentrated on the surgeon(17).

Experienced doctors in the ICU are generally very good at predicting the probability of survival of their critically ill patients (18). However, it is sometimes very difficult to predict the probability of survival. In addition, doctors often disagree about the prognosis of survival of individual patients (19), and it could be said that the role of subjective assessment of a patient's outcome is a neglected issue, even though occasionally this subjective assessment could be the most powerful indicator of the outcome in comparison to the APACHE II system (20). This could be due to an experienced clinician's ability to evaluate certain factors which current scoring systems do not take into account. Therefore, it is necessary to undertake further studies in this direction. Scoring systems which give reliable prediction of patient outcome, with good agreement between the expected and the observed mortality rates, are useful tools for controlling the quality of treatment and assessment of care (21). The neural network is projected to use all possible data available. The advantage of the neural network, i.e. its applicability suitable of obtaining the assessment from available data, demands further testing of this prognostic model.

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Objective assessment of diagnostic tests validity: a short review for clinicians and other mortals. Part I

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The whole point of a diagnostic test is to use it to make a diagnosis, thus the obvious need is to know how accurately a particular diagnostic test detects patients with or without a disease. In order to know it, a clinician or a researcher should have a basic understanding of the principles of objective appraisal of diagnostic tests. In this short review, the author presents the most common biostatistical methodology for assessment of the validity of diagnostic tests. Definitions and interpretations of sensitivity, specificity, positive and negative predictive values are also provided together with how they are calculated.

Key words: Sensitivity, Specificity, Positive predictive value, Negative predictive value.

Introduction

The very first time I encountered the concept of sensitivity, specificity, positive and negative predictive value, in the end I had a huge question mark above my head. Those somewhat vague parameters, which have an important role in describing the diagnostic performance of a particular diagnostic test, seemed to have a logic that is beyond my grasp.

However, later, when I became much more interested in biostatistics, I realized the importance of measures deriving from a work of a British mathematician and Presbyterian minister Thomas Bayes (1702–1761) which is known today as Bayes' theorem(1).

Why is it so important? The most common use of this approach will be when your patient has an abnormal lab test result and you wonder "What does this really mean?" or in other words, "How likely it is that this patient really has the disease in question?" Bayes' theorem allows us to evaluate the diagnostic performance of each particular test and also to compare several of them.

A two-by-two table

The best way to understand this concept is by example. Having a piece of paper, pen and calculator nearby, while reading this text is advisable.

Usually, a diagnostic test is validated by comparison against an established gold standard in an appropriate group of subjects. In order to make the analysis, we need

to make something called *a 2–by–2 table*, which is displayed in a Table 1. The Table is self–explanatory, but be sure always to label the table with the test results on the left side and the disease status on the top as shown.

Table 1 A two–by–two table notation for expressing the results of validation study for diagnostic or screening test

	Disease present	Disease absent
Test positive	True positives (TP)	False positives (FP)
Test negative	False negatives (FN)	True negatives (TN)

Now, let us set the stage for our hypothetical problem. Liver biopsy is currently considered to be the gold standard in the assessment of the presence and degree of liver fibrosis in various liver diseases such as viral hepatitis etc. (2). However, it is associated with the possibility of severe complications and serious discomfort for the patient (3). Therefore, our hypothetical researchers decided to evaluate a non-invasive marker of liver fibrosis comparing it with the gold standard (liver biopsy).

The researchers recruited 189 patients. After performing liver biopsy, 43 of them had liver fibrosis, while 146 did not. On the other hand, after performing a non-invasive test for liver fibrosis, 61 patients were positive for the presence of liver fibrosis, while 128 of them were negative. Now, let us make a 2-by-2 table from this data (Table 2).

Table 2 Two by two table showing the results of a validation study of non-invasive liver fibrosis test against the gold standard

	Liver biopsy positive	Liver biopsy negative	Total
Test positive	43 (TP)	18 (FP)	61
Test negative	0 (FN)	128 (TN)	128
Total	43	146	189

TP-true positive; TN-true negative; FP-false positive; FN-false negative

Sensitivity

The sensitivity or true positive rate (positive for disease) answers the question "How good is this test in picking up people who have the condition?" In other words sensitivity is the probability of a positive test among those who actually do have the condition (4, 5). It is calculated by using the formula

Sensitivity=TP/(TP+FN)

Sensitivity for our diagnostic test would be 43/(43+0)=1=100%. So, one could say that this non-invasive test for liver fibrosis will detect all patients that actually have it. The higher the value of sensitivity, the higher the proportion of those with the actual condition among those that test positive.

Specificity

The specificity or true negative rate (negative in health) answers the question "How good is this test at correctly excluding people without the condition?" Specificity is the probability of a negative test in those without the condition. (4, 5) The higher the specificity, the higher the proportion of those without the actual condition among those that test negative. Specificity is calculated by using the formula

Specificity=TN/(FP+TN)

Therefore, in our example the specificity would be 128/(18+128)=0.88=88% which means that our test will correctly classify 88% of those that actually do not have liver fibrosis. Yet, there will still be 12% of those who will test positive despite the fact that they do not have liver fibrosis.

Pre-test and post-test probabilities

Sensitivity and specificity are one way to evaluate the diagnostic ability of a test. Since they are dealing with probabilities before ac-

tually performing a test they are also called pre–test probabilities. From a clinical point of view, all we have is the result of a test, so clinicians are much more interested in knowing what proportion of patients with an abnormal test are truly abnormal or vice versa – the proportion of patients with a normal test who do not have the condition (6). These questions are answered by using so called, post–test probabilities and positive and negative predictive values, since we are dealing with numbers after actually performing a test.

Pre–test probabilities are of great use when we have several diagnostic tests at our disposal and we need to select the one with the best chance of detecting the condition. On the other hand, the whole point of a diagnostic test is to use it to make a diagnosis, so we need to know the probability that the test will give a correct diagnosis; sensitivity and specificity do not provide us with this type of information. (6) Predictive values however, do have one important limitation: they are measures that we calculate from a defined population with a defined prevalence. If we change the prevalence, the predictive values also change, therefore they do not necessarily apply to another population. Post-test probabilities do not have this particular limitation.

So, what are positive and negative predictive values and how do we calculate them?

Positive predictive value

Positive predictive value (PPV) answers the question "In group of patients with positive test, what is the proportion of those with the condition?" or in other words, it is the proportion of patients with positive test results that are correctly diagnosed (4, 6). The higher the PPV, the higher is our certainty that patient with a positive test really has the condition. It is calculated by using the formula

$$PPV=TP/(TP+FP),$$

or in our hypothetic research PPV = 43/(43 + 18) = 0.70 = 70%. This practically means that when we have a patient tests positive for liver fibrosis by our non-invasive test, he will actually have a 70% probability of really having liver fibrosis. This number tells us that our test is not particularly reliable in detecting the presence of the disease.

