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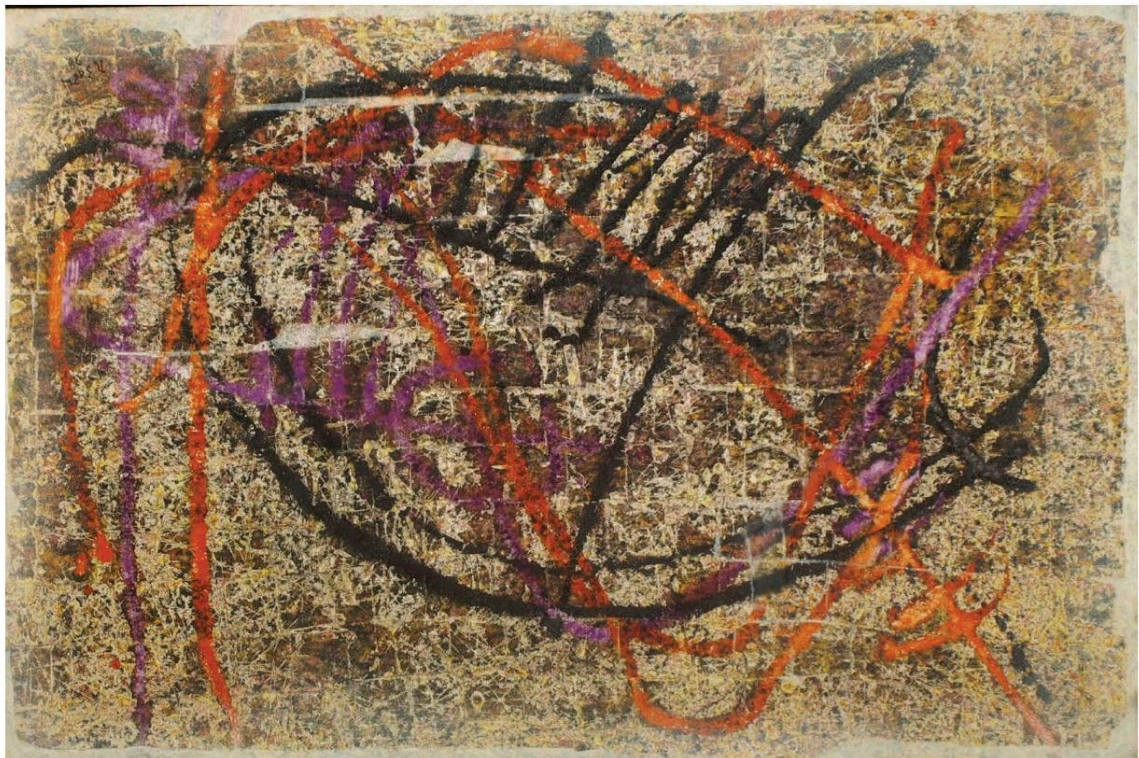
RUDOLF SABLIĆ
PAINTING WITH ACCENTUATED DRAWING, 1982.
OIL ON CANVAS

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Rudolf Sablić, slikar rođen u Vinkovcima, diplomirao je 1939. na Akademiji likovnih umjetnosti u Zagrebu te završio specijalni tečaj za slikarstvo kod Vladimira Becića.

Isprva je postimpresionističkim načinom slikao mrtve prirode i krajolike, potom je pronašao vlastiti izraz i od 1952. priklonio se lirskoj apstrakciji. Rano je pokazao istančan osjećaj za materiju slike i kromatiku, što je ostalo njegovom značajkom i u kasnijim razdobljima.

Radovi na papiru (kolaži, frotaže) suptilne su kompozicije nadahnute glazbenim oblicima; u crtežima naglašavao dinamizam pokreta u prostoru, a u potonjim djelima figurativni crtež u sklopu kromatski rasvijetljenih površina.

Bavio se ilustriranjem i opremom knjiga, kazališnom i filmskom scenografijom. (Hrvatska enciklopedija)

Contents

Original article

Experimental Liver Peroxidation Against the Background of Limb Ischemia - Reperfusion Injury – Is There a Pathogenic Difference Between Its Modifications?

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Abstract

Introduction: Use of the haemostatic tourniquet is an important step in providing first aid in open bleeding injuries. Tourniquet pressure on the extremity triggers local lipid peroxidation. However, the systemic influence of tourniquet thereof has not been fully studied.

Aim: This study aimed to evaluate the changes that occur against the background of ischemia-reperfusion syndrome (IRS) and modifications of trauma in the main gland for detoxification – the liver.

Material and Methods: In order to estimate the liver condition under the effects of a haemostatic tourniquet, animals were divided into five experimental groups, where modifications of hypoxia were performed as a result of bleeding, IRS and trauma due to mechanical fracture of the thigh.

Results: Biochemical study of the liver has shown that each type of such interventions caused the activation of lipid peroxidation in this organ. The highest increase of the malonic dialdehyde rate was observed in response to haemostatic tourniquet combined with blood loss. Additionally, its content was higher in the group combining mechanical trauma and the tourniquet compared to isolated trauma.

Conclusion: All types of interventions caused hypoxia – as a result of isolated bleeding and cessation of blood flow due to the tourniquet. However, the release of overconcentration of toxic derivatives of rhabdomyolysis, which entered the blood stream after limb release, activated the pathological mechanisms of IRS, which included intensified lipid peroxidation in the liver.

(Volotovska N. Experimental Liver Peroxidation Against the Background of Limb Ischemia - Reperfusion Injury – Is There a Pathogenic Difference Between Its Modifications? SEEMEDJ 2020; 4(2); 1-11)

Introduction

Nowadays, as in the past, the use of the haemostatic tourniquet is an important step in providing first aid [1, 2]. However, a careful study of the mechanisms of ischemia-reperfusion syndrome due to the use of haemostatic bandage is required [3]. The latter was proved to be an important cause of oxidative stress [4, 5], which promotes lipid peroxidation in the limb region under tourniquet pressure, and affects metabolism and protective reactions in the entire organism [6, 7].

The essence of ischemia-reperfusion syndrome (IRS) is that after the release of the tourniquet, a large amount of toxins enters systemic blood circulation [8, 9], which, in turn, causes an increase in functional liver activity [10-13]. However, against the background of hypemic hypoxia, full liver repair is impossible. At the moment, there is not enough data that could comprehensively explain the correlation between the IRS and the liver, especially lipid peroxidation (LPO) in the liver in the condition of IRS

Material and Methods

The experiments were performed on 260 white non-linear male rats 5-5.5 months old. The animals were removed from the experiment at the 1st hour after intervention, and on the 1st, 3rd, 7th and 14th day after trauma on the basis of thiopental-sodium anaesthesia (40 mg/kg of body weight intraperitoneal) by total bleeding from the heart. Such number was based on the need to obtain statistically significant data in each group, as well as at each time point – in order to see the stages of development of posttraumatic disease, in particular lipid peroxidation activity, caused by haemostatic tourniquet. The high number of experimental animas used is also due to higher mortality of rats in EG3 and EG5 groups (explained bellow) compared to other experimental groups Animals were divided into 5 groups (N=10 animals per group): control group (CG), where rats were administered only thiopental-sodium

anaesthesia (40 mg/kg of body weight intraperitoneally); EG1 (rubber tourniquet was applied to the upper third of the thigh for 2 hours, reperfusion lasted 1 hour (isolated IRS); EG2 (simulated blood loss in the amount of 40% of the volume of circulating blood from the femoral vein); EG3 (tourniquet on thigh was combined with 40% blood loss from the femoral vein on the other lower limb), EG4 (mechanical trauma that caused fracture of femur), EG5 (tourniquet on thigh was combined with fracture of femur of the other lower limb). Given the onset of severe pain in EG4 and EG5, an injection of a 2%-solution of lidocaine was administered for 7 days in the posttraumatic period. Animals in other groups were administered analgesics twice – on the day of intervention and on the following day.

The experiments were performed in the vivarium of I. Horbachevsky TNMU in the morning. The special room had a stable temperature (18-22 °C), relative humidity (40-60%) and illumination of 250 lux.

All experimental stages of work were performed in accordance with the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1986), the resolution of the First National Congress on Bioethics (Kyiv, 2001) and the Order of the Ministry of Health of the Ukraine No. 690 of 23 September 2009.

The activity of active thiobarbituric acid derivatives in 10% of liver homogenate samples was determined using a method based on the ability of secondary products of lipid peroxidation (LPO), especially malonic dialdehyde, during a reaction with thiobarbituric acid at high temperatures and in acidic pH, to form a coloured complex with optic density that can be registered by spectrophotometry on waves of 532 nm [14].

Statistical analysis

Statistical analysis of the obtained data was performed using Excel (Microsoft, USA). The statistical significance of the differences between independent indices was determined using Student's t-test at a normal distribution

and by non-parametric methods in other cases. The correlation coefficient was significant at $p < 0.05$.

Results

As can be seen in the data in Figures 1 and 2 against the background of ischemia modelling, the content of TBA-active products increases significantly.

Thus, in isolated ischemia-reperfusion injury (EG1) after the 1st hour, on the 1st, 3rd and 7th day, there was an increase of the index by 76.7% ($p < 0.05$), 2.8 times ($p < 0.05$), 2.5 times ($p < 0.05$) and 51.6% ($p < 0.05$), respectively, compared to the control. On the 14th day, the indicator did not return to baseline, exceeding the control by 25.9% ($p > 0.05$).

Against the background of isolated blood loss (EG3), the content of TBA-active derivatives of lipid peroxidation after the 1st hour was 2 times higher than said index in CG ($p < 0.05$), on the 1st day after intervention 4 times ($p < 0.05$), on the 3rd day 3.5 times ($p < 0.05$), on the 7th day 2.8 times ($p < 0.05$) and on the 14th day 2.4 times higher ($p < 0.05$).

As for EG3 – tourniquet on thigh was combined with 40% blood loss from the femoral vein on the other lower limb – that group included the most obvious increase in peroxidative activity compared to other groups: after the 1st hour, the index exceeded the control 2.4 times ($p < 0.05$), on the 1st, 3rd, 7th and 14th day, it was 3.8 times ($p < 0.05$), 4.9 times ($p < 0.05$), 3.6 times ($p < 0.05$) and 2.7 times higher ($p < 0.05$), respectively.

Figure 1 – Dynamics of TBA-active derivatives of lipid peroxidation in the liver (in % compared to the control level) after ischemia-reperfusion of limb and blood loss
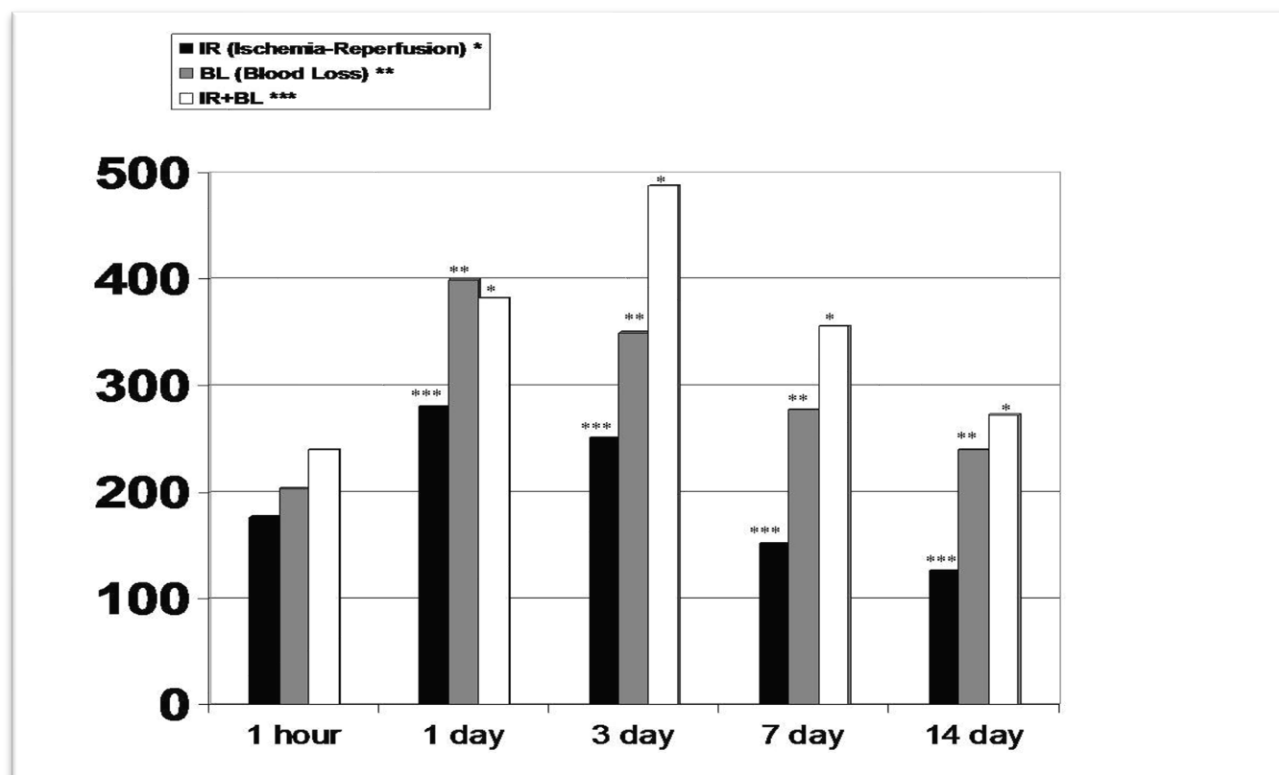
Notes: statistical differences between the 1st day, 3rd day, 7th day and 14th day in comparison with the 1st hour, 1st day, 3rd day and 7th day, respectively are significant, $p < 0.05$)
In the 1st group – * statistical significance compared to previous day
In the 2nd group – ** statistical significance compared to previous day
In the 3rd group – *** statistical significance compared to previous day

Comparison of the following groups with the effect of isolated ischemia-reperfusion of the limb on the LPO in liver tissue confirmed its pathogenic effect. Thus, against the background of isolated mechanical trauma (MT) of the thigh (EG4) in the 1st hour after the intervention, the activity of the LPO increased by 32.8% (p > 0.05). Significant increase was observed on the 1st, 3rd and 7th day after trauma, when, compared to the control, the index was higher by 65.6% (p > 0.05), 86.2% (p < 0.05) and 54.5% (p < 0.05), respectively.

On the 14th day, the index did not differ significantly from the control.

At the same time, its activity against the background of MT combined with the tourniquet (EG5) was higher. Thus, after the 1st hour, the index was higher than in CG by 66.1% (p < 0.05). On the 1st and 3rd day, it remained at the same level – it was higher compared with the control by 2.8 times (p < 0.05) and 2.9 times (p < 0.05); on the 7th and 14th day, it exceeded it by 86.2% and 31.8%, respectively.

Figure 2 – Dynamics of TBA-active derivatives of lipid peroxidation in the liver after IR of limb and mechanical trauma

Notes: Data are presented in % compared to the control level. statistical differences between the 1st day, 3rd day, 7th day and 14th day in comparison with the 1st hour, 1st day, 3rd day and 7th day, respectively, are significant, p < 0.05)
In the 3rd group – *statistical significance compared to previous day
In the 4th group – **statistical significance compared to previous day
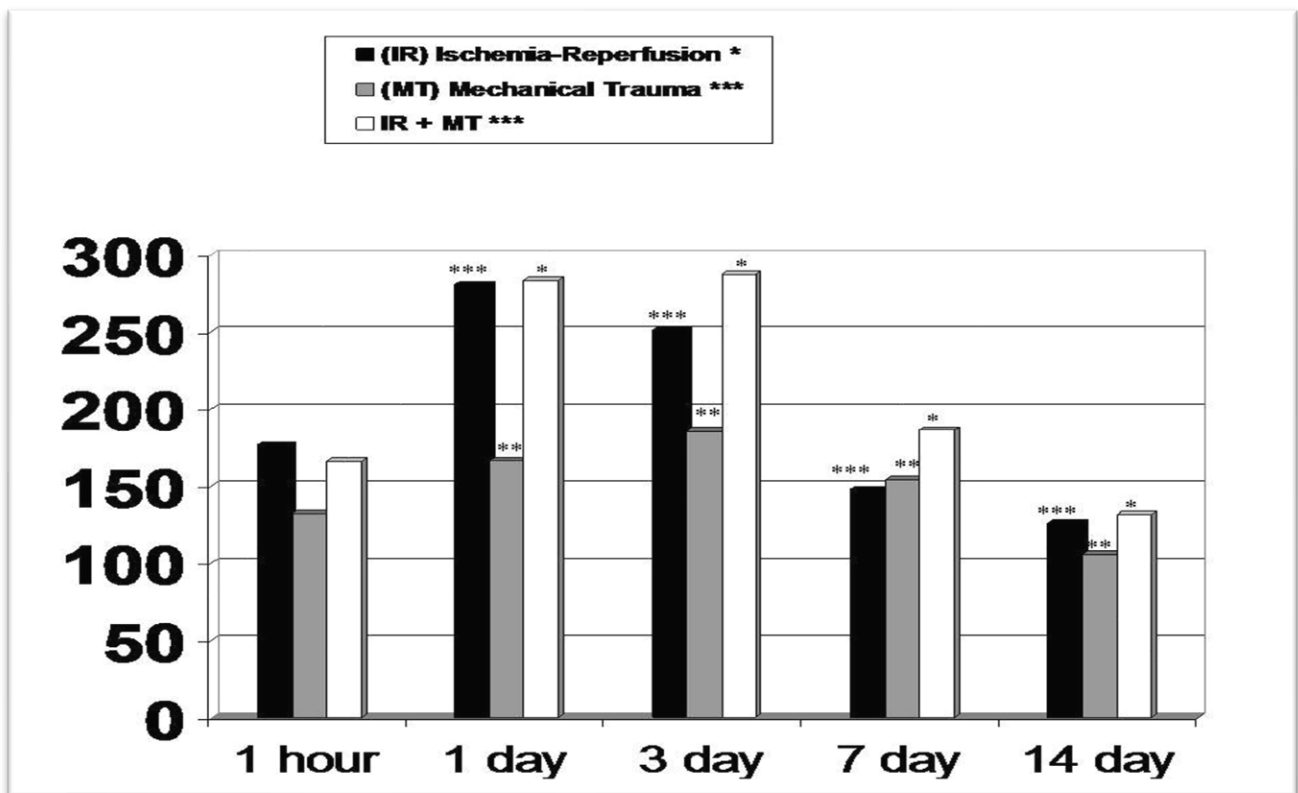In the 5th group – ***statistical significance compared to previous day

The dynamics of changes in the studied index had specific patterns caused by both ischemia and additional effects of mechanical trauma. With varying intensity, depending on the combination of pathogenic effects, the activity of LPO increased by the 3rd day, but decreased by the 14th day, although it did not reach the normal level of CG.

Thus, there was a significant increase of the index in EG1 – on the 1st day after the intervention, it remained increased by 58.8% (p < 0.05), compared with the activation of the POL in the 1st hour, after which a decrease was noted. At the same time, on the 3rd day it exceeded the activity in the 1st hour by 42.2% (p < 0.05). On the 7th and 14th day, the index was lower than the most acute period of the 1st day by 46% (p < 0.05) and by 1% (p < 0.05), respectively, and significantly lower than the 1st hour index.

In EG2, the dynamics of activity of LPO derivatives was similar, with the highest score on the 1st day, when the index exceeded the data of the 1st hour by 96.1% (p < 0.05), after which it decreased markedly. Thus, activity on the 7th day was lower by 30.5% (p < 0.05) and by 20.6% (p < 0.05) than indices of the 1st and 3rd day, respectively.

With regard to the value of the index in EG3 on the 1st day, compared to the 1st hour, the index increased significantly by 58.8% (p < 0.05), on the 3rd day it increased by 27.6% (p < 0.05) compared to the 1st day, and 2 times compared to the 1st hour. The 7th day index was 27% lower than the 3rd day index (p < 0.05), and the 14th day index was 23% lower (p < 0.05) compared to the 7th day period.

