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Original article

Examination of miners' immune response to coal dust and their quality of life

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Summary

Introduction. Inhalation of coal dust during blasting in brown coal mines has been shown to lead to a lung disease called pneumoconiosis. There is very little data in the literature on the direct impact of coal on the quality of life of people who work in coal mines as well as the body's immune response to the effects of coal dust. The aim was to examine the immune response to exposure to coal dust in miners in a brown coal mine and whether mine workers have poorer quality of life compared to those not exposed to coal dust.

Methods. This is a cross-sectional study among 100 employees in the Brown Coal Mine in Ugljevik, of which 50 of them are exposed to coal dust on a daily basis. Blood samples were taken from all subjects to test for the presence of cytokines IL-2, 4, 5, 9, 10, 13, 17A, 17F, 21, 22, IFN-γ and TNF-α. The quality of life of employees was measured using a questionnaire for self-assessment of physical and mental health (36-item Short-Form Health Survey, SF-36).

Results. Group of miners had a significantly ($p<0.05$) higher concentrations of pro-inflammatory cytokines IL-6, IFN-γ, IL-17A and IL-22 when compared to the control group. Subjects from the control group had significantly ($p<0.05$) higher concentrations of anti-inflammatory cytokines IL-4 and IL-10 when compared to the group of miners. The quality of life was significantly ($p<0.05$) better in the control group when compared to the group of miners.

Conclusion. Physical functioning, general health, mental health and Physical component summary were significantly poorer in the group of miners. Exposition to coal dust led to a significant increase in the production of pro-inflammatory cytokines and a decrease in the production of anti-inflammatory cytokines.

Keywords: immune response, cytokines, coal dust

Introduction

As one of the most important energy resources in the world, coal makes a great contribution to the world economy. Coal mining and processing involves multiple dust generation processes including coal cutting, transportation, crushing and grinding, etc. Coal dust is one of the main sources of danger to the health of coal workers. Exposure to coal dust can be prevented through

administrative and engineering controls. Ineffective control of exposure to coal dust can harm the health of workers in coal mines [1]. Given that there has been an unexpected increase in miners' pneumoconiosis (CWP) in the Appalachian Basin (USA) in recent years, Ting Liu has reviewed the literature on the importance of coal dust to miners' health. They pointed out that the impact of coal dust on the health of miners is not yet well understood and that further improvements are needed [1]. There is a large number of conducted researches related to environmental problems caused by mining in coal mines, processing, combustion and similar problems such as acid rain, smog occurrence, gas emissions, etc. In contrast, there is very little data in the literature on the direct impact of coal on the health of people who work in coal mines and who use coal on a daily basis [2].

Inhalation of coal dust during blasting in brown coal mines leads to the development of lung dust diseases caused by coal dust (CMDLD). The most common manifestation of CMDLD is pneumoconiosis of the lungs. Coal worker's pneumoconiosis (CWP) is a parenchymal lung disease caused by the accumulation of coal dust in the lungs and the consequent reaction of lung tissue to the formation of fibrous nodular lesions [3]. Other CMDLD diseases include progressive pulmonary fibrosis, chronic bronchitis, pulmonary emphysema, and diffuse pulmonary fibrosis associated with coal dust [4]. Workers in coal mines can also get chronic obstructive pulmonary disease, especially workers who are smokers [5]. The risk of developing lung diseases increases in underground mines [6].

The pathophysiological mechanisms of pneumoconiosis in miners have not been fully elucidated, but it is known that cytokines produced by alveolar macrophages also play a significant role in pathogenesis [7]. The role of IL-1, TNF-α, IL-6, TGF-β in patients with pneumoconiosis was investigated [7,8,9,10,11]. However, there are no data in the literature on the concentration of these and other important cytokines, such as IL-2, 4, 5, 9, 10, 13, 17A, 17F, 21, 22 and IFN-γ, measured in the serum of patients with CWP. In their study, Attfield and co-workers concluded that there is a negative effect of exposure to mine dust on pulmonary function that occurs even in the absence of radiographically detected pneumoconiosis [12]. In this sense, it is assumed that among the miners exposed to coal dust there are those who have triggered the pathophysiological mechanisms of pneumoconiosis and other health disorders associated with coal dust, but it has not been proven radiologically. Therefore, a cytokine immune response is expected in the exposed. The combination of a number of risk factors in brown coal mines can lead to a reduced quality of life for these workers due to exposure to a large amount of occupational hazards, including silica dust, coal dust, noise, vibration and heat [13]. The aim of the study was to examine the immune response to coal dust by testing the concentration of cytokines IL-2, 4, 5, 9, 10, 13, 17A, 17F, 21, 22, IFN-γ and TNF-α, as well as to examine the quality of life of miners in the Brown coal mine in Ugljevik.

Methods

A cross-sectional study was conducted between two groups of employees in the Brown Coal Mine and Thermal Power Plant in Ugljevik, the Republic of Srpska, Bosnia and Herzegovina from April 2018 to December 2020. The research included 100 respondents in the Brown Coal Mine in Ugljevik, of which 50 were exposed to coal dust and 50 were not exposed to coal dust. The first group was represented by employees who were exposed to coal dust, and the second by employees who were not exposed. Respondents were selected by random selection with prior informed consent. All respondents completed a questionnaire, which contained demographic and health data.

To examine the quality of life of workers in the brown coal mine, we used a standard questionnaire for self-assessment of physical and mental health (36-item Short-Form Health Survey, SF-36), which is widely used to assess the quality of life of people in many fields and studies.

Cytokine concentrations were determined in the serum of subjects using fluorescent beads labeled with anti-cytokine antibodies (Biolegend, San Diego, CA, USA) on a flow cytofluorimeter (Attune Acoustic Focusing Cytometer, Applied Biosystems, Themo Fisher, USA) in a Center for biomedical research at the Faculty of Medicine in Foca, University of East Sarajevo.

The methods of descriptive and analytical statistics were used in the paper. Among the methods of descriptive statistics, measures of central tendency and measures of variability were used, namely arithmetic mean with standard deviation and relative numbers for categorical variables. Among the methods of analytical statistics, Student's t-test and non-parametric alternative, Mann-Whitney U test were used for bound samples. The chi-squared test and Fisher's test, non-parametric tests, were also used to assess the difference between the groups. The usual value of $p<0.05$ was taken as the level of statistical significance of differences, while the values of $p<0.01$ were considered highly statistically significant. Results were statistically analyzed in GraphPad Prism software (GraphPad, La Jolla, CA, USA) and SPSS software package version 21.0 (Statistical Package for Social Sciences SPSS 21.0 Inc, USA).

Results

The study involved 100 respondents, half of them were miners working in the brown coal mine, while the remaining 50% of respondents were a control healthy group, which was not exposed to coal dust. Table 1 shows that a high statistically significant difference in relation to gender was observed between the two groups of respondents ($p=0.001$), with a significantly higher percentage of men working in the brown coal mine. Subjects belonging to the group of miners have breathing difficulties in a statistically significantly ($p=0.017$) higher percentage (26%) compared to the control group (8%), and also, the group of miners (28%) significantly more often ($p=0.046$) has cough compared to the control group of subjects (12%). Differences between groups of subjects in relation to the smoking status, occurrence of expectoration of dark sputum, present lung diseases, hospitalization due to pneumonia, heart disease, rheumatic diseases and hypertension were not observed (Table 1).

Table 1. Socio-demographic and clinical characteristics of the respondents

Variables	Group of miners, % or M ± SD	Control group, % or M ± SD	p
Gender, male	88	24	**0.001***
Age, years	44.5±10.6	43.0±13.72	0.543#
Dyspnea	26	8	**0.017***
Cough	28	12	**0.046***
Smoking	17	12	0.342*
Coughing up dark sputum	*8*	0	0.117**
Lung diseases	18	6	0.065**
Hospitalization for pneumonia	6	0	0.242**
Heart diseases	2	8	0.362**
Rheumatic diseases	16	10	0.371*
Hypertension	8	16	0.371*
Total, number (%)	**50 (50%)**	**50 (50%)**	

**Chi-square test, **Fisher's test;#t test for independent samples; # #M – mean, SD – standard deviation*

Respondents who work in the brown coal mine have significantly lower average values of the domains of physical functioning (p = 0.005), general health (p = 0.001) and mental health (p = 0.041) compared to the control group of subjects. Also, the average values of the common physical component of quality of life were significantly (p = 0.007) lower in the group of miners compared to the control group of respondents (Table 2). Differences in other domains of the SF-36 questionnaire between groups of respondents were not observed (Table 2).

There was no statistically significant difference between the groups of respondents in the average values of the pro-inflammatory cytokines IL-2, IL-5, IL-9, IL-21 and TNF-α in serum (Figure 1).

Subjects from the miners' group had statistically significantly (p <0.05) higher average values of the pro-inflammatory cytokines IL-6, IFN-γ, IL-17A and IL-22 in serum compared to the subjects from the control group. There was no statistically significant difference in the average values of the pro-inflammatory cytokine IL-17F in serum between the groups of subjects (Figure 2).

Subjects from the miners' group had statistically significantly (p <0.05) lower values of the anti-inflammatory cytokines IL-4 and IL-10 in serum, compared to the subjects from the control group, while a statistically significant difference in the average values of the anti-inflammatory cytokine IL-13 in serum was not observed between the groups of subjects (Figure 3).

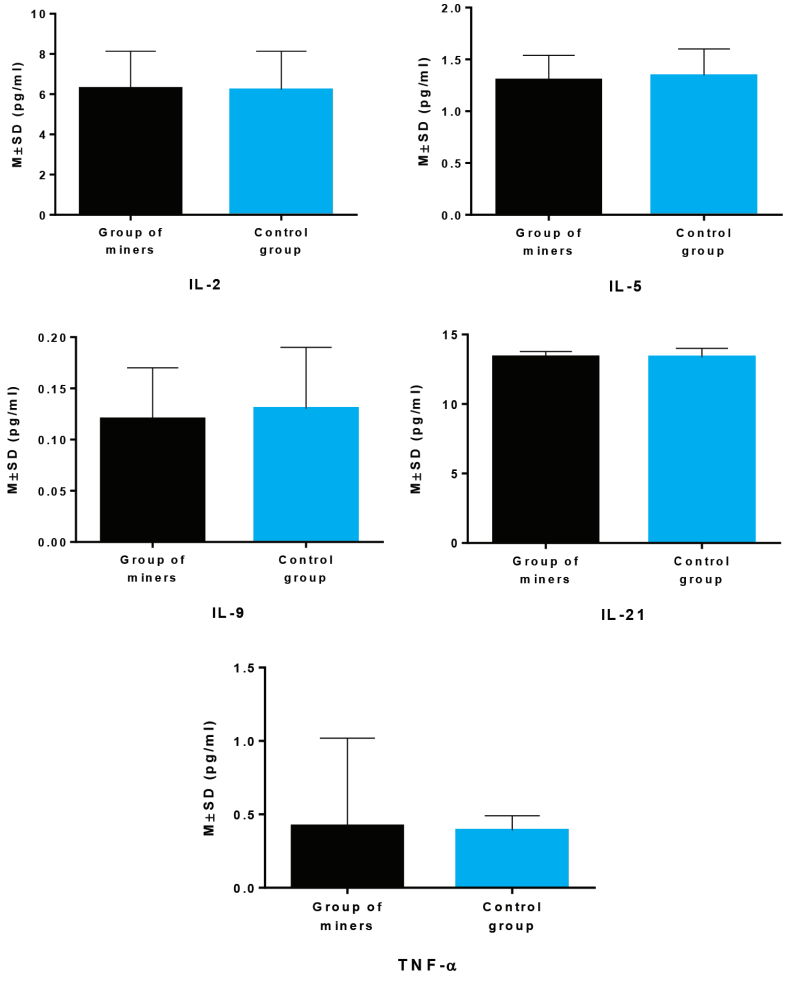

Figure 1. Average values of pro-inflammatory cytokines IL-2, IL-5, IL-9, IL-21 and TNF-α in serum of coal miners and control group of respondents. M – mean, SD – standard deviation; Mann-Whitney U test

Table 2. Respondents' quality of life measured by SF-36 questionnaire

Variables	Group of miners, M ± SD	Control group, M ± SD	p
Physical functioning	66.4±35.9	82.7±18.6	0.005**
Role physical	66.8±40.6	76.12±34.4	0.199**
Bodily pain	72.8±33.4	75.8±29.6	0.640*
General health	43.6±22.3	56.8±17.2	0.001*
Vitality	47.4±19.9	47.8±10.5	0.900*
Social functioning	80.8±54.8	81.4±20.1	0.944**
Role emotional	84.6±59.9	76.3±38.2	0.406**
Mental health	54.3±20.1	69.1±12.9	0.041*
Physical component summary	54.3±30.7	69.1±21.9	0.007**
Mental component summary	62.4±23.7	66.8±12.5	0.245*
Total, number (%)	**50 (50%)**	**50 (50%)**	

t test for independent samples;Mann-Whitney test; #M – mean, SD – standard deviation*

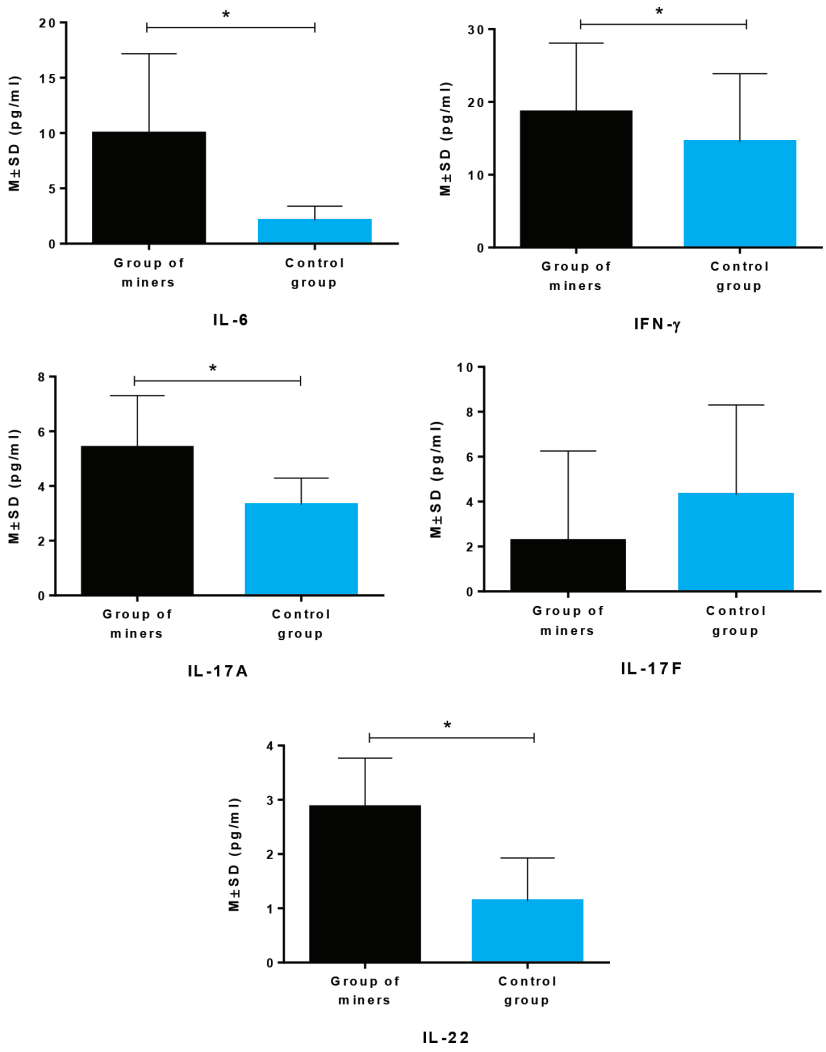

Figure 2. Average values of pro-inflammatory cytokines IL-6, IFN-γ, IL-17A, IL17F and IL-22 in serum of coal miners and control group of respondents. M – mean, SD – standard deviation; Mann-Whitney U test

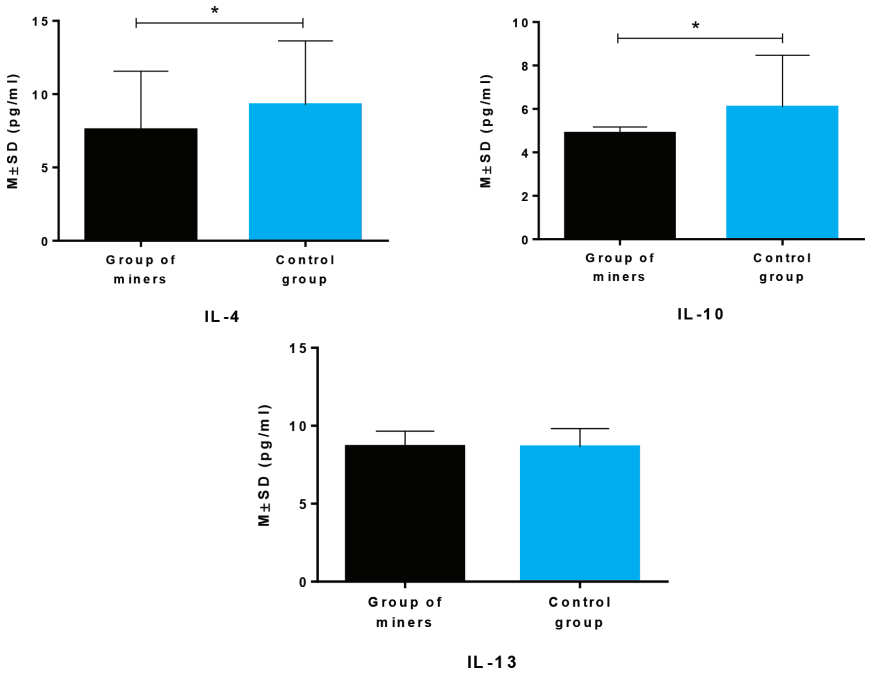

Figure 3. Average values of anti-inflammatory cytokines IL-4, IL-10 and IL-13 in serum of coal miners and control group of respondents; M – mean, SD – standard deviation, t test for independent samples

Discussion

Research on the quality of life and the immune response to coal dust was conducted at the Ugljevik mine and thermal power plant. The exploitation of brown coal in this mine is superficial. It is a mine in the eastern part of the Republic of Srpska, Bosnia and Herzegovina. The mine produces about 1.8 million tons of coal, of which about 95% ends up in the furnace of the thermal power plant, and the rest goes to commercial sale. The reserves of the coal basin amount to about 430 million tons of coal. The company has a special service for safety and health at work, which takes care of the personal protection of workers exposed to coal dust and which regularly organizes preventive health examinations [14].

The workers in the Ugljevik mine and thermal power plant are mostly male. In our sample, men were in the majority in the group of exposed workers (88%), and women were in the majority in the group of non-exposed workers (76%), which was statistically significant ($p = 0.001$). The group exposed to coal dust more often had symptoms of respiratory diseases, such as dyspnea ($p = 0.017$) and cough ($p = 0.046$).

When it comes to indicators of quality of life of employees in the mine and thermal power plant, our groups differed statistically in the following parameters: Physical functioning ($p = 0.005$), General health ($p = 0.001$), Mental health ($p = 0.041$) and Physical component Summary ($p = 0.007$). Quality of life is a multidimensional concept, as an important indicator of medical care. A study conducted by Han et al. [15] in China found that there is a difference in the domain of physical health problems between surface miners and underground miners ($p = 0.005$). Compared to the normal population, their subjects had lower scores in both the physical health and mental health components, and many factors contributed to this, including the duration of work due to dust exposure, chronic illness, health

insurance, and so on. Differences in the components of physical and mental health were also found in our study by comparing workers who were exposed to coal dust and those who were not exposed [15].

In order to study the impact of social factors on the quality of life of workers in the coal industry, Petrov [16] devised a new method for assessing the individual quality of life of miners, based on the WHO quality of life assessment. According to the research, the value of quality of life on average was 27.7%. According to the scale shown, the average quality of life of miners is low. The variability in the quality of life of miners is mainly determined by the index of social satisfaction and the index of satisfaction with health [16]. Concerns about health and the quality of social protection are the main factors in the low quality of life of workers in the coal industry. Ivoilov and co-workers [17] examined the quality of life in the Kamelovo region of Russia. It turned out that the parameters of the quality of life of workers decreased with the age of 20 to 64 years. The parameters of quality of life on the scales of pain, physical functioning and general health are inversely correlated with the age and length of service in dangerous working conditions for workers in a coal mine [17].

Yu and co-workers [18] conducted a cross-sectional study in China among 305 workers who are exposed to coal dust and 200 workers exposed to coal dust from five coal mines in Shanxi Province with the aim of examining the quality of their life. They used a short version of the Quality of Life Questionnaire of the Chinese Health Organization (WHOQOL-BREF). All functional domains of the Chinese WHOQOL-BREF were significantly worse in workers who are exposed to coal dust compared to workers who are not exposed to coal dust, except for psychological health. They pointed out that appropriate health policies should be developed to improve quality of life of coal dust workers [18].

It is known that components from coal dust react with cells in the lungs causing cell membrane damage which is accompanied by lipid peroxidation. The pathogenetic mechanism of CWP takes place in three phases. Initially, there is an accumulation and activation of inflammatory cells in the lungs. Damaged cells release intracellular enzymes, which provoke further tissue damage, resulting in scarring or destruction of alveolar septa. Coal dust phagocytized by alveolar macrophages stimulates the formation of reactive oxygen species which then stimulate the secretion of cytokines and chemokines. These inflammatory cytokines act as chemoattractants that attract polymorphonuclear leukocytes and macrophages from the pulmonary capillaries to the alveoli, resulting in chronic inflammation [19]. Current concepts of CWP pathogenesis suggest that alveolar macrophages play a key role because of their ability to release various mediators such as proteolytic enzymes and growth and differentiation factors. In the chronic phase of CWP leading to pulmonary fibrosis in pneumoconiotic lungs, it is clear that cytokines produced by alveolar macrophages play a significant role in the pathogenesis of CWP [7].

In our study, we did not find statistically significant differences in values of pro-inflammatory cytokines IL-2, IL-5, IL-9, IL-21 and TNF-α in serum between the groups of respondents. According to a study by Vanhee et al., after in vitro exposure of alveolar macrophages to coal dust, coal dust particles caused significant secretion of TNF-α and IL-6 [7]. Accordingly, in our study, we found a statistically significantly higher level of IL-6 in serum of the workers exposed to coal dust. Even though it has been shown that coal dust exposure stimulates pro-inflammatory response leading to increased release of cytokines from monocytes such as TNF-α [20, 21]. However, in our study we found no differences in serum TNF-α concentration between the groups of respondents.

In a study by Yao et al. [22] to examine the expression of type 1 and type 2 (Th1 and Th2) cytokines from serum of coal miners, 630 coal miners were studied. Authors concluded that the median level of pro-inflammatory cytokines IL-1β, IL-8, IFN-γ and IL-6 in cases group (groups suspected with CWP or with diagnosed CWP) were significantly higher ($p<0.05$) than that of non-cases group (control group of healthy miners) [22]. Our results coincides with this study, where IFN-γ concentration was significantly higher in a group of miners when compared to the control group ($p<0.05$). In our study we did not measured concentrations of IL-1β and IL-8, but we measured other pro-inflammatory cytokines such as IL-17A and IL-22 and we determined that both of them were significantly higher in the group of miners when compared to the control group. The IL-17A has been linked to chronic inflammation and the genetic association between single nucleotide polymorphisms of IL-17A and CWP in Chinese population is proven. In a case-control study by Han et al. [22] conducted among 1,391 subjects logistic regression analysis showed that the genotypes of rs3748067 AA (OR = 0.43, 95 % CI = 0.23-0.83) and rs8193036 TT (OR = 0.59, 95 % CI = 0.40-0.86) were associated with a decreased risk of CWP [23].

Yao et al. [21] also determined that the level of anti-inflammatory cytokine IL-10 (654.08 pg/ml) was significantly lower in CWP than that of control group (596.64 pg/ml) [19], which corroborates our results, where anti-inflammatory response was significantly higher in the control group of our respondents (higher concentrations of IL-10 and IL-4) when compared to the group of miners.

The limitation of our study is the relatively small number of cases. Therefore, further randomized and double-blind studies, involving larger numbers of miners, may contribute to better understanding of the additional role of inflammatory and immunomodulatory pathway in mine workers exposed to coal dust. Also, further limitation is the significant difference of respondents by gender distribution. However, this difference does not affect the results because gender is not expected to play a significant role in the results of this study.

Respiratory symptoms, such as dyspnea and cough were significantly more often present in the group of miners when compared to the control group of respondents. Physical functioning, general health, mental health and physical component summary were significantly poorer in the group of miners. Exposition to coal dust led to a significant increase in the production of pro-inflammatory cytokines (IL-6, IFN-γ, IL-17A and IL-22), and to decrease in the production of anti-inflammatory cytokines (IL-4 and IL-10). These results suggest that exposition to coal dust induce pro-inflammatory and decrease anti-inflammatory immune response.

Conclusion

Physical functioning, general health, mental health and physical component summary were significantly poorer in the group of miners. Exposition to coal dust led to a significant increase in the production of pro-inflammatory cytokines and a decrease in the production of anti-inflammatory cytokines.

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Ethical approval. The Ethics Committee of the Faculty of Medicine Foca approved the study and informed consent was obtained from all individual respondents. The research was conducted according to the Declaration of Helsinki.

Conflicts of interest. The authors declare no conflict of interest.

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Ispitivanje imunološkog odgovora rudara na ugljenu prašinu

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Kratak sadržaj

Uvod. Pokazalo se da udisanje ugljene prašine tokom miniranja u rudnicima mrkog uglja dovodi do bolesti pluća koja se zove pneumokonioza. U literaturi postoji vrlo malo podataka o direktnom uticaju uglja na kvalitet života ljudi koji rade u rudnicima uglja, kao i o imunskom odgovoru organizma na dejstvo ugljene prašine. Cilj je bio da se ispita imunski odgovor na izloženost ugljenoj prašini kod rudara u rudniku mrkog uglja i da li radnici u rudniku imaju lošiji kvalitet života u poređenju sa onima koji nisu izloženi ugljenoj prašini.

Metode. Ova studija presjeka je obuhvatila 100 zaposlenih u Rudniku mrkog uglja u Ugljeviku, od kojih je 50 svakodnevno izloženo ugljenoj prašini. Svim ispitanicima su uzeti uzorci krvi radi ispitivanja prisustva citokina IL-2, 4, 5, 9, 10, 13, 17A, 17F, 21, 22, IFN-γ i TNF-α. Kvalitet života zaposlenih mjeren je upitnikom za samoprocjenu fizičkog i mentalnog zdravlja (Short-Form Health Survey, 36 stavki, SF-36).

Rezultati. Grupa rudara je imala značajno ($p<0,05$) veće koncentracije proinflamatornih citokina IL-6, IFN-γ, IL-17A i IL-22 u poređenju sa kontrolnom grupom. Ispitanici iz kontrolne grupe imali su značajno ($p<0,05$) veće koncentracije antiinflamatornih citokina IL-4 i IL-10 u poređenju sa grupom rudara. Kvalitet života bio je značajno bolji ($p<0,05$) u kontrolnoj grupi u poređenju sa grupom rudara.

Zaključak. Fizičko funkcionisanje, opšte zdravlje, mentalno zdravlje i zajednička fizička komponenta zdravlja bili su značajno lošiji u grupi rudara. Izlaganje ugljenoj prašini dovelo je do značajnog povećanja proizvodnje proinflamatornih citokina i smanjenja proizvodnje antiinflamatornih citokina.

Ključne riječi: imunološka reakcija, citokini, ugljena prašina

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Original article

The importance of physical activity in diabetes

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Summary

Introduction. Obesity is becoming a global urgent issue that is considered a health problem in developed and developing countries. Obesity is a major risk factor for many non-communicable diseases such as cardiovascular diseases, type 2 diabetes mellitus (DM2), hypertension, coronary heart disease, or certain types of cancer. Physical activity is crucial for a healthy lifestyle. The aim of this study was to determine whether there is a difference in the physical activity of obese people with type 2 diabetes and obese people without type 2 diabetes.