Negative predictive value

The negative predictive value (NPV) answers the question "In the group of patients with negative test results, what is the proportion of those without the condition?" This is the proportion of patients with negative test results who are correctly diagnosed (4, 6). The higher the NPV, the higher is our certainty that a patient with negative test results does not have the condition. Negative predictive value is calculated by using the formula

$$NPV=TN/(FN+TN),$$

or in our case NPV = 128/(128+0) = 1 = 100%. This is an excellent NPV which means that we can be fairly certain that our patient with a negative non-invasive liver test really does not have liver fibrosis. There are particular clinical situations when it is important to know that our patient does not have the condition. These are settings where tests with high NPV have their significance, regardless of their PPV.

Sensitivity, specificity, PPV and NPV are looking at a one side of the coin. But, what if we want to look at both sides of coin, or in other words, if we want to assess the overall accuracy of a test, taking into account true positive and true negative cases? Also, the positive and negative predictive values depend crucially on prevalence; when we change the prevalence, PPV and NPV change also. How do we avoid this problem?

These are issues to be addressed in the next part of our series.

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International publications of authors from Bosnia and Herzegovina in Current Contents indexed publications in 2008

1. Imamović G, Batuman V, Sinanović O, Trnačević S, Mesić E, Zerem E, Osmanović E. Microalbuminuria as a possible marker of risk of Balkan endemic nephropathy. Nephrology (Carlton). 2008;13(7):616-21.

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AIM: To evaluate whether microalbuminuria could be a marker of early tubular damage in individuals at risk of developing Balkan endemic nephropathy (BEN). METHODS: A cross-sectional study was used to determine urinary albumin-to-creatinine ratio (UACR) in a test group of 61 participants from a BEN endemic region and control group of 64 participants from a nearby non-endemic region, both recruited from the general population of Bosnia and Herzegovina. The correlation between UACR and urinary b2 microglobulin-to-creatinine ratio (UBCR) and the receiver operating characteristic curve for UACR were analyzed in the test groups of 58 participants. The correlation analysis was also performed in a subset of nine subjects with elevated UBCR. RESULTS: Medians, interquartile ranges and confidence intervals (CI) for medians of UACR in the test and control groups were 2 mg/mmol, 0.975-8.247 mg/mmol, 1.3472-3.2691 mg/mmol and 1 mg/mmol, 0.695-1.41 mg/mmol, 0.8466-1.2053 mg/mmol, respectively (P = 0.0001). Microalbuminuria was found in 30 of the 61 examinees in the test group, in contrast to six of the 64 examinees in the controls (P < 0.0001). Participants from the endemic region had 9.3 times the odds of having microalbuminuria in contrast to participants from the non-endemic region. Pearson's correlation coefficients r of the log-transformed ratios and Kendall-tau coefficients of rank correlation in the group of 58 and in a subset of nine subjects with elevated UBCR were: 0.55 (P < 0.0001); 0.317 (P = 0.0005) and 0.59 (P = 0.045); 0.48 (P = 0.037), respectively. The area under the curve for UACR was 0.882 (P = 0.0001), sensitivity 100% and specificity 67.3%. CONCLUSION: Microalbuminuria may be a useful marker of early tubular injury in individuals at risk of developing BEN.

2. Martinović Z, Valjan V, Kvesić A, Kristo B, Vuckov S, Bakula B.War surgical care--experience from Franciscan Hospital "dr. fra Mato Nikolić" in Nova Bila during conflict in Central Bosnia (1993-1994). Coll Antropol. 2008 ;32(4):1221-7.

Croatian Hospital "dr. fra Mato Nikolić", Nova Bila, Bosnia and Herzegovina.

This report presents experience in treatment of war injuries in Franciscan hospital "dr. fra Mato Nikolić" in Nova Bila, during the war in Central Bosnia from 1993 to 1994, in conditions of encirclement and typhoid fever outbreak. Descriptive-retrospective analysis of organization, implementation and outcomes of surgical care for patients treated from January 1, 1993 till August 20, 1994. In this period, the hospital took care of 2500 wounded persons, 2286 (91.4%) of them male and 214 (8.6%) female, their the average age being 31.5 +/- 12.8. There were 1412 gunshot injuries (56.5%), 1022 explosive injuries (40.9%), and 66 blunt injuries (2.6%). There were 1250 injuries to extremities (50.0%), 349 injuries to head and neck (14%), 233 chest injuries (9.3%) and 193 injuries to abdomen

(7.7%). There were also 475 multiple injuries (19%). Surgical operations were performed in 1498 patients (60%), with surgical mortality rate of 4.5%. Total hospital mortality rate was 11.4 percent (n = 286). Despite extremely difficult conditions of work and lack of doctors, we achieved a low hospital mortality rate. The hospital continued to work after the war. Today, it is a modern health institution in Lasva Valley, Central Bosnia.

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3. Huskić J, Mulabegović N, Alendar F, Ostojić L, Ostojić Z, Simić D, Milicević R, Naletilić M. Serum and tissue angiotensin converting enzyme in patients with psoriasis. Coll Antropol. 2008;32(4):1215-9.

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Recent evidence suggests that the angiotensin converting enzyme (ACE) is present in skin. The real value of the determination of ACE activity as a clinical-biochemistry test for the diagnosis of psoriasis has not been attained. Serum and tissue ACE were measured in 60 patients with psoriasis, 20 patients with lichen planus, 20 patients with seborrhoic dermatitis and in 20 healthy individuals. The serum and tissue ACE activity was determined before and after therapy, using the spectrophotometric method and hippuryl-l-histidyl-l-leucine as a substrate. The results showed that serum ACE activity before therapy was significantly increased in both groups--patients with psoriasis ($p < 0.001$) and patients with lichen planus ($p < 0.001$) in comparison to healthy individuals. However, there were no significant differences in serum ACE activity among patients with seborrhoic dermatitis and healthy individuals. After therapy, serum ACE activity significantly decreased in both groups of patients with psoriasis and patients with lichen planus comparing it to the level found in the control group. The values in both were similar. The tissue ACE activity in altered skin was significantly increased only in the patients with psoriasis in comparison to uninvolved skin of these patients, as well as the skin of healthy individuals. After therapy, there were no significant differences in tissue ACE activity between the treated skin and the healthy skin. In conclusion, determination of tissue angiotensin converting enzyme activity can be used in the differential diagnostic of indistinct clinical forms of psoriasis.

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4. Novo A, Jokić I. Medical audit of diabetes mellitus in primary care setting in Bosnia and Herzegovina. Croat Med J. 2008;49(6):757-62.