A significant increase in LPO activity was recorded on the 1st day in EG4, when the index exceeded the data obtained in the 1st hour after the intervention by 25.7% (p < 0.05). The highest activity score was on the 3rd day, when the index exceeded the data of the 1st hour by 40.4% (p < 0.05), after which it gradually decreased, becoming lower than the analogous index after the 1st hour, the 1st, 3rd and 7th day by 20.2% (p < 0.05), 36.5% (p < 0.05), 43.1% (p < 0.05) and 36.5% (p < 0.05), respectively.

LPO activity in EG5, in contrast to EG1 and EG4, in which the 1st day was a critical period, continued rising on the 3rd day, when it exceeded the level of the 1st hour and 1st day by 70.4% (p < 0.05) and 72.8% (p < 0.05), respectively. After that, LPO activity decreased sharply – on the 7th day it decreased by 35.1% (p < 0.05) compared to the 3rd day, and on the 14th day it decreased by 29.3% (p < 0.05), compared to the 7th day.

As can be seen in Table 1, by comparing the values of the studied index in groups with different severity of ischemia, it was found that in the 1st hour, in EG3 the index was higher than in EG2 and EG1 by 26.5% (p < 0.05) and by 15.3% (p < 0.05), respectively. In addition, the index in EG1 was significantly higher than in EG4 – by 25% (p < 0.05). The results in EG5 exceeded EG4 by 20.2% (p < 0.05). Thus, dynamics of lipid peroxidation were as follows: on the 1st day, the estimated index was lower than in EG2 and EG3 by 29.8% (p < 0.05) and by 26.5% (p < 0.05), respectively. Also, in EG1, which was almost identical to EG5, it was higher than in EG4 (isolated trauma) by 40.6% (p < 0.05). On the 3rd day, there was a further increase in the activity of LPO in EG3 and EG5 and a decrease in the groups EG1, EG2 and EG4. Index of EG3 was higher by 48.5% (p < 0.05) and by 28.3% (p < 0.05) than in EG1 and EG2, respectively. A comparison between the indices in EG1, EG4 and EG5 found that the index of EG1 was slightly lower than in EG5 – by 12.4% (p < 0.05) and higher than in EG4 by 26% (p < 0,05). On the 7th day, a sharp decrease of index dynamics was observed in all studied groups. The activity of LPO in EG3 remained higher than in EG2 and EG1 by 22% (p < 0.05) and by 57.4% (p < 0, 05), respectively, while the score in EG5 remained higher than in EG1 by 17% (p < 0.05). On the 14th day, the index in EG3 exceeded the indices in EG2 and EG1 by 11.9% (p < 0.05) and 2.2 times (p < 0.05), respectively. At the same time, the index of EG5 on the 14th day remained statistically significantly higher than in group 4, by 19.7% (p < 0.05), which confirms the attachment of tangential pathogenic effects caused by ischemia-reperfusion syndrome.

Table 1. Content of TBA-active derivatives of lipid peroxidation in 10% of rat liver homogenates based on variants of ischemia-reperfusion syndrome and isolated blood loss and trauma (micromolkg-1)

Group	Reperfusion period				
	1st hour	1st day	3rd day	7th day	14th day
			Control = 1.89 (n = 10)		
Group 1 isolated ischemia-reperfusion	3.34 (n = 10)	5.22* (n = 10)	4.75* (n = 10)	2.87* (n = 10)	2.38 (n = 10)
Group 2 blood loss	3.85* (n = 7)	7.55* (n = 7)	6.61* (n = 6)	5.25* (n = 7)	4.54* (n = 7)
Group 3 ischemia-reperfusion + blood loss	4.55* (n = 6)	7.22* (n = 6)	9.22* (n = 6)	6.73* (n = 6)	5.15* (n = 5)
p_{1-3}	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
p_{2-3}	< 0.05	> 0.05	< 0.05	< 0.05	> 0.05
Group 4 trauma	2.51 (n = 10)	3.13* (n = 10)	3.52* (n = 10)	2.92* (n = 10)	2.00 (n = 10)
Group 5 ischemia-reperfusion + trauma	3.14* (n = 9)	5.35* (n = 9)	5.43* (n = 8)	3.52* (n = 9)	2.49* (n = 9)
p_{1-5}	< 0.05	> 0.05	< 0.05	< 0.05	> 0.05
p_{4-5}	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05

Notes: 1.* – differences in comparison with the control group are statistically significant ($p \leq 0.05$);
2. p1-3 – the probability of differences in relation to experimental groups 1 and 3;
3. p2-3 – the probability of differences in relation to experimental groups 2 and 3;
4. p1-5 – the probability of differences in relation to experimental groups 1 and 5;
5. p4-5 – the probability of differences in relation to experimental groups 4 and 5

Discussion

This experimental study was dedicated to identifying the effects of ischemia-reperfusion syndrome of the limb on indices of lipid peroxidation in the liver tissue. It was estimated that even with isolated use of a tourniquet, as well as with isolated blood loss or injury, peroxidation processes in the liver were intensified. However, the addition of IRI statistically significantly complicated the course of injuries, indicating a side effect of the use of the tourniquet. Knowledge of the periodization of traumatic disease against the background of the studied pathology is important for the development of sanogenic effects in order to minimize this pathogenic factor.

According to the latest research, haemostatic tourniquet causes not only temporary ischemia, but is accompanied by complications such as: ischemia and nerve damage in the pressure zone of the tourniquet, limb oedema, skin pallor, muscle weakness without paralysis and pain [9,

15]. In our experiment, we paid attention to the processes of peroxidation in the liver. Although the tourniquet is an effective tool for rapid cessation of critical bleeding, its use for 2-3 hours can trigger irreversible processes of tissue death due to absence of blood supply and, in severe cases, can lead to limb amputation [16-18].

Correlation analysis showed that in the development of ischemia-reperfusion syndrome, caused by the application of a haemostatic tourniquet, obviously insufficient liver function could play a great role [19]. Formation of the active hepatic response is a predictable reaction, which is part of the syndrome of multiple organ failure. Contribution of the IRS as the reason for such pathological effects to the organism was already demonstrated [20 - 25]. In addition to that, increased lipid peroxidation is manifested in certain liver dysfunction, especially bile formation [26].

Present results confirmed the increase of liver lipid peroxidation in the presence of different conditions that cause ischemia: tourniquet and bleeding, combination of blood loss with haemostatic plait during the period, which is considered to be safe, but unfortunately further increases the degeneration of cell membranes. Thus, the results of different scientists examined the concentration of glycolytic metabolites and the content of high energy phosphates in skeletal muscles, which were analysed at various times of tourniquet ischemia during operation in bloodless field. Against the background of moderate growth of phosphocreatine in the ischemic limb, a pronounced increase of lactate (4-5 times) was observed. It is obvious that the reason for a moderate increase of glucose and glucose-6-phosphate was the activation of glycogenolysis. Since ischemia lasted from 30 to 90 minutes in these cases, the changes in indices were detected after 5 minutes [27]. Present study did not directly show a sharp increase of LPO in the tissue possibly due to depletion of the antioxidant system in the liver.

Similar results, extraordinary increase (14 times) of lactate, were obtained by another group [28]. They likewise determined a decrease of skin temperature from 35.9 to 33.5 degrees, which quickly dropped to that level in the first 15 minutes, after which it remained stable at this level [28].

Summing up, the main causes for activation of pathogenesis branches of ischemia-reperfusion injury became known as the following: rhabdomyolysis and hypoxia. The former resulted in hyperejection of toxins into the bloodstream after release of limb from tourniquet. Its basic pathogenesis – muscle oedema with subsequent development of hypovolemia, haemoconcentration and massive release of myoglobin, potassium and biologically active substances that could trigger multiorgan failure from compressed tissue [29]. Increased concentration of myoglobin in renal tubules in the conditions of acidic pH promotes intratubular obstruction and kidney dysfunction [30-32]. Another factor is hypoxia, which is known to be the cause that stimulates lipid peroxidation

[33] – and in our experiment it was activated by two causes – bleeding and ischemia. The latter provided not only the lack of oxygen, but also local compression with subsequent rhabdomyolysis. Thus, a pathological circle was formed.

In turn, liver function was recognized in this study as a protective reaction. The most severe period of LPO activity decrease was the 1st day – in the groups with isolated IRI or isolated blood loos or isolated trauma (EG1, EG2, EF4) – and the 3rd day – in the groups with combined effects – ISI with blood loss (EG3) and IRI with mechanical trauma (EG5). Present study suggests that, something caused peroxidation of lipid membranes of hepatocytes, e.g. toxins and hypoxia.

In previously published results, the active response of the liver antioxidant system was represented by increased and subsequently depleted activity of antioxidant enzymes [34]. This studies coincide with other authors' results. Orlova E. et al. estimated that SOD activity is highest in the liver and advised a correction of its insufficiency with "Vin-Vita" [35]. Liver tissue damage as a result of application of tourniquet signalled the possibility for development of multiple organ failure due to the ischemia-reperfusion limb syndrome [36, 37].

To sum up the above, the highest increase of malonic dialdehyde rate was observed against the background of a combination of haemostatic tourniquet on the thigh with blood loss. Additionally, malonic dialdehyde content was higher in the group with a combination of trauma and the tourniquet in comparison with isolated mechanical trauma. This fact confirms the role of the tourniquet as a factor that complicates the course of traumatic disease due to the development of the ischemia-reperfusion syndrome. All of our interventions caused hypoxia – as a result of isolated bleeding and cessation of blood flow due to haemostatic tourniquet. Among the causes that activated pathological mechanisms of ischemia-reperfusion injury, attention should be paid to the influences of toxic derivatives of rhabdomyolysis, which entered the

bloodstream in excessive concentrations after limb release from tourniquet. Intensification of the liver function was the result of a protective response to pathogenic effects.

The knowledge gained is very important, as in recent years the number of situations accompanied by bleeding from large blood vessels increased significantly. In addition, the use of haemostatic tourniquets is still one of the most efficient and easy way to control blood loss. Thus, their importance cannot be denied, but side effects and their pathogenic effects on the systemic level require more detailed research.

Conclusion

Intensification and decrease of liver function was the result of a protective response to pathogenic effects. Periods of lipid peroxidation activity allowed us to find 2 periods of exhaustion of wounded organisms against the background of blood loss, combined with the use of haemostatic tourniquets and consequently affected by ischemia-reperfusion syndrome – they are on the 3rd and 14th day against the background of bleeding combined with IRS. Knowledge of this will help to better understand the pathogenesis of traumatic disease and with a new form of oxidative stress and development of the best treatment.

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Original article

Stature Estimation from the Right External Ear of Undergraduate Students in South-East Nigeria

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Abstract

Background: Ethnicity, stature and gender influence the anthropometric characteristics of the right ear, thus creating variations which are helpful for sex identification and medico-legal purposes in forensic examinations. This study has produced anthropometric data on the right external ear among the Ikwo people in southeastern Nigeria.

Materials and Methods: Ear and lobular indices were obtained from the dimensions of the right external ear of 240 Ikwo adults aged 18-35. Also, three predictive models (equations) were produced in the study.

Results: Descriptions of the right external ear for the study population were obtained. Aside from ear indices, which were higher in females than in males, males were taller in stature than females ($P < 0.001$). Regarding correlation, right ear parameters in both sexes appeared positive and strongly correlated with stature. Regression models strongly predicted stature based on external ear measurements.

Conclusion: Medical applications (monitoring diseases, forensics, industrial design and apparel design) of this study cannot be overemphasized in this world of medical sciences, in particular when it comes to understand the human external ear in terms of hearing and communication.

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Introduction

Hearing, balance and communication are an integral part of human life (1). Medical sciences, especially communication medicine, are raising medical standards to understand not only how the external ear works (hearing, balance and communication), but also to explain clinical disorders (2). By applying biochemical methods, forensic investigators collect information at a crime scene, such as fingerprints, hair strands, blood, even from notebooks, and trace the details of the person (3). In fact, the application of anthropometry to body structures has proved to be useful in forensics, genetics, agriculture, industry and neurosciences (4). Anthropometry has been dealing with the measurement of physical features and functions of the human body, including linear dimensions, weight, volume and range of movement (5, 6). So, measuring the human ear is not an exception in this contemporary science. In another setting, scientists and anthropologists apply anthropometry to study human body measurements using parameters such as height, weight, triceps skinfold, subscapular skinfold, arm circumference, abdominal and calf circumference, knee height and elbow breadth (7-9).

In terms of the anatomy, the ear is an advanced and very sensitive organ of the human body (10, 11). The ear's function is to transmit and transduce sound to the brain through the parts of the ear: the outer ear, the middle ear and the inner ear (11, 12). Diseases and abnormalities of the ear are enormous. There are a number of different infections, diseases and even cancers that can affect the ear and ultimately lead to hearing loss (otosclerosis, otitis media and Ménière's disease) (13-15). Stature estimations also include body height, usually estimated by applying anatomical and mathematical techniques using long bones (foot dimensions, hand and head measurements). It has been observed that in males, total height is sometimes greater and correlates more with stature than in females (11,16-18). The study using ear indices, lobular indices and stature of the Ikwo people in southeastern Nigeria will be useful in forensic science, ear health and clinical sciences.

Material and Methods

Study location

Ebonyi State is located in southeastern Nigeria and inhabited primarily by the Igbo people, with the city of Abakaliki as its capital. There are other major townships, including Afikpo, Onueke, Ezzamgbo, Edda, Effium, Aba Omege, Amasiri, Unwana, Echara Ikwo, Egu-Ubia, Ụbụrụ, Onicha. The state covers a large area of 5,533 km². Its latitude is 6˚10'40.7"N and its longitude is 7˚57'33.43"E. Ebonyi has a tropical climate, with the summers much rainier than the winters. When it comes to agriculture, it is one of the most important states in Nigeria, known for its popular brand of rice Abakaliki and other food crops grown in large quantities, such as yam, cassava, maize, cocoyam, cowpea and groundnut.

Study design

In the study, about 240 students (male = 120 and female = 120) from Alex Ekwueme Federal University Ndufu-Alike in the 2018/2019 academic session of aged 18-35 years were recruited. The informed consent was obtained and signed. Also, ethical approval (FUNAI/RE/120) was obtained prior to the study. A digital anthropometric method was applied to measure ear length and breadth, base of auricle, lobe length and breadth while stature measured using a stadiometer (Figure 1 and Figure 2). Also, the ear and lobular indices, lobe attachments, and ear shape were calculated and from this, differentiating sex based on external ear variables was achieved. Ear length was measured as the perpendicular distance between the tangents to the highest point on the helix and the lowest point on the lobule. Ear index was measured as distance from the ear width/Ear length ×100. Lobular index was measured as the lobular width/Lobular length ×100.

Figure 1. The stadiometer used for measuring the stature of the participants from Alex Ekwueme Federal University Ndufu-Alike, Ebonyi State, Nigeria

Stature was measured from the foot to the vertex of the head. All measurements were made by two research assistants using a scale to the nearest millimetre.

Figure 2. Points used for anthropometric measurements of the right external ear among the subjects from Alex Ekwueme Federal University Ndufu-Alike, Ebonyi State, Nigeria
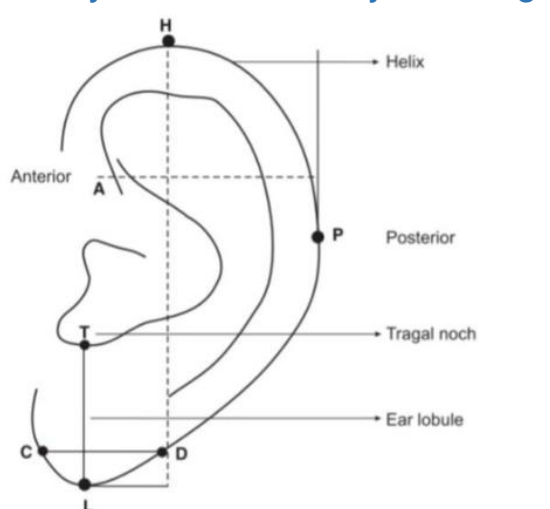
*****Total ear height= L-H, ear width= A-P, lobular height= L-T, lobular width (C-D).*

Inclusion criteria

All participants recruited for the study must belong to the same ethnic group (Igbo). Must be enrolled as students in Alex Ekwueme Federal University Ndufu-Alike Ikwo, Ebonyi State, southern part of Nigeria in the 2018/2019 academic session. Also, subjects must belong to the age bracket of 18-35 years.

Exclusion criteria

Subjects that are non-Igbo in the university were excluded from the study.

Statistical analysis

The data was collected using Excel spreadsheets (MS Office, 2013). The statistical analysis of the data was performed using SPSS Version 20 (IBM Corp., Armonk, NY). Student's t-test was performed after determining the normality of data (P<0.001). A Pearson's product moment correlation coefficient was applied on the right ear for both sexes. Also, the linear regression analysis was used to generate regression models to ascertain the level and strength of association between stature and right ear morphometry.

Results

Right ear indices (see Table 1) were higher in females than in males (54.8±2.53 mm and 51.52±2.51 mm respectively), while males were taller in stature than females (161.42±7.81 mm and 160.21±7.01 mm respectively). Lobular indices (99.45±8.62 mm and 99.43±8.61 mm) appeared to be the same in both males and females (P<0.001). All parameters appeared to be significant (P<0.001).

Table 1. Anthropometric measurements using the t-test to measure the right ear and stature among male and female adults of the Ikwo people (mm)

Parameters (mm)	Males N = 120	Females N = 120	P-values
EI	51.52±2.51	54.81±2.53	0.001
LI	99.45±8.62	99.43±8.61	0.001
S	161.42±7.81	160.21±7.53	0.001

*** EI: Ear indices, LI: Lobular indices, S: Stature

The analysis (see Table 2) showed a positive and stronger correlation in males than in females regarding the three parameters (P<0.001). In terms of correlation, ear indices were positive and stronger in males than in females (r = 0.92 and r = 0.82 respectively), while the value of lobular indices was r = 0.90 and 0.86 respectively. Furthermore, correlation between the ear variables with stature were positive and strong in both males and females (r = 0.89 and r = 0.80 respectively). In the regression analysis (see Table 3 and 4), R2 adjusted values were 0.94, 0.91 and 0.85 for ear indices, lobular indices and stature for males, and 0.90, 0.89, and 0.65 for females respectively. In this case, the study appeared strongly predictive regarding the three ear measurements with stature for both sexes and statically significant (P<0.001).

Table 2. Correlational analysis of the right ear and stature among male and female adults of the Ikwo people

Parameters	Male Stature		Female stature	
	r	P-value	r	P-value
EI	0.92	0.001	0.82	0.001
LI	0.90	0.001	0.86	0.001
S	0.89	0.001	0.80	0.001

***EL: Ear length, EW; Ear width, LL: Lobular length, LW: Lobular width, EI: Ear indices, LI: Lobular indices, S: Stature

Predictive equations for ear indices, lobular indices and stature were S = 89.14+(1.61×EI), 88.71+(1.71×LI) and 74.45+(1.68×S) for males, and S = 88.12+(1.60×EI), 86.27+(1.68×LI) and 74.45+(1.66×S) for females respectively.

Discussion

Scientists studying human ears are adding new dimensions to future discussions about the external ear in humans (1, 2). Our study showed that males were taller in stature than females. This also corroborated the sexual dimorphic features studied in anthropology. Moreover, ear indices appeared to be higher in males than in females, while the lobular indices were the same in both sexes. The human ear gives a clue to estimating stature at the time of criminal investigations and legal discussions (2, 5). In northern Nigeria, a study of the Hausa ethnic group aged 16-35 used the digital and face-arth software and observed sexual dimorphism in ear

analyses (12, 17). Also, a comparative study of the external ear involving medical students showed that all ear and lobular indices were higher in males than in females (13, 18, 19). The morphometric variables of the right female ear were somewhat inconsistent with our study, as they were higher compared to the right ear in males, although the difference was not significant (20, 21). The reason for the differences observed in the study can be attributed to the influence of ethnicity, genetics, climate and nutrition (22-24).

Our study follows the application of Krogman's principle to determine regression formulae used to estimate stature from bones, fragments of bones or measurements of body parts (2), and it was reported that regression formulae presents a better estimation and association between ear indices and stature. Our study (Table 3, 4) produced three prediction models (ear indices, lobular indices and stature).