Methods. This cross-sectional study included 50 obese patients with type 2 diabetes and 57 obese patients without type 2 diabetes. All patients went through the questionnaire, anthropometric measurements and laboratory tests. Type 2 diabetes was diagnosed in accordance with the American Diabetes Association. Data on physical activity were collected using the IPAQ (International Physical Activity Questionnaire), which was composed of questions on various physical activities in the previous 7 days.

Results. Activities at work and on the way to work in patients with type 2 diabetes were significantly lower compared to these activities in patients without type 2 diabetes ($p < 0.001$). Also, leisure time physical activities were lower in people with DM2 ($p = 0.001$). Just in case of household chores, subjects with DM2 had more utilized metabolic equivalent (MET) minutes whose utilization rate was close to 1700 MET minutes compared to 1500 MET minutes in subjects without DM2.

Conclusion. The results of this study indicate that obese subjects with DM2 are less active than obese subjects without DM2. Therefore, they should be recommended regular physical activities for at least 150 minutes per week to overcome the problem of obesity and the problem of DM2.

Key words: physical activity, type 2 diabetes mellitus, obesity, IPAQ

Introduction

Obesity is a growing problem worldwide [1] and is considered a health problem in both developed and developing countries [2]. In 2014, more than 39% of adults were overweight and 13% were obese [3]. Today, being overweight and obese cause higher mortality than malnutrition [3]. Obesity is accompanied by increased economic costs, social problems, increased morbidity and mortality from various diseases, so it is important to take measures and stop this pandemic [4,5]. Obesity is known to be a major risk factor for a number of non-communicable diseases such as

type 2 diabetes mellitus (DM2), hypertension, coronary heart disease, or certain types of cancer [6,7]. Overweight and obesity occur in 44% of cases of diabetes, 23% of patients with ischemic heart disease and about 7–41% of certain cancers (8,9). DM2 is most strongly associated with obesity, and the prevalence of obesity-related diabetes is expected to double to 300 million by 2025 [10]. Together, obesity and DM2 increase the risk of mortality in individuals 7 times [11]. It is estimated that by 2030, overweight and obesity will reach 89% and 85% in men and women, respectively [12,13]. This will result in an increase in the incidence of obesity-related coronary heart disease by 97%, cancer by 61% and DM2 by 21%. In addition, direct healthcare costs will increase significantly. It is estimated that if the body mass index (BMI) of the population were to decrease by 5% by 2030, obesity-related health care costs would be reduced by € 495 million over 20 years [12].

Physical activity is a key component of a healthy life. Aerobic exercise is the best way to lose fat. It has been shown that patients with DM2 have lower energy expenditure, fewer steps taken and shorter duration of physical activity [14], lower cardiorespiratory fitness [15,16], as well as lower muscle strength compared to subjects without this disease [17,18].

The aim of this study was to determine whether there is a difference in the level of physical activity of obese people with DM2 and obese people without DM2.

Methods

This cross-sectional study included 50 obese patients with DM2 and 57 obese patients without DM2 who were on medical nutrition therapy at the nutrition counseling institute of the Institute of Hygiene with Medical Ecology of the Faculty of Medicine in Belgrade. All patients were surveyed and underwent anthropometric measurements and laboratory tests.

A standardized questionnaire was used to estimate basic demographic data. In addition to basic demographic data (gender, age), the questionnaire also collected data on tobacco smoking.

In order to assess the nutritional status, all patients underwent anthropometric measurements to determine weight, height and waist circumference.

Body mass (BM) and body height (BH) were measured in the morning using a calibrated anthropometer after which the BMI (kg/m2) was determined. The recommendation of World Health Organization (WHO) was used to assess nutritional status [19]. The percentage of body fat was determined using bioelectrical impedance.

Waist circumference was measured in the middle of the distance between the lowest point of the costal arch (arcus costalis) and the anterior upper femoral spine (spina iliaca anterior superior), while the patients were in a standing position. Abdominal obesity was determined based on the value of the waist circumference, also according to WHO recommendations [19]. The following limit values were used to determine obesity: 1) waist circumference equal to or greater than 102 cm for men, or greater than or equal to 88 cm for women; 2) percentage of body fat equal to or greater than 25% for men, or equal to or greater than 33% for women [20].

The diagnostic criteria for DM are fasting glucose ≥7 mmol/l or plasma glucose value ≥ 11.1 mmol/l 120 min after the oral glucose tolerance test (OGTT) [21].

Data on physical activity were collected using the IPAQ (International Physical Activity Questionnaire), a questionnaire composed of questions about different physical activities in the past 7 days. The questionnaire is composed of 27 questions which are divided into 5 parts. The first part is composed of questions related to physical activities at work. The second part is composed of questions related to transportation. The third part consists

of questions related to housework. The intensity of physical activity was estimated based on MET minutes. A healthy adult while at rest is considered to consume 3.5 ml of O2/kg body weight per minute, which represents an energy expenditure of 1 kcal and is referred to as MET minute [22]. The fourth part is consisted of questions about recreation and the last fifth part are the questions related to sitting. Physical activity scores were calculated according to the instructions in the Scorecard calculation manual on the International Physical Activity Questionnaire (IPAQ).

IBM SPSS 23 program was used for data processing. The average, standard deviation, minimum, maximum, and coefficients of skewness and kurtosis were calculated from the descriptive parameters. A Chi-square test was used to test the association between the presence of diabetes and obesity. To determine the existence of differences between patients who do not have diabetes and those who have diabetes according to different activity parameters, the Mann-Whitney U test was used, and the Eta-squared (h2) coefficient was shown as a size of the effect.

Results

107 respondents participated in this research, of which three quarters (a total of 80) are female. Out of a total of 50 respondents who have diabetes, 80% are female, while out of 57 respondents who are not diagnosed with diabetes, 70% are female. The average age of patients without diabetes is 47 ± 13 years, while the patients with diabetes are slightly older and their average age is 54 ± 10 (p <0.001), (Table 1).

Comparing body height, weight and waist circumference, no statistically significant differences were found between people with and without diabetes, while the percentage of body fat was significantly higher in people with diabetes (p <0.001), (Table 2).

Activity at work, as well as activity during transport of patients with diabetes were significantly lower compared to the activities of the patients without diabetes (p <0.001) (Figure 1).

Also, activities during free time had lower values in the subjects with diabetes than in the subjects without diabetes (p = 0.001) (Figure 2).

Table 1. Basic demographic data in relation to the presence of diabetes

Parameters		Groups		P value
		D	N	
Gender	40 (80 %)	40 (70.2 %)	40 (80 %)	0.272
	10 (20 %)	17 (29.8 %)	10 (20 %)	
Age – average value ± SD		54.34 ± 9.63	46.56 ± 12.53	< 0.001

Table 2. Anthropometric indicators in the examined groups

Parameters	Groups (average values ± SD)		P value
	D	N	
Body height (cm)	165.3 ± 8.8	168.6 ± 10.8	0.082
Body weight (kg)	97.1 ± 20.1	96.6 ± 20.8	0.913
Waist circumference (cm)	118.9 ± 14.2	115.4 ± 15.1	0.216
Body fat (%)	38.9 ± 6.5	34 ± 6.4	<0.001

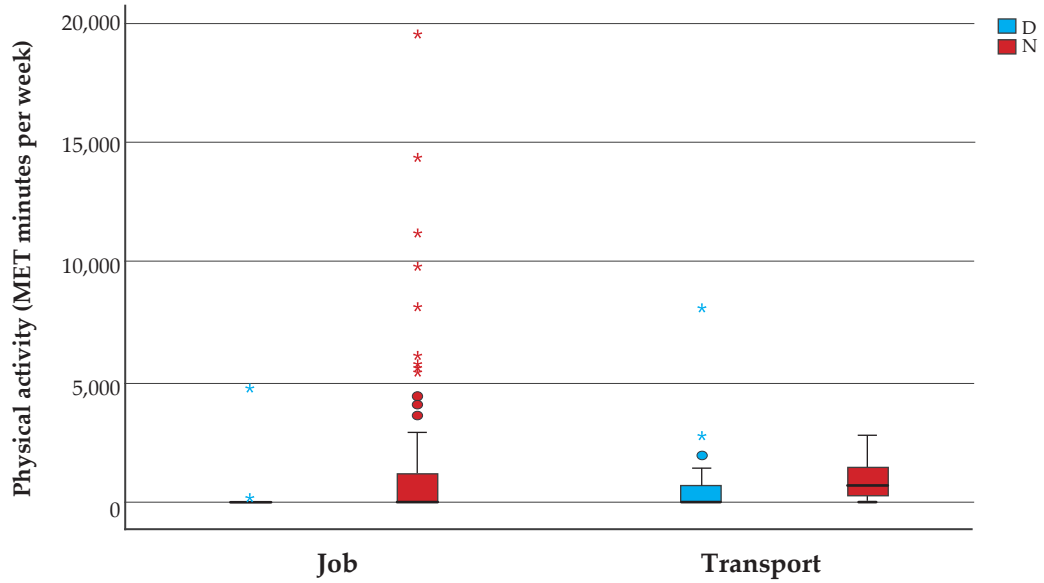

Figure 1. Comparison of activities at work and transport activities between the tested groups. D - subjects with diabetes, N - subjects without diabetes. ** - p <0.01

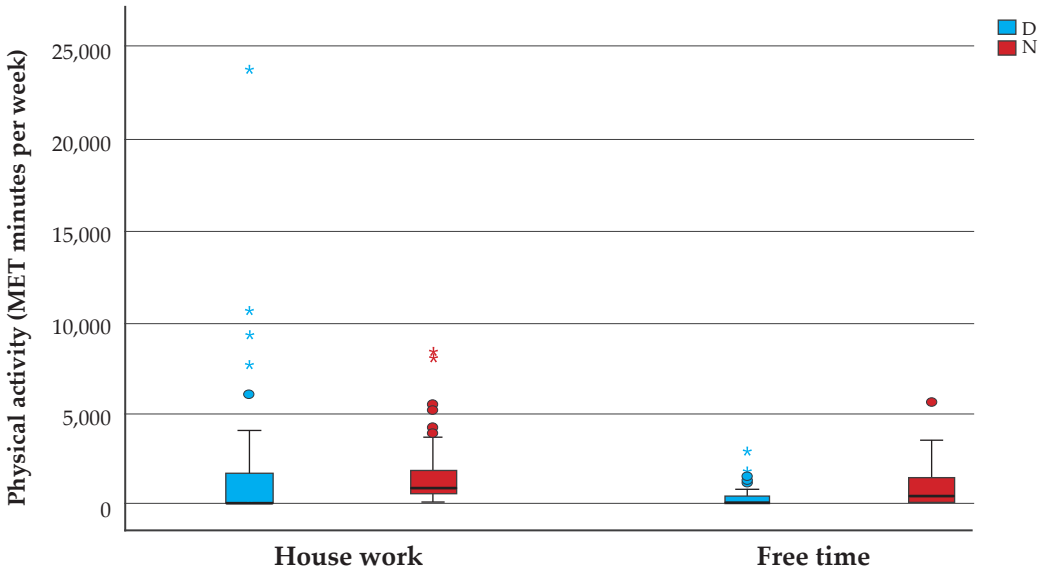

Figure 2. Comparison of activities during housework and free time between the tested groups. D - subjects with diabetes, N - subjects without diabetes. ** - p <0,01

When it comes to the intensity of activities, regardless of whether they are walking, moderate or vigorous activities, people with diabetes on average have fewer MET minutes for these activities than peple without diabetes ($p < 0.001$, $p < 0.001$, $p = 0.001$, respectively). Moreover, none of the subjects who have diabetes engaged in any of the vigorous activities during the week (Figure 3). Namely, patients with diabetes are active on average about 2900 MET minutes, in contrast to patients who do not have diabetes, who are active on average about 5100 MET minutes (Figure 3).

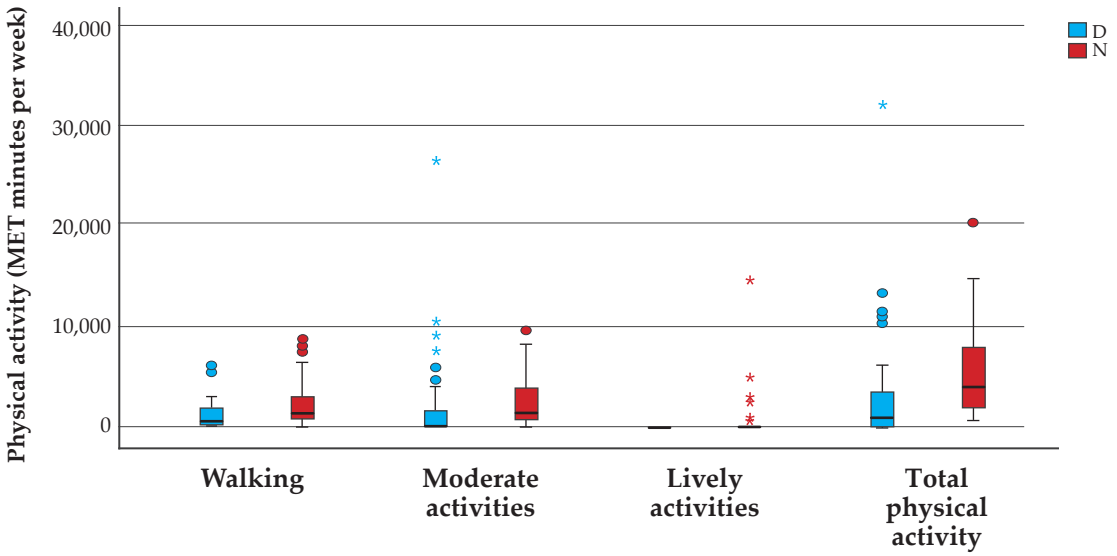

Figure 3. Comparison of the intensity of physical activities between the tested groups. D - subjects with diabetes, N - subjects without diabetes ** - p <0.01

When testing the differences between non-diabetic and diabetic patients in terms of different activity measures, the results show that by all parameters, non-diabetic patients are more active than the diabetic patients. When it comes to differentiation according to activities in different contexts (work, transportation, household chores, leisure time), the greatest effect occurs when comparing these two groups by activities at work, where 22% of variability of activities at work can be covered by differences between patients who do not have diabetes and patients who have this disease. The effect sizes of activities in other contexts are lower and range from 0.10 to 0.13.

When it comes to the intensity of activities, the sizes of the effect of walking (η^2 = 0.18) and moderate activities (η^2 = 0.17) are slightly larger than the size of the effect of vigorous activities (η^2 = 0.11). Finally, looking at total physical activity, the size of the effect is about 20% of the shared variance, or about 20% of the variability in total physical activity can be attributed to differences between non-diabetic and diabetic patients (Table 3).

Discussion

The test results show that the tested group of DM2 patients was older than the group without DM2. Comparing differences in genders, it was determined that women were represented more in both groups.

Subjects of both tested groups weighed on average about 97 kg. Based on the measurement of the percentage of body fat, it was determined that the respondents who do not have diabetes have 34% of body fat, while in people who have diabetes that value is slightly higher and amounts to 39%. Body fat is a metabolic organ that performs many functions such as lipid storage, protective and thermal insulation, immune response, endocrine functions and thermoregulation [23,24]. Recent research has concluded that body fat acts as an endocrine organ [25,26]. Obesity is often a precursor to DM2 and it is important to take steps to regulate obesity to prevent DM or reduce the complications of this disease. Body composition and body fat distribution are risk factors and can be considered a cause of DM2 [27]. An analysis of the literature has

Table 3. Differences in types of physical activity in relation to the presence of diabetes

Dependent variable (type of activity)	U	P	η^2	Distinguishing groups			
				The presence of diabetes	N	Average rank	Sum of ranks
Job	817.00	< 0.001	0.22	(D)	50	41.84	2092.00
				(N)	57	64.67	3686.00
Transport	835.50	< 0.001	0.13	(D)	50	42.21	2110.50
				(N)	57	64.34	3667.50
Housework	889.50	< 0.001	0.11	(D)	50	43.29	2164.50
				(N)	57	63.39	3613.50
Free time	922.00	< 0.001	0.10	(D)	50	43.94	2197.00
				(N)	57	62.82	3581.00
Walking	738.00	< 0.001	0.18	(D)	50	40.26	2013.00
				(N)	57	66.05	3765.00
Moderate activities	758.50	< 0.001	0.17	(D)	50	40.67	2033.50
				(N)	57	65.69	3744.50
Lively activities	1125.00	< 0.001	0.11	(D)	50	48.00	2400.00
				(N)	57	59.26	3378.00
Total physical activity	681.00	< 0.001	0.20	(D)	50	39.12	1956.00
				(N)	57	67.05	3822.00

U – Mann-Whitney test; η^2 - measure the size of the effect

shown that insufficiently intense aerobic exercise does not have a high probability of losing weight [28]. A ten-year cohort study from the early 1970s to the early 1980s, when obesity was a health problem at the beginning, suggests the following conclusion. Moderate activity, according to the American National Health and Nutrition Examination Survey, was associated with a 3-fold greater risk of large increase of body mass in men and almost 4-fold in women [29]. In a three-year study, in 34,079 women with an average age of 52.2 it is noticed an 11% higher risk of weight gain in those who had less than 7.5 MET hours of physical activities per week compared to the group of women who had more than 21 MET hours per week, or who had about 300 minutes of moderate physical activities per week [30]. Body exercise with a load or a combination of this type of exercise with running or walking significantly reduces risk factors for DM2 such as waist circumference, abdominal adiposity, HDL level and others [31,32,33]. In this paper, the statistical difference in age between two groups of respondents can be problematic. What may be correlated with this difference is the association of aging, obesity, and DM2 with declining pancreatic beta cell function with age [34]. Aging is also associated with decreased mitochondrial function and cartilage degradation, which contributes to DM2 [35-39]. Numerous impairments occur with obesity, aging and DM2 as a consequence of a sedentary lifestyle and poor eating habits. Physical activity is the only intervention that can positively affect all impairments, including the pathophysiology of all consequences and symptoms [40]. A Spanish study of 412 patients with DM2 indicates that a small number of respondents adhere to the principle of a healthy lifestyle. Namely, less than a quarter adhere to a proper diet, and less than half exercise regularly [41].

Compared with obese patients without DM2, obese patients with DM2 have a less favorable distribution of body fat with an

increase in visceral fat [42]. Excess visceral adipose tissue is known to worsen insulin resistance [42] and, therefore, increases the risk of complications of diabetes. A 5% reduction in body fat percentage in patients with DM2 was associated with improved glycemic control as assessed with glycosylated hemoglobin [43]. In this study, there was no difference in abdominal obesity estimated based on waist circumference between the two groups tested. However, when obesity was assessed on the basis of body fat percentage, the ratio of obese and non-obese individuals differed significantly between subjects with diabetes and subjects without diabetes. About 90% of patients with diabetes are obese according to the criterion of body fat percentage, while only 58% of patients who do not have diabetes are obese according to this criterion.

Obesity is known to significantly increase the risk of developing metabolic disorders, hypertension, cardiovascular diseases, stroke and cancer. However, up to 30% of obese patients are metabolically healthy. These individuals have preserved insulin sensitivity and a lower visceral fat content compared to most metabolically "unhealthy" obese patients [44]. Fluctuations in insulin sensitivity occur throughout the life cycle. For example, insulin resistance is observed during puberty, pregnancy and aging [45]. In addition, lifestyle variations, such as increased carbohydrate intake and decreased physical activity, have been associated with fluctuations in insulin sensitivity [46]. Insulin sensitivity is also determined by the distribution of fat in the body. Individuals who have more peripheral distribution of fat are more sensitive to insulin than those who have a central fat distribution [45]. Visceral fats have a greater ability to secrete various proteins and hormones [46].

Both DM2 and obesity are associated with insulin resistance. The basic factor that affects insulin resistance is the release of non-esterified fatty acids. Increased release of non-esterified fatty acids has been observed in DM2 and obesity, and has been associated with insulin resistance in both conditions [46]. Shortly after the acute increase in plasma non-esterified fatty acid levels, humans begin to develop insulin resistance. In contrast, when the level of non-esterified fatty acids in plasma falls, as in the case of the use of antilipolytic agents, the value of peripheral insulin and the level of glucose improves [47]. There is evidence that BMI, central adiposity, and weight gain indicate an increased risk of developing DM2 [48,49]. A meta-analysis of prospective studies has provided evidence that an increase in upper-body adiposity increases the risk of metabolic syndrome and DM2 development [50]. The duration of obesity in younger people compared to older individuals is also associated with a higher risk of DM2 [51]. Weight gain, especially between the ages of 25 and 40, increases the risk of developing DM2 [52]. Weight loss is obviously helpful in reducing the risk of developing diabetes. In a diabetes prevention program, it was found that a medium weight loss of 5.5% over 2.8 years reduced the risk of progression from prediabetes to diabetes by 58% [53].

Although exercise is an important component of any effective weight loss strategy, several studies have reported additive effects on weight loss when exercise is combined with reduced food intake. This can be achieved either by reduced fat intake, or by reduced carbohydrate intake or by the Mediterranean diet [54,55,56]. The Mediterranean diet has been shown to have beneficial metabolic effects, as well as to delay the need for antihyperglycemic drugs in patients with newly diagnosed DM2 [57].

The results of our study show that in all parameters, people without diabetes are more active than people with diabetes. By comparing total physical activities, it was found that obese people without DM2 are more active than people with DM2. Namely,

patients who have DM2 are active on average about 2900 MET minutes, unlike patients who do not have DM2 who are active on average about 5100 MET minutes. Decreased physical activity is known to be one of the risk factors for DM. Lack of physical activity and a sedentary lifestyle are risk factors for cardiovascular diseases, DM2, and overall mortality [58,59]. The American Diabetes Association has recommended that patients with DM spend a maximum of 90 minutes a day on a sedentary basis [60]. Changing a sedentary lifestyle to a more active lifestyle is a key to better DM2 management. Patients with DM2 have lower physical and cardiorespiratory fitness, lower energy expenditure, fewer steps taken, and shorter duration of physical activity compared with subjects without this disease [14,15,16]. By comparing muscle strength, it was found that people with DM also have less muscle strength compared to people who did not get sick [17,18]. By testing limb muscle strength and the connection of this parameter with DM complications, it was shown that muscle strength was negatively connected with the degree of DM complications [61]. This explains that due to the progression of DM, a decrease in physical activity can occur more and more, and a decrease in physical activity leads to an even greater progression and complications of DM. Physical activity plays a major role in the prevention and treatment of DM2. Studies have shown that aerobic exercise (walking, running, cycling) or strength training reduced the absolute value of hemoglobin A1c by about 0.6% [62], while the incidence of microvascular complications decreased by 37% [63]. It is assumed that the decrease in hemoglobin A1c would be significantly higher if, in addition to aerobic exercise, strength exercises were applied [62]. One large study showed that DM patients who performed low-intensity physical activity (90 min per week) had a significantly lower (14%) risk of mortality from all causes. In these subjects, there was an increase in life expectancy by 3 years [64]. Since the often recommended planned physical activity for patients with DM is a burden, it is important to emphasize that performing daily activities of low to moderate intensity is an important treatment for this group of people, because it has been shown to have positive effects [64]. Daily activities include various activities that lead to increased energy expenditure and that are carried out in work and leisure time. Those are most often: walking, working at the desk, washing, cooking and recreational sports. They can be of varying intensity, and can sometimes be of the same intensity as a planned structured exercise [67]. The results of our study show that walking was significantly less prevalent in people with DM2 compared to those without DM2. Although walking should be the most common daily activity, one study found that 55% of patients with DM2 reported that walking is not their regular physical activity [68]. Other studies have shown that walking at moderate speeds reduced the risk of DM2 by 20-30% in women who did not do any other intense physical activity [69]. Patients with DM who walked for at least 2 hours per week had a 39% reduction in all-cause mortality and a 34% reduction in mortality from cardiovascular compared with sedentary patients [70]. Also, moderate-intensity physical activity such as brisk walking, at least 150 minutes per week, reduced the incidence of diabetes by 58% after less than 3 years of follow-up [71]. In the United States, it has been found that about 40% of patients with DM use exercise therapy [72], however, only 28% of patients achieved the recommended level of physical activity [73].

Increasing aerobic physical activity reduces visceral fat, increases lean mass, reduces depression, and improves glucose tolerance, insulin sensitivity, and physical fitness. It is therefore not surprising that all scientific guidelines recommend at least 150 min/week

of moderate aerobic exercise in combination with resistance training to increase muscle strength done three times a week [1,74,75,76]. According to these recommendations, no form of activity should last less than 10 minutes [77]. Applied physical activity will lead to a decrease in fat mass, and thus to an improvement in DM and a slowdown in the development of DM complications.

This study had limitations, of which the design of the study itself should be especially emphasized, as well as the relatively small number of respondents.

Conclusion

The results of our study show that obese people with diabetes are less physically active than obese people who are not diagnosed with diabetes. As obesity and diabetes represent a growing epidemic in modern society, it is suggested that the simultaneous treatment of these two metabolic disorders would lead to significant improvements. Therefore, all people, especially obese people who also have type 2 diabetes, should be recommended to exercise regularly, for at least 150 minutes a week.

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Ethical approval. The Ethics Committee of the Institute of Hygiene with Medical Ecology of the Faculty of Medicine in Belgrade approved the study and informed consent was obtained from all individual respondents. The research was conducted according to the Declaration of Helsinki.

Conflicts of interest. The authors declare no conflict of interest.

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Značaj fizičke aktivnosti kod obolelih od šećerne bolesti

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Uvod. Gojaznost postaje globalno urgentno pitanje koje se smatra zdravstvenim problemom u razvijenim i zemljama u razvoju. Gojaznost je glavni faktor rizika za mnoge nezarazne bolesti poput kardiovaskularnih bolesti, dijabetes melitus tipa 2 (DM2), hipertenziju, koronarnu bolest srca, ili određene vrste karcinoma. Fizička aktivnost je presudna za zdrav način života. Cilj ove studije bio je da se utvrdi da li postoji razlika u fizičkoj aktivnosti gojaznih osoba sa šećernom bolesti tipa 2 i gojaznih osoba bez šećerne bolesti tipa 2.

Metode. U ovoj studiji preseka uključeno je 50 gojaznih pacijenata sa šećernom bolesti tipa 2 i 57 gojaznih pacijenata bez šećerne bolesti tipa 2. Svi pacijenti su prošli upitnik, antropometrijska merenja i laboratijska ispitivanja. Dijabetes tipa 2 je dijagnostikovan u skladu sa Američkim udruženjem za dijabetes. Podaci o fizičkoj aktvnosti prikupljeni su pomoću IPAQ upitnika (International Physical Activity Questionnaire), koji je bio sastavljen od pitanja o raznim fizičkim aktivnostima u prethodnih 7 dana.

Rezultati. Aktivnosti na poslu i na putu do posla kod pacijenata sa šećernom bolesti tipa 2 bile su značajno niže u poređenju sa ovim aktivnostima kod pacijenata bez šećerne bolesti tipa 2 ($p < 0.001$). Takođe, fizička aktivnost u slobodno vreme je manja kod osoba sa DM2 ($p = 0.001$). Samo u slučaju kućnih poslova, pacijenti sa DM2 imali su više iskorišćenih metaboličkih ekvivalenata (MET) minuta čija je stopa iskorišćenosti bila blizu 1700 MET minuta u odnosu na 1500 MET minuta kod osobe bez DM2.

Zaključak. Rezultati ove studije ukazuju na to da su gojazne osobe sa DM2 manje aktivne od gojaznih osoba bez DM2. Samim tim, trebalo bi im preporučiti redovnu fizičku aktivnost najmanje 150 minuta nedeljno u prevazilaženju problema bolesti gojaznosti i DM2.