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AIM: To assess the quality of diabetes care provided by family medicine teams in primary health centers in Bosnia and Herzegovina (BH) through a medical audit, addressing the extent to which clinical practice complied with pre-determined explicit criteria of long-term management. METHOD: Retrospective analysis included randomly selected medical records of patients with type 1 or 2 diabetes mellitus treated by 18 family medicine teams at 5 locations in BH, included in the Canadian International Development Agency/World Health Organization project "Strengthening health care systems in BH with focus on primary health care/family medicine model." Audit record form contained 24 questions on sex, age, diabetes type, body mass index (BMI), hypertension, family anamnesis, annual examinations (HbA1C, BMI, lipid profile or total cholesterol, blood creatinine, neurological examination, urinalysis for albuminuria, foot care, and examination of ocular fundus), smoking habits, alcohol consumption, patient education, prescribed insulin and other drugs, and patient's health care-seeking behavior. Standardized and record forms were returned anonymously with 99.3% response rate. RESULTS: Records of 536 patients with diabetes were analyzed (64% women and 87% patients with diabetes mellitus type 2). Family medicine teams showed poor compliance with established criteria for diabetes control. Metabolic control (69.5%) was acceptable, but the level of monitoring complications of diabetes (foot and ocular fundus examined in 53.4% and 53% of patients, respectively) was low. There were also considerable variations in diabetes management between different centers as well as between the teams in the same center. CONCLUSION: The audit revealed deficiencies in the quality of diabetes care and variations in care provision between primary care teams. Clinical guidelines and continuing education about acceptable diabetes care should be developed and implemented in BH.

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5. Muratbegović A, Zukanović A, Marković N. Molar-incisor-hypomineralisation impact on developmental defects of enamel prevalence in a low fluoridated area. Eur Arch Paediatr Dent. 2008;9(4):228-31.

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AIM: This was to study the impact of molar-incisor-hypomineralisations on developmental defects of enamel (DDE) prevalence. Additionally, to present the prevalence of DDE and molar-incisor-hypomineralisations (MIH) in 12-year old children in Bosnia and Herzegovina (BH) who live in an area with low

natural fluoride content in the drinking water (less than 0.1 ppm). METHODS: As a part of the oral health study of 12-year-olds, information about DDE and MIH were collected from a random sample of 560 children. To study the impact of MIH on DDE, a secondary database was developed to determine if and to what extent did MIH affect epidemiological parameters of DDE. The analyses focused on differences in DDE values between the main sample and sub-sample of participants in which those diagnosed with both MIH and DDE were excluded. Research results were analysed using percentages, arithmetic mean value, standard deviation and linear regression. RESULTS: In the main sample MIH prevalence was 12.3%. The DDE prevalence was 32.8% with the highest frequency being demarcated opacities. DDE prevalence, calculated without MIH examinees, was 21.4%. A strong positive correlation was found between MIH and DDE prevalence in different geographic locations (r=0.9, p=0.0008). CONCLUSIONS: Molar-incisor-hypomineralisation prevalence has a strong positive correlation with prevalence of developmental defects of enamel. Prevalence of DDE after excluding MIH examinees fell from 32.8% to 21.4%, which was a noticeable difference. Separate registration of these two conditions should be considered.

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6. Marjanović D, Pojskić N, Kapur L, Haverić S, Durmić-Pasić A, Bajrović K, Hadziselimović R. Overview of human population-genetic studies in Bosnia and Herzegovina during the last three centuries: history and prospective. Coll Antropol. 2008;32(3):981-7.

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Modern Bosnia and Herzegovina is a multi-ethnic and multi-religion country, with a very stormy history. Certain archaeological findings indicate continuous population of its territory since the Paleolithic. In time, vast number of different factors jointly influenced fascinating diversity of local human populations. A great number of small, more or less isolated, indigenous populations, make this area quite attractive for population-genetic surveys of different levels and approaches. Austro-Hungarian military physicians conducted the very first known bio-anthropological analyses of Bosnia-Herzegovina population at the end of the 19th century. Thus, the first step towards resolving the genetic structures of local B&H human populations was made. The studies that followed (conducted throughout most of the 20th century) were primarily based on the observation of various phenotypic traits. This stage was followed by the examination of various cytogenetic and fundamental DNA based molecular markers. The efforts undertaken over the last three centuries revealed "human genetic treasure" in Bosnia and Herzegovina. However, even now, after all the studies that were conducted, many interesting features remain to be discovered and described within the existing local human populations.

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7. Biscević M, Ljuca F, Biscević A, Gavrankapetanović I, Smrke BU, Ozturk C, Smrke D. Morphometric alteration of femoral condyles due to knee osteoarthritis. Coll Antropol. 2008;32(3):875-9.

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Aim of this study was to estimate how knee osteoarthritis (OA) affects the shape of femoral condyles by comparing the radiuses of condylar curves between healthy and OA knees. Seventeen female and five male patients with established diagnosis of knee OA were included in the study. Radiuses of medial and lateral condylar curves were calculated from the side view knee X-ray by original mathematical equation and compared to referent values of healthy knees, after adjusting to body height. The average radiuses of condylar curves were between 52.6 +/- 6.2 and 17.6 +/- 3.5 mm medially, and between 43.3 +/- 8.4 and 15.4 +/- 3.7 mm laterally for 0 degrees and 90 degrees femoral flexion contact points, respectively The OA knees had longer curve radiuses medially and laterally at 0 degrees, 10 degrees, and 20 degrees femoral flexion contact points in comparison to the healthy sample ($P < 0.001$; t-test). Our results suggest that the shape of the femoral condyles in OA knees is changed. It should be aware not only in researching of OA etiology, but also in designing of knee endoprostheses, in a manner to achieve better individual sizing.

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8. Prohić A, Alendar F, Simić D, Mašić I. The first dermatovenerologic institution in Bosnia and Herzegovina. Arch Dermatol. 2008t;144(10):1366.

Department of Dermatology, University Clinical Center Sarajevo, Sarajevo, Bosnia and Herzegovina.

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9. Tomić M, Galesić K, Markota I. Endothelin-1 and nitric oxide in patients on chronic hemodialysis. Ren Fail. 2008;30(9):836-42.

Department of Internal Medicine, Mostar University Hospital Center, Mostar, Bosnia and Herzegovina.

AIM: To establish the role of endothelin-1 and nitric oxide in the pathogenesis of hypertension in patients

on chronic hemodialysis by correlating endothelin-1 and NO plasma concentrations to the level of arterial hypertension with respect to angiotensin-converting enzyme (ACE) inhibitor therapy. METHODS: We determined plasma concentrations of endothelin-1 and NO in patients on chronic hemodialysis (CHD) before and after hemodialysis treatment. The study included 30 CHD patients and 20 healthy participants as controls. Correlation to blood pressure was determined, as well as the effect of ACE inhibitors on the relationship between both endothelin-1 and NO in correlation with arterial hypertension. MAIN FINDINGS: Endothelin-1 plasma concentration was significantly higher in CHD patients before hemodialysis treatment than in healthy controls. Endothelin-1 plasma concentration was also significantly higher in CHD patients after hemodialysis than in healthy controls. There was a significant decrease in endothelin-1 plasma concentration after hemodialysis in comparison with its values before hemodialysis. In CHD patients, a positive correlation was found between endothelin-1 plasma concentration and systolic blood pressure after hemodialysis, irrespective of ACE inhibitors therapy. In CHD patients taking ACE inhibitors, systolic blood pressure increased with increasing endothelin-1 plasma concentration before as well as after hemodialysis. In patients taking ACE inhibitors, there was a tendency for diastolic blood pressure to increase with an increase in endothelin-1 plasma concentration after hemodialysis and to decrease with an increase in NO plasma concentration. CONCLUSION: NO and endothelin-1 play a significant role in etiology of the hemodynamic changes of blood pressure during the dialysis.