Table 3. Regression equations for predictions of stature from the right ear among male adults of the Ikwo people

Model			Male
	R^2	P-value	R-equation
EI	0.94	0.001	$S = 89.14 + (1.61 \times EI)$
LI	0.91	0.001	$S = 88.71 + (1.71 \times LI)$
S	0.85	0.001	$S = 74.45 + (1.68 \times S)$

*** EI: Ear indices, LI: Lobular indices, S: Stature

Table 4. Regression equations for predictions of stature from the right ear among female adults of the Ikwo people

Model			Female
	R^2	P-value	R-equation
EI	0.90	0.001	$S = 88.12 + (1.60 \times EI)$
LI	0.89	0.001	$S = 86.27 + (1.68 \times LI)$
S	0.65	0.001	$S = 74.45 + (1.66 \times S)$

*** EI: Ear indices, LI: Lobular indices, S: Stature

All the regression-adjusted values (R2) for ear indices, lobular indices and stature were positive and higher in males than in females. All the anthropometric parameters were significant (P<0.001). Some studies (25-28) were consistent with our study that predicted stature from both ears in a simple linear regression analysis. Furthermore, some studies implicated the evolutionary changes to have influenced ear measurements (29-30). Again, interracial marriages have become the norm in many cultures and have been producing genetically similar population (31). Apart from ear

dimensions, some studies deal with other human body parts to estimate stature using regression models (32). The similarity in lobular indices in our subjects (Table 1-2) may result from the fact that they all belong to the same generation, with great grandparents who carried the same genetic interpretation.

Regarding sexual dimorphism, there reference to the correlation and estimation of stature from other body parts, for instance in a study of estimation of stature from length of fingers, estimation of stature from index and ring finger

16

length and estimation of stature from hand and phalange length (32-33). Also, studies on the estimation of stature from craniofacial measurements, sex differences in morphometry of North Indian acetabula (34-35). In particular, in some cases, it has been pointed out that the reason behind the association between stature and anatomical parts could be traceable to protein intake in early childhood with body composition, insulin-like growth factor in mid-childhood and early adolescence, environmental and genetic factors (21, 26). Importantly, human beings are considered to be bilaterally symmetrical in both thumb and fingers of both hands, irrespective of sex or handedness, allowing for an estimation of stature of an individual, which plays an important role for individuality (33, 35). To further support our study, one research discussed the right and left ears as mostly asymmetrical with respect to ear length, ear breadth and base of auricle, which were larger in males than in females, while the lobe length and breadth were larger in females as compared with males (chi square test, p ⩽ 0.001), concluding that the ear parameters gave a moderate to good sex identification accuracy (36). This study could be useful for human embryology and monitoring gestational age, for industrial designs and forensic education.

Conclusion

Ear and lobular indices appear to be correlated with stature. Using the three regression models, a correlation between stature and ear measurement can be predicted, meaning that ear length can be used to identify individual stature. This study is helpful for communication medicine, forensic study and basic medical science.

Disclosure

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Original article

Neoadjuvant Chemotherapy Affects TFF3 Peptide Expression in Luminal B Subtype of Breast Cancer – A Pilot Study

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Abstract

Aim: Trefoil factor family 3 (TFF3) peptide is normally expressed by epithelial cells in breast ducts, but it is also associated with different pathological conditions, including breast cancer. It is considered a marker of poor prognosis and associated with increased resistance to chemotherapy. Data on the effect of chemotherapy on TFF3 peptide expression are scarce. The aim of this pilot study was to assess suitability of research on this topic for large-scale studies.

Methods: Formalin-fixed, paraffin-embedded samples of core biopsies and of surgically removed tumors from patients with luminal B subtype of breast cancer were used for immunohistochemical analysis. Changes in TFF3 peptide and Ki-67 expression and microvessel density (MVD) values before and after chemotherapy were analyzed, as well as the association between TFF3 peptide expression and Ki-67 expression and MVD values.

Results: Significant reduction in TFF3 and Ki 67 expression was observed after chemotherapy, while MVD values did not differ significantly before and after chemotherapy. The association of TFF3 peptide expression and Ki-67 expression and TFF3 peptide expression and MVD values was not significant before or after chemotherapy.

Conclusion: The data obtained in this pilot study suggest that a large-scale study is justified, and it other breast cancer subtypes should be included.

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Introduction

Early detection of breast cancer enables better treatment. Also, adequate methods for monitoring response to treatment help in planning further treatment. Modern breast cancer diagnostics include core biopsy with immunohistochemical characterization of tumor tissue. According to St. Galen consensus, breast cancer is classified into following surrogate subtypes: luminal A, Her2-positive luminal B, Her2-negative luminal B, Her2-enriched and triple negative breast cancer. Such classification helps with prognosis and choice of therapy and is based on expression of estrogen receptor, progesterone receptor, Her2 and Ki 67 in the tumor [1]. Still, there is a need for further characterization of oncogenes and molecular markers connected to the onset, progression and relapse of breast cancer [2].

In modern breast cancer therapy, neoadjuvant chemotherapy is considered a standard approach in an increasing number of clinical settings (e. g. in patients with tumor subtypes that are highly sensitive to chemotherapy and in patients that have tumors > 2 cm and/or metastases in axillar lymph nodes), also allowing for cancer down-staging and helping with breast conservation [3,4]. It also alters expression of different markers in cancer cells, such as hormone receptors, Her2, claudin-1 and claudin-3 [5,6].

Trefoil Factor Family (TFF) is a group of three small secretory peptides – TFF1, TFF2 and TFF3 peptide, mostly secreted by epithelial cells of various mucosal sites. Their major role is mucosal protection, and they are known to affect cell migration, apoptosis, angiogenesis and immune response [7]. Of the three peptides, TFF3 peptide is the most widespread in the human organism, and is normally expressed by epithelial cells in breast ducts [8,9]. However, presence of TFF peptides is also associated with different pathological conditions, including breast cancer. TFF3 peptide is expressed in different subtypes of breast cancer, most predominantly in hormone receptor-positive subtypes and is considered a marker of poor prognosis because it promotes metastatic seeding and neoangiogenesis [10,11]. Our previous research showed that TFF3 peptide expression was the highest in luminal B subtype, moderately differentiated cancers (grade II) and in tumors with moderate expression of Ki 67, implying that TFF3 peptide might be considered a possible marker for determining tumor status, which might influence the choice of therapy [12]. Presence of TFF3 peptide in breast cancer cells is associated with increased resistance to chemotherapy, while cancers with complete response to chemotherapy have lower or no TFF3 peptide expression [13]. Despite the growing interest in TFF3 peptide research in the context of breast cancer diagnostics and therapy, there are no data on the effect of chemotherapy on TFF3 peptide expression in breast cancer tissue. Therefore, we launched a pilot study in order to assess the suitability of a large-scale study.

Material and Methods

Study design and material

This was a cross-sectional pilot study performed on tissue samples archived at the Department of Pathology and Cytology, University Hospital Centre Zagreb. Tissue samples of luminal B subtype of breast cancer were used in the study. They were taken during routine diagnostic and therapy procedures from women whose treatment included neoadjuvant chemotherapy. There were 22 tumor samples overall; 10 were Her2-negative, and 12 were Her2-positive. Also, samples from 5 normal breast tissue controls were included for comparison of TFF3 peptide expression. For each tumor, a core biopsy sample (pre-chemotherapy) and a sample of surgically removed tumor (post-chemotherapy) were used. Chemotherapy protocols of each patient are available in the Supplemental table. Changes in TFF3 peptide and Ki-67 expression and in microvessel density (MVD) values before and after chemotherapy were analyzed, as well as the association between TFF3 peptide expression and Ki-67 expression and MVD values.

Immunohistochemical staining

For each patient, archived immunostained slides (hormone receptor positivity, Ki-67 status and Her2 status) were revised in order to confirm the cancer subtype. Paraffin blocks of samples that met the criteria to be included in the study (luminal B subtype) were cut on a microtome (Leica, SM2000R, Leica Biosystems, Nussloch, Germany) into 3-5-μm-thick slides and mounted onto adhesive slides suitable for immunohistochemistry. Slides were stained for TFF3 peptide and CD34 endothelial marker, and Ki-67 staining scores (share of immunopositive nuclei) were obtained from the aforementioned archived slides. Antigen retrieval was performed using the Dako PT Link device (Dako, Agilent Technologies, Santa Clara, USA) with the EnVision FLEX Target Retrieval Solution, Low pH for TFF3 peptide and Ki-67 staining, and the Dako EnVision FLEX Target Retrieval Solution, High pH for CD34 staining. After that, the slides were stained in the Dako TechMate device using standard protocols as per the manufacturer's instructions. Purified monoclonal mouse anti-human primary antibody to TFF3 peptide was used at 1:1000 dilution (Sigma-Aldrich, St. Louis, USA), for Ki-67 monoclonal mouse, anti-human antibody was used at 1:75 dilution (Dako), and for CD34 monoclonal mouse, anti-human antibody was used at 1:50 dilution (Dako). For immunostaining visualization, the Dako EnVision FLEX kit was used. All slides were counterstained with hematoxylin, dehydrated and coverslipped using the Entellan covering medium (Merck, Darmstadt, Germany). For TFF3 peptide staining, appendix tissue was used as positive control, while placenta tissue and the appendix tissue with primary antibody omitted were used as negative control. The appendix tissue was used as positive and negative control for CD34 (primary antibody omitted on negative controls).

TFF3 peptide tissue expression level was assessed using a modified Quick score method that incorporates the share of immunopositive cells as well as staining intensity and reports the staining on a scale of 0 to 7 [12,14]. CD34 staining was used to obtain MVD (number of small blood vessels per surface unit under a 10 × objective)

[15]. Digital photographs were taken using the Axiocam 305 color camera mounted on a Zeiss Axio imager A2 light microscope and ZEN 2.3 lite (blue edition) software (Carl Zeiss Microscopy GmbH, Jena, Germany).

The investigations were carried out following the rules of institutional and national ethical standards and the rules of Helsinki Declaration of 1975, revised in 2013. The study has been approved by the ethical committees of the University Hospital Centre Zagreb (Class: 8.1-18/256-2, No.: 02/21 A6) and the Faculty of Medicine Osijek (No. 2158-61-07-19-10).

Statistical analysis

Patient age was expressed using mean values and standard deviation, while all other data were expressed using the median and interquartile range. For comparison of dependent samples, the non-parametric Wilcoxon test was used, and the Kendall's Tau test was used for the analysis of the association of numerical variables. Coefficient of determination was obtained by linear regression after correlation testing. For comparison of independent samples, the Mann-Whitney U test was used.

Data on Ki 67 expression after chemotherapy were unavailable for two patients, and these were excluded from the analyses that included Ki 67 expression. The statistical analysis was done using the MedCalc software (18.11.6, MedCalc Software bvba, Ostend, Belgium) and a P-value less than 0.05 was considered statistically significant.

Results

The mean age of cancer patients was 53.1 years (st. dev. 13.2) at the time of diagnosis; the mean age of subjects with no cancer was 55.6 years (st. dev. 9.07). TFF3 peptide staining was detected both in breast cancer tissue and in normal breast tissue.

The analysis of Quick score values for TFF3 peptide expression in patients with luminal B subtype of breast cancer showed a significant

reduction in TFF3 peptide immunostaining after chemotherapy (Table 1, Figure 1).

Table 1. TFF3 peptide expression, Ki 67 expression and MVD values in luminal B breast cancer subtype before and after chemotherapy

	N	Before chemotherapy	After chemotherapy	P-value*
TFF3 Quick score	22	6 (6 – 7)	5 (4 – 6)	0.005
Ki-67 score (%)	20	31 (25 – 38.5)	11.5 (4.8 – 21)	< 0.001
MVD value (/mm²)	22	79.9 (62.3 – 96.3)	71.3 (54.8 – 99.4)	0.59

* Wilcoxon test

Figure 1. Immunohistochemical staining of luminal B breast cancer tissue for TFF3 peptide
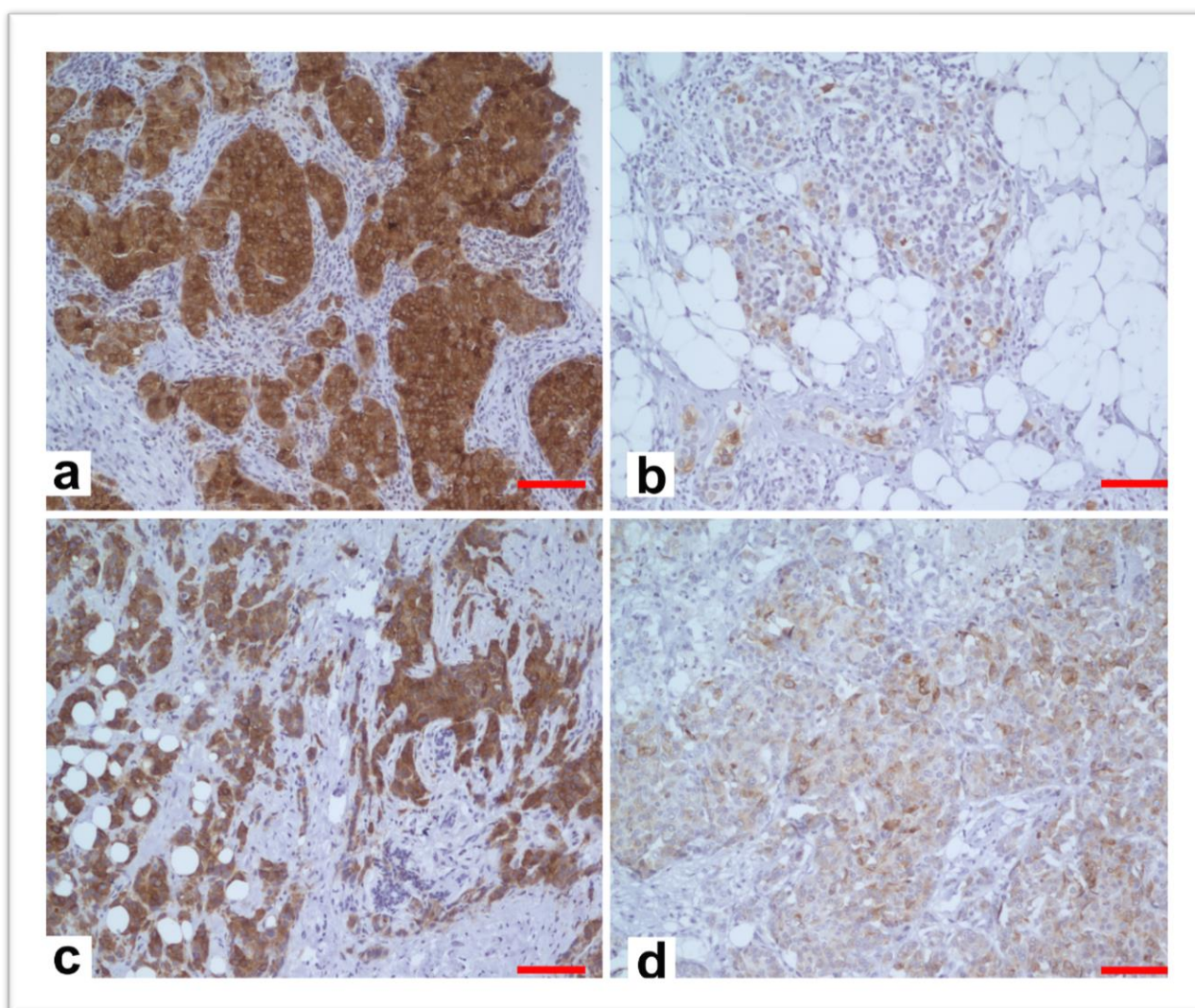
a) Her2-positive cancer before chemotherapy (core biopsy), Quick score = 7; b) the same tumor after chemotherapy (surgical excision), Quick score = 3; c) Her2-negative cancer before chemotherapy (core biopsy), Quick score = 6; d) the same tumor after chemotherapy (surgical excision), Quick score = 4. Scale bar: 0.1 mm

Compared to normal breast epithelium, Quick score values did not differ significantly in breast cancer before chemotherapy, while after chemotherapy, the median Quick score value was lower and close to significant values (P = 0.92 and 0.08, respectively; Mann-Whitney U test). Ki 67 expression in patients with breast cancer was significantly lower after chemotherapy (Table 1). Microvessels were identified by CD34 staining. On several slides, tissue fibroblasts caused some false positivity, which was excluded from MVD calculation. MVD values did not differ significantly before and after chemotherapy (Table 1).

Supplemental Table 1. The table shows all cancer patients with their corresponding neoadjuvant therapy regimens and durations used in this study.

Patient code	Neoadjuvant therapy regimen (type and duration; notes)
2 HER2-	AC-T 6 months
3 HER2-	AC-T 6 months
4 HER2-	AC-T 6 months
5 HER2-	AC-T 6 months
6 HER2-	AC-T 5 months
7 HER2-	AC-T 7 months
9 HER2-	AC-T 6 months
10 HER2-	AC-T 6 months
11 HER2-	AC-D (3 x AC + 1 x D) 2 months; discontinued due to intolerance
12 HER2-	AC-D 2 months
1 HER2+	ACdd 2 months + PHP
2 HER2+	ACdd-THP* 6 months
3 HER2+	AC-T 5 months**; trastuzumab 7 months
4 HER2+	AC-T 6 months
6 HER2+	AC-THP 6 months
8 HER2+	AC-PHP 6 months
9 HER2+	AC-PHP 5 months
10 HER2+	AC-TH 7 months
11 HER2+	AC-PHP 6 months
12 HER2+	AC-T + 4 PH 7 months
13 HER2+	AC-T + PH duration unknown
14 HER2+	AC-THP 6 months

* trastuzumab taken for only 3 months

** regimen discontinued after the 4th paclitaxel dose

Abbreviations: AC-D – doxorubicin, cyclophosphamide, docetaxel; ACdd – dose-dense AC regimen; AC-T – doxorubicin, cyclophosphamide, paclitaxel; AC-PH – doxorubicin, cyclophosphamide, pertuzumab, trastuzumab; AC-PHP: doxorubicin, cyclophosphamide, paclitaxel, trastuzumab, pertuzumab; AC-TH – doxorubicin, cyclophosphamide, paclitaxel, trastuzumab; AC-THP – doxorubicin, cyclophosphamide, docetaxel, trastuzumab, pertuzumab

The association of TFF3 peptide expression and Ki 67 expression, as well as of TFF3 peptide expression and MVD values, was not significant before or after chemotherapy, although the P-value for the association of TFF3 peptide expression and MVD values before chemotherapy was close to the significance level (Table 2).

Table 2. Association of TFF3 peptide with Ki-67 expression and MVD values in luminal B breast cancer subtype before and after chemotherapy

		Tau	95% CI of Tau	P-value*	R²
Before chemotherapy					
TFF3	Ki-67	0.04	-0.27 – 0.40	0.84	0.017
	MVD	0.28	-0.19 – 0.58	0.07	0.090
After chemotherapy					
TFF3	Ki-67	-0.25	-0.52 – 0.16	0.11	0.031
	MVD	0.25	-0.11 – 0.53	0.11	0.074

* Kendall's Tau test

Discussion

This pilot study revealed a significant reduction in TFF3 peptide and Ki 67 expression after neoadjuvant chemotherapy, while no significant changes in MVD values were found. Until now, no data have been available on the effect of chemotherapy on TFF3 peptide expression in breast cancer cells or its association with Ki-67 and MVD before and after chemotherapy. Since it is known that overexpression of TFF3 peptide affects proliferation of breast cancer cells, stimulates angiogenesis and contributes to chemotherapy resistance, information about the effect of chemotherapy on TFF3 peptide expression should be helpful in understanding its role in breast cancer [11,13].

Although expression of TFF3 peptide increases the chances of local invasion and metastatic seeding, its role in breast cancer is still not fully understood [10]. Some researchers argue that it should not be considered an oncogene, but a normal breast protein "abused" by cancer cells, and similar conclusions could be drawn from other diseases involving TFF3 peptide expression [9,16]. Due to its effect on disease progression and chemotherapy results, TFF3 peptide is considered a potential therapeutic target in certain types of breast cancer, and current research looks promising [13,17,18]. Nevertheless, since TFF3 peptide has important

physiological roles in the human organism, a careful risk/benefit assessment is needed. For example, TFF3 peptide is important for maintenance of intestinal mucosa, which is often affected by chemotherapy, hence targeting TFF3 peptide might worsen certain chemotherapy side-effects [19].