Ključne reči: fizička aktivnost, dijabetes melitus tip 2, gojaznost, IPAQ

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Original article

Comparison between radiographic and ultrasound angle measurements in the assessment of idiopathic scoliosis

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Summary

Introduction. Radiological assessment is still being considered a golden standard when it comes to detection, follow-up and treatment of idiopathic scoliosis. However, it has universally been proven that radiation is cumulative and that it has oncogenic effects. For this reason, nowadays it is becoming increasingly popular to perform spinal ultrasounds using the Scolioscan® device. Relevant research has shown diagnostic potential of the device and its application in the assessment and monitoring of idiopathic scoliosis. The aim of our study has been to compare angle measurements in ultrasound and radiological spinal images and to determine the role of ultrasound in the assessment process and follow-up of patients with idiopathic scoliosis.

Methods. This cross-sectional study has been conducted on a sample of 172 patients. Its participants are boys and girls, patients of the Team for Scoliosis that operates within the Department for Habilitation and Rehabilitation of Children in the Institute for Physical Medicine and Rehabilitation "Dr Miroslav Zotović", Banja Luka, the Republic of Srpska. Radiography and ultrasound of the spine have been performed on every patient on the same day. Three specially trained operators administrated the ultrasound scanning, while four raters (i.e. doctors) measured the radiographic Cobb angle and the ultrasound Scolio angle. Patients have been divided into groups according to gender, curve location and curve severity.

Results. In general, ultrasound angles are shown to be smaller compared to Cobb angles, which has been confirmed in previous studies as well. The mean difference between Cobb and Scolio angle is statistically, but not clinically significant (3.62±4.39°, $p<0.001$). There is a statistically significant good positive correlation between the Cobb and the Scolio angle ($r=0.675$, $p<0.001$). According to groups, our results indicate a better correlation in the girls group ($r=0.688$, $p<0.001$) as opposed to the boys group ($r=0.632$, $p<0.001$). The same holds true for the thoracic group ($r=0.736$, $p<0.001$), compared to the (thoraco) lumbar group ($r=0.654$, $p<0,001$). A stronger correlation can also be seen in the group with a Cobb angle that is equal to or higher than 20° ($r=0.518$, $p<0.05$) than in the group with a Cobb angle lower than 20° ($r=0.462$, $p<0.001$).

Conclusion. The results of our study confirmed a good validity of the ultrasound method using the Scolioscan® device, compared to conventional radiography, taking into account clinically insignificant differences in angle measurements. Using only B-mode ultrasound images – with no additional software analysis, nor 3D reconstruction of spinal deformities – proved to be sufficient for a follow-up of scoliosis, with respect to other parameters, such as clinical assessment, back surface topography, etc.

Key words: idiopathic scoliosis, ultrasound, radiography, imaging

Introduction

Idiopathic scoliosis is a three-dimensional spinal deformity with unknown etiology [1] that occurs in seemingly healthy children during growth, prominently progressing while in puberty and adolescence. This condition occurs more frequently in girls than in boys, especially during their growth spurt [2]. If scoliosis exceeds a Cobb angle of 30° –and particularly 50° [3] – there is a higher risk of health problems in adulthood [4]. A spinal deformity is structural and manifests in the form of vertebral changes with lateral deviation, rotation and impaired sagittal profile. Idiopathic scoliosis can be diagnosed exclusively via AP or PA radiography of the entire spine. According to SRS, the diagnosis of scoliosis can be established if the Cobb angle is 10° at minimum, with an axial rotation [1].

Radiological assessment is still being considered a golden standard when it comes to the detection, follow-up and treatment of scoliosis [5]. However, it is a proven fact that radiation is cumulative and has oncogenic effects, thereby increasing the risk of breast cancer in girls [6], as well as significantly contributing to leukemia and prostate cancer [7]. Consequently, using devices with a low dose of radiation (EOS) or no radiation at all (ultrasound, back surface topography) has become more frequent lately. Even though such methods still fail to replace conventional radiography in the treatment of idiopathic scoliosis, in recent years they have been used more frequently due to growing evidence of their reliability and validity.

Performing spine ultrasounds via the Scolioscan® device has become more popular and accessible worldwide. Relevant studies have shown its diagnostic potential and application in the assessment of idiopathic scoliosis [8,9,10,11]. It has also been demonstrated that the ultrasound can be used for the assessment of spinal flexibility, as well as for the prediction of in-brace correction [12]. The main advantage of ultrasound, compared to conventional radiography, is a complete absence of radiation. This enables its unlimited application, without possible health consequences in patients. Nevertheless, there are differences between these two methods, particularly in angle measurement assessments. Compared to conventional radiography, ultrasound imaging is unable to capture the patient's pelvic area. It is therefore inadequate in assessing the skeletal maturity according to the Risser sign and, consequently, the risk of progression. Also, it is not possible to assess cervical and upper thoracic curves, as ultrasound scanning reaches only up to the first thoracic vertebra. A 3D spine reconstruction using additional software (ScolioStudio) is possible, but it is more time-consuming and requires further education of clinicians.

Previous research has demonstrated a good to very good validity and reliability for the ultrasound assessment of scoliosis. It has been stated that ultrasound can reduce the need for radiographs during follow-ups and could additionally be used for scoliosis screening [13].

The aim of our study has been to compare angle measurements in ultrasound and radiological spine images and to determine the role of ultrasound when it comes to the assessment and follow-up of patients with idiopathic scoliosis.

Methods

This cross-sectional study has been conducted with the approval of the competent Ethical Committee. The sample consists of 172 patients, boys and girls, who have been patients of the Team for Scoliosis that operates within the Department for Habilitation and Rehabilitation of Children in the Institute for Physical Medicine and Rehabilitation ''Dr Miroslav Zotović'' in Banja Luka, the Republic of Srpska. The Team for Scoliosis consists

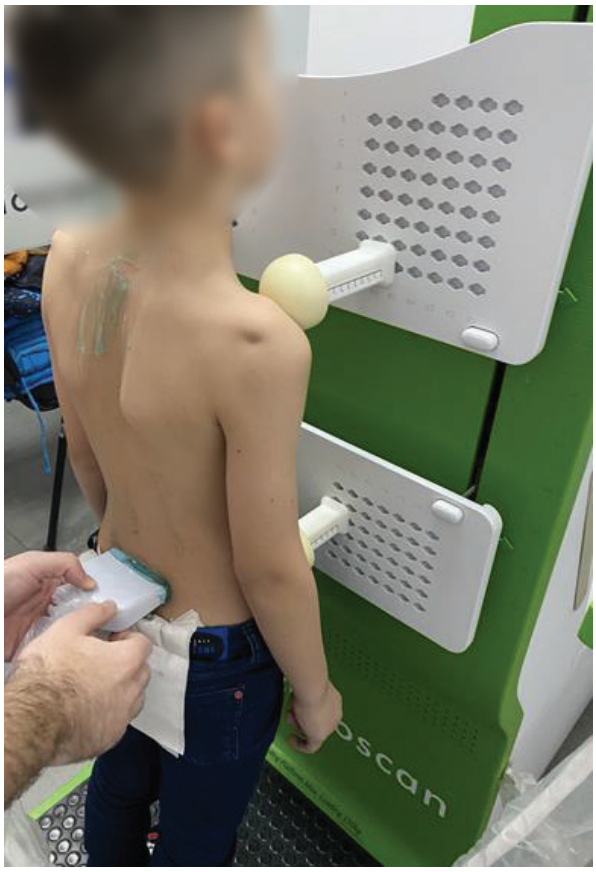
of doctors – specialists of physical and rehabilitation medicine – therapists and orthotists who conduct conservative treatment for all types of scoliosis, especially idiopathic scoliosis, according to SOSORT guidelines [1].

Radiography and ultrasound scans of the spine were performed for each patient on the same day. If, during the first examination in our facility, a spinal deformity is suspected, or a clinical deterioration is observed amidst a regular follow-up, spine radiography is performed. Indication for radiography is based on anamnestic data, the presence of risk factors and a detailed clinical assessment of the patient, including measurements of the angle trunk rotation with a scoliometer.

Our facility administers a digital radiography of the entire spine for the detection of scoliosis with possibility of spine measurements using TraumaCad® software. The diagnosis of scoliosis is established if the measured Cobb angle is higher than 10°, with rotation aspects of the vertebrae.

The Scolioscan® device system enables ultrasound imaging of the spine. Scolioscan® is manufactured by Telefield Medical Imagining Ltd. in Hong Kong, China and is comprised of a hardware system that enables the scanning process, as well as a software solution (Scoliostudio) for additional adjustments and 3D spine reconstruction. Its most important feature is a radiation-free scoliosis assessment. The short comings of Scolioscan® include patient's weight limit of up to 150 kg, as well as the presence of metal and magnet implantants in patients (i.e. pacemaker, defibrillator, cochlear implantant).

The scanning process is fast and effortless. The patient only needs to maintain a stable, but relaxed posture for about 45 seconds to one minute, for the duration of the scanning process (Picture 1). The device is adjustable to the patient's height and width. Three operators (technicians), with a successfully completed special training, administer the ultrasound scanning. Due to the absence of radiation, one can repeat the procedure as many times as needed, without causing any harm to the patient.

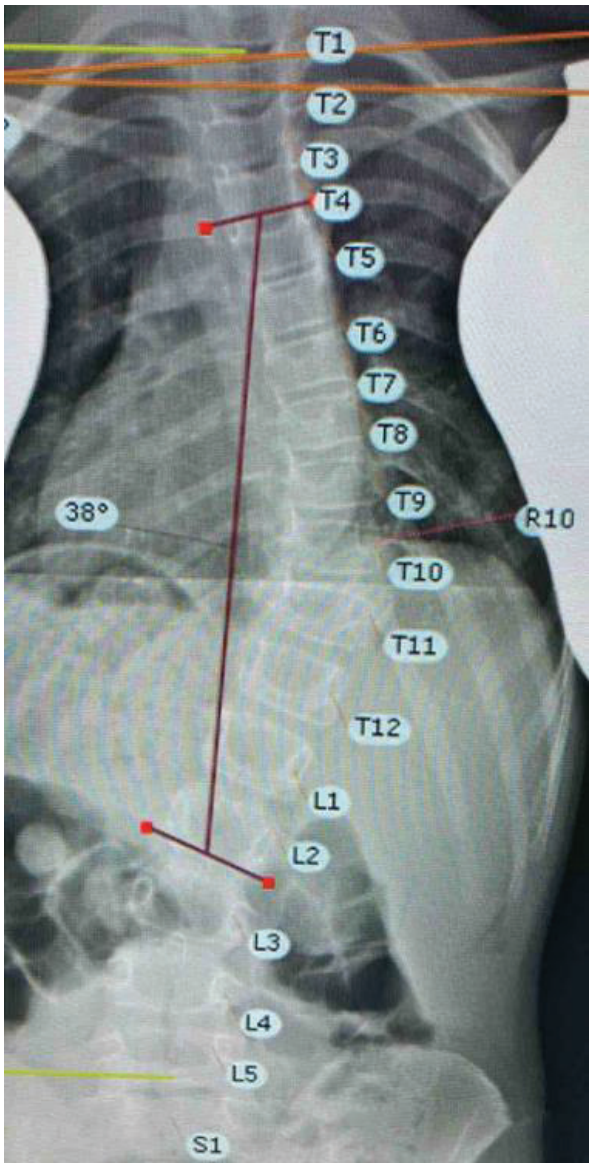


Picture 1. Scanning process on Scolioscan® device

After the scanning process, a B-mode image of the spine is produced, which has been used in this study. Additional analysis and 3D spine reconstruction that are available in the Scoliostudio software program have not been employed in this study. Different raters (i.e. doctors) have been in charge of the measurement of radiographic and ultrasound angles, in order to avoid subjectivity and its possible impact on the measurement process.

A Scolio angle is an ultrasound angle that is defined in our study by the same neutral vertebrae as a radiographic Cobb angle. In order to minimize the difference in angle measurements – that are possible due to a pronounced vertebral rotation in some curves – we have

applied manual ultrasound measurements with transverse processes as reference points. Cobb angle is defined on digital radiography by superior endplate of the upper neutral vertebra and inferior endplate of lower neutral vertebra which are chosen by the person who takes measurements (Picture 2). Both Cobb and Scolio angles are measured in degrees.



Picture 2. Radiographic Cobb angle measured on digital radiography

Factors that can affect these differences in image quality are: high BMI, significant vertebral rotation, impaired sagittal profile, and prominent spinal processes or scapulae.

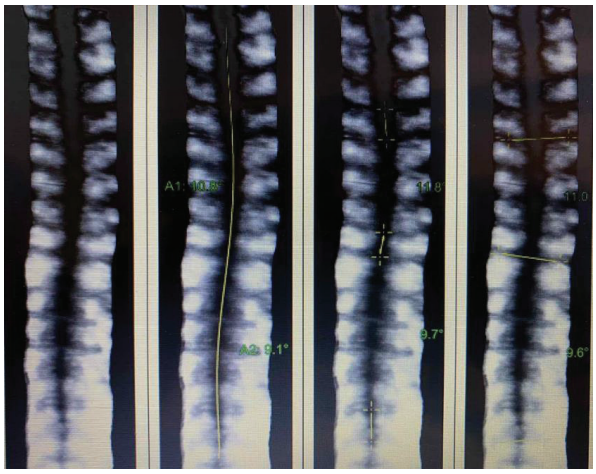
The inclusion criteria for this study concern patients with idiopathic scoliosis, with whom our Team has developed a good cooperation during the clinical and diagnostic assessment. While the ultrasound scanning is being performed, it is required of patients to maintain an upright position for up to one minute, for the duration of the scanning, without any movement whatsoever.

Non-cooperative patients, patients with secondary scoliosis, patients with high thoracic and cervicothoracic curves, as well as patients with a BMI higher than 85 percentiles have been excluded from the study. The reason for the latter is that the ultrasound scans only up to the first thoracic vertebra (Th1), which makes it impossible for the curves to be visible. Patients with a high BMI result in having low quality ultrasound images, making the assessment difficult and inaccurate, thus requiring additional adjustments and reconstruction in the Scoliostudio software system.

When it comes to patients with multiple scoliotic curvatures, only the primary curve has been considered in the measurement process.

There are three types of Scolio angle measurements: automatic, manual with a spinous process (SP) as a measurement reference point and manual with a transverse process (TP) as a measurement reference point (Picture 3). In order to provide greater accuracy and increase the comparability between angles, we have been using manual measurements, as well as the same neutral vertebrae that have been identified in X-ray and in ultrasound imaging. Landmarks that have been used for the measurement are transverse processes. Namely, curves with greater rotation have displaced spinous processes, which can lead to imprecise measurements and increase the discrepancy between radiological and ultrasound angles. Patients have been divided into three groups according their gender, primary curve location and curve severity. The group created based on the primary curve

location has additionally been divided into two sub-groups: thoracic and (thoraco) lumbar group. The latter includes thoracolumbar and lumbar curves. This division is based on findings indicating that there are differences in the measurement of ultrasound angles in thoracic and (thoraco) lumbar curves.



Picture 3. Automatic, manual SP and TP ultrasound measurements

Based on the severity of their curve, patients have been divided into two groups: having a Cobb angle below 20° and having a Cobb angle equal or above 20°. This threshold is in accordance with the recommendations for brace treatment, i.e. a Cobb angle that is equal to or higher than 20°.

Statistical data analysis has been performed using the program „SPSS for Windows 21". To determine the correlation between Cobb and Scolio angle – in total and according to groups – a Pearson's correlation test has been conducted. To examine whether there are statistically significant differences between variables, a t-test with unequal variance has been performed. The level of significance was set to $p < 0.05$. Logistic regression analysis has been used to test the impact of the predefined predictors (gender, curve location and curve severity).

Results

Our sample consists of 172 patients with juvenile and adolescent idiopathic scoliosis (94 females and 78 males), with a mean age of 12.01 years (SD ± 2.35, range 5 to 16).

The mean Cobb angle is 12.48° (SD ± 5.95°, range 4 to 43°) and the mean Scolio angle is 8.86° (SD ± 3.98, range 2.2 to 27.1°), as presented in Table 1.

The mean difference between Cobb and Scolio angle is 3.62° (SD ± 4.39), which is statistically significant ($p<0.001$). When expressed in percentages, the value is 23.55% (SD ± 29.50).

Table 1. Mean values and range of Cobb angle and Scolio angle in total

	X-ray (° Cobb)				Ultrasound (° Scolioscan)		
	n	M	min	max	M	min	max
TOTAL	172	12.48±5.95	4	43	8.86±3.98	2.2	27.1

Table 2. Mean values of Cobb angle and Scolio angle according to gender

	X - ray (° Cobb)			Ultrasound (° Scolioscan)		
n	boys	girls	total	boys	girls	total
172	10.99±4.19	13.72±6.86	12.48±5.95	8.16±3.56	9.44±4.22	8.86±3.98

Table 2 presents the data according to gender. In the boys group, the mean Cobb angle is 10.99° (SD±4.19, range 4 to 28°) and the mean Scolio angle is 8.16° (SD±3.56, range 2.2 to 24.9°). In the girls group, the mean Cobb angle is 13.72° (SD±6.86, range 4 to 43°) and the mean Scolio angle is 9.44° (SD±4.22, range 2.7° to 27.1°).

According to the location of the primary curve, the thoracic group consists of 47 patients, while the (thoraco) lumbar group includes 125 patients. In the thoracic group, the mean Cobb angle is 12.53° (SD±7.45, range 4 to 43°), while the mean Scolio angle is 8.43° (SD±4.18, range 2.8 to 24.9°). In the (thoraco) lumbar group, the mean Cobb angle is 12.46° (SD±5.31, range 5 to 33°) and the mean Scolio angle is 9.02° (SD±3.90, 2.2 to 27.1°). This data is presented in Table 3.

Table 3. Average Cobb and Scolio angle according to curve location

	Cobb angle		Scolio angle
T	47	12.53±7.45	8.43±4.18
(T)L	125	12.46±5.31	9.02±3.90
Total	172	12.48±5.95	8.86±3.98

Table 4 illustrates data of the groups defined according to curve severity. In the group with a Cobb angle lower than 20°, there were 149 patients with a mean Cobb angle of 10.54° (SD±3.47, range 4° to 19°) and a mean Scolio angle of 8.05° (SD±2.98, range 20° to 43°). In the group with a Cobb angle greater than 20°, there were 23 patients with a mean Cobb angle of 24.13° (SD±5.83, range 20° to 43°) and a mean Scolio angle of 14.34° (SD±5.16, range 8.7° to 27.1°).

There is a good positive correlation between angle measurements in radiography and ultrasound imaging (r=0.675, statistically significant p<0.001), as shown in Figure 1.

Based on gender, there was a slight difference in correlation in the girls group (r=0.688), compared to the boys group (r=0.632, p<0.001), in favor of girls.

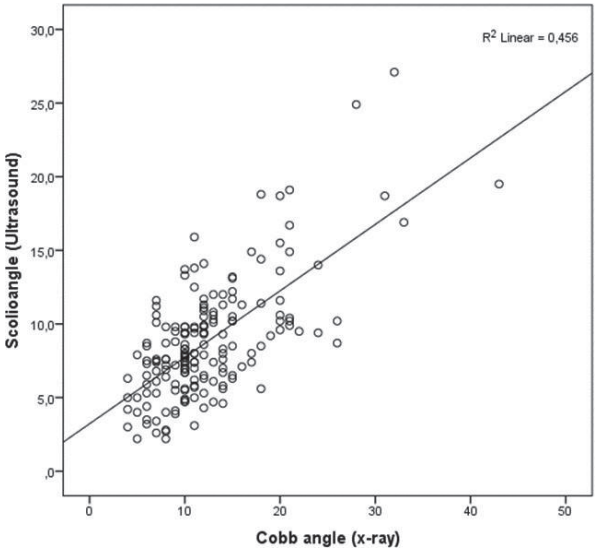


Figure 1. Correlation between radiographic Cobb and ultrasound Scolio angle

According to curve location, the correlation in the thoracic group is r=0.736 and in the (thoraco) lumbar group it is r=0.654 (p<0.001).

According to curve severity, in the group with a Cobb angle lower than 20°, the correlation is r=0.462 (p<0.001), while in the group with a Cobb angle equal or higher than 20° it is r=0.518 (p<0.05).

Table 4. Mean values of Cobb and Scolio angle according to curve severity

	n	X-ray (° Cobb)			Ultrasound (° Scolio angle)		
		M	Min	max	M	min	max
°Cobb<20°	149	10.54±3.47	4	19	8.05±2,98	2.2	18.8
°Cobb≥20°	23	24.13±5.83	20	43	14.34±5.16	8.7	27.1
Total	172	12.48±5.95	4	43	8.86±3.98	2.2	27.1

Two out of three predictors (gender, curve location and curve severity) have a statistically significant contribution to the entire regression model (curve severity and location). The best predictor is shown to be the Cobb angle (odds ratio 1.24).

Discussion

Considering that the ultrasound imaging method has only recently started to become more widely used on patients with scoliosis, the results of our study are very promising, confirming the validity of ultrasound in the detection and follow-up of spinal deformities in children, compared to conventional radiography. Furthermore, it is possible to predict that radiography might be used less frequently in the future, since our results confirm a good correlation in measurements between radiological and ultrasound spine images.

Our study did not question the reliability of the Scolioscan®, since we relied on results from previous studies that proved Scolioscan® to be feasible and reliable, with a mean ICC value of 0.94±0.04 (in the range from 0.88 to 0.97) between two operators and among three raters [13]. Our study was blinded, three operators conducting the scanning process, while four raters being blinded.

The obtained results show the mean difference between radiological and ultrasound angle being 3.62±4.39° ($p<0.001$). Even though there is a statistical significance, the difference is not considered notable in clinical settings. The Cobb angle measurement error lies within the interval of ±5° [14,15], in which no important clinical decisions regarding the treatment options are being made. Hence, one can conclude that the difference between Cobb and Scolio angle cannot be considered significant for the follow-up of patients with idiopathic scoliosis, especially when taking into account additional parameters regarding the treatment decision (i.e. scoliometer readings, back surface topography, etc).

Our results indicate a statistically significant good positive correlation between angle measurements in radiography and ultrasound imaging ($r=0.675$, $p<0.001$). This is significant, since other radiation-free scoliosis assessment methods indicate that a correlation coefficient of at least 0.55 represents a moderate to good correlation [16].

The results according to groups demonstrate a slightly better correlation in the girls group than in the boys group ($r=0.688$ vs. $r=0.632$). Furthermore, a better correlation has been detected in the thoracic group than in the (thoraco) lumbar group ($r=0.736$ vs. $r=0.654$). Additionally, a better correlation could be seen in the group with a Cobb angle equal to or higher than 20°, compared to the group with a Cobb angle lower than 20° ($r=0.518$ vs. $r=0.462$).

The contrast in results for groups categorized according to gender can be explained by the difference in sagittal profile development of girls and boys according to their age. It has been proven that girls enter growth spurt during postural instability [17], which is why spinal deformities and impaired sagittal profiles are more prevalent in girls. Changes in sagittal profile (reduced thoracic kyphosis and lumbar lordosis) can affect the scanning process, the image quality and, consequently, the measurement precision. A better correlation in thoracic curves, compared to (thoraco) lumbar curves, is possibly achieved due to a different anatomical structure of the spine in these two regions. Different sagittal profiles and vertebral rotations (which incorporates the ribs in the thoracic region) can affect the measurement precision.

According to the severity of the curve criteria, we have observed a better correlation in a Cobb angle that is equal to or higher than 20°, which can be explained by a smaller curve range, since there have only been 23 patients in this group.

Ultrasound angles are generally lower than Cobb angles, which has been confirmed in previous studies [13,18]. This can be explained by the fact that ultrasound measurements include more posteriorly located structures (spinous and tranverse processes), while radiography uses more anteriorly located structures (vertebral bodies).

The results of our study confirm a good correlation between ultrasound and radiological angle measurements, although not as high as observed in previous studies. Zheng et al. [13] demonstrated a moderate to strong correlation (R2=0.72) between Scolio and Cobb angles for both the thoracic and the lumbar regions. Nevertheless, the difference between Scolio and Cobb angles is shown to be 4.7°and 6.2°, with and without the correlation respectively, using the overall regression equation, which is consistent with our results. Brink et al. [18] found excellent linear correlation between Cobb and Scolio angles – for the thoracic region R2≥0.987 and for the (thoraco) lumbar region R2≥0.970. In the same study, the authors found no significant difference between various ultrasound angle measurements (automatic SP, manual SP, and manual TP). Therefore, our choice of manual measurements with transverse processes (TP) taken as reference points should not have interfered with the results.

The study of Tin-Yan Lee et al. [19], observed very good correlations between Cobb and Scolio angles – R2=0.893 in the thoracic region and R2=0.884 in the lumbar region, with an angle difference of no more than 3.0° for thoracic, and 1.5° for (thoraco) lumbar curves. Compared to this study, our results indicate a greater angle differences, which can possibly be ascribed to the difference in Cobb angle intervals of patients (8-70° vs.4-43°).

In their prospective study with a large number of patients (n=952), Wong et al. [20] confirmed a very good correlation between Scolio and Cobb angles – measured using EOS radiography – that is statistically significant ($p < 0.001$). As it was the case in our study, as well as in previously mentioned studies, a better correlation has been observed in upper spinal curves (r=0.873) than in lower spinal curves (r=0.740).

When comparing the results obtained in our research with other studies, we can observe a contrast in Cobb and Scolio angle correlations. This can be explained by the difference in image quality standards for measurements, as well as differences in the level of staff training and experience. Our study used B-mode ultrasound images for measurements, which are obtained directly after the scanning process without further software analysis or 3D reconstruction. Such a decision has been made for various reasons. Firstly, the reconstruction process is time-consuming and requires additional training. Secondly, in accordance with the previously stated observation, our aim was to examine whether a high quality B-mode image is sufficient for the detection and follow-up of scoliosis. In other studies, additional software adjustments have been conducted with a 3D reconstruction of the spine model, which certainly enables a better visibility and more precise measurements.

Recently, Scolioscan Air has been introduced, the world's first portable ultrasound scoliosis assessment system. It has proven to be sufficiently comparable to Scolioscan® in the assessment of scoliosis, while overcoming its shortcoming of space limitation, and expanding its indications for its application [21].

The usage of ultrasound imaging in the detection and follow-up of scoliosis has become more prevalent worldwide due to its non-radiation feature. Curve measurements obtained via Scolioscan® prove to be highly reliable, with good to excellent correlation with the conventional radiographic Cobb method.

Conclusion

Ultrasound assessment via the Scolioscan® device represents a great step forward in the process of detection and follow-up of patients with idiopathic scoliosis. Despite some shortcomings, the predominant advantage of the ultrasound method is a radiation-free assessment. Scoliosis patients who need long-term monitoring and treatment will be able to avoid radiation-related health issues.

The results of our study confirm a good validity of the ultrasound method via the Scolioscan® device compared to conventional radiography, considering the clinically insignificant differences in angle measurements.

Using B-mode ultrasound images only with no additional software analysis or 3D reconstruction of spinal deformities – proved to be sufficient for the follow-up of scoliosis patients, with respect to other parameters, such as clinical assessment, back surface topography, etc. Nevertheless, at present it is not yet possible to diagnose scoliosis using the Scolioscan® device alone.

It is expected that further research will investigate additional software tools and thereby provide more accurate ultrasound measurements, as well as forecast whether it will be possible to diagnose scoliosis via ultrasound exclusively. Since we are the first facility in our region that uses ultrasound imaging for the assessment of scoliosis, our next objective will be to focus on further ultrasound software analysis in order to reduce radiation of our patients as much as possible.

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Ethical approval. The Ethics Committee of the Institute for Physical Medicine and Rehabilitation "Dr Miroslav Zotović", Banja Luka, approved the study and informed consent was obtained from all individual respondents. The research was conducted according to the Declaration of Helsinki.