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10. Zukanović A, Muratbegović A, Kobaslija S, Marković N, Ganibegović M, Beslagić E. Relationships between socioeconomic backgrounds, caries associated microflora and caries experience in 12-year-olds in Bosnia and Herzegovina in 2004. Eur J Paediatr Dent. 2008;9(3):118-24.

Department of Preventive and Paediatric Dentistry, Faculty of Dentistry, University of Sarajevo, Bosnia and Herzegovina.

AIM: To present the prevalence of dental caries in Bosnia and Herzegovina (BH), to estimate the levels of salivary mutans streptococci and lactobacilli and compare them with caries prevalence in 12-years-old children from different socioeconomic backgrounds. STUDY DESIGN AND METHODS: A survey was carried out in 8 cantons of the Federation of BH (FBH) and in Republic Srpska (RS) in 2004. The final sample included 560 12-year-olds. The clinical examinations focused on dental status, expressed as DMFT index, and they were carried out by one examiner, following WHO standard methodologies. Additionally, the study involved 109 12-years old children from Sarajevo, divided in three groups, based on their socioeconomic background. For measuring lactobacillus and mutans streptococci (MS) count in saliva Dentocult LB and Dentocult SM-Strip Mutans were used. Levels of MS and lactobacilli were expressed as a score between 0 and 3, indicating very low to very high levels of SM and lactobacilli. RESULTS: The average DMFT of the 12-year-olds was 4.16+/-2.92. On average, 91% of the 12-year-olds were affected with dental caries. The SiC Index was 7.41+/-3.31. Investigating the relationship between caries associated microflora and caries experience in children of different socioeconomic status showed the following: significant difference in caries prevalence was found in children with different living conditions, where children with high socioeconomic status had better oral health compared to the other two groups. For mutans streptococci, 25.7% of the children had mutans class 0, 24.8% class 1, 34.9% class 2 and 14.6% class 3. The mean DMFT for mutans class 0 was 3.50, for class 1 was 4.30, for class 2 was 5.62 and for class 3 was 6.0. For lactobacilli, 38.5% of the children had lactobacilli class 0, 25.7% class 1, 23.9% class 2 and 11.9% class 3. The mean DMFT for lactobacilli class 0 was 4.3, for class 1 was 4.9, for class 2 was 4.8 and for class 3 was 6.0. No significant differences in the level of mutans streptococci and lactobacilli were found between the groups. CONCLUSION: There is moderate caries prevalence among BH 12-year-olds (DMFT 4.16+/-2.92). Caries experience varies between children with different living condition but no relation between levels of salivary mutans streptococci and lactobacilli and socioeconomic status of children could be found.

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11. Curić I, Curić S, Bagarić I, Bradarć N. Alimentary infections during war conditions: Mostar and Tomislavgrad, Bosnia and Herzegovina, 1992-1995. Coll Antropol. 2008;32(2):571-5.

Department for Infectious Diseases, Clinical Hospital Mostar, Mostar, Bosnia and Herzegovina.
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The aim of this study was to assess the outcome of sanitary and epidemiologic measures undertaken in relation to alimentary infections in the military corps of the Croatian Defense Council (Hrvatsko vjeće obrane) and civilian population in Mostar and Tomislavgrad regions during the 1992-1995 War in Bosnia and Herzegovina. A total of 25 (4.8%) of soldiers and 7 (7.1%) of non-military personnel were not being granted medical clearance to be employed in the food

provision services. We recorded a total of 68 alimentary infections cases in military personnel (with an incidence of 536.2 per 100,000 persons), and 436 in civilian population (573.9 per 100,000 person), without significant difference between them (p=0.647). We did not record any alimentary infection outbreak in the military personnel, while two smaller epidemics of the abdominal typhus were recorded among civilian populations, but without lethal outcomes. The results of this study suggest that even the most basic adherence to the principle of standard sanitary and epidemiologic preventive measures may substantially reduce the probability of alimentary infections outbreaks, even in the highly disruptive, warfare environment.

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12. Džubur Kulenović A, Kučukalić A, Malec D. Changes in plasma lipid concentrations and risk of coronary artery disease in army veterans suffering from chronic posttraumatic stress disorder. Croat Med J. 2008;49(4):506-14.

Department of Psychiatry, University Clinical Center Sarajevo, Bolnicka 25, 71000 Sarajevo, Bosnia and Herzegovina.
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AIM: To test the differences in serum lipid concentrations between veterans with chronic posttraumatic stress disorder (PTSD) and veterans without PTSD. METHODS: We determined plasma lipid parameters and calculated risk factors for 50 veterans in the PTSD group and 50 veterans in the non-PTSD group. Trauma exposure, coping strategies, and quality of life were assessed with Life Stressor List, Manchester Short Assessment of Quality of Life Scale, and Folkman-Lazarus Coping Strategies Questionnaire. RESULTS: There was no difference between the groups in the exposure to combat trauma. PTSD group had significantly lover education than non-PTSD group (10.6+/-1.8 vs 12.4+/-2.6 years, P=0.007) and lower monthly income per family member (euro67.8+/-51.3 vs euro281.9+/-208.2, P<0.001). PTSD group had significantly higher levels of all plasma lipid parameters (cholesterol: 6.54+/-1.24 vs 5.40+/-1.09 mmol/L, P<0.001; triglycerides: 2.55+/-0.68 vs 1.73+/-0.77 mmol/L, P<0.001; very low density lipoprotein-cholesterol: 1.14+/-0.32 vs 0.78+/-0.35 mmol/L, P<0.001; low density lipoprotein-cholesterol: 4.49+/-1.06 vs 3.46+/-0.93 mmol/L, P<0.001). High-density lipoprotein cholesterol concentration was significantly lower in PTSD group (0.96+/-0.18 vs 1.15+/-0.24 mmol/L, P<0.001). Established risk factor for arteriosclerosis (6.96+/-1.19 vs 4.71+/-0.88, P<0.001) and Adult Treatment Panel III ten years risk for coronary disease (19.44+/-7.27% vs 9.74+/-4.10%, P<0.001) were significantly higher in the PTSD group. Secondary traumatization was significantly more frequent in the PTSD group (3.8+/-5.7 vs 1.3+/-4.7 events; P<0.001). CONCLUSIONS: Chronic PTSD is associated with dyslipidemia, leading to an increased risk of coronary artery disease. Environmental factors and coping strategies should be considered as important factors for the occurrence and persistence of PTSD.