Newly-formed small blood vessels can be an early predictor of metastatic potential, and CD34 is one of the most important endothelial markers used for small vessel detection [20]. MVD values did not change significantly in this research, and the correlation between MVD and TFF3 Quick score was weak. Hence, although TFF3 peptide stimulates angiogenesis, it seems that a reduction in TFF3 expression after chemotherapy did not affect tumor vascularity. A reduction in Ki 67 expression was expected, although it was interesting to see that there was no significant association between TFF3 peptide and Ki 67 expression before or after chemotherapy, probably due to different mechanisms regulating their expression.

Limitations to this pilot study were the analysis of only one subtype of breast cancer and sample size. Nevertheless, based on the data obtained, we believe a large-scale study including other breast cancer subtypes is justified. Since TFF3 is a secretory protein found in different body liquids, there is also a possibility

of a quantitative evaluation of TFF3 levels (e.g. in serum) by diagnostic methods. Such sampling is less invasive than a core or surgical biopsy and could be used as a screening method in certain conditions, which should be further investigated in the future.

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[1]

Original article

The Need for Systematic Monitoring and Improved Surveillance of Hepatitis C Patients in Croatia

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Abstract

Aim: The aim of this study was emphasizing the need for a more systematic monitoring of patients diagnosed with HCV in Croatia.

Methods: From 2014 to 2018, at the University Hospital for Infectious Diseases, sera from 23,524 patients were tested for HCV. Confirmatory testing was performed by Western Blot. Adult patients with an anti-HCV positive screening test were analysed. HCV RNA was quantified by real-time PCR, while HCV genotypes and subtypes were determined by PCR and the reverse hybridization method.

Results: A total of 428 anti-HCV ELISA-positive adults were analysed (68.7% males, 31.3% females, median age 43 years, range 19-88 years). Hepatitis C was confirmed by WB in 390, while 28 patients had borderline WB results. Anti-HCV was not confirmed by WB in 10 patients. HCV RNA was tested in 331 patients and viremia was detected in 218 patients. There was no data on HCV RNA in 97 patients (22.66%). HCV genotypes/subtypes were determined in 185 of 218 anti-HCV WB positive patients. Genotype 1 was detected in 97/185 (52.43%), genotype 2 was detected in 3/185 (1.62%), while subtype 3a was detected in 76/185 (41.08%) and genotype 4 in 9/185 patients (4.86%).

Conclusion: In a five-year period, the HCV seroprevalence rate in subjects tested at the University Hospital for Infectious Diseases was 1.81%. According to the data analysed, almost one quarter of patients with detected anti-HCV antibodies were not treated further, which indicates the need for a systematic monitoring of patients diagnosed with HCV. It is necessary to determine viremia after a positive anti-HCV screening result in order to initiate treatment and prevent HCV-related complications.

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Introduction

Both a general practitioner and a secondary-care specialist are involved in the diagnosis of chronic viral hepatitis and in the clinical management of infected patients [1]. The initial diagnosis and management of chronic hepatitis relies on primary-care physicians to identify and screen patients who were in contact with hepatitis C, since most people with chronic hepatitis are asymptomatic until the development of cirrhosis or hepatocellular carcinoma [2]. The WHO recommends education of primary care physicians about the risks factors of acquiring HCV infection and encourages increased activity in finding new patients in screening programs.

Approximately 71 million people worldwide have chronic hepatitis C, of which 15 million in Europe, and about 400,000 people in the world die from the effects of hepatitis C per year. Croatia is a country with a low incidence rate of hepatitis C. It is estimated that 40,000 people are anti-HCV positive in Croatia. In May 2016, the World Health Assembly endorsed the Global Health Sector Strategy on viral hepatitis, which proposed to eliminate viral hepatitis as a public health threat by 2030. Elimination of viral hepatitis as a public health threat requires 90% reduction in incidence and 65% reduction in mortality in comparison with the 2015 baseline, along with the improvement of viral hepatitis diagnostic coverage up to 90% and treatment of 80% of eligible patients [4]. To reach these targets, the WHO Regional Office for Europe is encouraging Member States to plan and strengthen national responses to viral hepatitis through awareness-raising, surveillance, prevention, strengthening of laboratory capacity and provision of guidance on testing and treatment [5]. Implementation of the Global Health Sector Strategy would prevent 7.1 million deaths between 2015 and 2030 [4].

Hepatitis C virus (HCV) diagnostics starts with determination of anti-HCV antibodies [3,6]. Each positive anti-HCV screening result requires further HCV RNA detection in order to confirm

actual contact with HCV in the past. If HCV RNA is negative, positive anti-HCV results should be confirmed by Western immunoblotting (WB) [7,8]. In 2019, WHO recommended offering treatment with direct acting antivirals (DAA) to all individuals diagnosed with HCV infection who are 12 years of age or older, irrespective of disease stage (with the exception of pregnant women) [9]. Priority for treatment is defined by the stage of fibrosis, the risk of progression to advanced disease, the presence of extrahepatic manifestations and comorbidities, patients with HBV or HIV coinfection and patients with indication for organ transplantation [10].

The aim of this study was emphasizing the need for a systematic monitoring of patients diagnosed with HCV. This must include the determination of viremia after a positive anti-HCV screening result in order to administer a corresponding therapy since, according to the data analysed, almost one quarter of patients with detected anti-HCV antibodies are not treated further.

Patients and Methods

From 2014 to 2018, at the University Hospital for Infectious Diseases in Zagreb, sera from 23,524 patients were tested for anti-HCV antibodies by using either enzyme immunoassay (ELISA) for simultaneous detection of anti-HCV antibodies and capsid antigen (HCV Ag-Ab) or only anti-HCV antibody determination (BioRad, France). As required, each of the 428 positive ELISA sera was confirmed by Western Blot (INNO-LIA HCV Score Fujirebio; recomLine HCV IgG Mikrogen). HCV RNA was quantified by real-time PCR (Abbott RealTime HCV) and HCV genotypes and subtypes were determined by PCR and the reverse hybridization (INNO-LiPA HCV Genotyping) method.

Results

This study included 428 anti-HCV ELISA-positive adults who were newly diagnosed in the period from 2014 to 2018 and over 18 years of age. Median age of patients was 43 years, ranging from 19 to 88 years. Regarding gender, 68.7% of

the patients were male and 31.3% were female. According to the available diagnoses from the referral, 293 (293/428; 68.45%) patients had chronic hepatitis or elevated hepatic lesion, 52 (52/428; 12.14%) patients were injecting drug users and 83 (83/428; 19.39%) patients had other diagnoses, for example, neurological and muscular diseases and factors affecting the health system.

Anti-HCV was confirmed by Western Blot in 390 patients (91.12%), while 28 patients (6.54%) had borderline Western Blot results. When it comes to borderline Western Blot results, negative result was confirmed with molecular testing in 16 patients, HCV infection was excluded in two patients with paired sera and infection status was unknown in 10 patients. Positive anti-HCV ELISA was not confirmed by Western Blot in 10 patients (2.34%). Molecular testing for HCV RNA was performed in 331 patients – 65.86% (218/331) of the patients had measurable viremia (Table 1).

Table 1. Results of Western Blot and HCV RNA in anti-HCV (ELISA) positive patients tested at UHID

| anti-HCV | | | |
Enzyme-linked immunoassay	Western Blot	HCV RNA	Patients N (%)
Positive	Positive	Positive	218 (65.86)
		Negative	87 (26.28)
Positive	Borderline	Negative	16 (4.83)
Positive	Negative	Negative	10 (3.02)
TOTAL			331 (100.00)

Median viremia was 456.024.5 IU/ml (range 78-43.041.938 IU RNA HCV/mL). HCV RNA was not detected in 87 patients with positive HCV WB, 16 with borderline and 10 with negative HCV WB.

There was no data on HCV RNA testing at the UHID for 85 patients with positive and 12 with borderline HCV WB (97/428; 22.66%) (Table 2).

Table 2. Results of Western Blot and HCV RNA in anti-HCV (ELISA) positive patients

| anti-HCV | | | |
Enzyme-linked immunoassay	Western Blot	HCV RNA	Patients N (%)
Positive	Positive	Positive	218 (50.93)
		Negative	87 (20.33)
		Unknown	85 (19.86)
Positive	Borderline	Negative	16 (3.74)
		Unknown	12 (2.80)
Positive	Negative	Negative	10 (2.34)
TOTAL			428 (100.00)

According to the available diagnoses from the referral, 48 of 97 patients with positive anti-HCV ELISA and unknown viremia were patients who had chronic hepatitis or elevated hepatic lesion (49.48%). Seventeen of 97 patients were injecting drug users (17.52%) and 32 of 97 patients had other diagnoses, for example, neurological and muscular diseases and factors affecting the health system (32.99%).

HCV genotypes and subtypes were determined in 185 of 218 anti-HCV WB positive patients with HCV RNA >1.000 IU/ml in the serum. Genotype 1 was detected in 97 (97/185; 52.43%) patients, genotype 2 in 3 (3/185; 1.62%) patients, subtype 3a in 76 (76/185; 41.08%) /185 and genotype 4 in 9 (9/185; 4.86%) patients. Subtypes 1a and 1b of genotype 1 were further distinguished. Subtype 1a was detected in 57 (57/97; 58.76%) patients and subtype 1b in 36 (36/97; 37.11%) patients. In four patients, the subtype of genotype 1 could not be determined (Figure 1).
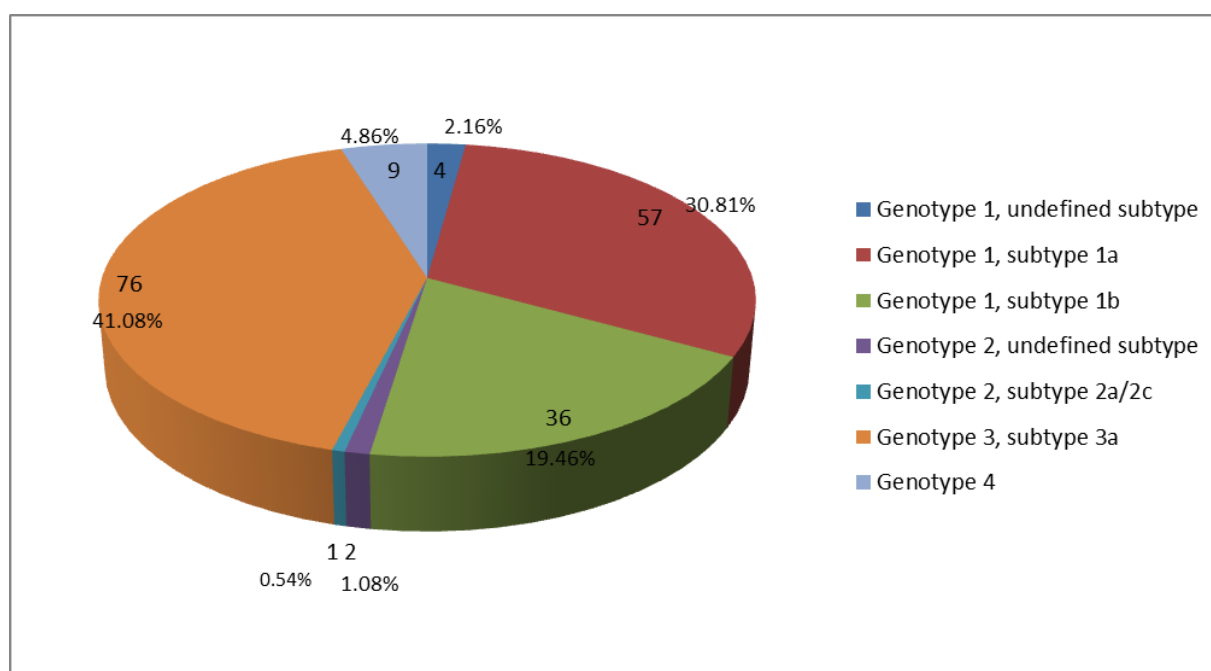
Figure 1. HCV genotype/subtype distribution in HCV RNA-positive patients (n = 185)

Discussion

HCV prevalence estimates range from 0.4% to 5.2% in the world, with countries in the north and west of Europe having lower estimates (0.9%) than countries in the east of Europe (3.3%) [11,12]. In a five-year period, HCV seroprevalence rate in subjects diagnosed at the UHID was 1.81%, which was slightly higher than the estimate (0.8-1.0%) for the Croatian population [13]. However, most of the data on prevalence was obtained through the serological testing of samples from voluntary blood donors, who were a strictly controlled group. HCV prevalence in blood donors continuously declined from 1.38% in 1992 to 0.0009% in the last decade. Therefore, it is to be expected that the actual prevalence in the general population is higher [13,14]. Nevertheless, this data suggests that HCV seroprevalence in Croatia is most similar to the seroprevalence rate in western European countries.

According to the latest analysis on the distribution of HCV genotypes and subtypes in Croatia, Croatian patients were mostly infected with HCV genotype 1 (56.63%), followed by

genotype 3 (37.23%), genotype 4 (4.21%) and genotype 2 (1.83%). This is very similar to our data, according to which genotype 1 was detected in 52.43% patients, genotype 2 in 1.62% patients, subtype 3a in 41.08% and genotype 4 in 4.86% patients [15]. However, genotype 1 subtyping showed 58.76% of subtype 1a infections and 37.11% subtype 1b infections in our study, while national studies suggest a higher prevalence of subtype 1b in Croatia [15,16].

Our cohort study tested 23,524 patients for anti-HCV antibodies to show how many adults with a positive anti-HCV screening result were accurately diagnosed, including determination of viremia, in the period from 2014 to 2018. Epidemiological and clinical data from the referrals and the UHID database suggest that 218 of 331 patients who were molecularly tested at the UHID had measurable viremia, while there was no data for 97 anti-HCV-positive patients. This suggests the possibility they were tested at another institution or that they were simply not treated further. This indicates the need for a more systematic monitoring and determination of viremia in potential patients in order to initiate treatment. According to the available diagnoses from the referral, 17.50% of the patients were people who injected drugs and, probably due to their lifestyle, were lost to secondary specialist care. Furthermore, 49.50% of the patients were people who had chronic hepatitis or elevated hepatic lesion and 33% of the patients were people with other diagnoses, for example, neurological and muscular diseases and factors affecting the health system. Since injecting drug use is one of the most efficient routes for HCV transmission, there is a very high prevalence of HCV in people who inject drugs in most European countries, while in Croatia it ranges from 29% to 65%, depending on the geographical region [13].

According to Becchini et al., general practitioners' role and referral back to primary care vary within and between countries – most general practitioners are rarely involved in monitoring clinical outcomes other than some side effects among patients undergoing antiviral treatment [1,8]. A lack of uniform practice suggests that in some patients with serologically confirmed presence of the infection, additional nucleic acid testing may not always be performed. Therefore, the role of the general practitioner and specialists involved in clinical management of chronically infected patients should be clarified in order to ensure that the patients are followed-up and accurately diagnosed [1]. The Croatian Reference Centre for Diagnostics and Treatment of Viral Hepatitis performs initial check-ups of patients with acute and chronic hepatitis B and hepatitis C infection, serological and molecular testing to detect parameters of virus infection, pretreatment evaluation of the patients (liver biopsy, Fibroscan) along with treatment and monitoring of patients after the end of treatment. National Reference Centre also provides strategies and guidelines for optimization of diagnostics and standardization of treatment of viral hepatitises [17]. However, in Croatia, there is no National Hepatitis Treatment Registry used by all physicians prescribing DAAs and patients are diagnosed and treated at various institutions across the country. Such decentralization enables easier access to care for patients, whereas an early diagnosis and successful treatment not only prevent HCV-related complications, but also stop infectiousness. On the other hand, it is possible that the monitoring of patients and overview of the treatment process is better in specialized, centralized settings than in unspecialized, decentralized settings [18].

Conclusion

It is necessary to improve the monitoring and surveillance of hepatitis C patients in Croatia since almost one quarter of patients with detected anti-HCV antibodies are not treated further. Determination of viremia after positive anti-HCV screening would ensure adequate treatment of the patients and prevent HCV-related complications.

Disclosure

Competing interests. None to declare.

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Original article

Sleep Disorders in Cervical Dystonia, Parkinson's Disease and Depression – What is the Difference?

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Abstract

Introduction: Sleep disorders are among the most common non-motor symptoms in patients with cervical dystonia (CD), Parkinson's disease (PD), and depression. The study aimed to assess the prevalence and characteristics of sleep disorders in patients with cervical dystonia compared to healthy controls, patients with Parkinson's disease, and patients with depression.

Methods: In this cross-sectional study, we evaluated 122 patients (30 control patients, 30 with cervical dystonia, 32 with Parkinson's disease, and 30 with depression). Demographic data were collected. All of them, except for the depression group, were tested for depression and anxiety using the Beck Depression Inventory (BDI) and Beck Anxiety Inventory (BAI). Sleep disorders were evaluated using the Pittsburgh Sleep Quality Index (PSQI) and Epworth Sleepiness Scale (ESS). Statistical significance was defined at α =0.05.

Results: Patients with cervical dystonia differed from the healthy control group in terms of PSQI score and some subscales. The depression group differed in most PSQI subscales when compared to the patients with Parkinson's disease and cervical dystonia, while the latter two groups of patients differed only in the duration subscale. Patients with Parkinson's disease differed from other groups of patients only in one subscale - daytime sleepiness.

Conclusion: Cervical dystonia patients suffer from more sleep disturbances when compared to healthy controls. There are differences in the frequency and extent of sleep disturbances with less pronounced symptoms in patients with cervical dystonia and Parkinson's disease, while patients with depression present the most pronounced symptoms. Symptoms of depression and anxiety correlate with sleep disturbances in patients with Parkinson's disease and cervical dystonia. Patients with cervical dystonia do not experience daytime sleepiness problems.

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Introduction

Circadian rhythm can be defined as changes in biological and behavioural conditions between the states of high and low activity during 24 hours. It is regulated by two paired nuclei located in the hypothalamus, called the suprachiasmatic nucleus (SCN). The process starts with retinal light stimulation that generates a signal through the retinohypothalamic tract to the SCN, which then generates a signal that stimulates the pineal gland. The process of stimulation involves a multi-synaptic link through the superior cervical ganglion, which releases noradrenaline during the night, inducing the activity of the serotonin-N-acetyltransferase (SNAT) enzyme via cyclic adenosine monophosphate (cAMP). This stimulates the pineal gland to produce the hormone melatonin during the night and release it into the circulation to facilitate sleep [1]. The neurotransmitters that are important in the process of wakefulness are serotonin, noradrenaline, and acetylcholine, and they are released by the neurons located in the ventrolateral preoptic nucleus [2]. Introduction to the rapid-eye-movement (REM) phase of sleep is characterized by a decrease in monoaminergic (serotonin, norepinephrine, and dopamine) tone with a parallel increase in cholinergic tone [3]. In non-REM sleep, glutamate signalling is important during thalamocortical slow oscillations [4]. The secretion of the hormone cortisol is decreased during normal deep sleep and hypercortisolaemia can induce insomnia [5]. Sleep disorders could be classified into several types: delayed sleep phase syndrome (DSPS), advanced sleep phase syndrome (ASPS), irregular sleep-wake pattern, and non-24-hour sleep-wake syndrome in blind and sighted persons. Delayed sleep phase syndrome is a sleep disorder where the patient has problems falling asleep and waking up at conventional times, and the assumption is that the pathophysiological basis lies in delayed endogenous melatonin secretion [6]. Advanced sleep phase syndrome is characterized by persistent early evening sleep onset and early morning awakening with no sleep-maintenance problems. It is an age-related problem, the pathophysiology of which is as a consequence of diminution in the output of the circadian pacemaker [7]. The syndrome of an irregular sleep-wake pattern is characterized by temporally disorganized and variable episodes of sleeping and waking behaviour.