Conflicts of interest. The authors declare no conflict of interest.

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Poređenje radiografskih i ultrazvučnih mjerenja ugla u procjeni idiopatske skolioze

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Uvod. Radiološka dijagnostika i dalje se smatra zlatnim standardnom u detekciji, praćenju i liječenju idiopatske skolioze. Međutim, dokazano je kumulativno i onkogeno djelovanje zračenja. Ultrazvuk kičmenog stuba pomoću Scolioscan® uređaja postaje sve popularniji i pristupačniji širom svijeta s obzirom na to da su relevantna istraživanja pokazala njegov dijagnostički potencijal i primjenu u procjeni i praćenju idiopatske skolioze. Osnovna prednost ultrazvuka u odnosu na standardnu radiografiju je potpuno odsustvo zračenja. Cilj našeg istraživanja je bio da uporedimo mjerenja ugla skoliotične krivine na ultrazvuku i radiografiji kičmenog stuba i da utvrdimo ulogu ultrazvuka za procjenu i praćenje deformiteta kičmenog stuba kod pacijenata sa idiopatskom skoliozom.

Metode. Studija presjeka se sastojala od 172 pacijenta, dječaka i djevojčica, koji su pacijenti Tima za skoliozu na Odjeljenju za habilitaciju i rehabilitaciju djece u Zavodu za fizikalnu medicinu i rehabilitaciju „Dr Miroslav Zotović“ u Banjoj Luci, Republika Srpska. Kod svakog pacijenta urađena je radiografija i ultrazvuk kičmenog stuba istog dana. Tri posebno edukovana tehničara su provodila ultrazvučna skeniranja, a četiri ljekara su mjerila radiografski Cobb-ov ugao i ultrazvučni Scolio ugao. Pacijenti su podijeljeni u grupe prema polu, lokaciji primarne krivine i veličini krivine. Korelacija između Cobb-ovog ugla i Scolio ugla na ukupnom uzorku i prema grupama utvrđena je Pearson-ovim korelacionim testom. Da bi se ispitala statistička značajnost u razlici između varijabli, korišćen je T-test ponovljenih mjerenja ($p<0,05$). Logistička regresiona analiza je urađena da se utvrdi uticaj prediktora (pol, lokacija primarne krivine i veličina krivine).

Rezultati. Ultrazvučni uglovi bili su uopšteno manji u odnosu na Cobb-ove uglove, što je potvrđeno u dosadašnjim studijama. Prosječna razlika između Cobb-ovog i Scolio ugla bila je statistički, ali ne i klinički značajna (3,62 ±4,39°, $p<0,001$). Postoji statistički značajna dobra pozitivna korelacija između Cobb-ovog i Scolio ugla ($r=0,675$, $p<0,001$). Prema grupama, rezultati pokazuju postignutu bolju korelaciju kod djevojčica ($r=0,688$, $p<0,001$), u odnosu na dječake ($r=0,632$, $p<0,001$). Takođe je prisutna bolja korelacija u grupi sa primarnim torakalnim krivinama ($r=0,736$, $p<0,001$) u odnosu na grupu sa (torako) lumbalnim krivinama ($r=0,654$, $p<0,001$). Bolja korelacija se uočava i u grupi sa Cobb-ovim uglom jednakim ili većim od 20° ($r=0,518$, $p<0,05$) u odnosu na grupu sa Cobb-ovim uglom manjim od 20° ($r=0,462$, $p<0,001$).

Zaključak. Rezultati našeg istraživanja su potvrdili dobru valjanost ultrazvučne metode korišćenjem Scolioscan® uređaja u odnosu na standardnu radiografiju, s obzirom na klinički beznačajne razlike u mjerenjima uglova. Korišćenjem samo B-mode opcije na ultrazvuku bez dodatne softverske analize i 3D rekonstrukcije deformiteta kičmenog stuba pokazalo se dovoljnim za praćenje skolioze, ako se uzmu u obzir i drugi parametri (klinička procjena, topografija leđa). Očekuje se da će dalja istraživanja sa upotrebom dodatnih softverskih alata omogućiti preciznija ultrazvučna mjerenja i pokazati da li će se dijagnoza skolioza u budućnosti moći postaviti isključivo ultrazvukom.

Ključne riječi: idiopatska skolioza, ultrazvuk, radiografija

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Original article

Seroprevalence of SARS-CoV-2 virus infection in employees in the health insurance sector

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Summary

Introduction. Serological testing for SARS-CoV-2 virus infection is a valuable method of estimating the extent of COVID-19 disease prevalence. The study aims to assess the seroprevalence of SARS-CoV-2 virus infection in a specific group of respondents employed in the health insurance sector, to determine the ratio of symptomatic and asymptomatic cases, as well as to examine susceptibility to COVID-19 in relation to the ABO blood group system.

Methods. This research was conducted among 150 randomly selected employees from the health insurance sector of the Republic of Srpska. All respondents completed the survey, voluntary consent to participate, and had a blood sample taken. Serum samples were tested for the presence of SARS-CoV-2 specific IgG antibodies.

Results. The seroprevalence of SARS-CoV-2 virus infection was 70.7%. Out of the 100 seropositive respondents, 48% had the presence of a symptom of COVID-19, while 52% had no symptoms of the disease in the period from 1 March 1 2020 until the day of testing. Examining the association between ABO blood group system and seropositivity in our study, we found that the highest seroprevalence of SARS-CoV-2 IgG antibodies among respondents was in blood group B (83.3%), followed by blood group AB (80.0%) and blood group A (75.0%), while the lowest seroprevalence was among respondents with blood group O (54.1%).

Conclusion. Among employees in the health insurance sector, SARS-CoV-2 seroprevalence of 70.7% was registered. Among respondents with a positive serological result on IgG, 52% were asymptomatic. The seroprevalence of SARS-CoV-2 virus infection is the lowest among subjects with blood group O.

Key words: COVID-19, seroprevalence, healthcare professionals, blood types

Introduction

Coronavirus disease 2019 (COVID-19), also known as novel coronavirus pneumonia (NCP), was first recorded in Wuhan, China in December 2019. At the beginning of March 2020, there were more than 80,000 infected people, of whom 3,200 died in China. According to the World Health Organization, from the beginning of the pandemic to 28 May 2021, 168,040,871 cases of COVID-19 and 3,494,758 deaths worldwide were confirmed. In Bosnia and Herzegovina, 203,658 cases and

9,184 deaths were confirmed in the same period [2]. In the Republic of Srpska, the first cases of COVID-19 were confirmed on 5 March 2020, and until 27 May 2021, 63,996 persons were tested positive, of whom 3,667 died [3].

COVID-19 infection rate and mortality rate vary between countries [4]. The exact reason for the different infection and mortality rates depending on the population is not clear. Several factors, such as age, gender, genetics, presence of comorbidities, and economic status, could be attributed to inequalities in susceptibility to COVID-19 disease and the severity of the clinical picture of the disease [4].

The beginning of epidemiological surveillance was accompanied by laboratory diagnostics. Over time, with the increase in the number of patients, both in the Republic of Srpska and around the world it was not possible to confirm all suspicious cases using RT-PCR test. In addition, as the epidemic progressed, asymptomatic cases of infection were found. According to Oran et al., asymptomatic infections account for approximately 40% to 45% of infections [5].

A study conducted by Korth and co-workers at the University Hospital of Essen between 25 March and 21 April 2020, found an overall IgG antibody prevalence among healthcare workers of 1.6%, being the highest in the group of healthcare workers with medium risk in relation to exposure [6].

A study conducted by Oliveira et al. investigated the prevalence of anti-SARS-CoV-2 antibodies in outpatients at a clinic in Sao Paulo, Brazil. This serological study included 439 patients from several outpatient services of which 61 patients were positive (13.9%). The prevalence of IgG was lower in patients who received a seasonal influenza vaccine [7].

In the midst of the 2019 SARS-CoV-2 pandemic, several brief scientific reports have been published examining the link between the ABO blood group system and the risk of SARS-CoV-2 virus infection [8]. Latz et al., as well as Zietz et al., found that individuals with blood group O were at the lowest risk for SARS-CoV-2 infection [9,10].

In December 2020, the Medical Faculties in Foca and Banja Luka and the Public Health Institute of the Republic of Srpska, with the approval of the Ministry of Health and Social Welfare of the Republic of Srpska, launched a national seroprevalence study of anti-SARS-CoV-2 antibodies. The study found that the seroprevalence in the Republic of Srpska was 40.3% [11].

So far, there have been no seroepidemiological investigations in specific groups in the Republic of Srpska, nor studies of the connection between the seroprevalence of SARS-CoV-2 and ABO blood groups. Therefore, this study aims to assess the seroprevalence of SARS-CoV-2 in a specific group of respondents employed in the health insurance sector, who do not work in the provision of health services, to determine the ratio of symptomatic and asymptomatic cases, and to examine the relationship between seroprevalence and ABO blood groups.

Methods

The study included 150 respondents (110 female and 40 male respondents) employed in the Health Insurance Sector of the Republic of Srpska, Banja Luka branch office, who do not work in the provision of health care services. After getting acquainted with the goals of the study, the respondents voluntarily accepted to participate in the study. The survey was conducted in March, 2021.

The collection of data and blood samples in the field was performed by trained health workers employed at the Faculty of Medicine in Foca. Laboratory analysis of samples was performed in the laboratory of the Faculty of Medicine Foca.

Participants answered questions from a two-part questionnaire. The first part of the questionnaire referred to the basic data on the

respondent. In the second part of the questionnaire, respondents answered questions related to COVID-19, symptom history, hospitalization, testing and PCR and/or rapid antigen or serological test results, vaccination, and the presence of symptoms in household members.

A blood sample was taken from all respondents by peripheral venipuncture. Blood samples were taken from 8 to 11 a.m. After venipuncture, the blood was left for 30-35 minutes at room temperature to coagulate spontaneously. The serum was separated after centrifugation at 3000 rpm for 10 min, the serum was separated. The sera were transported in the cold chain regime to the Faculty of Medicine in Foca on the same day.

Serum samples were tested for the presence of specific antibodies to SARS-CoV-2 IgG using ELISA method by commercial tests EUROIMMUN Medizinische Labordiagnostika AG, Germany. The measurement was performed on a EUROIMMUN ELISA Analyzer I-2P ("Euroimmun AG"). EUROIMMUN ELISA SARS-CoV-2 At is an ELISA test for the semi-quantitative detection of IgG antibodies to SARS-CoV-2 virus in human serum or plasma. The cut-off value recommended by the manufacturer for positivity is >1.1. The test has a sensitivity of 94.36% and a specificity of 100%.

The seroprevalence of SARS-CoV-2 virus infection was assessed as the proportion of individuals who had positive results to a serological test for the presence of specific IgG antibodies. Descriptive statistical measures are presented for the basic characteristics of the respondents. Pearson's chi-square test was used to determine the difference between categorical variables, while the parametric Student's t-test was used to determine the difference between quantitative variables. Pearson's chi-square test was used to determine the difference in the frequency of seroprevalence between blood groups. Student's t-test was used to determine the difference in antibody levels of seropositive respondents according to age and in relation to the presence and/or absence of symptoms. All tests refer to two-way testing. The cut-off value for determining the existence of a statistically significant difference is $p<0.05$.

Results

The study involved 150 respondents, of whom 40 (26.7%) were male and 110 (73.3%) were female. The educational structure showed that 122 respondents (83%) were highly educated, while 25 (17%) respondents had secondary education. Respondents were divided into two categories according to age. In the age group up to 40, there were 74 respondents (49.7%), while in the group of respondents older than 40 there were 75 of respondents (50.3%). The youngest respondent was 21 and the oldest 64 years old. The seroprevalence of SARS-CoV-2 virus infection in healthcare employees was 70.7%. The seroprevalence in men was higher and amounted to 77.5%, and in women it was 68.2%.

Regarding the mean values of the levels of specific IgG antibodies to SARS-CoV-2, no statistically significant difference was found between the category older than 40 (4.55) compared to the category younger than 40 (3.55), ($t=1.811$, $DF=98$, $p=0.073$). Out of 100 seropositive subjects, 48% of them had the presence of some symptoms of COVID-19, while 52% of them did not have any symptoms of the mentioned disease in the period from 1 March 2020 until the day of testing. The mean value of the titer of specific antibodies to SARS-CoV-2 IgG in respondents with the presence of symptoms was 3.37, while the mean value of the titer of specific antibodies to SARS-CoV-2 IgG in respondents without the presence of symptoms was 4.68 (Figure 1). The analysis of these data indicates that the mean values of the titer of specific antibodies to SARS-CoV-2 IgG of seropositive respondents were statistically significantly higher in those who did not have symptoms of COVID-19, compared with respondents with symptoms of COVID-19 ($t=2.724$,

DF=96 , p=0.008). Out of the total number of respondents, 130 of them mentioned their blood group. The results of the study of the distribution of seropositive and seronegative subjects and seroprevalence by blood groups are shown in Table 1. From the results shown in Table 1, it can be seen that the highest frequency of seropositive respondents was in respondents with blood group A, while the largest number of respondents with seronegative results belonged to blood group O.

Out of the total number of respondents, 46 (33.6%) were tested by RT-PCR test, while 54 (41.5%) were tested by rapid antigen or serological test. These tests results are shown in Table 2.

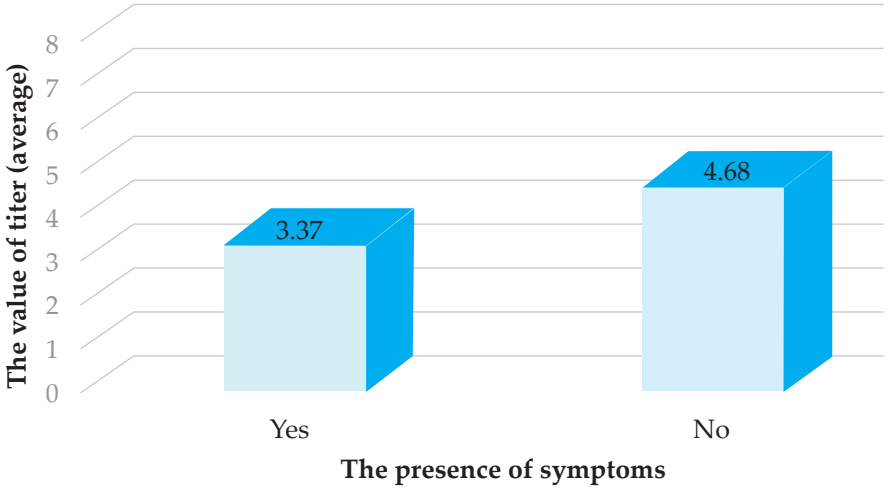

Figure 1. Mean values of the titer of specific antibodies to SARS-CoV-2 IgG in seropositive respondents in relation to the symptoms of COVID-19

Table 1. Ratio between blood groups and serological testing results, IgG seroprevalence for SARS-CoV-2

Blood group	The result of serological testing		Chi-square	p-value	IgG seroprevalence to SARS-CoV-2
	Positive	Negative			
A	36 (40.0%)	12 (30.0%)	8,649	0.034	75.0%
B	20 (22.2%)	4 (10.0%)			83.3%
AB	8 (8.9%)	2 (5.0%)			80.0%
O	26 (28.9%)	22 (55.0%)			54.1%
Total	**90 (100.0%)**	**40 (100.0%)**			**69.2%**

Table 2. Results of previous testing by RT PCR test and/or rapid antigen or serological test

Type of previous testing	Number of tested out of the total number of respondents N (%)	Previous test result N (%)	
		Positive	Negative
COVID-19 RT-PCR test	46 (33.6)	23 (16.3)	23 (16.3)
Rapid antigen or serological test	51 (40.2)	26 (18.4)	25 (17.7)

Out of the total number of respondents, 46 (33.6%) of them stated that they were tested by PCR test in the period from 1 March 2020 until the day of testing for SARS-CoV-2 IgG. Comparing the frequency of seropositivity between the group of PCR positive and PCR negative subjects, a statistically significant difference was found (x2 = 4.487, DF = 1, p = 0.028) (Table 3).

Table 3. Results of RT PCR testing in relation to the qualitative value of serological testing for SARS-CoV-2 IgG

			Qualitative value of IgG (positive or negative)		Total
			Positive	Negative	
Was the PCR test positive?	Yes	N	19	4	23
		%	61.3	26.7	50.0
		% out of Total	41.3	8.7	50.0
	No	N	12	11	23
		%	38.7	73.3	50.0
		% out of Total	26.1	23.9	50.0
Total		**N**	**31**	**15**	**46**
		%	**100.0**	**100.0**	**100.0**
		% out of Total	**67.4**	**32.6**	**100.0**

Out of the total number of respondents in our study, 9 (6.4%) have received the first dose of a vaccine, 3 respondents (33.3%) have received the second dose of a vaccine. Among our respondents, 64 (46.7%) stated that some members of their family had some of the symptoms of COVID-19 from 1 March 2020 until the day of testing, out of which 39 (60.9%) were proven by RT-PCR test (Table 4).

Table 4. Presence of symptoms in respondents and their family members and vaccine coverage

	Yes N (%)	No N (%)	Total
Have you had any symptoms of COVID-19 from 1 March 2020 until the day of testing?	69 (46.3)	80 (53.7)	149
Have any of your family members had any of the symptoms of COVID-19 since 1 March 2020?	64 (46.7)	73 (53.3)	137
If yes, has their infection been confirmed by PCR?	39 (60.9)	25 (39.1)	64
Have you received the first COVID-19 vaccine dose?	9 (6.4)	132 (93.6)	141
If yes, which vaccine have you received?	Sputnik V	Pfizer-BioNTech	9
	7 (77.8)	2 (22.2)	
Have you received the second vaccine dose?	3 (33.3)	6 (66.7)	9

Discussion

Our study was conducted in late March 2021, in order to assess the seroprevalence of SARS-CoV-2 virus infection in a specific group of respondents employed in the health insurance sector, who by the nature of their work do not work in the provision of healthcare services, and to determine the ratio of symptomatic and asymptomatic cases, and to examine susceptibility to COVID-19 in relation to the ABO blood group system.

A total number of 150 randomly selected respondents participated in the study, of which 40 (26.7%) were male and 110 (73.3%) were female. The seroprevalence of SARS-CoV-2 virus infection in the study group was 70.7%.

Having in mind the importance of assessing the seroprevalence of SARS-CoV-2 virus infection for the purposes of public health activities and planning, a number of studies on this topic have been conducted worldwide. Thus, in the period from 25 March to 21 April, 2020, Korth et al. conducted a study in Germany among 316 health professionals who have direct contact with COVID-19 patients. In the study population, the seroprevalence was 1.6%, as evidenced by a semi-quantitative ELISA test [6].

Iversen et al. conducted a seoprevalence study among specific categories of the population, such as voluntary blood donors and health-care workers. The study was conducted in Denmark in the period from 15 April to 23 April, 2020. The seroprevalence among healthcare workers was higher than that of voluntary blood donors and amounted to 3.04% [12].

In a study conducted between 5 May and 15 May 2020 in Northern Italy among 6,075 subjects, the seroprevalence of SARS-CoV-2-IgG was 23.1% [11]. In April 2020, a screening of employees (1,500 respondents) was performed in 22 nursing homes in Stockholm with a rapid COVID-19 test to detect specific antibodies to SARS-CoV IgM and IgG. Seropositive employees were found in 21 out of the 22 nursing homes. The seroprevalence of SARS-CoV-2 IgG antibodies in the study population was 23%, while 14.3% of the respondents were positive for IgM antibodies (alone or in combination with IgG), indicating the recent presence of infection. Of those who were seropositive, 46.5% reported no clinical symptoms indicating asymptomatic infection [14].

Grant et al. conducted a seroprevalence study in healthcare workers in London in the period May-June 2020, the seroprevalence of SARS-CoV-2-IgG was 31.6% [15].

A systematic literature review conducted by Rostami et al. included 47 studies, in which 399,265 people from 23 countries participated. Heterogeneity was observed among the studies ($I2=99.4\%$, $p<0.001$). The seroprevalence of SARS-CoV-2 in the general population varied from 0.37% to 22.1%, with a cumulative estimate of 3.38% (95% CI 3.05-3.72%; 15 879/399 265) [16].

The seroprevalence of SARS-CoV-2 infection registered in our study has higher values than those published so far, both among the groups exposed to patients and among the unexposed. Our respondents do not work on providing health care, but in the front offices they have the possibility of contact with patients who come to the institution for various certificates in order to exercise their rights in the field of healthcare protection. The higher values of SARS-CoV-2 infection seroprevalence can be explained by the fact that our research was conducted during the third wave of the pandemic, while the other mentioned studies were conducted during the first or the second wave, almost a year earlier than our research. In addition, at the time of conducting the cited studies, immunization against COVID-19 had not yet been initiated, which in our case could have had an effect on increasing seroprevalence. Out of the total number of seropositive respondents in our study, 52% of them did not report any symptoms of COVID-19, which is in accordance with a study conducted by Johanna et al. in Stockholm during April 2020 [14].

Previous reports have shown an association of ABO blood group systems with

susceptibility to a wide range of infections such as severe acute respiratory syndrome infection with SARS-CoV-2 virus, West Nile virus, Human Immunodeficiency virus, Hepatitis B and Norwalk virus [14]. The possible association between the ABO blood group system and COVID-19 infection and mortality was highlighted. Blood group A has been shown to be a risk factor for the development of COVID-19 disease, while blood group O has a lower incidence rate than the mentioned disease [17]. Similar results were obtained in a study by Latz et al. [9], as well as Zietz et al. [10]. The study conducted by Latz et al. found the results showing that blood group A did not correlate with positive COVID-19 test results (AOR:1.00, CI:0.88-1.13), while blood groups B and AB were associated with a higher probability of a positive COVID-19 test result (AOR:1.28, CI:1.08-1.52; AOR:1.37, CI:1.02-1.83). Blood type O is associated with a lower probability of a positive COVID - 19 test result (AOR: 0.84, CI: 0.75-0.95) [9].

The study conducted by Zietz et al. showed a slightly increased prevalence of Coronavirus infection in people who were not blood group O. The risk of intubation was lower in people with blood group A, and increased in people with blood groups AB and B compared to people with blood group O. A higher probability of mortality was found in people with blood group AB, and lower in people with blood groups A and B [10].

Several pathophysiological mechanisms of association between the ABO blood group system and SARS-CoV-2 virus infection have been proposed [18]. Anti-A and/or anti-B antibodies, which are, for example, present in individuals with O group, can bind to A and/or B antigens expressed on the viral envelope, thus preventing infection of target cells, or these naturally occurring antibodies could function as antibodies to neutralize the virus [18]. If these assumptions are correct, differences in initial susceptibility to SARS-CoV-2 infections could be explained in this way.

Zhao et al. found that the incidence of COVID-19 infection was higher in people with AB blood group [19], but Zietz and Tatonetti found that blood group AB was associated with reduced COVID-19 infection [10]. Moreover, during 2020, Zhao et al. found that blood group A was associated with higher mortality of COVID-19, and blood group O with lower mortality [19].

Examining the association between ABO blood group system and seropositivity in our study, we found that the highest seroprevalence of SARS-CoV-2 virus infection was among respondents with blood group B and it was 83.3%, while lower blood values were recorded in other blood groups. Thus, the seroprevalence in respondents with blood group AB was 80%, while respondents with blood group A had a seroprevalence of 75%. Respondents with O blood group had the lowest seroprevalence (54.1%), which is in accordance with research in France and Turkey. Gallian et al. in a study conducted in France showed that people with blood group O have a lower seroprevalence rate of SARS-CoV-2 virus infection [20], which was also shown by a study conducted by Göker et al. in Turkey in April 2020. [21]. Further research is needed to explain the reasons for the protective role of blood group O.

Zeng et al. in their study did not find any association between ABO blood types and mortality from COVID-19. The contradiction of the obtained results could be due to the small size of the sample, the residual conclusion from the heterogeneity of the population, the difference in the region, etc. [22].

While the diagnosis of acute SARS-CoV-2 infection is performed by RT-PCR test in respiratory samples, there is a growing demand for serological tests for population-based epidemiological studies and for the assessment of infection in individuals [23]. Recent studies have confirmed the suitability of various commercial immunotests including a high-throughput platform with a random approach for determining SARS-Cov-2-IgG in patients with COVID-19 [23].

Determination of IgG antibodies to SARS-Cov-2 is the method of choice for assessing the seroprevalence of SARS-CoV-2 virus infection. Measurement of SARS-CoV-2-IgG using automated immunoassays allows rapid testing of a large number of samples [24].

Study limitations

The study has certain limitations, because it did not include the examination of cellular immunity. In addition, memory bias in responding to the presence of COVID-19 symptoms is possible. Also, other respiratory infections may have been present during the observed period, so the reported symptoms may be attributed to the other infections. However, the study certainly provided important findings and contributed to a better understanding in this field.

Conclusion

The research showed that the frequency of seroprevalence of SARS-CoV-2 virus infection among the population of employees in the healthcare sector is 70.7%. Out of the total number of seropositive subjects, 52% were asymptomatic, and seropositivity was the lowest in people with O blood group.

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Ethical approval. The Ethics Committee of the Faculty of Medicine Foca approved the study and informed consent was obtained from all individual respondents. The research was conducted according to the Declaration of Helsinki.

Conflicts of interest. The authors declare no conflict of interest.

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Seroprevalencija infekcije virusom SARS-CoV-2 kod zaposlenih u sektoru zdravstvenog osiguranja

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Uvod. Serološka ispitivanja infekcije virusom SARS-CoV-2 su dragocjena metoda procjene obima raširenosti COVID-19 oboljenja. Studija ima za cilj da procijeni seroprevalenciju infekcije virusom SARS-CoV-2 u specifičnoj grupi ispitanika zaposlenih u sektoru zdravstvenog osiguranja, da odredi omjer simptomatskih i asimptomatskih slučajeva, kao i da ispita osjetljivost na COVID-19 u odnosu na AB0 sistem krvnih grupa.

Metode. Ovo istraživanje je sprovedeno među 150 slučajno odabranih zaposlenih radnika iz sektora zdravstvenog osiguranja Republike Srpske. Svi ispitanici su popunili anketu, dali dobrovoljni pristanak za učešće i uzet im je uzorak krvi. Uzorci seruma su testirani na prisustvo specifičnih IgG antitijela na SARS-CoV-2.

Rezultati. Seroprevalencija infekcije virusom SARS-CoV-2 je bila 70,7%. Od 100 seropozitivnih ispitanika, njih 48% je imalo prisustvo bilo kog simptoma COVID-19, dok 52% nije imalo nijedan simptom navedenog oboljenja u periodu od 1. marta 2020. godine do dana testiranja. Ispitivanjem povezanosti između AB0 sistema krvnih grupa i seropozitivnosti u našem ispitivanju došli smo do rezultata da je najveća seroprevalencija SARS-CoV-2 IgG antitijela među ispitanicima bila kod krvne grupe B (83,3%), zatim kod krvne grupe AB (80,0%) i krvne grupe A (75,0%), dok je najmanja bila među ispitanicima 0 krvne grupe (54,1%).

Zaključak. Kod zaposlenih iz sektora zdravstvenog osiguranja registrovana je seroprevalencija infekcije virusom SARS-CoV-2 od 70,7%. Među ispitanicima sa pozitivnim serološkim rezultatom na IgG 52% je bilo asimptomatsko. Seroprevalencija infekcije virusom SARS-CoV-2 je najmanja među ispitanicima sa 0 krvnom grupom.

Ključne riječi: COVID-19, seroprevalencija, zdravstveni radnici, krvne grupe

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Original article

Association between metabolic syndrome and homocysteinemia in ischemic stroke

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Summary

Introduction. Stroke is one of the leading causes of morbidity and mortality worldwide. The relationship between metabolic syndrome (MetS) and homocysteinemia (Hcy) as risk factors for ischemic stroke (IS) is not completely clear. The aim of the study was to determine the frequency of MetS, serum level of Hcy and the frequency of hyperhomocysteinemia (HHcy), as well as their association in patients with IS.