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13. Klarić M, Francisković T, Klarić B, Kvesić A, Kastelan A, Graovac M, Lisica ID. Psychological problems in children of war veterans with posttraumatic stress disorder in Bosnia and Herzegovina: cross-sectional study. Croat Med J. 2008;49(4):491-8.

Psychiatric Department of Clinical hospital Mostar, Kardinala Stepinca bb, 88000 Mostar, Bosnia and Herzegovina.
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AIM: To assess psychological problems in children as reported by their veteran fathers with war-related posttraumatic stress disorder (PTSD). METHOD: The study group consisted of 154 veterans with war-related PTSD who were treated at the Mostar University Hospital. The control group consisted of 77 veterans without war-related PTSD who were selected from veteran associations by the snowball method. General Demographic Questionnaire, the first and fourth module of the Harvard Trauma Questionnaire-Bosnia and Herzegovina version, and the Questionnaire on Developmental, Emotional, and Behavioral Problems in Children, created specifically for the needs of this study, were used to collect data on veterans' perception of psychological problems in their children. RESULTS: In comparison with veterans without PTSD, veterans with PTSD reported significantly more developmental (odds ratio [OR], 2.37; 95% confidence interval [CI], 1.51-3.73), behavioral (OR, 3.92; 95% CI, 1.53-10.03), and emotional problems (OR, 17.74; 95% CI, 2.40-131.10) in their children. CONCLUSION: Veterans with war-related PTSD more often reported developmental problems in their children. Father's PTSD may have long-term and long-lasting consequences on the child's personality.

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14. Radoncić F, Hudić I, Balić A, Fatusić Z. Perinatal outcomes during 1986-2005 in Tuzla Canton, Bosnia and Herzegovina. J Matern Fetal Neonatal Med. 2008;21(8):567-72.

Clinic for Gynecology and Obstetrics, University Clinical Centre, Tuzla, Bosnia and Herzegovina.

AIM: To determine the incidence rate and causes of perinatal mortality and make a comparison between the incidence rate and causes of perinatal mortality in

the prewar (1986-1991), war (1992-1995) and postwar (1996-2005) periods in Tuzla Canton, Bosnia and Herzegovina. METHODS: We retrospectively collected data from the databases of University Department for Gynecology and Obstetrics Tuzla. Data on the number of live births, stillbirths, early neonatal deaths, causes of death, gestational age and birth weights were collected. RESULTS: There were 101712 deliveries all together in the above mentioned period, out of which 101638 resulted in liveborn children. Perinatal mortality gradually declines in the period of 1986-2000. The decline owns mostly to early neonatal mortality more than to fetal which also shows the trend of decrease. Statistically significant difference in perinatal mortality was found between period 2001-2005 and another analysed periods, and the most difference was found between 1991-1995 and 1996-2000 ($p < 0.01$). Statistically significant difference in fetal mortality was found between period 2001-2005 and 1986-1990. Statistically significant difference in early neonatal mortality was found between period 2001-2005 and 1986-1990 ($p = 0.005$; $p < 0.005$). CONCLUSION: Perinatal mortality in Tuzla Canton were significant higher during the war, mainly due to lower adequacy and accessibility of perinatal health care. During the peace period a significant decline of perinatal mortality is registered, due to early neonatal death.

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15. Pavlović-Čalić N, Salkić NN, Gegić A, Smajić M, Alibegović E. Crohn's disease in Tuzla region of Bosnia and Herzegovina: a 12-year study (1995-2006). Int J Colorectal Dis. 2008;23(10):957-64.

Department of Gastroenterology, Internal Medicine Hospital, University Clinical Centre Tuzla, Tuzla, Bosnia and Herzegovina.

BACKGROUND: Bosnia and Herzegovina (B&H) is one of the Eastern European countries with lacking data on Crohn's disease (CD) epidemiology. GOAL: We aimed to assess incidence of CD in Tuzla Canton of B&H during a 12-year period (1995-2006). METHODS: We retrospectively evaluated hospital records of both CD inpatients and outpatients residing in Tuzla Canton of B&H (total of 496,280 inhabitants) between 1995 and 2006. Patient that satisfied previously described criteria were included in the study. Incidence rates were calculated with age standardisation using European standard population. Trends in incidence were evaluated as moving 3-year averages. RESULTS: During the observed period, 140 patients met the diagnostic criteria for CD. Mean annual incidence was found to be 2.3/10(5) (95% CI=1.6-3.0) inhabitants ranging from 0.20 to 6.45 per 10(5). Mean annual crude incidence during the last 5 years of study (2002-2006) was 4.15/10(5) (95% CI=3.35-4.95). The prevalence of CD, at the end of the observed period was found to be 28.2/10(5) (95% CI=23.5-32.9). CD incidence increased dramatically from 0.27/10(5) in 1995-1997 to 4.84/10(5) in 2004-2006, as well as did the number of colonoscopies performed; from 29 in 1995 to 850 in 2006. We observed almost constant trend of around three new cases of CD per 100 colonoscopies performed. CONCLUSIONS: (1)Our area is the region of moderate incidence of CD with the trend that remains toward continuing increase in the rates of CD, which is most likely a direct consequence of the growing number of performed colonoscopies. (2) We believe that in the future years, CD incidence in our region will probably further increase and stabilise at a level of around five cases per 10(5) inhabitants.

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16. Šapčanin A, Sofić E, Tahirović I, Salković-Petrišić M, Hoyer S, Riederer P. Antioxidant capacity in rat brain after intracerebroventricular treatment with streptozotocin and alloxan-a preliminary study. Neurotox Res. 2008;13(2):97-104.

Department of Chemistry, Faculty of Science, University of Sarajevo, Sarajevo, Bosnia and Herzegovina.

Intracerebroventricular (icv) administration of betacytotoxic drug streptozotocin (STZ) produces long-term and progressive cognitive deficits in rats, as well as deficits in cerebral glucose and energy metabolism. These changes resemble those found in the brain of patients with sporadic Alzheimer's disease (sAD), and therefore, STZ-icv treated rats have been proposed as an experimental model of sAD. In this study the antioxidant capacity (AC), using manual oxygen radical absorbance capacity (ORAC) assay, was measured in the rat brain frontoparietal cortex (FC) and brainstem-cerebellum region (BS-CB) after administration of STZ and another betacytotoxic drug alloxan (AL). Region-specific differences of AC were found, which were more expressed when hydroxyl radical (ORAC(-OHo)) generator was used in the assay. AC against ORAC(-OHo) was significantly lower in BS-CB than in FC of the control rats. Furthermore, ORAC(-OHo) significantly decreased in BS-CB 3-months following the icv administration of AL, but significantly increased following the TG+AL combined treatment in comparison with the controls. However, 3-months following the icv treatment of AL combination with a different glucose transport inhbitor, 3-O-methyl-D-glucose, ORAC(-OHo) values in BS-CB and ORAC(-ROOo) values in FC were significantly decreased in comparison to the controls. Our results suggest that betacytotoxic-icv treatment alters antioxidant defense systems in the brain, which particularly regarding the STZ-icv treatment, could be a useful tool in search for possible new antioxidant treatments of the neurodegenerative disorders such as sAD.