Focal dystonia is characterized by sustained or intermittent muscle contraction causing abnormal, often repetitive movement, posture, or both. Cervical dystonia (CD) is a focal dystonia type where neck muscles are involved [8]. The aetiology of CD is still unknown. Recent articles suggest miscommunication between the basal ganglia and cerebellar loops [9]. In CD, besides typical motor symptoms, patients also experience non-motor symptoms, such as depression, anxiety, cognitive decline, pain, and sleep disorders [10,11]. The aetiology of sleep disorders in this patient group remains unresolved. There is no evidence that it is related to motor symptoms or that the relief from botulinum toxin treatment used for motor symptoms does not improve sleep disorders [12]. Video-polysomnographic recordings in CD patients showed that the activity over cervical muscles disappeared during all sleep stages and thus could not influence sleep impairment [13]. One of the theories is that sleep disturbance is related to the dysfunction of some brain regions, such as the basal ganglia, with dopaminergic system disturbance. Another theory is that it is related to depression [14].

Parkinson's disease (PD) is a neurodegenerative disorder with alpha-synuclein inclusions as the main hallmark of disease pathology. During the disease course, α-synuclein pathology spreads from the brain stem to higher cortical regions, with consequential neuron degeneration. As a result of degeneration, there is a loss of many neurotransmitters in the brain, such as dopamine, serotonin, noradrenaline, and acetylcholine. This causes many motor and non-motor symptoms, including sleep disorders, among many other non-motor symptoms described [15]. Multiple factors could influence sleep disturbances, such as age-related

changes in sleep, nocturnal motor symptoms (rigidity, resting tremor, akinesia, tardive dyskinesia, and the 'wearing-off' phenomenon), non-motor symptoms (pain, hallucination, and psychosis), nocturia, and medication. Besides that, as part of PD pathology, there are changes in the neurotransmitter systems (dopamine, norepinephrine, serotonin, and acetylcholine) responsible for regulating sleep structure and the sleep/wake cycle [16].

Major depressive episodes are characterized by a period of depressed mood or anhedonia lasting for 2 or more weeks, with at least three additional signs, i.e. weight change or change in appetite, psychomotor agitation or retardation, feeling of worthlessness or guilt, diminished ability to concentrate, suicidal ideations or attempts, and insomnia or hypersomnia. Changes in neurotransmitter levels, such as a decrease of serotonin, norepinephrine and dopamine, and hypercortisolaemia are possible aetiologic factors of depression. Sleep disturbance is one of the most consistent symptoms. It can precede the symptoms of depression or persist after the disease remission and it is not related to depression itself. There are several theories on the aetiology of sleep disturbance in depression. One of them refers to monoaminergic level disruption, another one to glutamate level decrease, and one to hypercortisolaemia. However, none of them includes a clear conclusion regarding its aetiology [4].

The aim of the study was to assess the prevalence and characteristics of sleep disorders in patients with cervical dystonia, compared to healthy controls, patients with Parkinson's disease, and patients with depression.

Subjects and Methods

In this cross-sectional study, we analysed 122 subjects, 30 of whom were healthy controls, 30 CD patients, 32 PD patients, and 30 were subjects diagnosed with depression. The study was conducted at the Department of Neurology and Department of Psychiatry, University

Hospital Centre Osijek, from February to May 2017. All the participants signed a written informed consent. The study protocol was reviewed and approved by the University Hospital Centre Osijek Ethics Board and it was in accordance with the Declaration of Helsinki. CD patients were recruited from the botulinum toxin clinic and tested during their regular follow-up examinations (without relation to their botulinum injections schedule). The control group included sex- and age-matched healthy relatives and friends of CD patients. PD patients were recruited from the movement disorders clinic. PD diagnosis was made according to the UK PD Society Brain Bank (UKPDSBB) diagnostic criteria and both early and advanced PD patients were analysed [17]. Depressed patients were recruited from the psychiatric ward where they were hospitalized due to depression problems. Control, PD, and CD groups were tested for symptoms of depression and anxiety by using the Beck Depression Inventory (BDI) and Beck Anxiety Inventory (BAI). Severity of depression and anxiety were not evaluated in depressed patients. All patients were tested for sleep disturbances using the Pittsburgh Sleep Quality Index (PSQI) for night-time disturbances and Epworth Sleepiness Scale (ESS) for daytime sleepiness problems. The PSQI has been designed to assess sleep quality and disturbances over a 1-month time interval. It is a self-rated questionnaire that can be filled in 10-15 minutes. There are 19 individual questions divided into 7 subscales assessing subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbances, use of sleeping medication, and daytime dysfunction. The sum of all 7 subscales provides the global PSQI score ranging from 0 to 21, where lower scores denote better sleep quality [18]. The ESS is a simple self-administered questionnaire designed to measure daytime sleepiness. It consists of 8 questions about how likely would it be for the subject to doze off in 8 different situations (sitting and reading; watching TV; sitting, inactive, in a public place; as a passenger in a car for an hour without a break; lying down to rest in the afternoon; sitting and talking to someone; sitting quietly after lunch without alcohol; and, in a car, while stopped for

a few minutes in the traffic). After scoring every situation on a scale from 0 (would never doze off) to 3 (high chance to doze off), the total sum is calculated. The maximum score is 24. The score is higher in patients having more problems with daytime sleepiness [19]. A demographic questionnaire was designed for the study. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Study was approved in 2016 by local Ethical Committee of Medical school in University of J. J. Strossmayer in Osijek. Informed consent was obtained from all individual participants included in the study.

Statistical analysis

Categorical data were expressed as absolute frequencies and percentages, while the differences between the groups were tested by Fisher's exact test. Numerical data were expressed as median and interquartile ranges or as mean and standard deviations, depending on whether the data indicated normal distribution, which was tested by the Kolmogorov-Smirnov test. Correlation between variables that did not indicate normal distribution was tested with Spearman's rank correlation coefficient. Differences between the groups in which the data did not indicate normal distribution were tested by the Kruskal-Wallis test. Statistical significance was defined as $\alpha = 0.05$. Post hoc analysis of differences between the two groups was done with the Mann-Whitney U test and, after the Bonferroni correction, statistical significance was defined as $P < 0.016$. Statistical analysis was conducted by using STATISTICA 13 (StatSoft Inc., Tulsa, Oklahoma, USA).

Results

There were differences among the patient groups in terms of age (PD patients were older than subjects from other groups), while the study groups were quite homogeneous in terms of sex (Table 1). There was no difference in disease duration among the three groups of subjects (Table 2).

Table 1 Demographic data regarding sex and age

	CONTROL	PARKINSON'S DISEASE	FOCAL DYSTONIA	DEPRESSION	P
	N/%	N/%	N/%	N/%	
SEX					
• male	10 (33.3)	20 (62.5)	10 (33.3)	11 (36.7)	
• female	20 (66.7)	10 (37.5)	20 (66.7)	19 (63.3)	0.052*
AGE					
• 20-30	1 (3.3)	0 (0)	1 (3.3)	0 (0)	
• 31-40	2 (6.7)	0 (0)	2 (6.7)	3 (10.0)	
• 41-50	3 (10.0)	3 (9.4)	3 (10.0)	4 (13.3)	
• 51-60	10 (33.3)	3 (9.4)	10 (33.3)	16 (53.3)	
• 61-70	8 (26.7)	18 (56.3)	8 (26.7)	4 (13.3)	
• 71-80	6 (20.0)	8 (25.0)	6 (20.0)	3 (10.0)	**0.004***

*Fisher's Exact Test

Table 2 Differences in disease duration, symptoms of depression and anxiety between groups

	CONTROL	PARKINSON'S DISEASE	FOCAL DYSTONIA	DEPRESSION	
	median (IQR) mean	median (IQR) mean	median (IQR) mean	median (IQR) mean	P
DISEASE DURATION	-	5.00 (2.00-9.75) 6.31	7.00 (2.25-12.25) 7.20	7.00 (2.75-15) 9.42	0.315†
BDI	1.00 (1.00-1.25) 1.37	2.00 (1.00-3.00) 2.19	1.50 (1.00-2.25) 1.83	-	**0.020†**
BAI	1.00 (1.00-1.00) 1.13	1.00 (1.00-2.00) 1.56	1.00 (1.00-2.00) 1.37	-	**0.021†**

BDI – Beck Depression Inventory; BAI – Beck Anxiety Inventory; †Kruskal-Wallis test
Post hoc analysis performed between groups by applying the Mann-Whitney U test indicated to the following significant differences: BDI between control and PD group (P < 0.008); BAI between control and PD group (P < 0.006).

There were significant differences in BDI and BAI scores between control, PD, and CD groups (BDI p<0.020 and BAI p<0.021). The post hoc analysis conducted between the groups by using the Mann-Whitney U test and the Bonferroni correction indicated a significant difference in BDI and BAI only between the PD and the control group (Table 2). A positive correlation between the BDI and PSQI scores in both patient groups (CD group rs 0.409, p<0.025; PD group rs 0.668,

p<0.001) was found, but not between BDI and ESS (CD group rs 0.191, p<0.312; PD group rs 0.093, p<0.612). When correlating BAI and PSQI, positive correlations both for PD and CD groups (PD group rs 0.604, p<0.001; CD group rs 0.370, p<0.044) was found, whereas for BAI and ESS a positive correlation only for the CD group was found (CD group rs 0.393, p<0.032; PD group rs 0.271, p<0.133). The frequency of severity in the PSQI scale, ESS, and PSQI subjective score are shown in Table 3.

Table 3 Frequency of severity of sleep disorders and subjective assessment between groups

	CONTROL	PARKINSON'S DISEASE	FOCAL DYSTONIA	DEPRESSION	
	N/%	N/%	N/%	N/%	*P*
ESS					
• lower normal DS	17 (56.7)	11 (34.4)	19 (63.3)	14 (46.7)	
• higher normal DS	9 (30.0)	9 (28.1)	6 (20.0)	10 (33.3)	
• mild excessive DS	2 (6.7)	2 (6.3)	3 (10.0)	1 (3.3)	
• moderate excessive DS	2 (6.7)	8 (25.0)	1 (3.3)	1 (3.3)	
• severe excessive DS	0 (0)	2 (6.3)	1 (3.3)	4 (13.3)	**0.035***
PSQI					
• normal	23 (76.7)	7 (21.9)	10 (33.3)	0 (0)	
• poor sleep quality	7 (23.3)	25 (78.1)	20 (66.7)	30 (100)	**0.001***
PSQI SUBJECTIVE					
• very good	14 (46.7)	9 (28.1)	8 (26.7)	3 (10.0)	
• fairly good	11 (36.7)	8 (25.0)	12 (40.0)	10 (33.3)	
• fairly bad	3 (10.0)	11 (34.4)	7 (23.3)	11 (36.7)	
• very bad	2 (6.7)	4 (12.5)	3 (10.0)	6 (20.0)	**0.004***

DS – daytime sleepiness; ESS – Epworth Sleepiness Scale; PSQI – Pittsburgh Sleep Quality Index; * Fisher's Exact Test

Post hoc analysis showed the following differences between the groups: control and CD (PSQI score P < 0.001); PD and CD (ESS score P < 0.017); PD and depression (PSQI score P < 0.007); CD and depression (PSQI score P < 0.001; subjective PSQI P < 0.042)

We found significant differences among the groups for both scales and subjective PSQI scores. Post hoc analysis showed differences between the two groups (Table 3). Table 4 shows PSQI subscale scores, while Figure 1 shows global PSQI scores for the groups.

Table 4 Differences in PSQI subscale scores between healthy control, Parkinson disease (PD), focal dystonia (FD) and depression groups

	CONTROL median (IQR)	PARKINSON'S DISEASE median (IQR)	FOCAL DYSTONIA median (IQR)	DEPRESSION median (IQR)	P
Latency	0.00 (0.00-0.00)	1.00 (0.00-1.00)	1.00 (0.00-1.25)	2.00 (1.00-3.00)	0.001‡
Effectiveness	0.00 (0.00-0.25)	1.00 (0.00-2.75)	0.00 (0.00-2.00)	2.00 (0.00-3.00)	0.001‡
Duration	1.00 (0.00-2.00)	1.50 (0.00-2.00)	0.00 (0.00-0.00)	2.00 (0.00-3.00)	0.001‡
Disturbances	0.00 (0.00-1.25)	0.00 (0.00-1.75)	2.00 (0.00-3.00)	2.00 (2.00-3.00)	0.001‡
Awakening	1.00 (0.00-2.00)	3.00 (1.00-3.00)	2.50 (1.00-3.00)	3.00 (2.00-3.00)	0.001‡
Toilet	1.00 (0.00-2.00)	3.00 (1.25-3.00)	2.00 (0.00-3.00)	3.00 (1.00-3.00)	0.001‡
Breathing	0.00 (0.00-0.00)	0.00 (0.00-1.00)	0.00 (0.00-0.25)	0.00 (0.00-3.00)	0.043‡
Snoring	0.00 (0.00-1.00)	2.00 (0.00-3.00)	0.00 (0.00-3.00)	3.00 (0.00-3.00)	0.001‡
Cold	0.00 (0.00-0.00)	0.00 (0.00-1.75)	0.00 (0.00-0.00)	0.00 (0.00-3.00)	0.029‡
Hot	0.00 (0.00-0.00)	0.00 (0.00-1.75)	0.00 (0.00-2.25)	2.00 (0.00-3.00)	0.079‡
Nightmares	0.00 (0.00-0.00)	1.00 (0.00-2.00)	0.00 (0.00-1.25)	2.00 (0.00-3.00)	0.001‡
Pain	0.00 (0.00-0.00)	2.00 (0.00-3.00)	2.00 (0.00-3.00)	1.00 (0.00-3.00)	0.001‡
Other	0.00 (0.00-0.00)	0.00 (0.00-0.00)	0.00 (0.00-0.00)	3.00 (0.00-3.00)	0.001‡
Hypnotics usage	0.00 (0.00-0.00)	0.00 (0.00-2.00)	0.00 (0.00-2.25)	3.00 (3.00-3.00)	0.001‡
Dysfunctionality	0.00 (0.00-0.00)	0.00 (0.00-1.75)	0.00 (0.00-1.00)	2.00 (0.00-2.00)	0.001‡
Vigilance	0.00 (0.00-0.00)	0.00 (0.00-1.00)	0.00 (0.00-0.00)	0.00 (0.00-1.25)	0.009‡
Enthusiasm	0.00 (0.00-0.00)	0.00 (0.00-1.00)	0.00 (0.00-1.00)	3.00 (0.00-3.00)	0.001‡

PSQI – Pittsburgh Sleep Quality Index; †Kruskal-Wallis test

Post hoc analysis of differences between two groups carried out by applying the Mann-Whitney U test yielded significant differences for this subscale, and they are as follows: control and CD (P<0.001); PD and depression (P<0.001); CD and depression (P<0.001); duration score between control and CD (P<0.001); PD and CD (P<0.001); CD and depression (P<0.001); disturbance score between PD and depression (P<0.001); snoring score between CD and depression (P<0.009); nightmare score between control and CD (P<0.004); CD and depression (P<0.003); pain score between control and CD (P<0.001); other score between PD and depression (P<0.001); CD and depression (P<0.001); hypnotics usage score between PD and depression (P<0.001); CD and depression (P<0.001); dysfunctionality score PD and depression (P<0.006); CD and depression (P<0.001); enthusiasm score between PD and depression (P<0.001); CD and depression (P<0.001).

The median score did not differ between some groups, but the interquartile range indicated to differences among them. Significant differences among the groups in terms of global scores were observed (Figure 1), but also with regard to many subscales. Post hoc analysis, following the application of the Bonferroni correction, showed differences between the two groups (Table 4).

Figure 1 Global PSQI score between groups
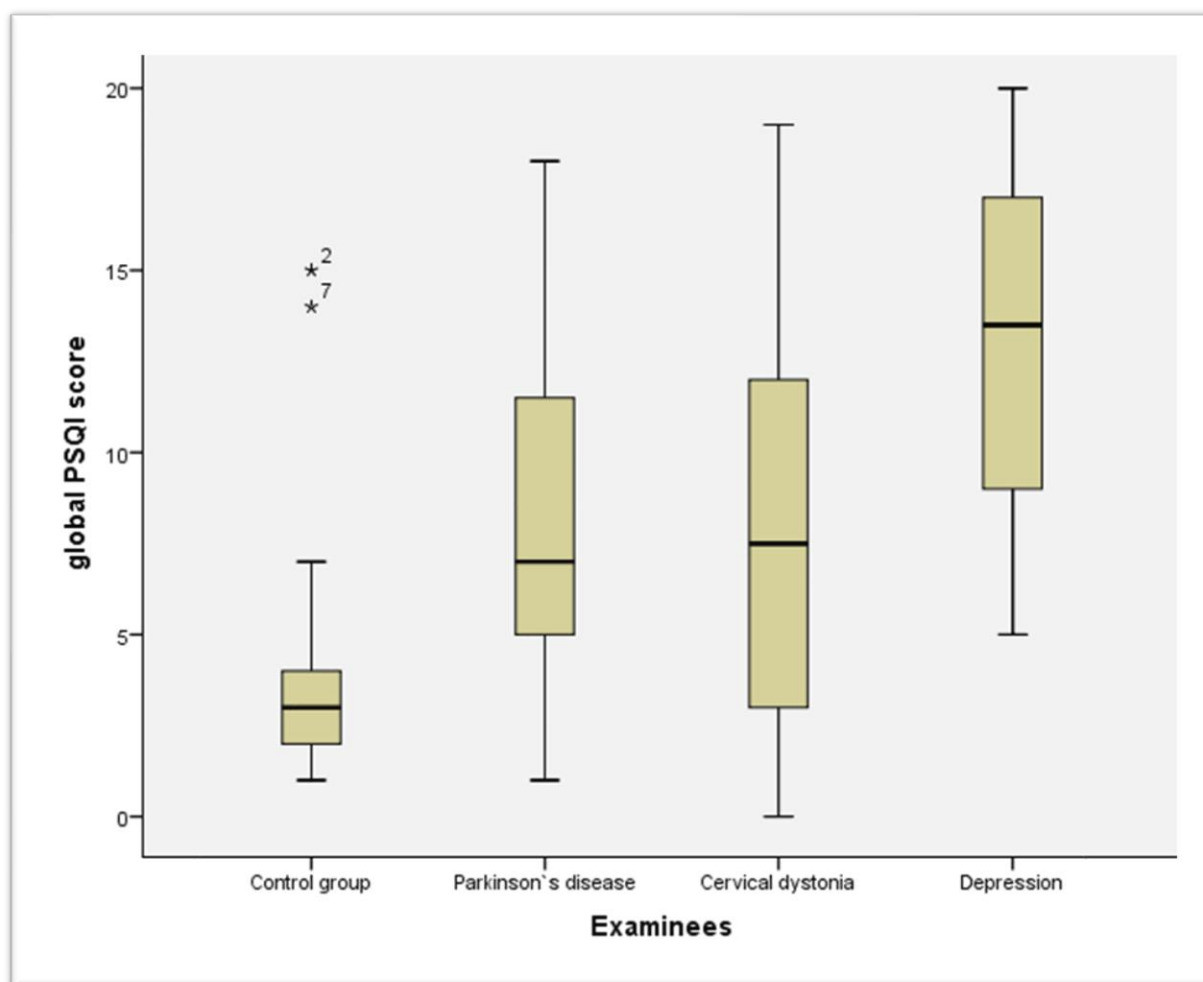
PSQI – Pittsburgh Sleep Quality Index.

Post hoc analysis consisting of the Mann-Whitney U test, caried out after Bonferroni correction in order to determine differences between two groups: PSQI score between control and CD groups (P < 0.004); PD and depression groups (P < 0.001); and CD and depression groups (P < 0.001).

Table 5 shows results pertaining to ESS subscale scores, while Figure 2 shows total ESS scores for all the groups. There were no significant differences in total ESS scores between the CD and control group, or between patient groups (after Bonferroni correction) (Figure 2). Only the subscale "sitting in a public place" presented lower results in the PD group when compared to CD and depression groups (Table 5).

Table 5 Differences in ESS subscale scores in between healthy control, Parkinson disease, focal dystonia, and depression groups.