Methods. The research included 53 subjects being in rehabilitation after IS and 40 subjects in the control group in rehabilitation due to the back pain problems aged 50-70 years. The diagnosis of the ischemic stroke was established by insight in the medical documentation. All subjects had to have a diagnosis of stroke confirmed by imaging (CT or MR of the endocranium). All subjects in the control group were excluded from the existence of previous stroke, myocardial infarction, angina pectoris and peripheral vascular disease. MetS was defined according to the joint statement from 2009.

Results. The frequency of MetS was significantly higher in patients with IS compared to the control group (88.7% vs. 70.0%, $p<0.05$). The level of Hcy and the frequency of HHcy were increased in the patients with stroke compared to the control group (15.0±5.50 μmol/L vs. 11.2±2.51 μmol/L, $p<0.01$ and 39.2% vs. 11.4%, $p<0.01$, respectively). Among patients with IS, those with MetS had higher frequency of HHcy (42.2% vs. 16.7%, $p<0.05$) and it increased with more individual components of MetS (11.1% in patients with 3 components, 36.8% in patients with 4 components and 64.7% in patients with 5 components, $p<0.05$). Hcy was also in positive correlation with serum triglyceride level.

Conclusion. Our results suggest that MetS and Hcy represent a significant risk factors for IS. It seems that there is an association between these risk factors in pathogenesis of the IS, but further analyses are needed to confirm this hypothesis.

Key words: ischemic stroke, metabolic syndrome, homocysteine, atherosclerosis, obesity

Introduction

Stroke is one of the leading causes of morbidity and mortality worldwide [1,2]. Metabolic syndrome (MetS) is a group of metabolic and hemodynamic disorders that multiplies risk of atherosclerotic cardiovascular diseases (CVD). Patients with MetS have several times higher morbidity and mortality from type 2 diabetes mellitus (DM2), stroke and myocardial infarction [3]. The presence of MetS also increases the risk of the recurrent stroke [4].

Most commonly described components of MetS are high blood pressure, dyslipidemia (high triglycerides (Tg) and low high-density lipoprotein cholesterol level (HDL)), insulin resistance (IR) with consequent hyperglicaemia and visceral obesity [3]. There were many different criteria for MetS diagnosis in the past but in 2009, criteria were internationally agreed [5].

Homocysteine (Hcy) is sulfur-containing amino acid derived from methionine. Folic acid, vitamins B12 and B6 have an important role in homocysteine-methionine metabolic cycle [6]. Many prospective studies established Hcy as an independent risk factor for CVD, including stroke [7]. It is estimated that 5-7% of whole population have mild to moderate hyperhomocysteinemia (HHcy) and the main reasons are vitamins deficiency, medication, kidney diseases and genetic disorders [8].

Interaction between MetS/IR and HHcy has been described, but nature of that link has not yet been established well and data of many studies are conflicting [9-14]. Some authors even suggest that HHcy should be an additional constituent of MetS [6].

In a study of Meigs patients with DM2, as well as experimental animals with IR, had HHcy which was associated with changes in key enzymes of Hcy metabolism. It is supposed that methionine elimination stimulated by insulin could explain these findings [15]. Homocysteine-thiolactone, active form of Hcy, inhibits insulin-induced tyrosine phosphorylation of beta-subunit insulin receptors and its substrate: insulin receptor substrate 1 and p60-70 in rat liver cells [10]. Paterson et al. showed that Hcy dose-dependently inhibits insulin releasing in pancreatic beta cells [16]. In insulin resistant experimental animals HHcy may be caused by hyperinsulinemia but Hcy may also cause IR through insulin receptor kinase inhibition. Thus, HHcy might be a cause or/and a result of IR [13]. The aim of this study was to determine the frequency of MetS, level of Hcy and frequency of HHcy, as well as their association, in patients with IS.

Methods

The cross-sectional study included 53 consecutive patients with IS recruited from the neurological department of the Institute of Physical Medicine and Rehabilitation ''Dr Miroslav Zotović'' in Banja Luka one to three months after the acute event. The study group consisted of 53 patients who had suffered an acute ischemic stroke, and were in rehabilitation at the above-mentioned Institute. The diagnosis of the disease was established by insight in the medical documentation. All subjects had to have a diagnosis of ischemic stroke confirmed by imaging (CT or MR of the endocranium). A control group consisted of 40 gender- and age-matched patients in rehabilitation due to degenerative spinal diseases hospitalized at the same time in the above-mentioned Institute. All subjects in the control group were excluded from the existence of previous stroke, myocardial infarction, angina pectoris and peripheral vascular disease by anamnesis and insight of previous medical documentation.

The waist circumference (WC) was measured in the horizontal plane at the midpoint between the last right rib and the iliac crest using an inelastic fiberglass tape. Blood pressure (BP) was measured by standard sphygmomanometer at least 10 minutes after rest. For each patient BP was measured in duplicate and mean value was used. Blood sample was collected after a 12-hour fasting period to determine HDL, Tg, glucose and Hcy levels. Enzymatic colorimetric method was used to determine glucose, HDL i Tg serum levels. Hcy serum level was measured by Chemiluminiscent microparticle immunoassay method (Architect Tacrolimus device, produced by Abbot Laboratories). Reference values for Hcy level in serum were defined by kit producer: 5.46 – 16.20 μmol/L for men and 4.44-13.56 μmol/L for women.

MetS was defined according to the joint statement from 2009 (5). At least three of five criteria are requested to fulfill diagnostic criteria:

1. Elevated waist circumference (≥ 94 cm for males and ≥ 80 cm for females);
2. Elevated Tg (≥ 1.7 mmol/L) or use of medication for hypertriglyceridemia;
3. Reduced HDL cholesterol (<1.0 mmol/L for males and <1.3 mmol/L for females);
4. Elevated blood pressure (systolic ≥130 mmHg and/or diastolic ≥ 85 mmHg), diagnosis of hypertension or use of medication for hypertension;
5. Elevated fasting glucose (≥5.6 mmol/L) or use of medication for hyperglycemia.

Normality of data was tested by the Kolmogorov-Smirnov test. For group comparisons, chi-square test, Mann-Whitney U test and Student t-test were used, as appropriate. Correlations were assessed using Spearman's rho. Significant testing was two-sided, with alpha set to 0.05 for statistical significance and 0.01 for high statistical significance.

Results

Table 1 shows descriptive statistics parameters of age, parameters of MetS (WC, systolic and diastolic BP, glycaemia, Tg and HDL) and homocysteinemia for both groups, such as statistic parameters of LDL and total cholesterol for examined group.

Mean homocysteinemia value among patients was 15.04 μmol/L, vs. 11.16 μmol/L among controls (p<0.01) (Table 1). MetS was found among 88.7% patients with stroke and table 2 shows prevalence of HHcy among examined and control group. HHcy was present among 39.2% examinees and 11.4% controls and the diference was statisticly significant (p<0.01). Patients from the examined group with MetS had HHcy in 42.2% cases, while patients without MetS had HHcy in 16.7% cases. The diference wasn't statistically significant

Table 1. Descriptive statistics parameters

	Group	Number	Mean	SD	Min-Max				
Age	Patients	53	63.28	5.97	61.68	64.89			
	Controls	40	58.48	5.43	56.79	60.16			
WC	Patients	53	97.75	12.34	94.43	101.08			
	Controls	39	98.92	9.65	95.90	101.95			
Systolic blood pressure	Patients	53	135.85	12.62	132.45	139.25			
	Controls	39	138.72	18.49	132.92	144.52			
Diastolic blood pressure	Patients	53	80.75	7.30	78.79	82.72			
	Controls	39	85.38	8.22	82.80	87.97			
Glycaemia	Patients	53	5.86	1.76	5.39	6.33			
	Controls	40	6.06	1.98	5.45	6.68			
Total cholesterol	Patients	43	6.05	1.37	5.64	6.46			
	Controls	-	-	-	-				
HDL	Patients	53	0.90	0.21	0.84	0.95			
	Controls	40	1.43	0.41	1.30	1.56			
LDL	Patients	12	3.31	1.14	2.67	3.96			
	Controls	-	-	-	-				
Tg	Patients	53	1.77	0.86	1.53	2.00			
	Controls	40	1.96	2.10	1.31	2.61			
Hcy	Patients	51	15.04	5.50	13.53	16.55	***t***	***df***	***p***
	Controls	35	11.16	2.51	10.33	11.99	***4.420***	***75***	***<0.01***

WC – Waist Circumference, HDL - High Density Lipoprotein, LDL - Low Density Lipoprotein, Tg - Triglycerides, Hcy - Homocysteine

most likely because of small numbers of patients without MetS (Table 3).

Prevalence of HHcy was increasing with the numbers of MetS components in the examined group. Patients with three MetS components had HHcy in 11.1% cases, those with four MetS components in 36.8%, while patients with all five MetS components had HHcy in 64.7% cases ($p<0.05$) (Figure 1).

Table 2. Prevalence of HHcy among examined group and control group

HHcy	Patients		Controls		Total	
	Number	%	Number	%	Number	%
Yes	20	39.22	4	11.43	**24**	**27.91**
No	31	60.78	31	88.57	**62**	**72.09**
Total	**51**	**100.00**	**35**	**100.00**	**86**	**100.00**

c2	*df*	*p*
7.966	**1**	**0.005**

HHcy - Hyperhomocysteinemia

Table 3. Prevalence of HHcy in regard to MetS among examined group

HHcy in regard to MetS	Patients with MetS		Patients without MetS		Total	
	Number	%	Number	%	Number	%
Yes	19	42.22	1	16.67	20	39.22
No	26	57.78	6	83.33	32	60.78
Total	**45**	**100.00**	**6**	**100.00**	**51**	**100.00**

c2	*df*	*p*
1.450	**1**	**0.228**

HHcy - Hyperhomocysteinemia, MetS - Metabolic Syndrome

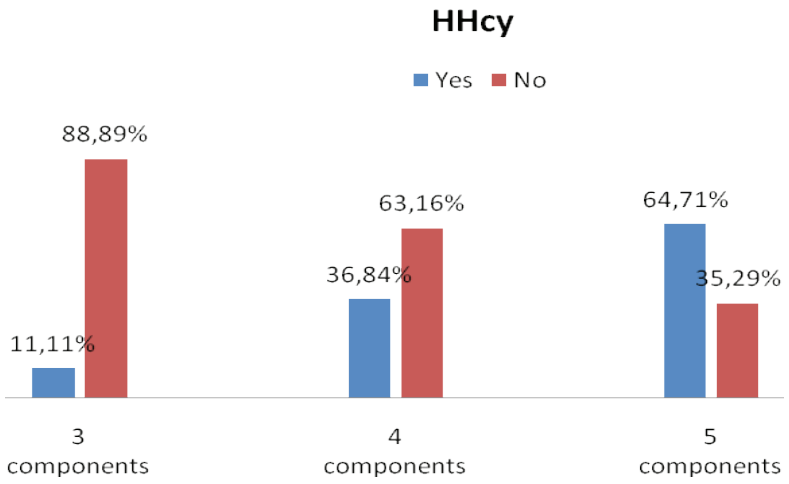

Figure 1. *HHcy prevalence in regard to number of MetS components in the examined group HHcy - Hyperhomocysteinemia, MetS - Metabolic Syndrome*

Table 4 shows Pearson's correlation of homocysteinemia with MetS components in the examined group. Homocisteinemia positively correlated with serum Tg level and it was statistically significant. Other MetS components had slightly positive correlation with Hcy, except HDL which had slightly negative correlation. These correlations weren't statistically significant.

Table 4. Pearson's correlation of homocisteinemia and MetS components

Pearson's correlation		Homocysteine	Waist circumference	Tg	HDL cholesterol	Systolic blood pressure	Diastolic blood pressure	Glycemia
Homocysteine	***r***	1.000	0.125	***0.288***	-0.223	0.190	0.136	0.241
	p		0.382	***0.041***	0.116	0.183	0.340	0.089
Waist circumference	***r***	0.125	1.000	0.141	***-0.371***	0.229	***0.358***	0.139
	p	0.382		0.314	***0.006***	0.099	***0.009***	0.321
Tg	***r***	***0.288***	0.141	1.000	***-0.369***	0.075	0.062	0.001
	p	***0.041***	0.314		***0.007***	0.593	0.658	0.997
HDL cholesterol	***r***	-0.223	***-0.371***	***-0.369***	1.000	***-0.315***	***-0.366***	-0.075
	p	0.116	***0.006***	***0.007***		***0.022***	***0.007***	0.596
Systolic blood pressure	***r***	0.190	0.229	0.075	***-0.315***	1.000	***0.661***	***0.280***
	p	0.183	0.099	0.593	***0.022***		***0.000***	***0.042***
Diastolic blood pressure	***r***	0.136	***0.358***	0.062	***-0.366***	***0.661***	1.000	0.177
	p	0.340	***0.009***	0.658	***0.007***	***0.000***		0.205
Glycemia	***r***	0.241	0.139	0.001	-0.075	***0.280***	0.177	1.000
	p	0.089	0.321	0.997	0.596	***0.042***	0.205	

Tg - Triglycerides, HDL-High Density Lipoproteins

Discussion

Most of the examinees of the present cross-sectional study had several metabolic impairments and thus fulfilled criteria for MetS diagnosis. Prevalence of the MetS was high in both examined groups yet it was significantly higher in patients with IS (89% vs 70%).

The data from other studies about MetS prevalence are heterogeneous taking into account different criteria used for its diagnosis, as well as influence of the gender, age and ethnicity of the examined population on MetS diagnosis. In general population older than 20-25 years MetS prevalence in men in urban areas varies from 8% in India till 24% in the USA and from 7% in France till 43% in Iran in women. MetS prevalence in general population increases with aging and it amounts from 7% in the examinees aged 21-29 and 44% in the examinees aged 60-69 years in American population [17]. The frequency of MetS is even higher in patients with manifest atherosclerotic diseases. Olijhoek et al. found that 43% of patients with stroke met criteria for MetS diagnosis [18] while in a study of Reffat

et al. that percentage was 57% for all types of stroke and 64% for ischemic stroke [19]. In all of these studies NCEP ATP III criteria were used for MetS diagnosis [20]. In the present study we used more strict criteria for MetS diagnosis and data from other authors that used the same criteria are limited. That fact as well as older participants could explain higher prevalence of MetS in this study compared to previous studies. Low level of physical activity and sedentary lifestyle due to chronic back pain could contribute to high prevalence of MetS in the control group.

In the present study the examinees with IS had significantly higher value of homocysteinemia as well as three times higher prevalence of HHcy in comparison to the control group. Fallon et al. found that increase of homocysteinemia of 4.7 μmol/L increases the risk for IS of 20-40% in male smokers in Finland [21]. Many studies showed that Hcy is independent risk factor for atherosclerotic vascular diseases [6,7,11,12] while some authors found positive correlation between Hcy level and vascular dementia occurrence after IS [22].

We also analyzed the association between MetS and HHcy in the patients with stroke. HHcy was present in 42% of patients with MetS and only in 17% of patients without diagnosis of MetS. This difference was not significant most likely because of the small number of patients without MetS. As the number of MetS components increased, the frequency of HHcy also increased which further indicates the association between MetS and HHcy in patients with IS.

The data from literature about the association between MetS and homocysteinemia are conflicting and they are limited when it comes to agreed criteria for MetS diagnosis that we used. Some studies did not find any connection between Mets and Hcy levels [9,10] or even found inverse correlation between IR and homocysteinemia [23], but the majority of the studies found positive correlation of MetS or its components and Hcy [6,11,12,13,15,24,25,26]. Bellia et al. showed that 67% of patients with CVD had MetS and HHcy simultaneously. The authors supposed that MetS could be significant pathogenetic factor through which HHcy induces vascular damage and increases CVD risk [27]. In Framingham's study HHcy had moderate positive correlation with hyperinsulinemia and serum Hcy levels were significantly higher in examinees with three or more MetS components compared with those without MetS or with single MetS component [15]. Setola et al. lowered Hcy and insulin serum levels and improved insulin sensitivity and endothelial function in patients with MetS by prolonged treatment with folic acid and vitamin B12 [28]. HHcy and MetS interact in atherosclerotic vascular diseases by promotion of oxidative damage, endothelial cell dysfunction, increased platelets aggregation, etc. [6,24,25,26].

When it comes to the correlation of MetS single components and homocysteinemia we found that only serum Tg levels had significantly positive correlation while other components had slightly positive correlation and levels of HDL had slightly negative correlation.

Positive correlation between serum Tg and Hcy levels that we found in the current study is in correlation with results of other authors [29] and could be explained by inhibition of fatty acid oxidation caused by Hcy which leads to serum Tg elevation [30]. Some in vitro experiments on human cells showed that Hcy induce endoplasmic reticulum stress which leads to higher expression of genes responsible for cholesterol and Tg biosynthesis, as well as their takeover and accumulation in cells [31].

We did not find a positive correlation between visceral obesity, measured by WC, and plasma homocysteine level. Conflicting data have been published on the association of Hcy and obesity. Most studies showed that body mass index (BMI), as a measure of obesity, did not correlate with Hcy level. On the other hand, abdominal adiposity was significant

predictor of plasma Hcy level [32,33]. Recent meta-analysis by Wang et al. showed that homocysteine concentrations were significantly elevated among obese patients [34]. These are in contradiction to findings of this study.

Conclusion

In conclusion, we can say that patients with IS had high prevalence of MetS and HHcy as significant risk factors for this disease. MetS and Hcy interact in promotion of atherosclerotic vascular diseases such as IS but nature of this association is not fully clear and understood. This was a cross-sectional study and we did not follow changes of plasma Hcy level in post-stroke period. Some studies showed that elevated Hcy during the convalescent phase of acute stroke was independently associated with an increased risk of recurrent ischemic stroke, especially in those patients with large-vessel atherosclerosis ischemia [35]. Further studies are needed to clarify the pathogenetic link between HHcy and MetS in stroke, as well as dynamic of plasma Hcy level in stroke and post-stroke period.

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Ethical approval. The Ethics Committee of the Institute for Physical Medicine and Rehabilitation "Dr Miroslav Zotović", Banja Luka, approved the study and informed consent was obtained from all individual respondents. The research was conducted according to the Declaration of Helsinki.

Conflicts of interest. The authors declare no conflict of interest.

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Povezanost metaboličkog sindroma i homocisteinemije kod ishemijskog moždanog udara

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Uvod. Moždani udar predstavlja jedan od vodećih uzroka morbiditeta i mortaliteta širom svijeta. Povezanost između metaboličkog sindroma (MetS) i homocisteinemije (Hcy) kao faktora rizika za ishemijski moždani udar (IMU) nije dovoljno rasvijetljena. Cilj istraživanja je bio da se ustanovi učestalost MetS, nivo Hcy u serumu i učestalost hiperhomocisteinemije (HHcy), kao i njihova međusobna povezanost kod oboljelih od IMU.

Metode. Istraživanjem su bila obuhvaćena 53 ispitanika na rehabilitaciji nakon IMU i 40 ispitanika kontrolne grupe koji su bili na rehabilitaciji zbog bola u leđima starosti od 50 do 70 godina. Dijagnoza IMU je postavljena uvidom u medicinsku dokumentaciju i svi bolesnici su morali imati dijagnozu potvrđenu „imaging" metodom (KT ili MR endokranijuma). Svim ispitanicima kontrolne grupe isključeno je postojanje ranijih moždanih i srčanih udara, angine pektoris i periferne vaskularne bolesti. Dijagnoza MetS je postavljena na osnovu usaglašenih, zajedničkih kriterijuma iz 2009. godine.

Rezultati. Učestalost MetS je bila značajno viša kod bolesnika sa moždanim udarom u odnosu na kontrolnu grupu (88,7% vs. 70,0%, $p<0,05$). Nivo Hcy u serumu i učestalost HHcy su bili viši kod bolesnika sa moždanim udarom u odnosu na kontrolnu grupu (15,0±5,50 µmol/L vs. 11,2±2,51 µmol/L, $p<0,01$ i 39,2% vs. 11,4%, $p<0,01$). Pacijenti sa IMU i MetS imali su veću učestalost HHcy u odnosu na one bez MetS (42,2% vs. 16,7%, $p<0,05$) i njena učestalost je rasla sa porastom broja pojedinačnih komponenti sindroma (11,1% kod pacijenata sa 3 komponente, 36,8% kod pacijenata sa 4 komponente i 64,7% kod pacijeneta sa 5 komponenti, $p<0,05$). Nivo Hcy u serumu je bio u pozitivnoj korelaciji sa nivoom triglicerida u serumu.

Zaključak. Naši rezultati sugerišu da MetS i Hcy predstavljaju značajne faktore rizika za nastanak IMU. Čini se da postoji povezanost između ovih faktora rizika u patogenezi IMU, ali su potrebna daljnja istraživanja da bi se potvrdila ova hipoteza.

Ključne riječi: ishemijski moždani udar, metabolički sindrom, homocistein, ateroskleroza, gojaznost

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Original article

CLIF-C AD score versus MELD score in predicting mortality in alcoholic liver cirrhosis patients

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Summary

Introduction. Alcoholic liver cirrhosis is an advanced stage of progressive liver failure with an often adverse outcome. Numerous scoring systems are used to predict outcomes. The results of MELD Score (Model For End-Stage Liver Disease) and CLIF Consortium Acute Decompensation score (CLIF-C ADs) were used in this paper to determine which one is more reliable in predicting mortality.

Methods. The value of CLIF-C AD and MELD scores using online calculator at the time of hospitalization was calculated. Follow-up has also started during hospitalization and control examinations in the next 3 months.

Results. This study included 145 patients of both genders, diagnosed with alcoholic liver cirrhosis. During the first 3 months from the moment of the calculation of the score, 39 patients (32 male and 7 female patients) passed away, which represents 82.1% versus 17.9%. The mean age of patients was 59.18 ± 9.19 years. All CLIF-C AD scores of 99 and above had a 100% probability of death in the first 3 months.

Conclusion. The CLIF-C AD score proved to be more reliable than the MELD score in predicting mortality in patients with alcoholic liver cirrhosis in the first 3 months.

Keywords: CLIF-C AD score, MELD score, alcoholic liver cirrhosis

Introduction

Generally, liver cirrhosis is a common disease caused by numerous etiological agents, individually or synergistically [1]. In the Balkans, given the habits of the population, the most common etiological agent is a long-term consumption of alcohol, which inevitably leads to the liver damage and progresses to liver cirrhosis [2]. If supportive measures or liver transplants are not applied, cirrhosis of the liver usually ends fatally [3].

In order to predict the severity of cirrhosis of the liver, the numbers of scoring systems that we use for monitoring were created [4]. MELD and CLIF-C AD scoring systems were used in

this paper. These are online calculators, where the known values were entered into a mathematical formula, calculating the probability of death within 3 months. Clinicians use both of the scores in predicting mortality in everyday practice with very similar confidence [5, 6].

Data on patient dialysis, at least twice in the last 7 days, creatinine, bilirubin, INR and sodium values were used to calculate the MELD score [7]. Age, sodium, creatinine, leukocyte and INR were used for the calculation of CLIF-C ADs [8].

The aim of this study was to determine whether CLIF-C AD score or MELD score were more confident in prediction of mortality within 3 months after the diagnosis of alcoholic liver cirrhosis was confirmed.

Methods

Patients have been hospitalized at the University Clinical Center of the Republic of Srpska for the last 3 years (from January 1, 2018 to January 1, 2021). This study included 145 patients of both genders, diagnosed with alcoholic liver cirrhosis. Medical history data from the clinical information system and discharge letters of patients were analyzed. The value of CLIF-C AD and MELD scores using an online calculator (mathematical formula) at the time of hospitalization was calculated. Follow-up has also started during hospitalization and control examinations for the next 3 months. The obtained results were analyzed in the SPSS program.

Results

In the mentioned period, out of 145 patients, in the first 3 months from the moment of the calculation of the score, 39 patients (32 male and 7 female patients) passed away which represents 82.1% versus 17.9% and 26.89% of mortality. The mean age of patients was 59.18 ± 9.19 years.

The lowest value of MELD score is 11, which represents a 6% probability of death in the first 3 months, while the highest value is 40, and represents 81.30% probability of death in the first 3 months.

The lowest value of CLIF-C AD was 39, which represents a 67% probability of death. The highest value of CLIF-C AD was 127, which represents a 100% probability of death within 3 months (Table 1).

Table 1. Alcoholic liver cirrhosis patients' characteristics

Characteristics	Count (N)	Percent (%)
Number of patients	39	100.00
Mean age	59.18 ± 9.19	
Gender		
Male	32	82.10
Female	7	17.90

SCORE

MELD	Values	probability of dying within 3 months
Minimum	11	6.00%
Maximum	40	81.30%

CLIF-C AD	Values	probability of dying within 3 months
Minimum	67	39.00%
Maximum	127	100.00%

Note, all values of CLIF-C AD score of 99 and above have a 100% probability of death in the first 3 months.

Linear regression analysis of the dependent variable mortality and independent variables of MELD and CLIF-C AD scores showed statistical significance in the prediction of early mortality in favor of the CLIF-C AD score ($p = 0.026$).

Discussion

Alcoholic liver cirrhosis as a dominant etiological agent of progressive liver failure in the Balkans is a special challenge for the health system. Treatment of patients is expensive, and liver transplantation is still very difficult to be achieved [2]. Given the habits of the inhabitants, we are talking about the predominance of male patients. The mean age of deceased patients was 59.18 years. The mean age of the patients in Bhattarai et al. [9] was 54 years, while in Bell et al. [10] was 58 years, and Sugimura et al. [11] was about 60 years. The mentioned authors also noted the predominance of the male in relation to the female patients.

The main goal of the liver cirrhosis treatment, if transplantation is unattainable, is symptomatic-supportive therapy [3]. In order to monitor the success of the therapy and the prognostic significance, corrected if necessary, numerous scoring systems were created. MELD and CLIF-C AD scores were used in this paper [5,6]. The essence is in the fact that these are online scoring systems, calculators, each of them uses 5 parameters, which we entered into a mathematical formula and thanks to it you get a value expressed in numbers, i.e. the percentage that is the probability of death within 3 months.

Retrospective analysis of discharge letters and the remaining necessary documentation in the clinical information system extracted the necessary parameter data at the time of hospitalization and calculated the score value. The mentioned patients were followed for the next 3 months and the reliability of one in relation to the other score was analyzed.

In our study, the statistical significance and much higher reliability of the CLIF-C AD score in relation to the MELD score were noted. Jalan et al. suggested that CLIF-C AD score is more accurate in predicting prognosis for 225 cirrhotic patients in their study [8] and Baldin et al. [12] confirmed the same statement for 266 patients with alcoholic liver cirrhosis reminding that high CLIF-C AD score is associated with higher organ dysfunction and increased short-term mortality. Perdigoto et al. noted that MELD score performed better for 3 months mortality prediction. In their study, 118 patients were enrolled, while 39 patients had higher 28-day and 90-day mortality suggested that CLIF-C AD revealed good accuracy when ACLIF is present, however MELD score performed better for 90-day mortality prediction [13].

Conclusion

The CLIF-C AD score proved to be more reliable than the MELD score in predicting mortality in patients with alcoholic liver cirrhosis in the first 3 months.

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Ethical approval. The Ethics Committee of the University Clinical Center of the Republic of Srpska approved the study and informed consent was obtained from all individual respondents. The research was conducted according to the Declaration of Helsinki.

Conflicts of interest. The authors declare no conflict of interest.

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CLIF-C AD skor u odnosu na MELD skor u predviđanju mortaliteta kod pacijenata sa alkoholnom cirozom jetre

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Uvod. Alkoholna ciroza jetre je uznapredovali stadijum progresivne jetrene insuficijencije sa često nepovoljnim ishodom. Za predviđanje ishoda koriste se brojni sistemi bodovanja od kojih su u ovom radu korišćeni rezultati MELD skora (Model For End-Stage Liver Disease) i CLIF Consortium Acute Decompensation skora (CLIF-C ADs) sa ciljem da se utvrdi koji je pouzdaniji u predviđanju ranog mortaliteta.