17. Sesar K, Živčić-Bećirević I, Sesar D. Multi-type maltreatment in childhood and psychological adjustment in adolescence: questionnaire study among adolescents in Western Herzegovina Canton. Croat Med J. 2008;49(2):243-56.

Dom zdravlja, J. Grubisica 11,
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AIM: To determine the prevalence and intercorrelation of different forms of childhood maltreatment and psychological problems in adolescents in Western Herzegovina Canton. METHOD: A questionnaire study was conducted in March 2003 on a convenient sample of 458 third-grade high-school students (39% boys) aged between 15 and 20 (median age, 17). Data were collected using a sociodemographic questionnaire, Family Adaptability and Cohesion Evaluation Scales III, Child Maltreatment Questionnaire, Youth Self-Report, and Rosenberg Self-Esteem Scale. Sociodemographic and family characteristics and exposure to maltreatment were analyzed as possible predictors of exposure to a particular type of abuse and subsequent psychological adjustment problems. RESULTS: Out of 458 students, 77% were emotionally abused, 52% physically abused, 30% neglected, 20% witnessed family violence, and 13% of girls and 21% of boys were sexually abused before the age of 14. Significant association between the maltreatment by a mother, father, and other adults were found for emotional and physical abuse and for neglect and witnessing family violence (r=0.413-0.541, P<0.001 for all). Significant correlation was found between all forms of abuse (r=0.163-0.594, P<0.05), except between sexual abuse and witnessing family violence (r=0.048, P=0.351). Almost two-thirds of students were exposed to multi-type maltreatment in childhood. Family characteristics and maltreatment scores significantly predicted anxiety/depression (R=0.456, R(2)=0.076), withdrawal (R=0.389, R(2)=0.049), somatic complaints (R=0.437, R(2)=0.059), social problems (R=0.417, R(2)=0.063), attention deficit and hyperactivity disorder (R=0.368, R(2)=0.045), rule-breaking behavior (R=0.393, R(2)=0.045), aggression (R=0.437, R(2)=0.078) (P<0.001 for all), as well as self-esteem (R=0.371, R(2)=0.035, P=0.003). CONCLUSION: Most third-grade high-school students in Western Herzegovina Canton were exposed to multi-type maltreatment in childhood, regardless of the war experience. Emotional and physical abuse were most frequently combined forms of maltreatment. Sociodemographic and family characteristics and exposure to some forms of abuse were significant predictors of exposure to other forms of abuse. Exposure to maltreatment in childhood predicted difficulties in psychological adjustment in adolescence.

18. Balić D, Latifagić A, Hudić I. Insulin-like growth factor-binding protein-1 (IGFBP-1) in cervical secretions as a predictor of preterm delivery. J Matern Fetal Neonatal Med. 2008;21(5):297-300.

Clinic for Gynecology and Obstetrics,
University Clinical Center, Tuzla,
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OBJECTIVE: The objective of this study was to investigate the level of insulin-like growth factor-binding protein-1 (IGFBP-1) in cervical secretions and Bishop score as predictors of preterm delivery in asymptomatic pregnant women. METHODS: This was a prospective study at the Clinic for Gynecology and Obstetrics at the University Clinical Center in Tuzla, on a sample of 80 healthy pregnant women at between 24 and 34 gestational weeks. After interview every woman underwent IGFBP-1 concentration measurement by the 'Actim Partus' test. The Bishop score was determined by the author (A.L) during vaginal examination. Rates among groups were compared using arithmetic mean and standard deviation, Student's t-test, Mann-Witney U-test, and Spearman-Rank correlation test. Statistical importance was determined at the variation levels of 5% and 1%. RESULTS: Eight (10.00%) women in the study group had a positive Actim Partus test and six (7.50%) of them had a preterm delivery. The positive predictive value was 44.44% and negative predictive value was 98.59%. The specificity of the Bishop score in the study group was 83.78% and the sensitivity was 50.00%. The positive predictive value of the Bishop score in this group was 20.00% and the negative predictive value was 95.36%. There was no correlation between the Bishop score and Actim Partus test (p = 0.15). CONCLUSIONS: If the concentration of IGFBP-1 is <10 microg/L (negative Actim Partus test) in asymptomatic pregnant women, the risk of preterm delivery is low. The Actim Partus test could be used as a screening test for preterm delivery in asymptomatic pregnant women.

19. Ceklić L, Mađar N, Neubauer AS. Optical coherence tomography fast versus regular macular thickness mapping in diabetic retinopathy. Ophthalmic Res. 2008;40(5):235-40.

Department of Ophthalmology, Clinical Center of Eastern Sarajevo, RS-Bosnia and Herzegovina
University of Sarajevo, Sarajevo, RS-Bosnia.

OBJECTIVE: The purpose of the study was to investigate if absolute values and reproducibility of thickness

maps obtained from 2 optical coherence tomography (OCT) scanning protocols, regular high-resolution and fast low-density mode, differ in patients with diabetic macular edema. METHODS: A total of 26 consecutive patients undergoing fluorescein angiography and Stratus OCT scanning for the evaluation of diabetic macular edema at the Departments of Ophthalmology in Munich and Vienna were included. RESULTS: Retinal thickness of the central field of the thickness map measured by fast retinal thickness protocol was 287 +/- 97 and 290 +/- 113 microm by the regular protocol. This difference as well as that for all other fields was not statistically significant. Three times repeated measurements applying both OCT scanning modes in 10 patients yielded very good intrasession correlation coefficients between 0.70 and 0.99, with corresponding intrasession standard deviations ranging between 6 and 16 mum. The fast mode yielded slightly less reproducible values than the regular mode. Visual acuity did not influence the results. CONCLUSION: In practice both scanning modes can be interchanged and absolute values can be compared directly. Best reproducibility is obtained with higher sampling density even in patients with reduced visual acuity due to diabetic macular edema. Copyright 2008 S. Karger AG, Basel.

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20. Zerem E, Imamović G, Omerović S. Symptomatic simple renal cyst: comparison of continuous negative-pressure catheter drainage and single-session alcohol sclerotherapy. AJR Am J Roentgenol. 2008;190(5):1193-7.