	CONTROL	PARKINSON'S DISEASE	FOCAL DYSTONIA	DEPRESSION	
	median (IQR)	median (IQR)	median (IQR)	median (IQR)	**P**
Watching TV	1.50	1.50	1.00	1.00	
	(1.00-2.00)	(1.00-2.00)	(0.00-2.00)	(0.00-2.25)	0.208‡
Sitting, inactive, in a public place	0.00	1.00	0.00	0.00	
	(0.00-1.00)	(0.00-1.00)	(0.00-1.00)	(0.00-1.25)	0.147‡
Passenger in a car	0.00	0.00	0.00	0.00	
	(0.00-1.00)	(0.00-2.00)	(0.00-1.00)	(0.00-1.00)	0.458‡
Lying down to rest in the afternoon	2.00	2.00	2.00	2.00	
	(1.00-3.00)	(1.00-3.00)	(1.00-2.00)	(0.75-2.25)	0.402‡
Sitting and talking	0.00	0.00	0.00	0.00	
	(0.00-0.00)	(0.00-1.00)	(0.00-0.25)	(0.00-1.00)	0.129‡
Sitting after lunch	0.00	1.00	0.00	1.00	
	(0.00-1.00)	(0.00-3.00)	(0.00-1.00)	(0.00-2.00)	**0.011‡**
Sitting in a public place	0.00	0.00	0.00	0.00	
	(0.00-0.00)	(0.00-1.00)	(0.00-0.00)	(0.00-0.00)	**0.005‡**
In a car, while stopped for a few minutes	0.00	1.00	1.00	0.00	
	(0.00-1.00)	(0.00-2.00)	(0.00-1.00)	(0.00-2.00)	0.390‡

ESS – Epworth Sleepiness Scale; †Kruskal-Wallis test

Post hoc analysis of differences between two groups carried out by applying the Mann-Whitney U test, which after applying the Bonferroni correction (P<0.016) yielded significant differences for this subscale, and they are as follows: sitting after the lunch for PD and CD (P < 0.042); sitting in public for PD and CD (P < 0.013); PD and depression (P < 0.005).

Figure 2 Total ESS scores between groups
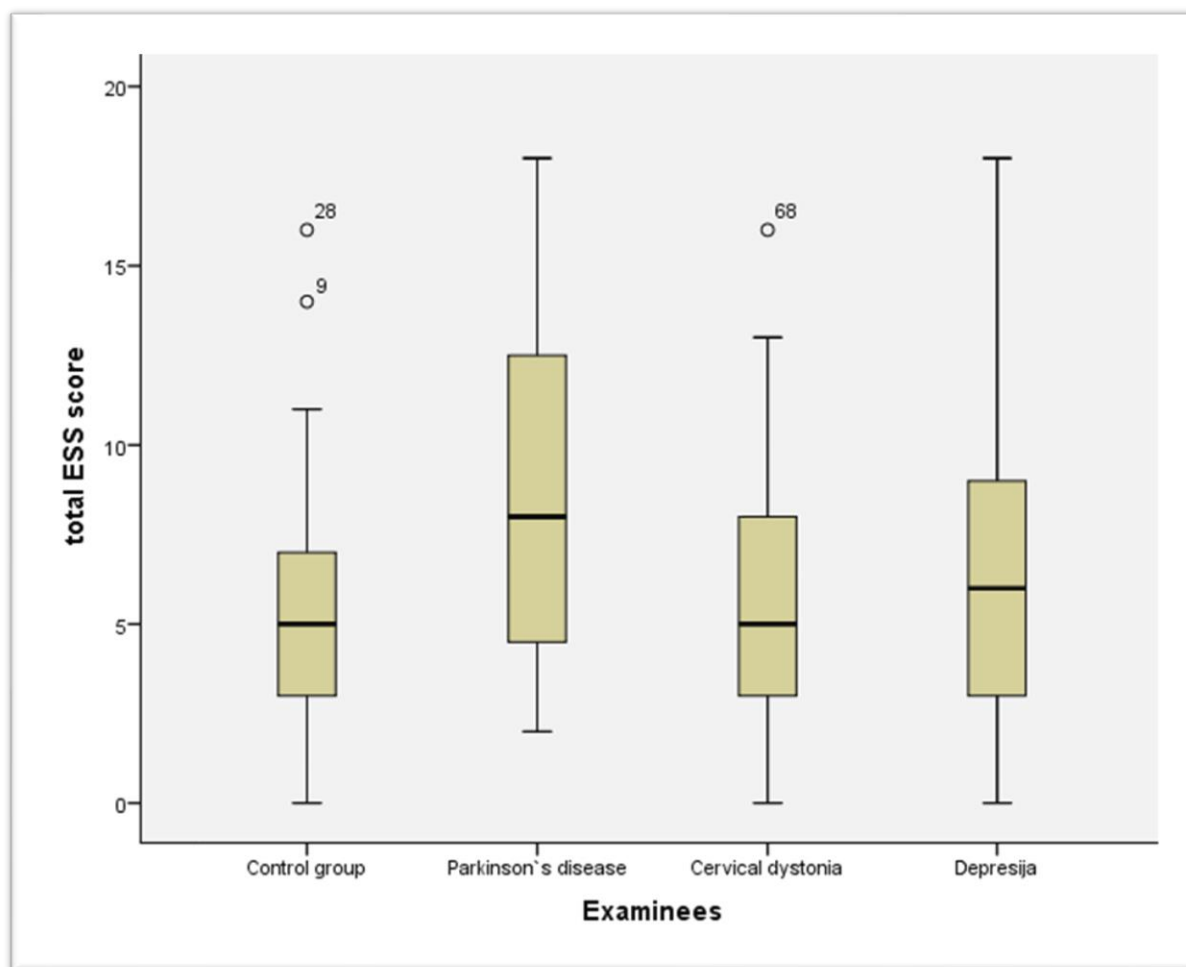
ESS – Epworth Sleepiness Scale.

Post hoc analysis consisting of the Mann-Whitney U test that was performed to determine the differences between two patient groups showed the following differences: ESS score for PD and CD groups (P < 0.031).

Discussion

Patients with CD were analysed for sleep disturbance relative to the control group, and all three patient groups were analysed for sleep disturbances relative to each other. When compared to the control group, CD patients displayed more frequent sleep problems that included higher PSQI scores. However, there was no difference with regard to daytime sleepiness problems. They have reported longer latency to fall asleep, lower duration of sleep during the night, more frequent nightmares, and more pain that disturbed their night-time sleep. Avanzino et al. reported impairment in sleep duration, latency, and efficiency of sleep in

patients with blepharospasm, but not in the CD patient group. Likewise, Antelmi et al. also found decreased sleep efficiency and increased sleep latency in video-polysomnographic recordings of CD patients [13,20]. Impairment in sleep latency has its pathophysiological basis in delayed endogenous melatonin secretion, probably due to lower noradrenaline and serotonin levels that are an important part of this process [1].

Although all three patient groups suffered from sleep disturbances, we still found differences among them. Sleep of PD patients during the night is of shorter duration when compared to the CD group. PD group was older than the CD group, so this difference could be related to age,

i.e. it could be a consequence of age-related diminution in the output of the circadian pacemaker [7]. The depression group differed from the CD group in terms of PSQI scores and most subscales. They reported prolonged latency, shorter duration of sleep, and worse quality of sleep resulting from snoring and nightmares. They also use hypnotics more often and are more likely to suffer from dysfunctionality and loss of enthusiasm during the day arising from their sleep problems. The depression group reported more of such similar problems when compared to the PD group (latency, duration of sleep, disturbances, and other problems and disturbances). They also used hypnotics more often and reported dysfunctionality and loss of enthusiasm during the day arising from their sleep problems. So, of the three patient groups, sleep problems were most pronounced in the depression group, followed by PD, and finally the CD group. Smit et al. published a paper about altered presynaptic serotonin transporter (SERT) binding in CD patients. They found that sleep disturbances were strongly linked to SERT binding in the raphe nuclei in CD patients [21]. Depression and anxiety in CD patients are related to serotonergic system impairment. Patients with CD accompanied by depression and anxiety present lower SERT (serotonin transport) binding in the midbrain/diencephalon [22]. We found that both anxiety and depression symptoms correlate with sleep disturbances in the CD group. This indicates that neurotransmitter impairment leading to depression and anxiety could be the aetiological factor of sleep disturbances in the CD group.

All three patient groups had problems with waking up and there was no significant difference among them. Dopamine-containing neurons are involved in the regulation of the waking process [23]. There is evidence that the dopaminergic system is disturbed in CD patients with depression. Zoons et al. found alterations of striatal DAT (dopaminergic transport) and D2/3 receptor binding in CD patients with depression [24]. In addition to dopamine, noradrenaline and acetylcholine are also important in the waking process. Noradrenaline levels are high during the waking state and low during sleep [25]. Noradrenergic and cholinergic cells in the pons increase firing to activate the waking pattern [26,27]. There is a case report about pathologic findings from an autopsy of a patient with primary segmental dystonia (Meige syndrome). The authors found moderate-to-severe neuronal loss in several brainstem nuclei, including the substantia nigra pars compacta, locus coeruleus, raphe nuclei, and pedunculopontine nucleus [28]. Another paper with histopathologic findings in CD patients did not report a neuronal loss in the pedunculopontine region, but described a pedunculopontine nucleus choline acetyltransferase deficiency with a functional cholinergic deficit [29]. This could explain the lower noradrenaline and acetylcholine levels in CD patients, which are an important part of the wake-sleep cycle.

There was no difference in ESS scores between the CD and control group, but the PD group reported lower ESS scores more frequently when compared to the CD patient group. In terms of subscales, and after the Bonferroni correction, a significant difference was found only with regard to the risk of falling asleep while sitting in public, where PD patients reported higher chances of falling asleep when compared to the CD and depression group. Although excessive daytime sleepiness has not been indicated in CD patients [10,20,30], Trotti et al. reported opposite results for their CD patients when compared to the control group. They explained that this could be attributed to the use of anticholinergics that can affect sleepiness during the day in a certain percentile of patients [14,32]. There is no evidence that impairment of sleep quality has an impact on daytime sleepiness [19,32].

Conclusion

Sleep disturbances are frequent non-motor symptoms in CD, PD, and depression patients.

According to our data, all three groups of patients had sleep disorders, but they differed in the frequency and extent of those disorders, with less pronounced symptoms found in CD and PD patients. Symptoms of depression and anxiety correlate with sleep disturbances in PD and CD patient groups. CD patients do not experience daytime sleepiness problems. The aetiology of sleep disturbances in CD patients is probably related to monoamine neurotransmitter system impairments similar to those of PD patients, but to a lesser extent than in patients with depression.

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[1]

Original article

The Role of Total Antioxidant Status in Cerebral Vasoreactivity of Chronic Obstructive Pulmonary Disease Patients

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Abstract

Introduction: Chronic obstructive pulmonary disease (COPD) is associated with an oxidant-antioxidant imbalance. COPD patients have impaired cerebral vasoreactivity (CVR). Impaired CVR could be correlated with total antioxidant status (TAS) in plasma.

Aim: To determine the role of systemic TAS in CVR of COPD patients.

Material and Methods: In this cross-sectional observational study, we included 120 participants (the mean age of 67±7.9, 87 males), 90 COPD patients categorized according to the severity of airway obstruction in mild, moderate, severe/very severe and 30 age- and sex-matched controls. We analyzed baseline mean flow velocities (MFV) of the middle cerebral artery (MCA) and the basilar artery (BA), the mean breath-holding index (BHIm) of those arteries (BHImMCA and BHImBA) by transcranial Doppler ultrasound and TAS in plasma. The level of significance was set to α =0.05.

Results: A significant negative correlation between TAS and BHImBA (Rho=−0.445, P=0.01) was found only in the mild COPD group. In COPD groups, baseline MFV BA is in a significant positive correlation with BHImMCA and BHImBA (Rho=0.336, P=0.001 and Rho=0.647, P<0.001). According to the severity of airway obstruction in COPD groups, a significant positive correlation between baseline MFV BA and BHImBA was found: in mild (Rho=0.731, P<0.001), moderate (Rho=0.574, P=0.001) and severe/very severe (Rho=0.398, P=0.03).

Conclusion: Systemic TAS was not correlated with CVR in all COPD groups. However, perfusion in the BA of COPD groups was in a significant positive correlation with the anterior and posterior CVR. The analysis of perfusion in the basal cerebral arteries should be part of a future study of CVR in COPD patients.

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Introduction

The coexistence of chronic obstructive pulmonary disease (COPD) and vascular disease is known, but the pathophysiology of this association has not been fully elucidated. The regulation of the cerebral blood flow (CBF) could be altered by some extrapulmonary consequences, such as arterial blood gas levels, acid-base imbalance, oxidative burden, endothelial dysfunction and autonomic disorders (1,2). The strongest regulator of the CBF is the arterial partial pressure of carbon dioxide ($PaCO_2$). Large changes in the flow could result even from its small fluctuations. The change in the CBF for a given increase in arterial carbon dioxide (CO_2) is greater than the change in the CBF observed for the same magnitude reduction in arterial CO_2 (3).

COPD patients have high oxidative stress associated with the severity of airway obstruction (4). Oxidative stress occurs when the resident antioxidants are insufficient or fail to upregulate sufficiently to neutralize an increased oxidant burden (5). In COPD, increased oxidative stress causes pulmonary inflammation. There is experimental and clinical evidence that pro-inflammatory mediators from the lungs overflow into the systemic circulation and cause the alteration of blood vessel structure. The vascular modelling and arterial stiffness lead to endothelial dysfunction. Those dysfunctional cells reduce the bioavailability of the vasoactive substance they secrete, such as nitric oxide (NO), which otherwise causes relaxation of smooth muscle cells lining in arterioles (6). The sensitivity of cerebral blood vessels to changes in $PaCO_2$ is termed cerebral vasoreactivity (CVR), where arterial hypercapnia induces vessel dilatation and causes an increase in cerebral perfusion (7). Damaged endothelial function is associated with impaired CO_2 reactivity, causing impairment of CVR. Therefore, CVR could be a surrogate of cerebrovascular endothelial function (8).

COPD patients have impaired CVR in the anterior and posterior cerebral circulation and the impairment increases with the airway obstruction severity (9). A study that analyzed moderate smoking-related COPD postmenopausal women showed altered cerebrovascular responses in the anterior cerebral circulation to hypercapnia. They exhibited increased oxidative stress, suggesting that it may be significant in the dysfunction of CVR observed during hypercapnic challenge in COPD (10). Different methods defining total antioxidant status (TAS) of different biological samples have been developed and some of them can determine TAS in the plasma of COPD patients (11). The aim of this study was to determine the role of systemic TAS in CVR of COPD patients.

Material and Methods

Study subjects

A cross-sectional observational study was conducted at the general hospital between March and August 2018. One hundred twenty participants were included in the study – 90 COPD patients (mild, moderate and severe/very severe group, each consisting of 30 participants) and 30 healthy volunteers with a mean age 67 ± 7.9, from which 87 males (Student's T test, P = 0.27). In terms of gender, there were no significant differences between the proportion of men and women in COPD patients and the control group (74.4 vs. 66.7 and 25.6 vs. 33.3% respectively; P = 0.41; Fisher's exact test). The baseline characteristics of COPD patients and controls were summarized in Table 1. The exclusion criteria were as follows: previous cerebrovascular disease, stenosis of internal carotid arteries (>50%), inadequate trans-temporal acoustic window/s, atrial fibrillation, active malignity, exacerbation of COPD in previous two months, specific therapies (nonsteroidal anti-inflammatory and antioxidant therapy, vitamins A, C and/or E supplementation). COPD patients with arterial hypertension, hyperlipidemia, ischemic heart disease and diabetes mellitus that represent vascular disease risk factors were included if they were under good therapy control.

Table 1. Baseline characteristics, lung functions, laboratory values and pack-years in COPD patients and controls

	Median (interquartile range)		Difference	95% confidence interval	P*
	Controls	COPD			
Age (years)	64 (4)	67 (9)	1.8[‡]	-5.1 do 1.5	0.27
FEV$_1$/FVC ratio [Median (interquartile range)]	0.80 (0.75 – 0.83)	0.61 (0.46 – 0.68)	0.20[‡]	0.15 do 0.25	<0.001[‡]
FEV$_1$ (%)	106 (12.7)	65 (25.5)	40.7	33.6 do 47.7	<0.001
BMI (kg/m²)	27.2 (3.2)	28.7 (6.7)	1.5	-4.03 do 0.97	0.23
Body temperature (˚C)	35.3 (5.7)	35.9 (3.3)	0.64	-2.3 do 1.04	0.45
Systolic blood pressure (mmHg)	130 (13)	135 (16)	5,2	-11.6 do 1.2	0.13
Diastolic blood pressure (mmHg)	80 (5)	81 (6)	0,8	-3.5 do 1.8	0.53
Hemoglobin (g/l)	137 (13)	143 (14)	5,7	-11.6 do 0.08	0.05
CRP (mg/l) [Median (interquartile range)]	2.2 (0.97 – 3.15)	2.6 (1.4 – 6.2)	0.6[‡]	-0.1 do 1.6	0.08[‡]
Pack-years [Median (interquartile range)]	41 (17.3 - 48)	43 (33 - 55)	9[‡]	-6.8 do 30	0.27[‡]

*Student T test; †Mann-Whitney U test; ‡Hodges-Lehmann median difference

FEV1/FVC – forced expiratory volume in one second / forced vital capacity; FEV1 – forced expiratory volume in one second; BMI – body mass index; CRP – C-reactive protein; pack-years = number of cigarettes per day x number of smoking years / 20

Study design

The participants were divided into four groups. The first three groups were COPD patients, divided according to spirometry findings and the Global Initiative for Chronic Obstructive Lung Disease (GOLD) classification of airflow obstruction severity based on forced expiratory volume in one second (FEV1) as mild; moderate; and severe to very severe (12). According to GOLD, COPD was defined by post-bronchodilator FEV1 to forced vital capacity (FVC) ratio (FEV1/FVC) of <0.70, confirming a persistent airflow obstruction (13). The fourth group were controls – healthy volunteers (FEV1/FVC >70% and FEV1 >80% of the predicted value).

Anthropometric measurements, comprising height and weight, were determined. Body mass index (BMI) was calculated and expressed as weight (kg) / height (m2). Pack-years were calculated as the number of cigarettes per day x the number of years smoked /20 (14). The participants were asked to abstain from caffeine, alcohol and intense physical activity at least 12 hours prior to participation in the study. For all participants, the exclusion of significant internal carotid stenosis was performed by the method of color and power Doppler flow imaging with a linear probe (VF 10-5 Linear Ultrasound Transducer, Siemens Acuson X300, Germany). For all participants, a specialized technician performed spirometry (Spiroscout^R Ganshorn, Germany) according to the American Thoracic Society/European Respiratory Society standardization (15). Baseline cerebral perfusion and CVR were determined by transcranial

Doppler (TCD) ultrasound (DigiLite™, Rimed, Industrial Park Raanana, Israel).

The study was approved by the Ethics Committee of General Hospital Našice (No. 01-497/3-2017) and by the Ethics Committee of the Faculty of Medicine Osijek, Josip Juraj Strossmayer University of Osijek, Croatia (No. 2158-61-07-17-209). All data were anonymized and the study was conducted in accordance with the amended Declaration of Helsinki. Informed consent was obtained from all individual participants included in the study. All participants signed an informed consent form before entering the study.

Biochemical analysis

Venous blood was taken for a biochemical analysis. We analyzed the complete blood count (Sysmax XN-1000 SA-01, Sysmax Europe GmbH), C-reactive protein (CRP) and TAS (Beckman Coulter DXC 700 AU analyzer, USA). The plasma antioxidant status was measured using a commercially available TAS kit (reagents Randox Laboratories Ltd, United Kingdom). The serum separated from blood was frozen at -20 °C. The samples were collected over a period of 14 days and analyzed in one day by using a colorimetric assay. The procedure was repeated until the target number of participants was reached. The reference range was set at 1.30 – 1.77 and the results were expressed as mmol/L (16). Other blood samples were analyzed immediately after being taken.