Metode. Izračunata je vrijednost CLIF-C AD i MELD skora korišćenjem onlajn kalkulatora prilikom prve hospitalizacije. Praćenje je takođe uslijedilo tokom hospitalizacije i kontrolnih pregleda u naredna 3 mjeseca.

Rezultati. Ovim istraživanjem obuhvaćeno je 145 pacijenata, oba pola, sa dijagnozom alkoholne ciroze jetre. Tokom prva 3 mjeseca od momenta izračunavanja inicijalnog skora, preminulo je 39 pacijenata (32 muškog i 7 ženskog pola), što predstavlja 82,1% prema 17,9%. Prosječna starost pacijenata bila je 59,18 ± 9,19 godina. Sve vrijednosti CLIF-C AD skora 99 i više imale su 100% vjerovatnoću smrtnog ishoda u prva 3 mjeseca.

Zaključak. Pokazalo se da je CLIF-C AD skor pouzdaniji od MELD skora u predviđanju mortaliteta kod pacijenata sa alkoholnom cirozom jetre u prva 3 mjeseca.

Ključne riječi: CLIF-C AD skor, MELD skor, alkoholna ciroza jetre

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Review

Quality Management – a basic instrument in Healthcare systems

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Summary

In industry and the public sector quality has become the important management challenge of the 21st century. Although quality should be self-understanding in healthcare the reality shows another picture. There are nowadays international standards and models of quality excellence which make the introduction and control of quality management even in healthcare easier and allow a better benchmarking. The Model of Business Excellence of the EFQM with the five enabler criteria and the four result criteria can be used as a basic guideline. Focusing on such main parameters like leadership, people, strategy and policy, resources and processes which are important background criteria for quality it can be easily adapted to the requests of quality in almost all healthcare fields. Definitions of the different quality aspects like quality assurance, quality management, total quality management, quality control show that there are still special quality parameters in healthcare which need to be recognized. Reality in healthcare still shows a lot of deficiencies concerning quality measurements. Quality in healthcare including the mentioned key criteria will only be effective if it is an integrated part of the daily work by all people who are concerned with healthcare.

Key words: Quality management in healthcare, quality control, EFQM, Model of Business Excellence, DIN EN ISO 9001

Introduction

Quality is a management challenge in the 21st century. While in former times quality mostly was a matter of industry and industrial products, in the last years new fields of application and new branches especially in the public sector like administration, finance, healthcare institutions and education make increasing use of the benefits of quality management. Although quality in healthcare seems to be self-evident, there are certain quality criteria which in view of the tremendous development in medical research might have been forgotten or lost. The focusing on quality measures in healthcare therefore seems to be necessary to remind all healthcare workers

to realize that especially in this field quality is a basic requirement. In the meantime, there are many institutions which sell a broad variety of methods which might support the implementation of QM systems. The long lasting experience with the establishment of QM systems has taught, that every institution which wants seriously to go into the matter of QM first has to analyse the own situation, to improve the offered methods and to adapt the chosen ones to the own environment. Quality is based on an ongoing process, it is customer-focused and supportive, and needs a continuous improvement: plan–do–check–act. The establishment of Quality needs time, and one cannot expect fast results [1,2].

Definitions of quality

How can Quality in healthcare with a special concern to the main focus, the patient, be defined? Quality in healthcare is a holistic approach which in its sum includes all parameters which guarantee an optimal requirement for healthcare. Quality Assurance (QA) in healthcare work stands for the continuous commitment of all persons involved in healthcare work to fulfil the expectancies of the stakeholders concerned with healthcare and the whole society. Quality Management (QM) stands for the entirety of measures (planning, managing, securing, improving) which are necessary to reach the goals of a healthcare quality policy. QM has to be realized by all leadership levels and have to be concerned with the development of strategies, distribution of money, regulation of quality structures, quality influencing parameters and assessment of quality. Total Quality Management (TQM) is defined as the leadership strategy depending on the cooperation of all members of an institution to put quality in the center of all activities, with the aim to satisfy the customers, for sustained successful results, for the advantage of the members of the institution and for the whole society. Quality Control (QC) includes the controlling of working methods and measures which have to be installed to fulfil the requirements of a high quality standard in healthcare [1,2,3].

Systems and models for establishing quality

Although the models of establishing quality primarily were focused on industry the main parameters of such models can be adapted to different working fields. This especially counts for the "Model of Business Excellence" of the European Foundation of Quality Management [1].

EFQM Model of Business Excellence

In 1988 the European Foundation for Quality Management (EFQM) was founded by 14 European industrial companies with the aim to support the quality of industrial products in Europe. It has now developed to a not-for-profit membership organization dedicated to increasing the competitiveness and effectiveness of European organizations, whatever their size, sector, function, or structure of incorporation (large international companies to all types of public sector organizations) are [2,3].

Since its founding in 1988, EFQM has developed a comprehensive TQM programme which encompasses the following key features:

- The EFQM Model for Business Excellence
- Self-assessment by EFQM members to identify and facilitate ongoing improvements within their organization in accordance with best practice procedures
- The European Quality Award and Prizes
- Training and educational support [1,2,3]

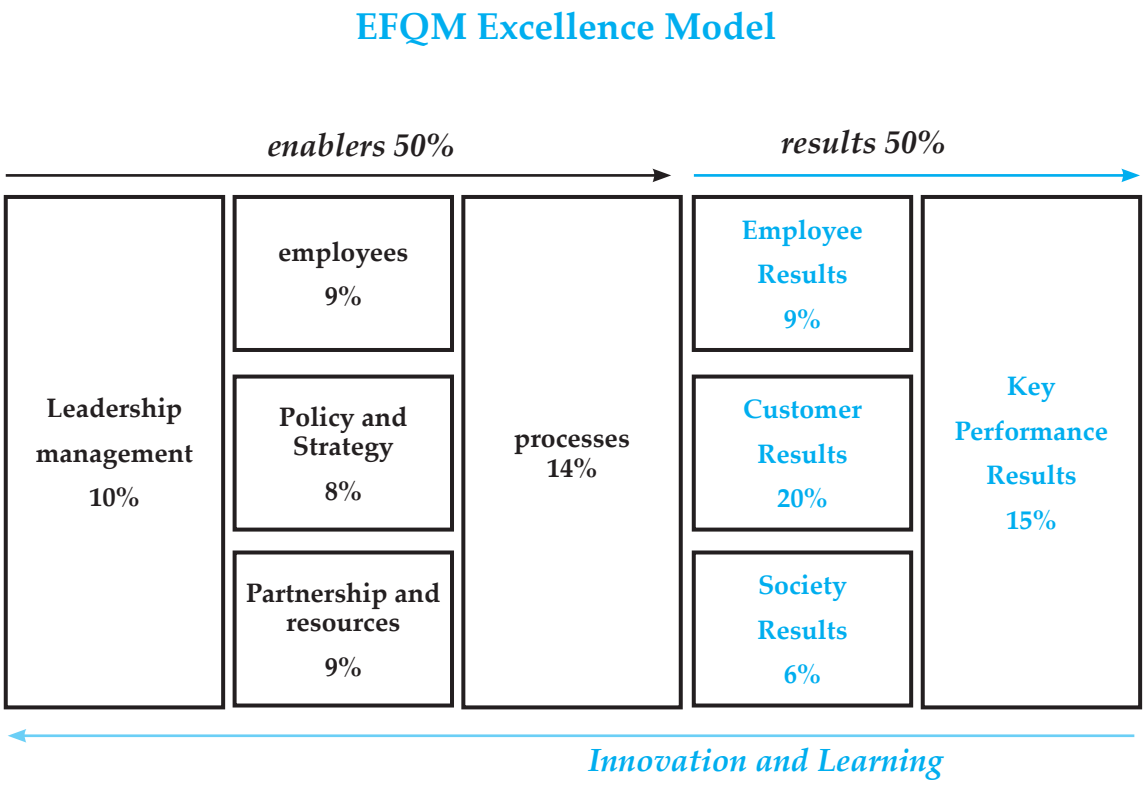

Figure 1. EFQM Model of Business Excellence

The EFQM model is widely recognized as one of the most cost-effective ways of improving performance: as the model is applied and monitored through self-assessment and can be administered by internal quality or change managers (EFQM-Assessors) [1,3].

The basic principle of the EFQM model is that customer and staff satisfaction and integration into society are achieved via the role of the organizational leadership in setting the policy and strategy and the management of staff, resources, and processes, culminating in excellence in business or organizational results [1,3].

The five criteria of the "Enablers" are concerned with how the organization performs various activities. The four criteria of "Results" are concerned with the results the organization is achieving with respect to different stakeholders [1].

The EFQM model is designed to be flexible enough to accommodate the requirements of the organizations at various stages of implementation. It provides controlled self-assessment with organizations having to carry out their own detailed self-evaluation which points out strengths and weaknesses and provides input for improvement. It also allows accreditation and certification processes. The fundamental concepts which underpin the EFQM model can be used to illustrate how the model is applied in the public sector and in healthcare [1,3].

DIN EN ISO 9000

The International Organization for Standardisation (ISO) is an international association of national Institutes for Standardisation. The standards are worked out by a technical committee and should be revised every 5 years. The latest fundamental revision was done in 2015 (ISO 9001:2015) and was based on a customer inquiry/survey [1,3,4].

The ISO 9001:2015 is based on the PDCA (Plan-Do-Check-Act) and is concerned with the model of processing and covers 10 parts [1,2,4]:

Use, notions, references

- Surrounding of organization, responsibility of the leadership, planning
- Management of the Finances, process management
- Evaluation, analysis and improvement

There are certain requirements, which have to be fulfilled by the organization which will be certified according to ISO 9001:2015. The organization has to lay down and to perform those processes which are necessary to satisfy the requirements of the customer. To guarantee this the organization has to establish a QM system which fulfils the requirements of the international standard. This QM system has to be evaluated and improved by the organization [1,4].

The guidelines of the ISO 9004:2000 are based on the following principles [1,4]:

Customer Orientation
Leadership
People Management
Process Oriented Performance
System Oriented Management
Continuous Improvement
Technical Statements for Decision Making
Relationship to Supplier for a common profit

The DIN EN ISO 9000 Family is more or less a catalogue of measures which have to be fulfilled to get a certification of the actual QM situation of the organization which can be used as a comparison with other organization (benchmarking) and with this as a matter of competition for the own products. QA can be reached by the accreditation according to those standards [1,4,5].

Other systems and models concerned with QM

Most of the numerous institutions worldwide which are concerned with the professional business of offering quality management systems are based on the EFQM Model of Business Excellence since this model really covers all the basic parameters which are necessary for the establishment of quality wherever it is needed [1,2].

General Criteria of Quality (mainly based on the EFQM Model of Excellence)

Leadership

Leadership is one of the most important parts on the way to establish quality and is more or less the figurehead in an organization or institution. The behaviour and the actions of the executive team and all other leaders of an institution are the background to inspire, support and promote a culture of TQM. Parameters of a convincing leadership are:

- visible commitment and consistent culture including the development of values and expectations for the organization, the ability to lead by example, making themselves accessible by listening and responding to the people from the organization, and reviewing and improving the effectiveness of their own leadership,
- support concerning definition of priorities, fund learning, facilitation and improvement activities, enabling people to participate in improvement activities and use of appraisal and promotion systems to support improvement and involvement,
- involvement with customers and suppliers with the areas of responding to needs, establishing and participating in partnership, participation in professional bodies,

addressing potential conflicts arising from customer-supplier relationship such as the balance between the priorities of different customer groups, recognition and appreciation of people`s efforts and achievements, especially of individuals and teams at all levels within and outside of the organization [1, 2, 6].

People Management

Quality management in this field covers all measures which are concerned with the release and the support of the full potential of the employees of an organization. In this context main parameters are:

- improvement of the resources especially using innovative strategies and methods,
- development and sustaining of people`s capabilities including the identification, classification and matching of people`s competencies with their needs, the establishing and implementation of training plans, the evaluation of the effectiveness of training, the development of team skills and the promotion of continuous learning,
- involvement and recognition of people especially through encouragement and support of individuals and teams participating in improvement,
- communication through an effective dialogue through the identification of communication needs, sharing information, evaluation and improving communication effectiveness, structuring top-down and bottom-up as well as lateral communication,
- caring for people like promoting awareness and involvement in health, safety and environmental issues as well as social and cultural activities, providing facilities and services [5,6].

Policy and Strategy

Quality management in the field of policy and strategy includes a variety of activities mostly concerned with:

- relevant and comprehensive information related to customers and suppliers, community, shareholders, internal performance indicators, benchmarking activities, performance of competitors and best in class organizations, social environment and legal issues, economic indicators, new methods,
- the development of policy and strategy concerning its values, mission and vision, balancing short and long term pressures and requirements, needs and expectations of its stakeholder, identification of present and future competitive advantages and reflecting the principals of TQM,
- the communication and implementation of policy and strategy especially to all levels of the organization using policy and strategy as a basis for planning of activities and setting off objectives throughout the organization and testing, evaluating, improving and prioritizing plans,
- the regularly updating and improving policy and strategy through evaluating the relevance and effectiveness as well as reviewing, updating and improving policy and exploitation strategy [4,5,6].

Resources

To reach QM in the field of resources evidence is needed for the following parameters:

- management of the financial resources by reviewing and improving financial strategies and practices, evaluating investments, managing risks, managing external controls on financial flexibility to allow a maximum of freedom within the organization,

- management of information resources through giving access to relevant information to appropriate users, structuring and managing information to support policy and strategy and assuring and improving information validity, integrity and security,
- management of supplier`s relationship and material by maximising the added value of suppliers, optimising material inventories, reducing consumption of utilities, reducing waste,
- managing buildings, equipment and other assets through optimising of exploitation, managing the maintenance and utilisation,
- managing new technologies, teaching methods, intellectual property by identifying and evaluation of alternative and emerging technologies, training modules, information systems, exploitation of intellectual property [5].

Processes

Process management is one of key measurements to establish and to improve QM in an organization. It is concerned with:

- the definition, the conduction of the identification, and the evaluation of the key processes,
- the systematically managing of processes like establishing and monitoring standards of operation, the performance of measurements in process management, implementation of system standards (ISO, health and safety systems), resolving interface issues inside the organization and with external partners,
- setting reviews and targets for improvement with identifying and prioritising methods of improvement, setting standards of operation priorities and targets for improvement according to benchmarking results from extern partners, relating current performance measurements and targets for improvement to past achievement,
- the improvement of processes using innovation and creativity by bringing to bear the creative talents of students and postgraduates in incremental and breakthrough improvements, discovering and use of new designs, technology and operating philosophies, changing organizational structures to encourage innovation and creativity and using feedback from customers and stakeholders to stimulate innovation and creativity in process management,
- the change and evaluation of benefits of the process including the agreement to appropriate methods of implementing change, the communication of process changes, the training of people prior to implementation, the review of process changes to ensure predicted results are achieved [4,5].

Result criteria

Result criteria are concerned with what the organization has achieved and is achieving and should be ideally assessed as trends over a period of at least three years including actual performance, targets, performance of competitors and performance of "Best in Class" organizations. Self-assessment should indicate the extent to which the activities of the organization are covered by, and the relative importance of the parameters chosen to measure results including relevance of the measurements to the various stakeholders. The results presented should include perception or direct feedback data as well as predictor or relevant organization performance measures. The reliability and validity of any survey results presented should be discussed. Results are mainly concerned with [2,5,6]:

- Customer satisfaction: main achievements in healthcare are concerned with the development of new diagnostic and therapeutic measures to treat sick people. Outcome of this development therefore is mainly concerned with the satisfaction of those people, suffering from

diseases which cannot be treated in a right way till then [2,4].

- People's (staff) satisfaction: as an example, motivated and committed healthcare workers are the basic potential of a health institution. Support of the healthcare workers and their satisfaction therefore play an important role for the establishment of QM in a healthcare institution. Achievements of the institution in relation to the satisfaction of its people should therefore include people's perception (e.g. surveys, structured appraisals, focus groups, etc) relating to motivation like career development, communication, empowerment, equal opportunities, involvement, leadership, recognition, target setting and appraisal, training and education, and relating to satisfaction like employment conditions, facilities and services, health and safety conditions, job security, pay and benefits, peer relationship, the organization's environmental policy and impact as well as its role in the community and society, working environment [3].

- Impact on Society: every organization needs evidence of its perception through the society and therefore has to achieve in satisfying the needs and the expectations of the local, national and international community at large [1].

- Business results: every organization is assessed according to what it is achieving in relation to its planned business, objectives and especially the quality of the outcome, taking into account the satisfaction, the needs and the expectations of everyone involved in its business. The success can be measured according to the additional measurements of the organizational performance like efficiency and effectiveness measurements, monitoring and evaluation of key services, external and internal audits inspections and evaluations, results of benchmarking measures with other comparable organizations, measurable increase in grants and impact factor points obtained [1,2].

Establishing of Quality in Healthcare

To establish the quality in healthcare institutions, the willingness of the leadership to accept and realize quality management and make it to the important measure in all parts of the healthcare institution is necessary. To reach the intended quality aims, a permanent effort is needed and will be possible if it is done in a holistic manner, which means that all levels of administration and departments will be integrated in the whole quality management concept. Quality in healthcare is the result of complex and all different levels of patient treatment parameters including levels which are determined through existing structures which aim to reach the expected quality standard. Quality with this aim needs a professional quality management which includes the function of a quality manager who is responsible for the leadership of the healthcare institution. Quality management should be realized in the cooperation with all coworkers including permanent analysis and the quality related adaption of all activities in all functional areas. Using this instrument, the quality manager will be able to realize quality measures in all functional areas of the healthcare institution. Very important in the healthcare institution is the establishment of a hygiene commission, consisted of members of all different departments of the healthcare institution. With this interdepartmental membership integrating all important persons responsible for the quality management in the healthcare institution, this commission can be a central instrument to establish, integrate and realize quality in the healthcare institutions [1,2,5].

Conclusion

Quality especially in healthcare is a management challenge in the new century. It has to be produced and not only controlled. Quality

reasoning cannot be conditioned and ordered, it has to be developed through all hierarchical levels and to imply a process which leads to a change of the behaviour. Quality, in spite of fast success in the beginning needs patience and a climate where it can develop. Quality is a continuous process and will only be effective if it is an integrated measure of a daily work by all people who are concerned with it, especially in healthcare.

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Upravljanje kvalitetom kao osnovnim instrumentom u sistemu zdravstvene zaštite

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U industriji i javnom sektoru kvalitet je postao važan upravljački izazov 21. vijeka. Iako kvalitet treba da bude samorazumijevanje u zdravstvu, realnost pokazuje drugu sliku. Danas postoje međunarodni standardi i modeli izvrsnosti kvaliteta koji olakšavaju uvođenje i kontrolu upravljanja kvalitetom, čak i u zdravstvu, i omogućavaju bolji benchmarking. EFQM model poslovne izvrsnosti sa pet kriterijuma mogućnosti i četiri kriterijuma rezultata može se koristiti kao osnovna smjernica. Fokusirajući se na takve glavne parametre kao što su liderstvo, ljudi, strategija i politika, resursi i procesi koji su važni pozadinski kriterijumi za kvalitet, lako se može prilagoditi zahtjevima kvaliteta u skoro svim oblastima zdravstvene zaštite. Definicije različitih aspekata kvaliteta kao što su osiguranje kvaliteta, upravljanje kvalitetom, upravljanje totalnim kvalitetom, kontrola kvaliteta pokazuju da još uvijek postoje posebni parametri kvaliteta u zdravstvu koje treba prepoznati. Realnost u zdravstvu još uvijek pokazuje dosta nedostataka u pogledu mjerenja kvaliteta. Kvalitet u zdravstvu, uključujući pomenute ključne kriterijume, biće efikasan samo ako je sastavni dio svakodnevnog rada svih ljudi koji se bave zdravstvenom zaštitom.

Ključne riječi: Upravljanje kvalitetom u zdravstvu, kontrola kvaliteta, EFQM, Model poslovne izvrsnosti, DIN EN ISO 9001

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Review

Pneumoconiosis among miners in coal mines

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Summary

Pneumoconiosis of workers in brown coal mines is an occupational disease, a global public health problem and a serious disease of the lung parenchyma. If it is not prevented, it leads to irreversible changes in the lungs with complications. The disorder occurs after prolonged exposure to coal dust containing high concentration of free crystalline silica. Data in literature regarding its health impact on people working in coal mines are relatively scarce. Recently, there has been an increase in miners' pneumoconiosis, which requires a stricter policy to protect workers in the mines. There are two classical types of CWP: simple and complicated. The main diagnostic method of CWP is based on a specific X-ray finding, and the auxiliary method of choice is spirometry. The pathophysiological mechanism of CWP formation is not fully known, although it has been shown that damage to the lung parenchyma goes through three phases caused by effect of lung cells exposure to coal dust. Studies show that cytokines play an important role in inflammation and the immune response as mediators of toxic and pathogenic effects in CWP. A link between exposure to coal dust in brown coal mines and the development of CWP has also been demonstrated, with a consequent reduction in the physical and psychological quality of life of workers in the mines.

Key words: pneumoconiosis, coal dust, miners

Introduction

Miners' pneumoconiosis in coal mines is a chronic and irreversible disease that represents a global public health problem. A large number of research is associated with environmental problems caused by mining in coal mines, processing, combustion and similar problems such as acid rain, smog, gas emissions and the like. In contrast, there is insufficient data in the literature on the direct impact of coal on the health of people who work in coal mines and who use coal as an energy source [1].

Inhalation of coal dust during blasting in brown coal mines leads to the development of coal mine dust lung disease (CMDLD). The most common manifestation of CMDLD is pneumoconiosis of the lungs. Coal Workers' pneumoconiosis (CWP) is a parenchymal disease caused by the accumulation of coal dust in the lungs and the consequent reaction of lung tissue, the formation of fibrous nodular lesions [2, 3], and the main cause is prolonged exposure to coal dust which contains high concentrations of free silica crystals [4]. Workers in brown coal mines are at high risk for respiratory morbidity and premature death. Given the global prevalence of mining in coal mines and the potential for severe respiratory diseases, the respiratory health of workers in coal mines is an important problem that should be prevented, given that CWP is an incurable disease.

The aim of this paper is to summarize research on pneumoconiosis among workers in coal mines.

Prevalence and mortality from pneumoconiosis

Considering the chronic nature of CWP, the assessment of the prevalence of this disease is mainly examined through prevalence studies. Blackely and collaborators estimated the prevalence of pneumoconiosis in U.S. miners working for at least 25 years. They conducted a prevalence study using radiograms collected from 1970 to 2017. Each radiogram was classified according to international standards. The prevalence was over 10%, and in central Appalachia as much as 21% [5].

In a study conducted in the Czech Republic, the authors compared the total and specific mortality of miners working in brown coal mines with and without pneumoconiosis, and the mortality of the general male population in the period from 1992 to 2013. The mortality of miners with CWP was higher compared to the general male population [6].

Coal mining and coal processing involves multiple dust generation processes, including cutting, transport, crushing and grinding of coal, etc. Coal dust is one of the main sources of health hazards for miners. Exposure to coal dust can be prevented by administrative and engineering controls. Ineffective control of coal dust exposure can harm miners' health. Although many efforts have been made to eliminate these threats, an unexpected increase in miners' pneumoconiosis (CWP) in the U.S. Appalachian Basin has been recorded in recent years. The authors hypothesized that nano-sized coal dust has contributed to an increase in the prevalence of CWP in recent years [7].

In Colombia, a study was conducted to assess the exposure to coal dust and the prevalence of pneumoconiosis in underground mining in three Colombian mines. The prevalence of pneumoconiosis was 33.8% (95% CI: 27.0 - 41.3%). Pneumoconiosis was significantly associated with the level of severe exposure to coal dust (PR=2.055, 95% CI: 1.043 - 4.048; p = 0.038) and work in underground mining for 25 years or more (for those with 25.0-29.9 years: PR = 2,199, 95% CI: 1,449 - 3,338; p = 0.001) [8].

A meta-analysis of the prevalence of pneumoconiosis among miners in China concluded that the prevalence of CWP remains high in China compared to the UK (0.8%, during 1998-2000) and the US (3.2% in 2000.). In addition, conditions in privately owned mines caused more CWP (9.86%; 95% CI: 1.25-25.17%) than in state-owned mines (4.83%; 95% CI: 2.35-8.13%) (P <0.05). The data clearly showed that regulatory agencies in China need to step up their efforts to implement more rigorous policies to protect miners, especially those in privately owned mines [9].

According to a study to determine the prevalence of CWP in China, in Jiangsu Province, from 2015, it was determined that out of the total number of respondents covered by CWP screening, 5.5% of workers had CWP. Out of the total number of workers working

in brown coal mines with a diagnosis of CWP, 71.1% are still in the first stage of CWP, and 90.7% of this number of workers work directly in blasting or in tunnels. Also, 7.3% of the total number of patients with CWP had a complication in the form of pulmonary tuberculosis, and the mortality of patients with stage III was the highest and amounted to 69.7%. According to the Chinese National Registry for Occupational Health and Poisoning, 23,152 new cases were diagnosed in 2013 [10].

Data on the frequency of CWP in the world indicate that it is necessary to introduce a stricter policy in order to protect workers in brown coal mines, especially for workers working in tunnels or directly in mining.

The introduction of preventive measures in brown coal mines can reduce the incidence of CWP. Thus, in the United States, after the introduction of preventive measures in brown coal mines, the prevalence of CWP was reduced from 30% to 2% [11].

Pathophysiology of pneumoconiosis

There are two classical types of CWP: simple and complicated.

The difference between these two is primarily in the severity of the disease. In simple CWP, fibrous lesions are centered around the respiratory bronchioles, especially in the upper lobes of the lungs, with radiological shadows 1–10 mm in diameter. With increased exposure to coal dust, macro-nodules are formed, which can be 1 to 2 cm in size, and severe symptoms appear at this stage. This stage is called complicated CWP or progressive massive fibrosis. The main diagnostic method of CWP is based on a specific X-ray finding, and the auxiliary method of choice for estimating the severity of obstruction is spirometry [12, 13].

Miners working in brown coal mines are generally divided into two basic groups. Miners who work in tunnels and directly on blasting belong to the group of underground miners. The second group consists of miners working at the surface of the mine. Underground miners are at a significantly higher risk for the development of CWP, due to higher and longer exposure to coal dust, compared to workers working at the surface of the mine [14].

A study conducted by Kurth and collaborators found a significant association between impaired airflow and pneumoconiosis. Impaired airflow was present in 7.7% of non-smoking miners, while in miners with pneumoconiosis it was present in 16.4% of them. The survey was conducted among non-smoking miners participating in the National Institute for Occupational Safety and Health (NIOSH) Coal Workers' Health Surveillance Program (CWHSP) [15].

Although the exact pathophysiological mechanism of CWP formation is not fully known, it has been established that coal dust components interact with cells in the lungs causing cell membrane damage accompanied by lipid peroxidation.

The pathogenic mechanism of CWP takes place in three phases. Initially, there is an accumulation and activation of inflammatory cells in the lungs. Damaged cells release intracellular enzymes, which provoke further tissue damage, resulting in scarring or destruction of alveolar septa. Coal dust phagocytosed by alveolar macrophages stimulates the formation of reactive oxygen species which then stimulate the secretion of cytokines and chemokines. These inflammatory cytokines act as chemoattractants that attract polymorphonuclear leukocytes and macrophages from the pulmonary capillaries in the alveoli and result in chronic inflammation [16].

The second phase of this pathological process consists of alveolar macrophages that stimulate the secretion of fibrogenic factors, which induce fibroblast proliferation and/or stimulate collagen synthesis resulting in the development of pulmonary

fibrosis, which is the main pathological indicator of CWP [17].