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OBJECTIVE: The purpose of this study was to evaluate whether continuous percutaneous catheter drainage with negative pressure yields better results than single-session alcohol sclerotherapy in the management of symptomatic simple renal cysts. SUBJECTS AND METHODS: Eighty-five patients with 92 simple renal cysts were randomly assigned to two groups in a prospective controlled trial. One group was treated with sonographically guided continuous catheter drainage with negative pressure and the other group with single-session alcohol sclerotherapy. Patient demographics, clinical characteristics, treatment outcome, and complications were analyzed. RESULTS: The initial volume of the cysts did not differ significantly between the groups, but the final volume was significantly smaller in the continuous drainage group ($p = 0.026$). During the 24-month follow-up period, 37 (40%) of the cysts disappeared completely: 24 (52%) of the 46 cysts in the drainage group and 13 (28%) of the 46 cysts in the sclerotherapy group ($p = 0.033$). In the sclerotherapy group, the probability of disappearance of the cysts was highly dependent on cyst size, being less for giant cysts ($p = 0.01$). Cyst size was not a significant factor in probability of disappearance in the drainage group ($p = 0.15$). The probability of disappearance of giant cysts (volume > 500 mL) differed significantly between the groups ($p = 0.009$), but there was no difference in probability of disappearance of moderately large cysts ($p = 0.16$). Three of 14 patients with giant cysts in the drainage group and 10 of 13 such patients in the sclerotherapy group had recurrences that necessitated additional treatment ($p = 0.007$). They were successfully treated with continuous catheter drainage. CONCLUSION: Continuous catheter drainage with negative pressure is more efficient than single-session alcohol sclerotherapy in the management of giant cysts. For moderately large cysts, the two methods have similar results.

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21. Šumanović-Glamuzina D, Čulo F, Čulo MI, Konjevoda P, Božić T, Robović A, Vuksić, I, Bilinovac Z, Kuzman Z. Vasodilatory prostaglandins in perinatal hypoxic brain damage. Coll Antropol. 2008;32 Suppl 1:183-7.

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Prostaglandin (PGE2 and PGI2) synthesis was determined in the cerebrospinal fluid (CSF) and serum of 19 hypoxic neonates at the age of 5-96 hours by using Enzyme Linked Immunosorbent Assay (ELISA) method. Control group consisted of 8 children of the same age whose samples were taken due to initial suspicion of neonatal meningitis. The prostaglandin concentrations in CSF were correlated with initial hypoxic-ischemic encephalopathy (HIE) stage and neurological findings of patients at the age of 12 months. The values of PGE2 and PGI2 in the CSF of children with perinatal hypoxia (PNH) were significantly higher than in the children from the control group. The values of PGI2 in serum were significantly higher than in "CSF" of patients with PNH. Although average values of PGE2 and PGI2 in the liquor were higher in children with advanced stage of HIE, the differences between different stages were not statistically significant. We did not find any significant correlation between average concentrations ofprostaglandins and neurological findings of the 12-month-old children.

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22. Prohić A. An epidemiological survey of tinea capitis in Sarajevo, Bosnia and Herzegovina over a 10-year period. Mycoses. 2008;51(2):161-4.

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The aim of this study was to determine the incidence and aetiological agents of tinea capitis in Sarajevo area, Bosnia and Herzegovina, during a 10-year period (1997-2006). A total of 707 patients with suspected dermatophyte infections of scalp was analysed. Tinea capitis was determined in 241 (34.1%) of these patients, in whom causative agents were identified in 209 (29.6%). Zoophilic dermatophytes (91.8%) prevailed over anthropophilic (7.2%) and geophilic (1.0%) dermatophytes. Microsporum canis was the most frequent dermatophyte isolated (90.4%), followed by Trichophyton schoenleinii (2.4%) and Trichophyton violaceum (1.9%). The majority of infections occurred in males (56.5%) and in children with age less than 10 years (52.6%).

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23. Zildžić M, Salkić N, Eminović I, Mesic D. Fractured rib as a foreign body of the colon. Gastrointest Endosc. 2008;67(1):186-8.

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24. Zerem E, Imamović G, Omerović S.Percutaneous treatment of symptomatic non-parasitic benign liver cysts:single-session alcohol sclerotherapy versus prolonged catheter drainage with negative pressure. Eur Radiol. 2008;18(2):400-6.

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To evaluate whether prolonged catheter drainage with negative pressure yields better results than single-session alcohol sclerotherapy in the treatment of symptomatic non-parasitic benign liver cysts. Forty patients were randomly assigned to two groups in a 24-month prospective controlled trial. One group was treated with ultrasound-guided prolonged catheter drainage with negative pressure (20 patients with 24 cysts) and the other group with single-session alcohol sclerotherapy (20 patients with 23 cysts). Patient demographics, clinical characteristics, treatment outcome, and complications were analyzed. The median volumes and 95% CI (confidence interval) for the medians and interquartile ranges of all 47 cysts before treatment and on last follow-up were: 389 ml, 143-1,127 ml, 136-1,300 ml, and 0 ml, 0-10 ml, and 0-23 ml, respectively ($P<0.0001$). The average volume reduction was 92.4% (range, 74.9-100%), 94.2 % (range, 74.9-100%) in the drainage and 90.2% (range, 76.9-100%)in the sclerotherapy group. Twenty-seven cysts (57.4%) disappeared completely, 16 (66.7%) in the drainage and 11 (47.8%) in the sclerotherapy group. No differences in average volume reduction, final volume and disappearance of the cysts between the groups were noted. The hospital stay was 1 day for all patients. Percutaneous treatment is safe and effective for hepatic non-parasitic cysts. Prolonged catheter drainage with negative pressure and single-session alcohol sclerotherapy had similar results.

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25. Zerem E, Delibegović S. Professor Marusić placed his editorial board at disposal of postgraduate students of Tuzla University School of Medicine. Croat Med J. 2008;49(1):101.

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Prepared by:
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Organization of the text. The text of observational and experimental articles is usually (but not necessarily) divided into sections with the following headings: Introduction, Methods, Results, and Discussion. This so-called "IMRAD" structure is not simply an arbitrary publication format, but rather a direct reflection of the process of scientific discovery. Long articles may need subheadings within some sections (especially the Results and Discussion sections) to clarify their content. Other types of articles, such as case reports, reviews, and editorials, are likely to need other formats.

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An abstract in English (up to 250 words each) should follow the title page. The abstracts should have titles (in English and in Bosnian/Serbian/Croatian), without authors' names and institutional affiliations. Its structure should be similar to that of the text. For original articles, the abstract should provide the context or background for the study; it should state the study's purposes, basic procedures (selection of study subjects or laboratory animals, observational and analytical methods), main findings, and principal conclusions. It should emphasize new and important aspects of the study or observations.

Following the abstract, authors provide, and identify as such, 3 to 5 key words or short phrases that capture the main topics of the article. Terms from the Medical Subject Headings (MeSH) list of Index Medicus should be used; if MeSH terms are not available, natural language terms may be used. MeSH terms are available from: www.nlm.nih.gov/mesh/.

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findings, then explore possible mechanisms or explanations for these findings, compare and contrast the results with other relevant studies, state the limitations of the study, and explore the implications of the findings for future research and for clinical practice.

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Acknowledge anyone who contributed towards the study by making substantial contributions to conception, design, acquisition of data, or analysis and interpretation of data, or who was involved in drafting the manuscript or revising it critically for important intellectual content, but who does not meet the criteria for authorship. List the source(s) of funding for the study, for each author, and for the manuscript preparation in the acknowledgements section.