TCD monitoring

TCD monitoring of the middle cerebral arteries (MCAs) that represent the anterior cerebral circulation was performed by previously described protocol (9,17,18). The breath-holding test was used as a hypercapnic stimulus. Using an original headband device (Rimed, Industrial Park Raanana, Israel), we secured both 2-MHz Doppler probes over the trans-temporal windows. An optimal insonation position during monitoring was maintained and escaped movement artifacts. Baseline mean flow velocity (MFVbaseline) was defined as a continuous mean velocity over 30 seconds during the

resting period in supine position and normal breathing of room air. Maximum MFV (MFVmax) was defined as the last 3 seconds of breath-holding after normal inspiration. The minimum breath-holding time was set at 15 seconds and the maximum at 30 seconds. The test was repeated two times, with a resting period of 2 minutes. Breath-holding index (BHI) was calculated as the difference in the increase of MFV (cm/s) occurring during breath-holding divided by the time (seconds) for which the participant held breath ([MFVmax -MFVbaseline] / breath-holding time). We were calculating BHI of each test on the right and left side and then the mean BHI (BHIm) for each participant. The testing of the basilar artery (BA) that represents the posterior cerebral circulation was performed in a seated position, using the same handheld suboccipital insonation probe. Testing was repeated twice to calculate BHIm of the BA. Among the Croatian population, normal BHI values range from 1.03 to 1.65 (17).

Statistical analysis

The sample size was calculated to be 120, effect size 0.35, level of significance 0.05 and power 0.9 (G*power software, version 3.1.9.2, by Franz Faul, University Kiel, Germany). Local ethics committees approved the study.

The category data are represented by absolute and relative frequencies. Differences between categorical variables were tested by the $\chi 2$ test, and, if necessary, by the Fisher exact test. The normality of the distribution of numeric variables was tested by the Shapiro-Wilk test.

Numerical data are described by the arithmetic mean and standard deviation in case of a normal distribution, and by the median and the limits of the interquartile range in cases where the distribution is not normal. The means of the numerical variables of interest were evaluated using a 95% confidence range. The Student's t-test and Mann-Whitney's U test (with Hodges-Lehmann median difference) were used to test the differences in the numerical variables between two independent groups of subjects, depending on the normality of the distribution. The Kruskal-Wallis (post-hoc Conover) test was

used to test for differences in the numerical variables between three or more independent groups. The correlation of the numerical variables was evaluated by the Spearman correlation coefficient ρ (rho). The differences in the numerical variables before and after testing were tested with the Wilcoxon test (19). All P values were two-sided. The level of significance was set at Alpha=0.05. MedCalc Statistical Software version 18.11.3 (MedCalc Software

bvba, Ostend, Belgium; https://www.medcalc.org; 2019) and SPSS (IBM Corp. Released 2015. IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp.) were used for all analyses.

Results

The baseline characteristics of COPD patients according to the severity of airflow obstruction were summarized in Table 2.

Table 2. Baseline characteristics, lung functions, laboratory values and pack-years in COPD groups

| | Median (interquartile range) | | | | P* |
	Mild	**Moderate**	**Severe/very severe**	**Total**	
Age (years)	66 (59 – 70)	68 (62 – 72)	71 (65 – 78)	68 (62 – 74)	**0.01**
‡**FEV$_1$/FVC ratio [Median (interquartile range)]**	0.68 (0.67 – 0.69)	0.645 (0.57 – 0.68)	0.41 (0.34 – 0.48)	0.68 (0.67 – 0.69)	**<0.001**
‡ **FEV$_1$ (%)**	93.5 (87.8 – 103.5)	64 (56 – 69.3)	37 (31 – 42.8)	64 (42 - 88)	**<0.001**
BMI (kg/m²)	29.4 (26.9 – 31.7)	31.7 (25.9 - 35)	24 (19.9 – 29.2)	28.6 (24.1 - 33)	**<0.001**
Body temperature (ºC)	36.2 (36 – 36.5)	36.1 (36 – 36.3)	36.3 (36 – 36.5)	36.2 (36 – 36.4)	0.26
Systolic blood pressure (mmHg)	140 (130 - 150)	132.5 (120 - 146)	130 (120 – 142.5)	132.5 (120 - 150)	0.21
Diastolic blood pressure (mmHg)	80 (80 - 90)	80 (80 - 85)	78 (70 - 80)	80 (80 - 80)	0.05
Hemoglobin (g/l)	136 (130.5 – 150.3)	147.5 (135 - 154)	144 (131.75 - 158)	144 (133 -151)	0.44
CRP (mg/l) [Median (interquartile range)]	2.6 (1.3 – 4.6)	2.5 (1.6 – 6.2)	2.95 (1.3 – 7.73)	2.6 (1.4 – 6.2)	0.69
Pack-years	40.5 (31.8 – 52.5)	43 (27.6 - 51)	49 (41.5 – 73.88)	43 (33.5 – 54.4)	0.15

*Kruskal-Wallis test (Post-hoc Conover); †on level P < 0.05 significant differences between mild vs. moderate, mild vs. severe/very severe, moderate vs. severe/very severe; ‡on level P < 0.05 significant differences between mild vs. severe/very severe, moderate vs. severe/very severe

FEV1/FVC – forced expiratory volume in one second / forced vital capacity; FEV1 – forced expiratory volume in one second; BMI – body mass index; CRP – C-reactive protein; pack-years = number of cigarettes per day x number of smoking years / 20

Arterial hypertension was significantly less observed in the severe/very severe COPD group (χ2 test, P = 0.003) and there were no differences in the presence of hyperlipidemia,

ischemic heart disease and diabetes mellitus among the COPD groups (data not presented).

Significant differences were found in baseline MFV of MCA. In COPD groups, higher velocity was on the left, and in controls on the right MCA.

Baseline MFV of MCA and BA and also BHImMCA and BHImBA were significantly lower in COPD patients than in controls. TAS was significantly higher in COPD than controls (Table 3).

Table 3. Baseline MFV, cerebral vasoreactivity and TAS in COPD patients and controls

	Median (interquartile range)				P*
	Controls	P*	COPD	P*	
MFV MCA right (cm/s)	37 (29.8 – 44.3)		30 (23 - 35)		0.004
MFV MCA left (cm/s)	34.5 (30 – 40.5)	<0.001	31 (23 – 39.25)	<0.001	0.01
MFV BA (cm/s)	29.5 (26 – 30.3)	-	24.5 (17 – 30.3)	-	0.003
BHI$_m$MCA	1.24 (1.16 – 1.39)	-	0.8 (0.7 – 0.9)	-	<0.001
BHI$_m$BA	1.07 (1.02 – 1.15)	-	0.7 (0.6 – 0.9)	-	<0.001
TAS (mmol/L)	1.6 (1.5 – 1.7)	-	1.7 (1.5 – 1.8)	-	0.03

*Mann-Whitney U test; †Wilcoxon test

MFV – mean flow velocity; TAS – total antioxidant status; MCA – middle cerebral artery; BA – basilar artery; BHIm – breath-holding index mean

In COPD groups, based on greater severity of the disease, there were significant reductions in baseline MFV of MCA and BA and we found significant impairment of BHImMCA and BHImBA (Table 4).

Table 4. Baseline MFV, cerebral vasoreactivity and TAS in COPD groups

	Median (interquartile range)						P*
	Mild	P§	Moderate	P§	Severe/very severe	P§	
MFV MCA right (cm/s)	32 (29.8 - 39)	0.78	30.5 (18 – 39.25)	0.53	26 (19 – 32.5)	0.91	0.02[‡]
MFV MCA left (cm/s)	32.5 (25.8 - 41)		31.5 (23 – 37.75)		24.5 (17.8 – 33.3)		0.04[‡]
MFV BA (cm/s)	28 (20.8 – 32.3)	-	22.5 (17 – 30.3)	-	19 (14.5 – 29.3)	-	0.04[‡]
BHI$_m$ACM	0.94 (0.89 – 1.06)	-	0.8 (0.7 – 0.9)	-	0.7 (0.6 – 0.7)	-	<0.001[‡]
BHI$_m$AB	0.83 (0.72 – 1.03)	-	0.7 (0.6 – 0.9)	-	0.6 (0.4 – 0.7)	-	<0.001[‡]
TAS (mmol/L)	1.6 (1.52 – 1.78)	-	1.7 (1.6 – 1.8)	-	1.7 (1.6 – 1.8)	-	0.52

*Kruskal-Wallis test (post-hoc Conover); †on level P < 0.05 significant differences between mild vs. severe/very severe; ‡on level P < 0.05 significant differences between mild vs. moderate, mild vs. severe/very severe, moderate vs. severe/very severe; §Wilcoxon test

MFV – mean flow velocity; TAS – total antioxidant status; MCA – middle cerebral artery; BA – basilar artery; BHIm – breath-holding index mean

Using the Spearman's rank correlation coefficient, a significant negative correlation was found between age and BHImBA in controls. In COPD groups, we found a significant negative correlation between age and BHImMCA and BHImBA. In the COPD group, baseline MFV of BA is in a significant positive correlation with BHImMCA and BHImBA (Table 5).

Table 5. Correlation between age and baseline MFV BA with BHIm MCA and BHIm BA in COPD patients and controls

	Spearman's rank correlation coefficient Rho (P value)	
	Age (year)	MFV BA (cm/s)
Controls		
BHI_mMCA	-0.089 (0.64)	0.051 (0.79)
BHI_mBA	-0.376 (0.04)	0.124 (0.51)
COPD		
BHI_mMCA	-0.375 (< 0.001)	0.336 (0.001)
BHI_mBA	-0.376 (< 0.001)	0.647 (<0.001)

MFV – mean flow velocity; BHIm – breath-holding index mean; MCA – middle cerebral artery; BA – basilar artery

TAS and BHImBA significantly negatively correlated only in COPD groups, but not in the control group (Figure 1).

Figure 1. The correlation between total antioxidant status (TAS) and mean breath-holding index of the basilar artery (BHImBA) in COPD patients and controls
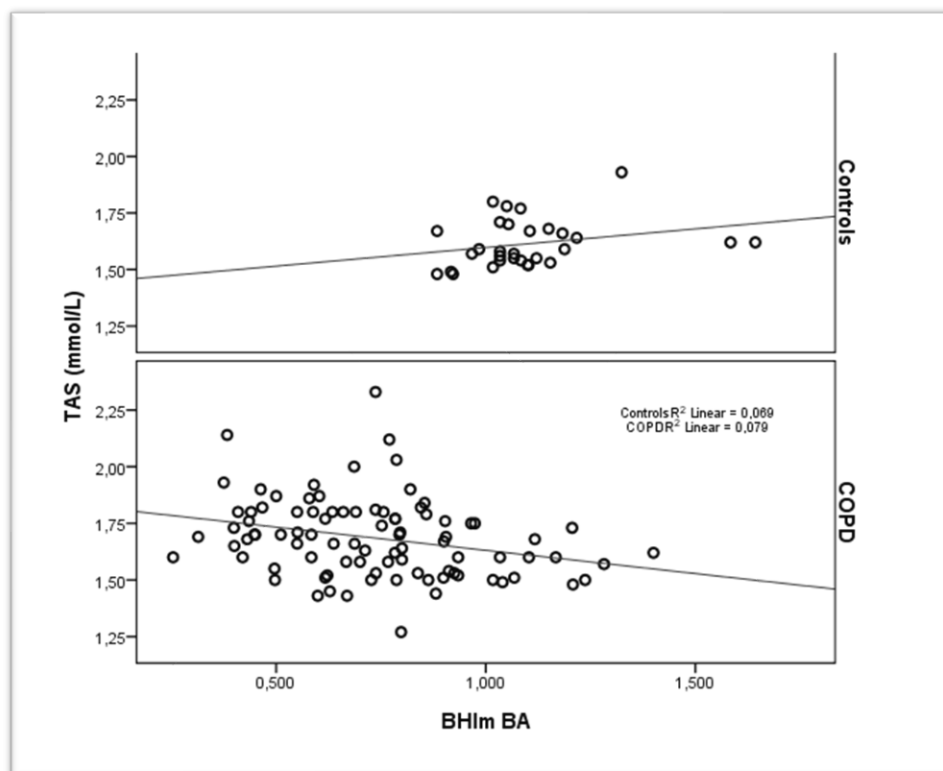
Using the Spearman's rank correlation coefficient Rho, we found a significant negative correlation between TAS and BHImBA (Rho= −0.327, P=0.002) in COPD patients and no significant correlation in controls.

Figure 2. The correlation between total antioxidant status (TAS) and mean breath-holding index of the basilar artery (BHImBA) according to COPD severity
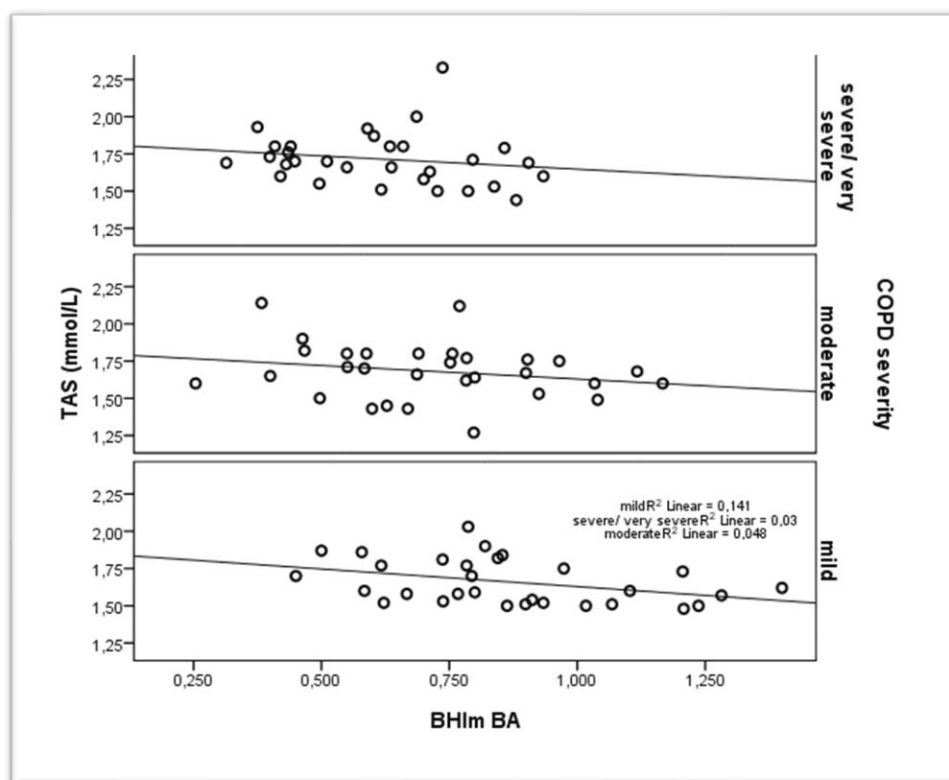
Using the Spearman's rank correlation coefficient Rho, we found a significant negative correlation between TAS and BHIm BA (Rho= −0.445, P=0.01) in the mild COPD groups and no significant correlations in other COPD groups.

There were no significant correlations between TAS and BHImMCA in COPD groups, nor between TAS and BHImMCA in the control group (data not presented). Using the Spearman's rank correlation coefficient Rho, based on the severity of COPD and age, a significant negative correlation was found between age and BHImBA (Rho= −0.398, P=0.03) in the moderate group. In the severe/very severe COPD group, a significant negative correlation was found between age and BHImMCA (Rho= −0.451, P= 0.01), but there were no significant correlations in other COPD groups (data not presented).

In terms of the severity of airway obstruction, we found a significant negative correlation between TAS and BHImBA only in the mild COPD (Figure 2). There was no significant correlation between TAS and BHImMCA in any COPD group (data not presented). In all COPD groups, a significant positive correlation waws found between baseline MFV BA and BHImBA (Figure 3), but there was no correlation between MFV BA and BHImMCA (data not presented).

Figure 3. The correlation between baseline mean flow velocity of the basilar artery (MFV of BA) and mean breath-holding index of the basilar artery (BHIm BA) based on COPD severity
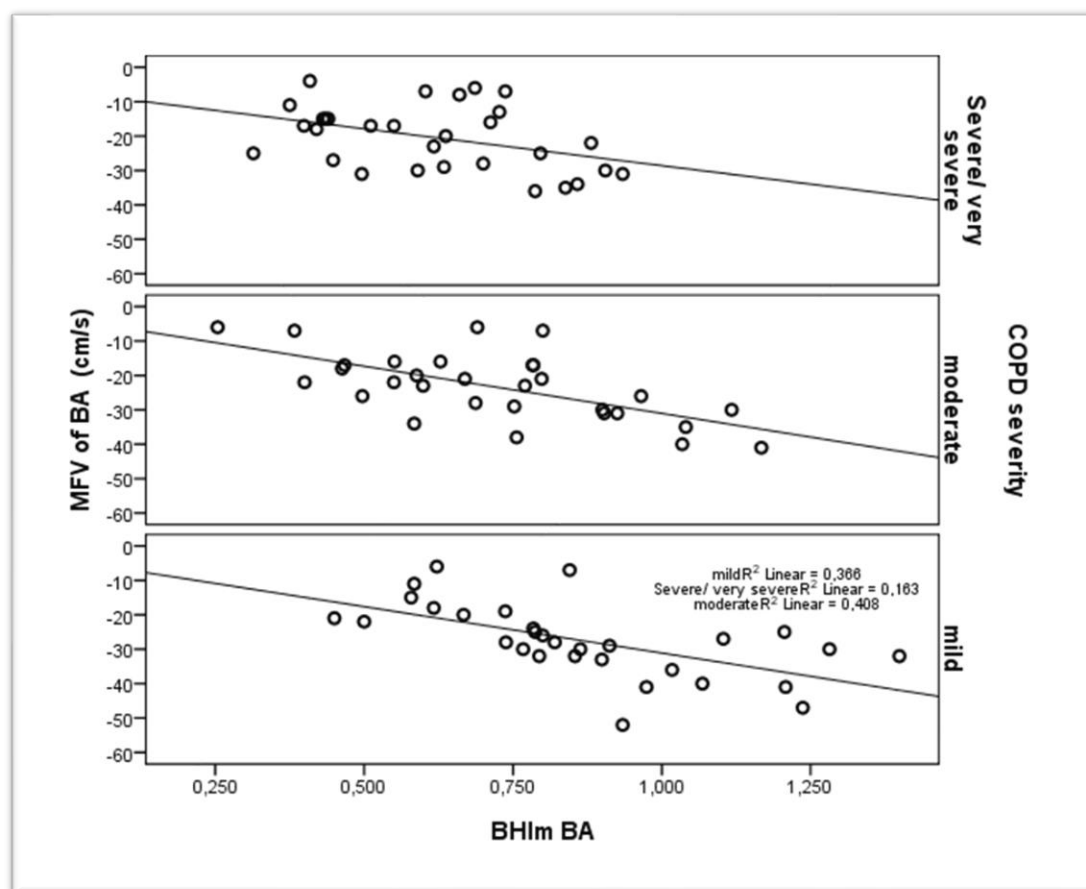
Using the Spearman's rank correlation coefficient Rho, we found a significant positive correlation between baseline MFV BA and BHImBA in all COPD groups: in mild (Rho=0.731, P<0.001), moderate (Rho=0.574, P=0.001) and severe/very severe COPD (Rho=0.398, P=0.03).

Discussion

The aim of the study was to analyze the relationship between CVR and TAS in the plasma of COPD patients and controls. In COPD patients, TAS was in a significant negative correlation with CVR in the posterior cerebral circulation, but in terms of the severity of airflow obstruction, we found a negative correlation between CVR and TAS in the posterior cerebral circulation only in the mild COPD group. There was no significant correlation between CVR and TAS in the anterior cerebral circulation. The results from this study do not allow conclusions that impaired CVR is associated with systemic changes in the antioxidative status of COPD patients.

A significantly higher TAS values were found in COPD patients than in controls, which contrasts the previously published data. The reason for that could be multifactorial, but determining the mechanisms for was in the scope of our recently published manuscript, where we demonstrated that TAS is a predictor of COPD (20).