The third phase in the pathogenetic mechanism of CWP formation is the increased synthesis of extracellular matrix components [18].

Current concepts of CWP pathogenesis suggest that alveolar macrophages play a key role because of their ability to release various mediators such as proteolytic enzymes and growth and differentiation factors. In the chronic phase of CWP leading to pulmonary fibrosis in pneumoconiotic lungs, cytokines produced by alveolar macrophages play a significant role in the pathogenesis of CWP [19].

Wang et al. did a review of the literature to evaluate the association between IL-1 gene polymorphism and susceptibility to pneumoconiosis. The study included 10 case-control studies. The conclusions of this review suggest that IL-1RA (+2018) may modify worker sensitivity to pneumoconiosis and silicosis. New large-scale replication studies need to be conducted and the link between IL-1RA (+2018) and the risk of pneumoconiosis and silicosis of coal workers needs to be reassessed [20].

Han and collaborators conducted research to investigate the genetic association between single nucleotide polymorphisms (SNPs) of IL-17A and CWP in the Chinese population. A total number of 1391 subjects were included in this study, including 694 subjects in the control group and 697 in the case group. TaqMan qRT-PCRs were performed for genotype rs2275913, rs3748067, rs4711998 and rs8193036 within the IL-17A gene. Luciferase assays were used to determine the effects of the rs8193036 C> T allele on IL-17A expression. The rs3748067 G> A and rs8193036 C> T polymorphisms reduce CWP risk. These findings could be helpful in identifying people at reduced risk of CWP, and further studies justify their validity [21].

Cytokines are also known to play a very important role in a wide range of CWP biological processes such as inflammation and immune responses and are crucial mediators of toxic and pathogenic effects in observed CWP patients [22].

It is well known that various cytokines and growth factors secreted from macrophages/monocytes play a key role in the pathogenesis of pneumoconiosis. They can act as biosensors to predict pneumoconiosis. Kim and collaborators measured tumor necrosis factor-alpha (TNF-alpha), interleukin-8 (IL-8) and platelet-derived growth factor-AA to assess which cytokines can be used as sensitive biomarkers in pneumoconiosis, monocytes with or without coal dust (5 mg / ml) and serum in 42 miners with pneumoconiosis from coal mines and ten healthy controls. The release of carbon-stimulated TNF-alpha and IL-8 from monocytes in the blood was significantly increased in patients with pneumoconiosis compared with controls. Serum TNF-alpha and IL-8 levels were higher in subjects with pneumoconiosis than in control groups [23].

The genes IL-4, IL-4 (IL4R) and IL-13 are key immune factors and can influence the course of various diseases. Wang et al. investigated the association between potential functional polymorphisms in IL-4, IL-4R, and IL-13 and the risk of CWP in the Chinese population. Six polymorphisms (C-590T in IL-4, Ile50Val, Ser478Pro and Gln551Arg in IL-4R, C-1055T and Arg130Gln in IL-13) were genotyped and analyzed in a case control study of 556 individuals with CWP and 541 individuals from the control group. The results of this study suggest that the IL-4 C-590T polymorphism is involved in the etiology of CWP and susceptibility to this disease. Larger studies are needed to confirm these findings [24].

Cytokines produced by macrophages, IL-1, and TNF-α are involved in coal dust-induced inflammation as proinflammatory cytokines. The presence of a constant stimulus and chronic secretion of these cytokines can result in the development of inflammatory diseases such as silicosis and CWP [17].

Inter-individual differences in spontaneous and stimulated IL-1 and TNF-α production give us data indicating that CWP severity is associated with the production of these cytokines. According to the research of Ates and collaborators, performed at the Turkish brown coal mines, the association of the gene polymorphism of the cytokines IL-1, TNF-α, IL-6 and TGF-β in patients with CWP and the severity of this disease was estimated. The results of this study showed that TNF-α (-238) is a risk factor in both the development and severity of CWP, while TNF-α (-308) is important only in the severity of CWP. In contrast, IL-6 had a protective effect on the development and severity of the disease in patients with CWP [25, 26]. According to data in recently published studies, it is clear that the inflammatory cytokines IL-1 and TNF-α are associated with the formation and development of CWP [27].

According to research by Vanhee et al. in vitro exposure of alveolar macrophages to coal dust, coal dust particles caused significant secretion of TNF-α and IL-6 [19].

However, there are few data in the literature on the concentration of these and other important anti and proinflammatory cytokines such as IL-2, 4, 5, 9, 10, 13, 17A, 17F, 21, 22 and IFN-γ, measured in the serum of patients with CWP.

In a study conducted in Foca in the Republic of Srpska, among the miners in the Brown coal mine in Ugljevik, the production of anti and proinflammatory cytokines IL- 2, 4, 5, 9, 10, 13, 17A, 17F, 21, 22 and IFN-γ was examined. It was observed that the average values of anti-inflammatory cytokines IL-6 ($p = 0.03$), IL-10 ($p = 0.02$), IL-4 ($p = 0.02$), IL-17A ($p = 0.02$), were significantly higher in the control group of subjects compared to the group exposed to coal dust [28].

The association between exposure to coal dust in brown coal mines and the occurrence of respiratory symptoms, lung function deficit and the development of CWP has been proven [29].

Cumulative exposure to coal dust has been associated with a decrease in FEV1 among workers in brown coal mines in the United States without radiographically proven CWP [30].

In a study performed on 3,380 British miners with documented CWP, exposure to coal dust was associated with abnormal pulmonary function with FEV1 <65%. Longitudinal studies performed in England [31] and in the United States [32] showed similar results, linking coal dust exposure with FEV1 values.

Differences in risks from exposure to dark and brown coal

It is known that the structure of dark coal is dominated by less sustainable woody structure, higher carbon content (65–80%) and higher calorific value (12.6 to 23.8 MJ/kg), compared to the carbon content (60–65%) and the calorific value (6-12.5 MJ/kg) of brown coal (lignite). There are a small number of papers in the literature that have examined the relationship between work in lignite mines and the occurrence of pulmonary dysfunction.

However, in one study, in a group of 904 young miners in lignite mines in Sardinia exposed to relatively low levels of coal dust, individual exposure to coal dust was associated with a significant decrease in FEV1 and FVC, and the ratio of carbon dioxide diffusion capacity to alveolar volume [33].

These data show that there is a risk of pulmonary dysfunction in lignite mines.

According to data, 4.7 million underground workers in coal mines worked in China between 2010 and 2014, who were constantly exposed to a large amount of occupational hazards, including image dust, coal dust, noise, vibration and heat, which can lead to a large number of occupational physical diseases, the most common of which is CWP [34]. Also, underground miners usually work in much worse conditions, with higher

risk and higher intensity, which together can increase occupational stress [35].

Therefore, the combination of all these factors cannot only lead to the occurrence of physical diseases in miners, but also to disorders in their mental health, which is why it is important to pay attention to the quality of life of underground miners working in coal mines.

Miners' quality of life

Quality of life is a multidimensional concept that encompasses the physical, psychological and social components of health, and is widely accepted as an important parameter of medical care [36].

The 36-item Short-Form Health Survey (SF-36) is widely used to assess the quality of life of people in many fields and studies [37], including the quality of life of workers in brown coal mines [35].

According to a study by Han et al. from 2017 in China on 612 underground miners working in brown coal mines, it was found that the physical component of quality of life was significantly better in surface miners compared to underground miners. Compared to other populations without exposure to mine pollutants, the physical and psychological component of quality of life was significantly better in the group of respondents who do not work in the mines [38].

Also, longer work experience in mines showed significantly poorer quality compared to miners who have shorter work experience [38].

Mijović and collaborators conducted a survey of the quality of life of workers working in the Brown coal mine in Ugljevik between two groups of respondents, one of whom was exposed to coal dust, and the other, controlled, was not. Subjects working in the brown coal mine have significantly lower average values of the domains of physical functioning ($p = 0.005$), general health ($p = 0.001$) and mental health ($p = 0.041$) compared to the control group of subjects.

Also, the average values of the common physical component of quality of life were significantly ($p = 0.007$) lower in the group of miners compared to the control group of respondents. Differences in other domains of the SF-36 questionnaire between groups of respondents were not observed [39].

Conclusion

Review shows that coal workers' pneumoconiosis (CWP) is an insufficiently researched disease. The input of data is especially warranted in fields of immunology and pathophysiology. It is known that there is a disorder at the level of the lung parenchyma in the form of pulmonary fibrosis, with a consequent violation of lung function and diffuse lung capacity, which leads to respiratory failure. Common complications include pulmonary tuberculosis and even lung cancer.

The physical and psychological component of the quality of life of these workers is further impaired in relation to the population of people who do not work in the mine, which imposes the need to work on prevention and improvement of their health.

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Pneumokonioza radnika u rudnicima mrkog uglja

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Pneumokonioza radnika u rudnicima mrkog uglja je profesionalno oboljenje, globalni javnozdravstveni problem i ozbiljno oboljenje plućnog parenhima. Ukoliko se ne prevenira, dovodi do ireverzibilnih promjena na plućima sa komplikacijama. Nastaje nakon produženog izlaganja ugljenoj prašini sa visokom koncentracijom slobodnih kristala silike o čemu je malo podataka u literaturi o direktnom uticaju na zdravlje ljudi koji rade u rudnicima uglja.

U posljednje vrijeme zabilježen je porast pneumokonioza rudara, što zahtijeva striktniju politiku zaštite radnika u rudnicima. CWP se dijeli na jednostavnu i komplikovanu u zavisnosti od težine bolesti. Glavna dijagnostička metoda CWP se zasniva na specifičnom rendgenskom nalazu, a pomoćna metoda izbora je spirometrija. Patofiziološki mehanizam nastanka CWP nije u potpunosti poznat, mada je pokazano da oštećenje plućnog parenhima prolazi kroz tri faze usljed reakcije ugljene prašine i ćelija u plućima. Istraživanja pokazuju da citokini imaju važnu ulogu u inflamaciji i imunskom odgovoru kao medijatori toksičnih i patogenih efekata u CWP. Dokazana je i povezanost između izloženosti ugljenoj prašini u rudnicima mrkog uglja i razvoja CWP sa posljedičnim smanjenjem fizikalnog i psihološkog kvaliteta života radnika u rudnicima.

Ključne riječi: pneumokonioza, ugljena prašina, rudari

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Review

The role of glutathione transferase polymorphisms in the development of diabetic nephropathy

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Summary

Genetic and environmental factors play an important role in the development of type 2 diabetes mellitus (DM2) and its complications. Diabetic nephropathy (DN) is one of the most common microangiopathic chronic complications of diabetes.

Oxidative stress occurs under condition of increased production of free radicals and/or decreased activity of antioxidant defense mechanisms and it is an important link in the complex mechanism of diabetic vascular changes. Glutathione transferases (GST) are enzymes involved in xenobiotic metabolism and they are part of complex antioxidant defense mechanisms. Numerous studies have found an association of GST gene polymorphism to a predisposition to various diseases, including diabetes and diabetic nephropathy.

Our and other authors' results suggest that genetic variations in enzymes involved in free radical metabolism are associated with the development of end-stage kidney disease in patients with diabetes, which could become the basis for the development of preventive and early therapeutic strategies in high risk people.

Key words: diabetes, diabetic nephropathy, glutathione transferase polymorphism

Introduction

Diabetes (DM) is one of the biggest public health problems today. The number of people suffering from DM is constantly increasing significantly, as lifestyle changes lead to a decrease in physical activity and an increase in obesity. According to estimates by the World Health Organization (WHO, 2006), high blood glucose is the third most important risk factor for premature death, after high blood pressure and cigarette smoking. Also, DM is among the 10 leading causes of death, and with three other non-communicable diseases (cardiovascular disease, malignancies and respiratory diseases) it is responsible for more than 80% of early deaths from

non-communicable diseases. It should be noted that 30% to 80% of people with diabetes still live with undiagnosed disease [1].

According to the data from the Renal Register for Bosnia and Herzegovina, until 31 December 2017, diabetes mellitus is one of the leading causes of chronic renal failure, immediately right after glomerulonephritis. The largest number of patients with diabetic nephropathy, according to the mentioned data, were patients aged 45 to 64, and slightly less patients aged 65 to 74. Also, a larger number of patients with diabetic nephropathy had type 2 diabetes mellitus [2].

Diabetes mellitus is defined as a complex metabolic disorder characterized by chronic hyperglycemia, with a disorder in the metabolism of carbohydrates, fats and proteins. The heart of diabetes is a reduced effect of insulin on target cells, which occurs due to reduced hormone secretion and/or inadequate tissue response to insulin (insensitivity of target cells to its action), and often both disorders exist in one patient. Also, it is not clear which of the abnormality is the primary cause of hyperglycemia. Type 2 diabetes is often a manifestation of a broader disorder that includes metabolic syndrome (a set of risk factors for cardiovascular disease accompanied by glucose intolerance, hyperinsulinemia, dyslipidemia, hypertension, visceral obesity, hypercoagulability/thrombophilia and microalbuminuria). The risk of complications of DM appears long before the onset of clinical signs of diabetes (when there is impaired glucose tolerance and impaired glycemic control). Obesity is one of the most important risk factors for development of type 2 diabetes, and contributes to the risk of cardiovascular disease and mortality in general [3,4,5,6].

Diabetes is a highly heterogeneous and multifactorial, polygenic disease in which many common gene variants, mostly with little effect, contribute to the overall risk of developing the disease. The risk of developing type 2 diabetes during life is 40% if one parent had this disease, and even higher if the mother had it. Over 130 variants of the type 2 DM-related gene have been identified, however, these variants explain less than 15% of its heritability. Genetic polymorphism is the appearance of two or more discontinuous genotypes or alleles in a population that determine the diversity of individuals in it. The gene that determines an individual's susceptibility to a certain disease is called a sensitive gene and its analysis can help in taking certain preventive actions, in efforts to establish the concept of personalized medicine based on insight into the nature of individual phenotypic differences. By knowing the sensitive gene in some individuals, they may be advised to reduce exposure to certain risk factors, in order to reduce the incidence of the disease. The risk of developing diabetic nephropathy is increased several times by inheriting risk alleles at susceptibility loci of various genes like ACE, IL, TNF-α, COL4A1, eNOS, SOD2, APOE, GLUT, etc [5,6].

Chronic hyperglycemia in diabetes is associated with long-term damage and dysfunction of various organs, especially the eyes, kidneys, nerves, heart and blood vessels. Glycoregulation has the greatest importance in the control of diabetes and the development of microvascular and macrovascular complications of the disease. Chronic hyperglycemia also leads to the development of specific microvascular changes in the retina, renal glomerulus and peripheral nerves, so diabetes is the leading cause of vision loss, terminal renal failure, and neuropathy. Many different mechanisms result in progressive loss of mass and function of β-cells, which is clinically manifested as hyperglycemia and DM (from autoimmune destruction of pancreatic β-cells with consequent insulin deficiency which is usually seen in DM type I, to resistance of target tissues to insulin action, which is usually seen in DM type II) [7–9].

Once hyperglycemia occurs, patients with diabetes have an increased risk of developing a

number of complications, which reduce quality of life and increase health costs. These complications are characterized by a fairly individual rate of development, and we can metaphorically say that DM would not be a disease if there were no complications [10–14].

Diabetic nephropathy

Diabetic nephropathy (DN) causes progressive decline in renal function and is considered a major cause of end-stage renal disease in people with diabetes worldwide. Diabetic nephropathy (DN) is a microvascular complication of diabetes mellitus, characterized by the presence of persistent proteinuria (> 300 mg / day) with concomitant retinopathy but without clinical or laboratory evidence for other kidney or urinary tract diseases. It develops in about a third of patients with type 1 and type 2 diabetes mellitus. Studies have shown that genetic predisposition contributes to the development of diabetic nephropathy. The main potentially modifying factors that contribute to the individual's onset and progression of diabetic nephropathy are persistent hyperglycemia and hypertension. Diabetic nephropathy will not develop if there is no hyperglycemia regardless of genetic predisposition and other present risk factors [15–19].

Microalbuminuria has been shown to be a predictor of early mortality in patients with type 2 diabetes and is a risk factor for cardiovascular disease and kidney disease in people with the disease, but also in those who do not have diabetes [15–17].

Complications such as peripheral autonomic neuropathy are commonly seen in patients with diabetic nephropathy and are associated with increased morbidity and mortality [7,9,11].

Prospective studies in patients with diabetes have shown that diabetic retinopathy is a predictor of later development of nephropathy, and in clinical practice, the diagnosis of diabetic retinopathy (DR) indicates possible diabetic nephropathy (DN) [7,9,11].

Increased production of reactive oxygen species (oxygen free radical) and reduced efficiency of the antioxidant protection lead to oxidative stress or oxidative modifications of molecules in our body. Numerous studies have shown that oxidative stress is an important component in the development of vascular damage in patients with type 2 diabetes and that hyperglycemia can be a link between diabetes mellitus and oxidative stress. Occurrence of oxidative stress, i.e. hyperproduction of superoxide anion in the mitochondrial transport chain, with reduced endogenous antioxidant protection, seems to be a link between pathological processes induced by hyperglycemia [20].

Oxidative stress, which occurs when the concentration of reactive oxygen species (ROS) exceeds the capacity of antioxidant protection of the organism, as well as the decrease of antioxidant protection of the organism have been shown to be significant events in the formation of DN. Hyperglycemia induces mitochondrial hyperproduction of superoxide anions and can activate various cell damage pathways. The superoxide ion is the initial oxygen free radical, formed mostly in the mitochondria, which can be converted into other more reactive oxygen species and which can damage the cell in a number of ways. Increasing the concentration of superoxide ions partially inhibits the activity of glycolytic enzyme GAPDH (glyceraldehyde 3 - phosphate dehydrogenase), which leads to an increase in the concentration of all compounds formed in glycolysis before the site where this enzyme acts. The conversion of dihydroxyacetone phosphate (DHAP) to diacylglycerol (DAG), a protein kinase activator - C (PKC), is increased, as also the conversion of triose phosphate to methylglyoxal is increased, the major intracellular precursor of glycation advanced end products (AGE). Excess fructose 6-phosphate is converted to

UDP -N-acetylglucosamine, which increases protein modification, and excess glucose is metabolized through the polyol pathway, where reduced coenzyme (NADPH) is consumed and glutathione (GSH) concentration decreases. Despite all research, the association between these processes remains unknown, and clinical studies on their inhibitors have been disappointing [21,22].

In the development of diabetic microvascular kidney disease, i.e. oxidative cell damage, as well as its progression, the most important role is played by hyperglycemia and activation of the renin-angiotensin-aldosterone system in kidney tissue. Several cell types in the nephron, including podocytes as well as mesangial cells and proximal tubular cells, express GLUT1 glucose transporters that cannot reduce glucose entry into the cell at its high concentrations in the extracellular space. Intracellular hyperglycemia, consequently, causes the formation of reactive oxygen species. Mitochondrial O2 - diffuses into the cytoplasm where together with hyperglycemia and/or free fatty acids (FFA) contributes to the development of many diabetes-related cell damages. Reactive oxygen species deplete antioxidant protection, using "scavengers" of free radicals and enzymes that detoxify them. If oxygen species are not detoxified, cellular components are damaged and signaling pathways change. The concentration of non-enzymatic antioxidants decreases, but also the activity of enzymes of antioxidant protection [23–25].

The subject of various researches was the examination of the activity of the enzyme glutathione S-transferase in patients with diabetes. In patients with diabetes, levels of glutathione (GSH), a key component of cellular non-enzymatic antioxidant defense system involved in the removal of free radicals and reactive oxygen species, are reduced. Pancreatic β-cells are a potential target for tissue damage induced by oxidative stress, as these cells are sensitive to stress due to low production of antioxidant protection enzymes. Kim et al. have shown that insulin and growth factors regulate gene expression for enzymes involved in the detoxification processes of electrophilic compounds, including cytochrome P450 (CYP), glutathione S-transferase (GST) and microsomal epoxy hydrolases, and this may partly explain progressive deterioration of β-cell function in DM type 2 [26].

Glutathione transferase

Glutathione transferases (EC 2.5.1.18) (GST) belong to a family of multifunctional enzymes that represent significant participants in the phases II in enzymatic detoxification in the cell. They catalyze the conjugation reaction of tripeptides, glutathione (GSH, γ-glutamyl-cysteinyl-glycine), a peptide compound with a free thiol group, with various electrophilic metabolites of endogenous or exogenous origin, including many carcinogens, drugs, and many products of oxidative metabolism, toxins and allow them to eliminate from the cell. GSTs have a strong antioxidant activity against reactive oxygen species and peroxides. In conjugation with glutathione they reduce the reactivity of electrophilic compounds to biologically important macromolecules, such as proteins and nucleic acids. GSTs also catalyze hormone biosynthesis, peroxide degradation, tyrosine degradation, dehydroascorbate reduction, and many other metabolic reactions. They also participate in cell apoptosis [24].

The distribution of GST isoenzymes is not the same in most tissues, i.e. certain isoenzymes that are highly present in one organ may be absent or present in very low concentrations in other tissues. Therefore, the detoxification processes and harmful effects of electrophilic compounds are largely determined by the appropriate isoenzyme profile of glutathione transferases of these tissues [24].

Isoenzymes represent different forms of one enzyme that catalyze the same reactions.

Different GSTs show different substrate specificity and catalyze different types of chemical transformations. Human GSTs are divided into three families: the cytosolic, mitochondrial, and microsomal families. The cytosolic family of GST is further divided into seven classes, named after the Greek letters, and abbreviated to capital letters: Alpha (GST A, five isoenzymes), Mi (GST M, five isoenzymes), Omega (GST O, two isoenzymes), Pi (GST P, one enzyme), Sigma (GST S, one enzyme), Theta (GST T, two isoenzymes) and Zeta class (GST Z, one enzyme). The classification of cytosolic GSTs was performed on the basis of the primary structure. Members of the same class of GST have more than 40% identical amino acid sequences, while GSTs from different classes have less than 25% identical sequences. Catalytically active proteins are dimers of subunits of the same class. Each subunit has two significant domains in its structure: the *thioredoxin-like N-terminal domain* (G-domain) and the α-helical domain (the so-called H-domain). The G-domain represents the site of reduced glutathione (GSH) binding and it is present in all classes. The sulfur atom from glutathione is linked by a hydrogen bond to the catalytic residues at the N-terminal end of the protein. This bond stabilizes activated GSH and plays the most significant role in the catalytic action of GST [24–27].

Glutathione transpherase gene polymorphism and risk of diabetes

GSTs are represented in unique forms in each organ. Different GST isoenzymes are products of GST gene expression, which differ from each other in their structural, physicochemical and immunological properties. Their synthesis is encoded by a number of genes in the human genome, which are grouped on different chromosomes and which are class-specific. So far, 16 genes encoding glutathione transferase of the cytosolic family have been identified in humans. Functional genetic polymorphisms are present in most of these genes. The most commonly described polymorphisms are genes encoding GSTM1-1, GSTT1-1, and GSTP1-1. The genes encoding the synthesis of all five M classes of GST are grouped on the shorter arm of chromosome 1 (1p13.3), and the genes encoding the synthesis of T class are located on chromosome 22 (22q11.2). Approximately half of members of the white and yellow races are without active alleles (GSTM1 - null genotype), while the lowest frequency of GSTM1 - null genotype (24%) is present in members of the black race. Homozygous deletion of the GSTT1 gene is present in about 20% of white and black people, while the GSTT1-null genotype is most prevalent in members of the yellow race [28,29].

Genetic deficiency of the GSTT1 enzyme appears to be a strong and independent predictor of early vascular morbidity and death in individuals with type 2 diabetes. A number of studies have been published on the association of GST polymorphisms and various diseases, especially in conditions associated with oxidative stress, such as obesity, type 2 diabetes, coronary heart disease, neurodegenerative diseases or smoking habits, cancer, and different responses to chemotherapy. With the discovery of allelic variants of GSTP1 encoding enzymes with reduced catalytic activity, the hypothesis that the combination of GST class M, P, T polymorphisms contributes to the development of the disease, together with environmental factors, has been the subject of many studies [30–36].

The association between GSTT1/GSTM1 deletion polymorphisms and predisposition to diabetes has been the subject of several studies, but there is inconsistency in the results of these studies. Yalin et al. show that this GSTM1 gene polymorphism may contribute to the development of diabetes as well as that the GSTM1 gene may be a useful marker for predicting susceptibility to diabetes mellitus. In their research, no association

was found between the GSTT1-null genotype and the GSTP1-null genotype with the onset of diabetes. Patients with type 2 diabetes mellitus, in the population of northern India, had a higher incidence of GSTM1-null genotype compared to controls, and the presence of GSTM1-null genotype doubled the risk of developing type 2 diabetes. In contrast, studies in some other populations have shown an association between the GSTT1-null genotype and the risk of developing type 2 diabetes, and that the presence of the GSTT1-null genotype and the GSTM1-null/GSTT1-null genotype combination was an independent risk factor for diabetes. Despite differences in literature data, a meta-analysis of the study concluded that GSTT1-null genotype as well as GSTM1-null/GSTT1-null genotype represent risk factors for the development of type 2 diabetes mellitus [37–47].

There is a small number of papers on the association of GST gene polymorphism and microvascular complications of diabetes, and their results are inconsistent. Deletions of GSTM1 and GSTT1 individually or both of them were associated with decreased GST activity and increased oxidative stress in individuals with chronic kidney disease caused by diabetes or non-diabetic etiology. People with the GSTT1-null genotype are more likely to have generalized vasculopathy, with an increased risk of developing diabetic nephropathy and retinopathy, and this association does not depend on smoking status [48,49].

In the human kidney, GSTM classes are mainly localized in the tubules. GSTM1 gene expression has been detected in about 50% of people. This genetic locus is highly polymorphic and the absence of GSTM1 is attributed to homozygous deletion of the gene (null genotype). Class T enzymes are active in the kidneys and liver. The genetic locus for GSTT1 is also polymorphic and the absence of GSTT1 was found in 15–30% of Caucasians and > 50% of Chinese people [35,36,39,40,42,47].

Cilenšek et al. showed that the frequency of GSTT1-null genotype was 2 times higher in patients with diabetic retinopathy, compared to patients without this complication. The absence of GSTM1 is thought to have protective effects on the development of diabetic retinopathy in patients with type 2 diabetes mellitus [47].

Datta et al. indicated that patients with type 2 diabetes with GSTT1 and GSTM1-null genotype have reduced activity of GST enzymes, which can affect the increased production of reactive oxygen species, and that these patients have a higher risk of developing diabetic nephropathy [48,49].

Yang et al. showed that the existence of GSTT1-null genotype is a risk factor for development of diabetic nephropathy, and that homozygous deletion of GSTT1 gene is a risk factor for development of terminal renal failure in patients with diabetes, but not in patients with hypertension as an etiology of this condition. In this study, no association was found between the deletion of the GSTM1 gene and the development of end-stage renal disease in both groups [50].

Kim et al. have demonstrated a positive association of GSTM1-null genotype with the occurrence of diabetic nephropathy, while Fujita et al. proved that the frequency of GSTM1-null genotype was not significantly higher in patients with type 2 diabetes with nephropathy compared to patients without nephropathy, and that the mentioned gene polymorphism did not contribute to the development of diabetic nephropathy in patients with type 2 diabetes mellitus [51,52].

Šuvakov and co-workers showed that people with GSTM1-null genotype had a 1.6-fold higher risk of developing end-stage renal disease than people with GSTM1-active genotype. When GST genotypes were analyzed in combination, individuals who were carriers of the GSTM1-null/GSTT1-null genotype had the highest risk of developing end-stage renal disease. The risk found in carriers of GSTM1-null

and GSTP1 low-activity genotype is also significant. Also, it was shown that certain GST polymorphisms affect increased oxidation of proteins and lipids, where the effect was most pronounced in carriers of GSTM1-null genotype. There was also a strong association between genotypes, with reduced or absent activity of GSTM1, GSTT1, GSTA1, and GSTP1 and susceptibility to oxidative or carbonyl stress in patients with end-stage renal disease [53].