References (separate page). Small numbers of references to key original papers will often serve as well as more exhaustive lists. Avoid using abstracts as references. References to papers accepted but not yet published should be designated as "in press" or "forthcoming"; authors should obtain written permission to cite such papers as well as verification that they have been accepted for publication. Information from manuscripts submitted but not accepted should be cited in the text as "unpublished observations" with written permission from the source. Avoid citing a "personal communication" unless it provides essential information. For scientific articles, authors should obtain written permission and confirmation of accuracy from the source of a personal communication.

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The titles of journals should be abbreviated according to the style used in Index Medicus. Consult the list of Journals Indexed for MEDLINE, published annually as a separate publication by the National Library of Medicine (available from: www.nlm.nih.gov/tsd/serials/lij.html).

Sample References

Articles in Journals

Standard journal article (List the first six authors followed by et al.):

Halpern SD, Ubel PA, Caplan AL. Solid-organ transplantation in HIV-infected patients. N Engl J Med. 2002;347(4):284-7.

More than six authors:

Rose ME, Huerbin MB, Melick J, Marion DW, Palmer AM, Schiding JK, et al. Regulation of interstitial excitatory amino acid concentrations after cortical contusion injury. Brain Res. 2002;935(1-2):40-6.

Organization as author:

Diabetes Prevention Program Research Group. Hypertension, insulin, and proinsulin in participants with impaired glucose tolerance. Hypertension. 2002;40(5):679-86.

No author given:

21st century heart solution may have a sting in the tail. BMJ. 2002;325(7357):184.

Volume with supplement:

Geraud G, Spierings EL, Keywood C. Tolerability and safety of frovatriptan with short- and long-term use for treatment of migraine and in comparison with sumatriptan. Headache. 2002;42(Suppl 2): S93-9.

Issue with supplement:

Glauser TA. Integrating clinical trial data into clinical practice. Neurology. 2002;58(12 Suppl 7):S6-12.

Issue with no volume:

Banit DM, Kaufer H, Hartford JM. Intraoperative frozen section analysis in revision total joint arthroplasty. Clin Orthop. 2002;(401):230-8.

Letters or abstracts:

Tor M, Turker H. International approaches to the prescription of long-term oxygen therapy [letter]. Eur Respir J. 2002;20(1):242. ; Lofwall MR, Strain EC, Brooner RK, Kindbom KA, Bigelow GE. Characteristics of older methadone maintenance (MM) patients [abstract]. Drug Alcohol Depend. 2002;66 Suppl 1:S105.

Article republished with corrections:

Mansharamani M, Chilton BS. The reproductive importance of P-type ATPases. Mol Cell Endocrinol. 2002;188(1-2):22-5. Corrected and republished from: Mol Cell Endocrinol. 2001;183(1-2):123-6.

Article with published erratum:

Malinowski JM, Bolesta S. Rosiglitazone in the treatment of type 2 diabetes mellitus: a critical review. Clin Ther. 2000;22(10):1151-68; discussion 1149-50. Erratum in: Clin Ther 2001;23(2):309.

Article published electronically ahead of the print version:

Yu WM, Hawley TS, Hawley RG, Qu CK. Immortalization of yolk sac-derived precursor cells. Blood. 2002 Nov 15;100(10):3828-31. Epub 2002 Jul 5.

Books and Other Monographs

Personal author(s):

Murray PR, Rosenthal KS, Kobayashi GS, Pfaller MA. Medical microbiology. 4th ed. St. Louis: Mosby; 2002.

Editor(s), compiler(s) as author:

Gilstrap LC 3rd, Cunningham FG, VanDorsten JP, editors. Operative obstetrics. 2nd ed. New York: McGraw-Hill; 2002.

Organization(s) as author:

Royal Adelaide Hospital; University of Adelaide, Department of Clinical Nursing. Compendium of nursing research and practice development, 1999-2000. Adelaide (Australia): Adelaide University; 2001.

Chapter in a book:

Meltzer PS, Kallioniemi A, Trent JM. Chromosome alterations in human solid tumors. In: Vogelstein B, Kinzler KW, editors. The genetic basis of human cancer. New York: McGraw-Hill; 2002. p. 93-113.

Conference paper:

Christensen S, Oppacher F. An analysis of Koza's computational effort statistic for genetic programming. In: Foster JA, Lutton E, Miller J, Ryan C, Tettamanzi AG, editors. Genetic programming. EuroGP 2002: Proceedings of the 5th European Conference on Genetic Programming; 2002 Apr 3-5; Kinsdale, Ireland. Berlin: Springer; 2002. p. 182-91.

Dissertation:

Borkowski MM. Infant sleep and feeding: a telephone survey of Hispanic Americans [dissertation]. Mount Pleasant (MI): Central Michigan University; 2002.

Other Published Material

Newspaper article:

Tynan T. Medical improvements lower homicide rate: study sees drop in assault rate. The Washington Post. 2002 Aug 12;Sect. A:2 (col. 4).

Dictionary and similar references:

Dorland's illustrated medical dictionary. 29th ed. Philadelphia: W.B. Saunders; 2000. Filamin; p. 675.

Electronic Material

CD-ROM:

Anderson SC, Poulsen KB. Anderson's electronic atlas of hematology [CD-ROM]. Philadelphia: Lippincott Williams & Wilkins; 2002.

Audiovisual material:

Chason KW, Sallustio S. Hospital preparedness for bioterrorism [videocassette]. Secaucus (NJ): Network for Continuing Medical Education; 2002.

Journal article on the Internet:

Abood S. Quality improvement initiative in nursing homes: the ANA acts in an advisory role. Am J Nurs [serial on the Internet]. 2002 Jun [cited 2002 Aug 12];102(6):[about 3 p.]. Available from: http://www.nursingworld.org/AJN/2002/june/Wawatch.htm

Monograph on the Internet:

Foley KM, Gelband H, editors. Improving palliative care for cancer [monograph on the Internet]. Washington: National Academy Press; 2001 [cited 2002 Jul 9]. Available from: http://www.nap.edu/books/0309074029/html/.

Homepage/Web site:

Cancer-Pain.org [homepage on the Internet]. New York: Association of Cancer Online Resources, Inc.; c2000-01 [updated 2002 May 16; cited 2002 Jul 9]. Available from: http://www.cancer-pain.org/.

Part of a homepage/Web site:

American Medical Association [homepage on the Internet]. Chicago: The Association; c1995-2002 [updated 2001 Aug 23; cited 2002 Aug 12]. AMA Office of Group Practice Liaison; [about 2 screens]. Available from: http://www.ama-assn.org/ama/pub/category/1736.html

Database on the Internet:

Who's Certified [database on the Internet]. Evanston (IL): The American Board of Medical Specialists. c2000 – [cited 2001 Mar 8]. Available from: http://www.abms.org/newsearch.asp

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