We conducted a study to examine the frequency of GST deletion polymorphisms in the population with diabetes in the eastern part of Bosnia and Herzegovina, and the possible association of these polymorphisms with the development of microvascular complications of diabetes. In this study, the distribution of GSTM1 polymorphic variants did not differ significantly in patients with diabetes compared to the control group, while the GSTT1-null genotype was significantly more common in the group of patients with diabetes compared to the control group. Patients with diabetes were significantly more often carriers of the combined GSTM1-active/GSTT1-null genotype compared to the patients from the control group. Also, the distribution of individual GSTM1 and GSTT1 as well as combined GSTM1/GSTT1 genotypes did not differ significantly in patients with diabetic nephropathy compared to the patients without this complication, while GSTM1 deletion polymorphism was not associated with the risk of developing diabetes. Patients with diabetes, carriers of the GSTT1-null genotype had a 3-fold higher risk of developing diabetes compared with patients carrying the GSTT1-active genotype. A small modifying effect of the GSTM1-active genotype was obtained in patients carrying the combined GSTM1-active/GSTT1-null genotype, in whom the risk of developing diabetes was 3.37-fold higher compared with patients carrying both reference alleles. Further modifying effect of GSTM1 and GSTT1 deletion polymorphisms at risk for diabetic nephropathy was not obtained, although patients with GSTM1-null genotype had a 1.47-fold higher risk of developing nephropathy compared with patients with GSTM1-active genotype, this increase was not statistically significant. No statistically significant risk for nephropathy in patients with diabetes was observed with respect to GSTT1 deletion polymorphism. No combination of GSTM1/GSTT1 genotypes was associated with a significant risk of developing diabetic nephropathy. Although patients with the GSTM1-null/ GSTT1-null genotype had a 2.07-fold higher risk of developing nephropathy, the risk was not statistically significant. In patients with diabetic nephropathy, stratified based on the severity of renal disease, it was shown that patients with end-stage renal disease were significantly more likely to be carriers of the combined GSTM1-null/ GSTT1-null genotype. In patients with diabetes, regardless of the presence of diabetic nephropathy, there is a significant positive correlation between the presence of both GSTM1-null genotype and GSTT1-null genotype and advanced glycation end products in the serum of these patients. Concentrations of late glycation end products (AGEs) were significantly higher in patients carrying either the GSTM1-null or GSTT1-null genotype compared to patients carrying active variants of these genes. Moreover, this association was also obtained in patients carrying the combined GSTM1-null/ GSTT1-null genotype. There were more overweight people in the group of patients with diabetes. Also, in the subgroups of patients with diabetes, the percentage of overweight people was significantly higher than the percentage in the control group. A larger number of subjects in the group of patients with diabetes had hypertension compared to subjects in the control group, which confirms the importance of these risk factors in the development of diabetes. Diabetes lasted significantly longer in patients with diabetic nephropathy compared to the patients without this microvascular complication [3,16].

Conclusion

Diabetic nephropathy (DN) is a microvascular complication of diabetes mellitus that develops in about a third of patients with DM. Numerous studies have linked the polymorphism of the GST gene to a predisposition to various diseases, including diabetes and diabetic nephropathy.

Studies indicate that genetic variations in enzymes involved in free radical metabolism are associated with the development of terminal renal failure in patients with diabetes. GSTT1-null genotype and GSTM1-null/GSTT1-null genotype are risk factors for the development of type 2 diabetes mellitus. These results allow the development of preventive and early therapeutic strategies in high-risk individuals in a modern concept of personalized medicine.

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Značaj polimorfizama glutation transferaza za nastanak dijabetične nefropatije

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Genetički faktori i faktori sredine imaju važnu ulogu u nastanku dijabetes melitusa tip 2 (DM2) i njegovih komplikacija. Dijabetesna nefropatija (DN) jedna je od najčešćih mikroangiopatskih hroničnih komplikacija dijabetesa.

Oksidativni stres nastaje u uslovima povećane produkcije slobodnih radikala i/ili smanjenja aktivnosti enzima antioksidativne zaštite i predstavlja bitnu kariku u složenom mehanizmu nastanka dijabetičnih vaskularnih promjena. Glutation transferaze (GST) su enzimi koji su uključeni u procese metabolizma ksenobiotika i dio su kompleksnih mehanizama antioksidativne zaštite. Brojna istraživanja povezuju polimorfizam gena za GST sa predispozicijom za nastanak različitih bolesti, uključujući dijabetes i dijabetičnu nefropatiju.

Naši i rezultati drugih autora ukazuju na to da su genetske varijacije u enzimima koje su uključene u metabolizam slobodnih radikala povezane sa nastankom terminalne bubrežne insuficijencije kod bolesnika sa dijabetesom, što bi moglo postati osnova za razvoj preventivnih i ranih terapijskih strategija djelovanja kod osoba sa visokim rizikom.

Ključne riječi: dijabetes, dijabetesna nefropatija, polimorfizam glutation transferaza

BIOMEDICINSKA ISTRAŽIVANJA
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Case report

Acute surgical abdomen due to duodenal perforation in an elderly COVID-19 patient

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Summary

Introduction. Since the announcement of the SARS-CoV-2 pandemic, the health system has been facing great challenges. Due to several uncertainties and concerns, surgeons face a huge challenge in solving urgent surgical conditions in order to save a life.

Case report. We present a patient with a coronavirus (COVID-19) infection and acute abdomen who was in poor general condition at the time of surgery, septic, all as a result of duodenal ulcer perforation. She did not have a positive RT-PCR at the time of surgery, but the lung CT scan showed highly indicative signs of COVID-19 infection. She had a positive nasopharyngeal swab RT-PCR test for the SARS-CoV-2 virus on the first postoperative day.

Conclusion. All suspected COVID-19 patients during surgery should be treated as positive for SARS-CoV-2 virus with the use of all staff protection measures.

Keywords: COVID-19, acute abdomen, RT-PCR, CT

Introduction

Since initially discovered in China, in the city of Wuhan, in December 2019, the SARS-CoV-2 coronavirus began to spread unstoppably around the world, so the pandemic was declared in March 2020 [1]. The consequences of this new disease are noticed at all levels of the health system, primarily due to the engagement of many health workers in the treatment of patients with COVID-19. Medical doctors of all specializations, including surgeons, are involved in the treatment of patients with COVID-19 disease.

A specific challenge is to treat patients who require urgent surgical intervention during this pandemic situation because the surgical treatment of COVID-19 patients requires more serious protection measures than treating non-COVID-19 patients. Waiting for the real-time polymerase chain reaction (RT-PCR) report for SARS-CoV-2 can further endanger life of the

patient who needs urgent surgical intervention. Radiological diagnostic methods such as X-rays and CT play a major role in diagnosing COVID-19 patients or at least raising the suspicion of the disease. Therefore, all cases of suspicious COVID-19 patients, who need emergency surgical intervention, it is necessary to be treated as COVID-19 patients, using safety protocol measures, until the arrival of negative RT-PCR result [2, 3].

We are presenting a COVID-19 patient with acute abdomen and perforated duodenal ulcer that required emergency surgery.

Case report

The 75-year-old patient checked in at the pre-admission surgical clinic due to epigastric pain that spreads to the right side of the chest, and nausea. The pain started a few hours earlier. On admission to the clinic the abdomen was soft, painfully sensitive to palpation in the epigastrium. Comorbidities - hypertension, previous treatment of rheumatic problems, and an amputated toe due to vascular issues. Following protocol for all patients who require hospital treatment, a rapid serological test, rapid antigen test and nasopharyngeal swab RT-PCR test were done in the admission clinic - the result was negative. Patient had negative epidemiological data for COVID-19. An ultrasound of the abdomen was performed - we discovered minimal collection of free fluid in the pouch of Douglas, other findings were normal. X-ray of the abdomen showed no signs of pneumoperitoneum, X-ray of the lungs showed very sharpened pulmonary bronchovascular pattern. Lung CT findings showed bilateral, multilobar, predominantly peripheral and posterior distribution of subpleural lines and fibrous bands with zones of consolidation mainly in the lower lobes suggestive of COVID-19 disease; free fluid present in the abdomen without signs of pneumoperitoneum. Due to radiologically verified lung changes indicating the possible SARS-CoV-2 virus infection, she was admitted to the COVID-19 department for further diagnosis and treatment. Initial laboratory findings were RBC 3.81, WBC 8.1, HGB 102, CRP 131, LDH 251, albumin 30. Treatment started according to the protocol for COVID-19. A nasopharyngeal swab PCR test on SARS-CoV-2 was taken twice, and the second one was positive. Despite the applied therapy, the general condition worsened as well as the laboratory parameters after 4 days of hospitalization (CRP 315.2, RBC 3.4, WBC 29.2, fibrinogen 15.5, albumin 18). In addition, abdominal distension with diffusely painfully sensitive palpation occurred, patient was febrile and exhausted. Control CT of the chest and abdomen was performed: changes in the lungs which are characteristic of COVID-19 were in progression compared to the previous images. A large amount of thick fluid content and pneumoperitoneum were present in the perihepatic region (Figure 1 and 2). Boey score of 2 set indications for surgery. Under general endotracheal anesthesia, an open abdominal operation was performed, with an upper medial incision. Perforation was documented on the front side of the duodenal bulb up to 1.5 cm in size, with fibrin deposits along the serosal surfaces. Perforation site suture was performed with perforation site omentoplasty. Diagnostic peritoneal lavage was performed, and two drains were placed - subhepatic and in the pouch of Douglas. After the surgery, the patient was transferred to the COVID-19 intensive care unit, where conservative treatment was continued with oxygen therapy (max 8 l O_2) via an oxygen mask. On the 4th postoperative day, she was transferred to the COVID-19 department, where conservative treatment with antibiotics (Imipenem, Vancomycin, and Metronidasole), anticoagulant therapy (Nadroparin calcium 0.6 ml sc twice a day), proton pump inhibitors (PPI), oxygen support (via a mask with a flow of up to 6 l O_2), corticosteroids was continued, as well as

vitamin therapy with fluid replacement and other symptomatic therapy. Patient was without oxygen support from the 10th postoperative day. On the 20th day of hospitalization, she was released for home treatment, in good general condition and with appropriate local findings, with inflammatory biochemical parameters within normal range and advice for regular examinations.

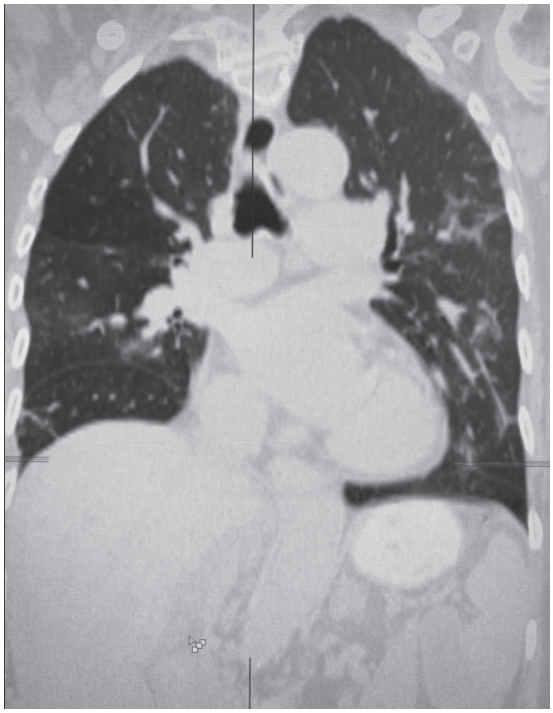


Figure 1a. Lung CT on a submission day

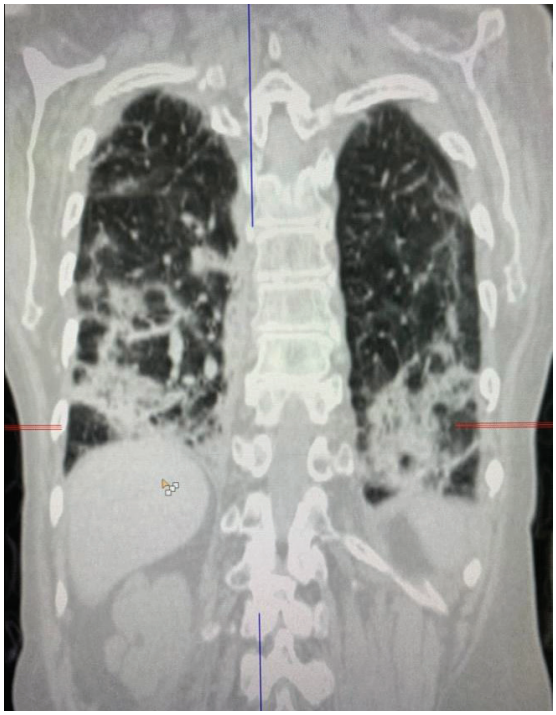
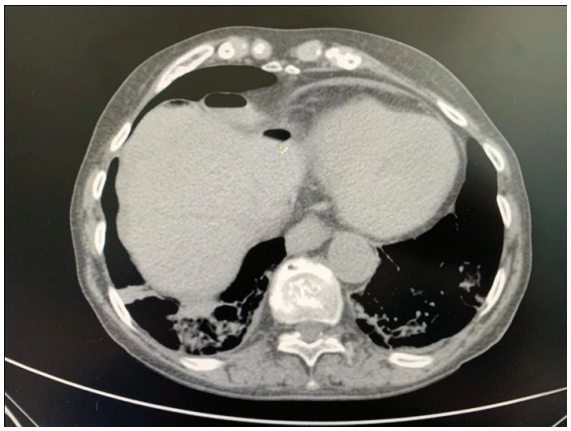


Figure 1b. Lung CT on a day of surgery



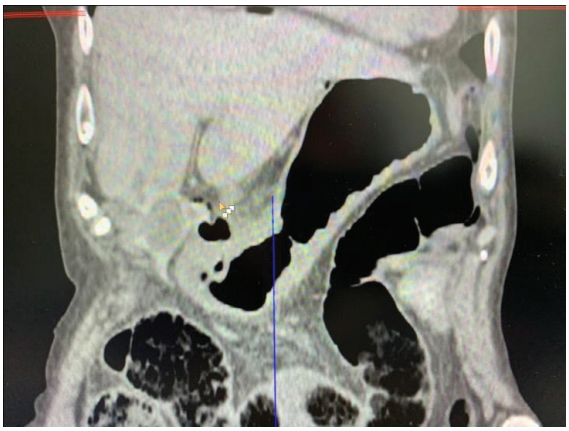


Figure 2a. and 2b. Abdominal CT - subdiaphragmatically and down the anterior abdominal wall (with the patient lying on his back) free gas collections indicating perforation of the hollow organ

Discussion

COVID-19 is typically characterized by respiratory tract symptoms, but sometimes may be presented with abdominal pain, without having findings of abdominal disease, and with no respiratory symptoms. In a retrospective study, 11.8% of patients were positive for SARS-CoV-2, without respiratory symptoms and with abdominal problems and verified changes on CT screening of the lungs, characteristic of COVID-19 [4].

In a COVID-19 patient, an acute abdomen may develop for multiple reasons. Although gastrointestinal symptoms may appear in up to 70% of the patients, they do not usually present a severe abdominal situation [5]. The mechanisms of abdominal pain during SARS-CoV-2 infection may be classified as pulmonary (with CT abnormalities), due to the involvement of lower pulmonary lobes, and extrapulmonary, as a result of direct damage of gastrointestinal (GI) epithelium by SARS-CoV-2 virus attack on angiotensin-converting enzyme 2 (ACE2) in GI cells, as a receptor for viral entry or as a complication of the drugs used [6, 7].

Since the declaration of the COVID-19 pandemic in our country, all patients presenting at the emergency surgical clinic are treated as potential COVID-19 suspects. According to the protocol in our hospital, examination in the surgical clinic requires the use of personal protective equipment (PPE), and if the patient's condition requires hospitalization, all patients are tested by rapid antigenic tests on SARS-CoV-2 before admission. If the result is negative, they are admitted to the surgical department, and if it is positive, they are admitted to the COVID-19 department, for further diagnosis and treatment.

In our case, on admission, the patient had no respiratory distress, with an initially negative rapid test for SARS-CoV-2 virus. Abdominal X-ray showed no pneumoperitoneum, ultrasound and CT of the abdomen was nonspecific, but the positive finding on chest X-ray and CT of the chest suggested that the patient had COVID-19, with no clear signs of acute abdominal surgical disease. That is why the patient was admitted to the isolation unit of the COVID-19 department, where the treatment began. Since the patient has not had previous gastrointestinal problems, we cannot say with certainty what caused the duodenal ulcer perforation. Several studies and associations state that whenever possible, non-surgical treatment should be applied and patients should be treated with conservative therapy for conditions that allow it (acute cholecystitis, appendicitis, diverticulitis, etc.) [5]. However, many authors argue that although COVID-19 increases mortality and morbidity in surgical patients, abdominal conditions should be surgically solved urgently. It is important to emphasize that the absence of pneumoperitoneum on X-ray of the abdomen is not an absolute sure sign of the absence of perforation of hollow organs of the abdomen [5, 8].

The data showed that only a quarter (25%) of patients who went to the operating room (OR) had an RT-PCR swab test performed. According to published studies, negative RT-PCR swab tests have been reported up to 34.7% of patients with chest CT findings suggestive of COVID-19 initially. Most of the patients with consistent CT COVID-19 findings and initial negative RT-PCR results will develop RT-PCR positivity after approximately 5 days [9]. Patients presenting with an acute abdomen are likely to undergo surgery regardless of the findings of CT of the lungs or the findings of swabs if their clinical condition requires urgent surgery [10]. On the 4th day of hospitalization, the patient underwent a control CT scan of the chest and abdomen after which an emergency surgery was scheduled.

On the day of surgery, the second RT-PCR was in progress, and the first one was negative (taken on the 2nd day of hospitalization). The second RT-PCR was positive, postoperatively.

The question arises, to use laparoscopic or open surgery in COVID-19 patients. The

SARS-CoV-2 virus is spread and transmitted by respiratory droplets and direct contact. The virus was also detected in fecal masses. Some authors state that they did not isolate the virus in peritoneal fluid, while other authors say they did [7, 11,12]. In patients with duodenal ulcer perforation, perforation site suturing with omentoplasty is a validated technique equally in laparoscopic and open surgery in terms of efficacy and safety, with somewhat easier early postoperative recovery after laparoscopic surgery [7]. In our case, we decided to do a laparotomy because there were no technical possibilities for laparoscopic surgery in the COVID-19 OR.

Many authors present data on the number of infected health workers, which puts additional pressure on the already burdened health system in all countries [13]. Therefore, according to the protocols, the number of elective surgeries in surgical wards has been reduced, but urgent surgical matters cannot wait and must be performed in order to save human lives [14]. The risk of exposure for the surgical staff is high, especially for anesthesia teams. Therefore, it is necessary to use the complete PPE as well as to take all precautions necessary related to the preparation of the OR and the instruments, before, during, and after the surgery.

Conclusion

This paper does not present a clinical rarity, and the situation of the occurrence of two simultaneous acute diseases with all the diagnostic and therapeutic dilemmas they carry is possible in everyday clinical practice. We should not forget about the possibility of non-infectious pathology, during pandemics, as well as the possibility of acute chronic disease. Stress ulcers can also occur, so the possibility of existing pharmacotherapeutic gastroprotection should be used.

We also emphasize the importance of multiple testing during COVID-19 in the case of early negative findings of serological and PCR tests, as well as to consider the possibility of indirect, non-etiological, diagnosis of the disease, e.g. by performing CT of the lungs with epidemiological data.

Acute surgical diseases in patients with COVID-19 should be treated surgically whenever possible, using laparoscopic or open surgery. The choice depends on the affinity and training of the surgeon with the aim of minimizing the duration of the operation and reducing exposure to the virus. Further research on the isolation of SARS-CoV-2 from the peritoneal cavity with urgent surgical conditions is needed to reconcile conflicting views.

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Ethical approval. This article does not contain any studies with human participants performed by any of the authors.

Conflict of interest. The authors declare no conflict of interest.

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Duodenalna perforacija sa akutnim hirurškim abdomenom kod pacijenta sa Covid-19

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Uvod. Od proglašenja pandemije SARS-CoV-2 virusa, zdravstveni sistem u svetu se nalazi pred velikim izazovom. Zbog niza nepoznanica i nedoumica, hirurzi se nalaze pred velikim izazovom prilikom rešavanja urgentnih hirurških stanja u cilju spašavanja života.

Prikaz slučaja. Predstavljamo pacijenta sa COVID-19 infekcijom i akutnim abdomenom koji je u trenutku operacije bio lošeg opšteg stanja, septičan, a sve kao posledica perforacije ulkusa duodenuma. U trenutku operacije nije imao pozitivan RT-PCR, ali sa radiološki visokoindikativnim znacima COVID-19 infekcije na CT-u pluća. Prvog postoperativnog dana je imao pozitivan RT-PCR bris nazofarinksa na SARS-CoV-2 virus.

Zaključak. Svi pacijenti suspektni na COVID-19 prilikom hirurške intervencije trebalo bi da se tretiraju kao da su pozitivni na SARS-CoV-2 virus uz upotrebu svih mera zaštite osoblja.

Ključne reči: COVID-19, akutni abdomen, RT-PCR, CT

BIOMEDICINSKA ISTRAŽIVANJA
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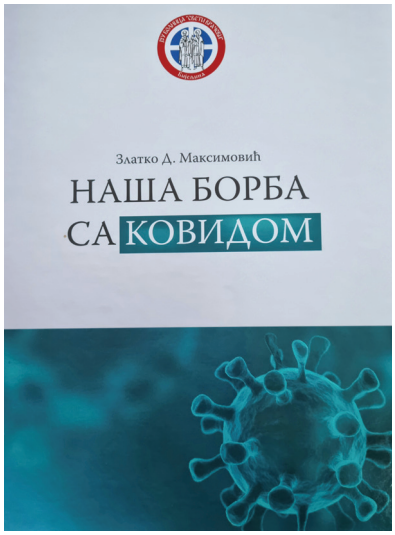
Prikaz knjige / Book review

Naša borba sa Kovidom



Naslov knjige: Naša borba sa Kovidom
Autor: prof. dr Zlatko D. Maksimović
Izdavač: Narodna biblioteka „Filip Višnjić", Bijeljina
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Zadovoljstvo mi je da predstavim jedinstvenu monografiju na našem jezičkom području "Naša borba sa Kovidom", autora prof. dr Zlatka Maksimovića, vanrednog profesora hirurgije Medicinskog fakulteta u Foči, Univerziteta u Istočnom Sarajevu. Monografija je rezultat analize funkcionisanja Javne zdravstvene ustanove ''Sveti Vračevi'' Bijeljina u pandemiji Kovid-19, a objavljena je u maju 2021. godine. Izdavač je NB "Filip Višnjić", Bijeljina.

Koliko je jedna sredina uspjela u kontroli pandemijske nemani saznaće čitalac ove monografije. Moje je mišljenje, kao neposrednog svjedoka i dijelom aktivnog učesnika u tim događajima, da je autor uspio dočarati ta dešavanja medicinskoj i opštoj nemedicinskoj javnosti na pristupačan i realističan način.

Autor ovu monografiju počinje citatom Viktora Igoa: „Nema ničeg moćnijeg od ideje čije je vrijeme došlo".

Monografija "Naša borba sa Kovidom" napisana je na 262 strane i prikladno je ilustrovana originalnim fotografijama iz arhive autora. Sastoji se iz 11 poglavlja. U njima se opisuju postojeća znanja o Kovid-19, hronologija događaja vezana za Kovid-19 u Bijeljini, prelomni trenuci u borbi protiv nje, otvaranje nove kovid bolnice i poliklinike, funkcionisanje operativnog bloka u navedenim pandemijskim uslovima, svjedočenja dobrovoljaca/medicinskih i nemedicinskih profesionalaca ove ustanove koji su radili na zbrinjavanju Kovid-19 pozitivnih ili pacijenata kod kojih se postavila sumnja na ovu bolest, utiscima gostujućih stručnjaka o funkcionisanju bolnice u ovom periodu, procesu vakcinacije zaposlenih, utiscima zvaničnika Republike Srpske o aktivnosti bolnice u borbi protiv pandemije, te o donacijama bolnici u ovom periodu.

U odvojenom poglavlju su sažeto prikazana iskustva i pouke koje su zaposleni i rukovodstvo bolnice naučili tokom borbe protiv Kovid-19 pandemije.

Recenzenti monografije su prof. dr Goran Stevanović, infektolog, direktor Klinike za infektivne i tropske bolesti „Prof. dr Kosta Todorović" Kliničkog centra Srbije u Beogradu i prof. dr Biljana Mijović, epidemiolog, redovni profesor Medicinskog fakulteta u Foči, Univerziteta u Istočnom Sarajevu. Recenzenti i autor ovog članka preporučuju monografiju studentima medicine i zdravstvene njege,

a takođe i stručnjacima različitih medicinskih i nemedicinskih profila u oblasti javnog zdravlja. Monografija sadrži interpretaciju multidisciplinarnog pristupa ovom još uvijek aktuelnom problemu javnog zdravlja.

Značaj ove monografije prevazilazi samu interpretaciju Kovid pandemije. Kao što je ''došao'' Kovid, uvijek se može desiti nešto drugo, na primjer infektivna bolest ili sasvim druga masovna nesreća. Ova knjiga i iskustva koja su u njoj opisana pokazuju kojim pravcem treba ići i kako treba odgovoriti na ovakve izazove.

Na kraju treba reći: "Verba volant, scripta manent (Desilo se samo ono što je ostalo i zapisano)" i zato čestitam autoru i izražavam svoju zahvalnost i poštovanje što je u toku još uvijek razbuktale epidemije našao vremena i želje da ovo vrijedno djelo napiše.

Prof. dr Siniša Ristić

Zahvalnost recenzentima

Zahvaljujemo recenzentima radova objavljenih u časopisu ''Biomedicinska istraživanja'' tokom 2021. godine na savjesnim i stručnim recenzijama, kao i korisnim savjetima i prijedlozima koji omogućavaju autorima da poboljšaju kvalitet svojih radova. Svojim savjesnim radom recenzenti značajno doprinose kvalitetu i naučnoj ozbiljnosti časopisa na čemu im uredništvo iskreno zahvaljuje.

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UPUTSTVO AUTORIMA ZA PISANJE ČLANKA ZA ČASOPIS „BIOMEDICINSKA ISTRAŽIVANJA“

„Biomedicinska istraživanja“ je časopis Medicinskog fakulteta Foča, Univerziteta u Istočnom Sarajevu, sa otvorenim pristupom i slijepom recenzijom, koji izlazi dva puta godišnje, u junu i decembru.

U časopisu „Biomedicinska istraživanja“ objavljuju se originalni naučni radovi, prethodna ili kratka saopštenja, pregledni i stručni radovi, radovi za praksu, edukativni članci, komentari na objavljene članke, prikazi bolesnika, pregledi iz literature, radovi iz istorije medicine, prikazi knjiga, izvještaji sa naučnih i stručnih skupova, novosti i pisma iz svih oblasti medicine, stomatologije, defektologije i zdravstvene njege.

Otvoreni pristup časopisu *Biomedicinska istraživanja* omogućava slobodan, besplatan i neograničen onlajn pristup svim člancima poslije njihovog onlajn objavljivanja. Korisnici mogu besplatno da preuzmu, čitaju, kopiraju i štampaju kompletne tekstove svih radova ili da ih koriste za svaku drugu zakonom dozvoljenu svrhu, bez prethodnog odobrenja izdavača ili autora.

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Knjiga:

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Book:

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

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Dissertation:

Radosavljević V. Faktori rizika za nastanak malignih tumora mokraćne bešike. Doktorska disertacija. Univerzitet u Beogradu, 1999.

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