In terms of gender, there were more men participating in the study than women, but they were equally represented in the COPD groups and in the control group. All women in the study have reached menopause. In that period, oxidative stress was reported to increase because of the decreasing levels of estrogen, which has beneficial vasoactive and antioxidative effects (21). A study of postmenopausal women with moderate COPD

related to smoking analyzed oxidative stress and CVR in the anterior cerebral circulation. The study showed an impaired cerebrovascular response to hypercapnia, significantly higher levels of oxidative stress, a higher level of glutation peroxidase that represents antioxidant enzyme activity and a higher ratio between oxidative stress and antioxidant activity than in controls. There was no significant correlation between the markers of oxidative stress/antioxidants and CVR measured by TCD. The only correlation was found between higher catalase activity, which represents antioxidant enzyme activity associated with higher ventilatory response sensitivity. In the context of higher oxidative stress caused by the decline of estrogen levels in menopause, it was concluded that that could be a possible explanation of the observed differences in cerebrovascular sensitivity to hypercapnia between COPD women and controls (10).

Hypoxemia is part of clinical features of COPD patients. A recent study analyzed how lifetime exposure to hypoxia (3,600 m above sea-level, La Paz, Bolivia) affects oxidative stress, CVR and cognitive function. Chronic mountain sickness is a maladaptation syndrome experienced at high altitudes, characterized by severe hypoxemia. The syndrome is related to a higher risk of stroke, migraine, increased morbidity and mortality. It has been confirmed that individuals with such syndrome have exaggerated oxidative stress and a corresponding decrease in vascular NO bioavailability. Oxidative stress was associated with an impaired cerebrovascular function. The authors of this study concluded that oxidative stress induced by hypoxemia is associated with blunted cerebral perfusion, impaired CVR to hypercapnia, accelerated cognitive decline and depression (18).

In our study, COPD patients had significantly impaired CVR. Impaired CVR, that is impaired CO2 vasoreactivity as a surrogate of endothelial dysfunction, results in a decline of NO (8). A recent study on an animal model showed that an increase in the bioavailability of NO through prostaglandin pathways causes vasodilatation; therefore, it increases oxygenation and

produces higher TAS. That way, the antioxidant – TAS eliminates free radicals from biological systems (22). This conclusion is based on a previous study suggesting that as the TAS value increases, free radicals decrease, which protects macromolecules from damage (23). A recent study analyzed age- and apolipoprotein E (APOE) gene-specific hemodynamic changes using the blood-oxygen-level-dependent (BOLD) functional magnetic resonance imaging (MRI) method. The breath-holding method was used for the examination of CVR and visual stimulation tasks to evoke functional hyperemia. After a three-day intake of nitrate as a NO source, there was no effect on CVR or functional hyperemia, but CVR significantly decreased with age and was dependent on the genotype of participants. That way, the response of vascular functions on breath-holding could be dependent on the APOE-genotype and independent of NO (24). Such an analysis far exceeds our technical capabilities, but it would certainly be useful to include a genetic analysis in the endothelial dysfunction analysis of COPD patients, in order to successfully manage therapeutic decisions, evaluate the response to target interventions and provide prognostic information.

In our study, there were differences between the baseline right and left anterior cerebral perfusion in COPD patients and controls. Determining the mechanism(s) involved in those differences was not the goal of this study. Our opinion is that the differences observed were not significant for the ultimate results of the study.

In COPD, the baseline anterior and posterior cerebral perfusion was significantly lower than in controls. Based on greater severity of COPD, a significant decline of perfusion was recorded in the anterior and posterior cerebral circulation. The baseline posterior cerebral perfusion was in a significant positive correlation with CVR of the anterior and posterior cerebral circulation in COPD, which was not found in controls. In terms of the severity of airway obstruction, all COPD groups had a significant positive correlation between the baseline posterior cerebral

perfusion and CVR in the posterior cerebral circulation. Decreased cerebral perfusion means reduced cerebral blood flow velocity. TCD perfusion analysis assumes that there is no change in the width of the artery lumen and that the blood flow is laminar. Despite that interpretation constraint of TCD, in the study, in cases when lower cerebral perfusion was recorded, that decrease correlated with an impaired ability of the cerebral arteries to respond to vasodilator stimuli, such as hypercapnia (18,25,26).

In our study, a significant negative correlation was found between age and CVR in the posterior cerebral circulation only in the moderate COPD group and between age and CVR in the anterior cerebral circulation in the severe/very severe COPD group. Therefore, the results do not allow a final conclusion on the correlation between the age of COPD patients and CVR in our sample. In the study that used functional TCD and hypercapnic stimuli as a marker of CVR, the cerebral perfusion changes were determined as a response to the language and arithmetic task in healthy young, healthy old and old subjects with the risk factors for atherosclerosis. Arterial hypertension was the most frequent risk factor and the breath-holding method was used as a hypercapnic stimulus. The cerebral perfusion changes were significantly lower during the cognitive task and hypercapnia in the group of old subjects with vascular risk factors compared to the healthy young and old subjects. These results showed that the presence of cardio- and cerebrovascular risk factors significantly reduces the ability of cerebral vessels to react to vasoactive stimuli. Aging alone, without such risk factors, could not be responsible for hemodynamic changes caused by neuronal activation (27). In the study that used the pulsed arterial spin labelling perfusion MRI and BOLD methods, the regional cerebral blood flow and CVR were assessed in young and elderly participants. Age-related decreases in the baseline CBF and CVR were observed in the cerebral cortex, which may be related to the vulnerability to neurological disorders in aging (28). Detection of early dysfunction of the cerebral vascular system in older people with

the risk of developing cognitive impairment ensure the possibility of vascular-specific procedures which help maintain cognitive function through a lifetime (29). The TCD ultrasound technique does not allow clear spatial resolution of CVR.

The main limitation of the study was that TAS was the only biomarker that we performed because of technical and financial reasons. In comparison with clinical characteristics and individual biomarker, the combination of COPD markers increases the prognostic value for proper outcome monitoring (30,31). We did not analyze any of the oxidants, so the increased oxidative burden in our population is not clearly proven. On the other side, the choice of peripheral blood among other biological samples is more appropriate because of the non-invasiveness of blood sampling and its property of easily allowing repeated measurements (11).

The main limitations of TCD, and therefore of our study, are its dependency on the operator, the presence of adequate insonation windows and the possibility of recording inaccurate mean flow velocities due to wrong insonation angles (32). The strength of TCD is that the estimation of cerebral blood flow velocity, that is cerebral perfusion and CVR by that method, using CO2 as a hypercapnic stimulus, has acceptable levels of reproducibility. It is an appropriate method for determining the effect of hypercapnia on cerebral haemodynamics (33).

The correlations between CVR and systemic levels of the antioxidant marker of TAS in the plasma have not been demonstrated in all COPD groups. However, based on the decrease in perfusion in the anterior and posterior cerebral circulation detected in COPD patients compared to controls, there is a possible correlation between CVR and the initial perfusion in the basal cerebral arteries.

According to previous studies, there are correlations between impaired CVR and the risk of stroke, progressive global vascular damage, an increased risk of mortality, especially

cardiovascular mortality, cognitive decline and dementia (34–38). Early detection of COPD patients at risk of these comorbidities presents an opportunity for early therapeutic interventions aimed at reducing such risks.

Conclusion

In our study, a negative correlation between CVR and the plasma level of TAS was found only in the posterior cerebral circulation in the mild COPD group. The results do not allow the conclusion that impaired CVR is associated with systemic changes in the antioxidative status of COPD patients. However, we found that decreased perfusion in the basilar artery of COPD patients was in a significant positive correlation with CVR of the anterior and posterior

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cerebral circulation, which was not observed in controls. The analysis of perfusion in the basal cerebral arteries should be part of a future study of CVR in COPD patients.

Disclosure

Competing interests. None to declare.

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Administrative, technical or logistic support: Hlavati M, Tomić S, Buljan K, Butković-Soldo S
Analysis and interpretation of data: Hlavati M, Tomić S, Buljan K, Butković-Soldo S
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Provision of study materials or patients: Hlavati M, Buljan K
Statistical expertise: Hlavati M, Tomić S, Buljan K, Butković-Soldo S

Abbreviations. COPD – chronic obstructive pulmonary disease; CBF – cerebral blood flow; $PaCO_2$ – arterial partial pressure of carbon dioxide; CO_2 – carbon dioxide; NO – nitric oxide; CVR – cerebral vasoreactivity; TAS – total antioxidant status; GOLD – Global Initiative for Chronic Obstructive Lung Disease; FEV1 – forced expiratory volume in one second; FEV1/FVC – forced expiratory volume in one second FEV1 to forced vital capacity FVC ratio; BMI – body mass index; TCD – transcranial Doppler ultrasound; CRP – C-reactive protein; MCA – middle cerebral artery; MFV – mean flow velocity; BHIm – breath-holding index mean; BA – basilar artery; APOE – apolipoprotein E; BOLD MRI – blood-oxygen-level-dependent magnetic resonance imaging method.

Short review article

Percutaneous Laser Disc Decompression in the Treatment of Lumbar Radicular Pain

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Abstract

Lumbar radicular pain is defined as pain in the lumbar spine with propagation to the lower extremities. It is a major public health, social and economic problem in the modern society, and is one of the most common reasons for visits to the doctor. Lumbar radicular pain is often the reason for absenteeism and occupational disabilities. It is estimated that about 70-85% of the world's population have experienced lumbar spine pain once in their lifetime. There are numerous modalities for the treatment of lumbar radicular pain, ranging from pharmacotherapy to surgery. In order to avoid systemic side effects of analgesics, anaesthesia and long-term and extensive surgery, minimally invasive procedures are increasingly used for treating lumbar radicular pain. Percutaneous laser disc decompression (PLDD) is one such procedure, first performed by Dr Choy and Dr Ascher in 1986. PLDD is an outpatient surgery performed under local anaesthesia, its success rate is high and the complication rate is low. This method therefore certainly attracts the attention of clinicians dealing with this issue.

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Introduction

Improper posture, sedentary lifestyle and frequent weightlifting are the most common causes of herniated intervertebral discs, which is the reason why young, working-age people suffer the most from such pain. It is estimated that 80% of people experience low back pain in their lifetime (1). Lumbar pain with or without a radicular component most commonly occurs in adults, but it may also occur in the paediatric population (2). Lumbar radicular pain is first treated conservatively, and if the pain persists, the gold standard is surgical microdiscectomy. In most patients who experience the first episode of lumbar radicular pain, it decreases to a level that does not impair daily functioning

after 6 weeks (3). The increase in incidence of low back pain caused by herniated intervertebral disc increased the attention given to minimally invasive pain management (1). Due to faster recovery than after surgical microdiscectomy, percutaneous laser disc decompression (PLDD) has attracted much attention. The first PLDD was performed in 1986 and the procedure was approved by the FDA in 1991 (4). Percutaneous laser disc decompression is a minimally invasive method in which the risk of damage to muscles, bones, ligaments and nerves is reduced. The aim of this study is to present PLDD as one of the treatment modalities of lumbar pain caused by disc herniation.

Mechanism of pain onset

Lumbar radicular pain can be caused by compression of the herniated disc on the nerve, but it may also be caused by local inflammation. Acute pressure on the nerve root causes numbness, paraesthesia, weakness and pain. Disc pressure on the nerve root leads to disruption of nutritional supply to the nerve, increased permeability of blood vessels, impaired ionic balance and changes in the conduction of the nerve impulse. When there is no compression of the herniated disc on the nerve root, the cause of pain are substances from the nucleus pulposus, proteoglycans and a lowered pH level. When the integrity of the annulus fibrosus is impaired, a substance leaks into the epidural space, producing a proinflammatory effect. Phospholipase A2 plays an important role in this process. Much higher phospholipase A2 levels were observed in disc extrusion than in intact disc; this substance is involved in the synthesis of prostaglandin and leukotriene, leading to a local inflammatory response (5).

Mechanism of action of percutaneous laser disc decompression

The mechanism of action of percutaneous laser disc decompression is based on the principle that the disc is viewed as a closed hydraulic system containing water, which is incompressible. The water content of the disc is about 50-89%, and decreases with age. Laser

energy warms the surrounding tissue. In this way, a small volume of nucleus pulposus water content is evaporated (Figure 1). A small change in the volume of water results in a disproportionate decrease in pressure inside the disk (6). A decrease in pressure within the disc causes the hernia to withdraw and leads to a decrease in pressure on the root of the involved nerve. This reduces lumbar radicular pain caused by disc herniation.

Figure 1. The laser probe is inserted into the needle. Evaporation of water from the nucleus pulposus (author's work).
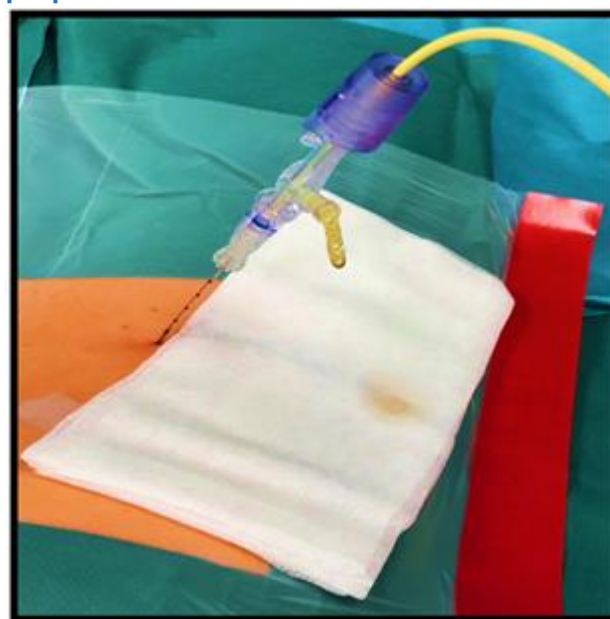
Anatomy of the intervertebral disc

The intervertebral disc is a complex structure; it is the largest avascular structure in the body and it enables spinal mobility (7). It consists of the outer part, annulus fibrosus, and the inner part, nucleus pulposus (8). Annulus fibrosus consists of 15-25 layers of crisscrossed fibres; the number of layers increases in the lumbar spine segment. Annulus fibrosus consists of about 60% collagen and 20% proteoglycan, and nucleus pulposus consists of 65% proteoglycan and 20% collagen (9). Annulus fibrosus is innervated by spinal nerve branches, while there is no innervation of the nucleus pulposus in an intact disc. The metabolism of the disc is mainly anaerobic and nutrients are diffused through the endplate. Degeneration of the endplate thus results in

insufficient nutritional supply of the intervertebral disc (10). Reduced amounts of proteoglycans, collagen, water, and calcified endplate lead to disc damage. Degenerative changes of the disc lead to a reduction in intervertebral distance and thus cause osteoarthritis of the facet joints. The incidence of degenerative disc changes increases with age, and if they occur in younger people, the cause is most often a genetic predisposition or injury. Some of the factors that accelerate degenerative changes of the disc include smoking, atherosclerosis, frequent lifting of heavy loads, and a sedentary lifestyle.

Technique of performing percutaneous laser disc decompression

PLDD is performed in the operating room. During the procedure, the patient lies in a prone position. The skin at the intended puncture site is sterilized, prepared and protected from the surrounding area with sterile compresses. After the relevant intervertebral space (disc) is visualized, the optimal position is found by lateral and craniocaudal angulation. Local anaesthetic infiltration is performed at the puncture site and an 18 G needle insertion is performed at the same place. The needle advances under fluoroscope control to be positioned in the middle of the disk, and the final position is confirmed by lateral projection and contrast application (Figure 2). The laser probe is then inserted into the needle, supplying laser energy according to default parameters, which depend on the device and the protocol of each institution. Laser energy leads to tissue heating and evaporation of a small volume of water within the nucleus pulposus (11, 12).

Figure 2. Confirmation of proper needle position in intervertebral disc after contrast administration. Intervertebral disc L5/S1 (author's work).

Indications and contraindications

An indication for PLDD is symptomatic disc protrusion. The pain may be localized in the lumbar spine or it may radiate into one or both legs. Discogenic pain is likewise an indication for PLDD. Discogenic pain is pain that is not of radicular origin, it occurs in the absence of spinal deformity, and it has no positive signs of nerve tension. The generators of discogenic pain are nociceptive fibres of the annulus fibrosus. The outer posterolateral portion of the annulus fibrosus is rich in sensory fibres.

First degree spondylolisthesis, mild scoliosis, and osteoarthritis are not contraindications for PLDD. Prior surgery is also not a contraindication, unless it was a vertebral fusion or there are nerve root adhesions. PLDD can also be performed if there is disc extrusion without sequestration. There are differing opinions, but the author of the method believes that PLDD can also be used in spinal canal stenosis exacerbated by disc herniation (13).

Contraindications for PLDD are acute pain that has not been treated conservatively, because 80-85% of acute pain disappears after resting,

relaxation, non-steroidal anti-inflammatory drugs, and epidural administration of steroids and local anaesthetic. PLDD is contraindicated in severe spondylolisthesis, severe scoliosis, metastatic cancer, vertebral compression fracture, compression of the nerve root with the bone, and the presence of free disc sequestration. Advanced age is not a contraindication, but in the elderly, the amount of water in the disc is reduced, which is more pronounced in males. Haemorrhagic diathesis, near-disc vertebral haemangioma, multiple sclerosis, demyelinating diseases, and systemic infections are also contraindications (14).

Postprocedural recommendations

After PLDD, the patient is recommended to rest and lie, as well as to abstain from sitting and walking. After the first day, it is recommended to limit sitting and walking to a maximum of 20 minutes. Wearing a lumbar orthosis for two weeks in order to reduce mobility is likewise recommended. Patients who do not do manual labour can return to work after 3 days, while those who do manual labour can return to work after 7-10 days. Physical therapy can be performed after 1 week. Antibiotic prophylaxis is recommended to prevent infection (14).

Complications of PLDD

Possible complications of PLDD include nerve root injury, cauda equina syndrome, bowel perforation, thermal injury along the needle path, and thermal necrosis of the endplate. Paraspinal muscle spasm that causes patients discomfort is also described, and in more severe cases, a physical examination will show lateral curvature of the spine with a concavity to the side of spasm. Muscle tension can be sensed by palpation. Muscle spasm disappears after 3-4 days and does not affect the outcome of the treatment. Benzodiazepine as a relaxant and local heat can be used to relieve the spasm. Aseptic or infectious discitis are more serious complications. The most common cause of infectious discitis is Staphylococcus aureus. Symptoms usually occur 3-5 days after surgery and manifest as fever and pain of the affected

disc and increased serum inflammatory parameters. If discitis is suspected, an emergency MRI of the affected disc should be performed. This condition requires antibiotic treatment. The incidence is less than 1%. The diagnosis of aseptic discitis is made by ruling out septic discitis. Signs and symptoms are the same as those for septic discitis, but there is no fever or elevated inflammatory parameters. The condition improves after several days of rest and administration of non-steroidal anti-inflammatory drugs.

Inflammation of the sacroiliac joint is likewise possible. It usually occurs several days after an excellent response to PLDD. The mechanism of pain onset is thought to be the cessation of lumbar radicular pain, which leads to the cessation of compensatory "locking" of the lumbosacral (LS) segment and sacroiliac (SI) joint. This condition is treated by infiltration of the SI joint with local anaesthetics and corticosteroids and non-steroidal anti-inflammatory drugs (14).

Discussion

Patients who experience pain caused by herniation of the intervertebral disc and who do not respond to conservative treatment are candidates for surgery (15). Surgical treatment is still the gold standard in the treatment of disc herniation, but classic surgery may impair spinal stability, so consideration should be given to minimally invasive treatments (16, 17). Compared to discectomy/microdiscectomy, PLDD has fewer postoperative complications and tissue injuries (18). A group of Iranian researchers observed the impact of PLDD on pain intensity as measured by the Visual Analogue Scale (VAS) and on disability as measured by the Oswestry Disability Index (ODI). The mean VAS before the procedure was 6.70, and it was 2.60 after the procedure. The smallest pain reduction was observed in a 27-year-old man and was 43%, while the highest pain reduction was 71%, observed in a 45-year-old female. The mean ODI before surgery was 31.03 and it was 20.60 after surgery, which is statistically significant. (p < 0.001) (11).

PLDD is a minimally invasive procedure and could in some cases be used as an alternative to surgical discectomy. The study compared the success rates and complications between PLDD and microdiscectomy. There was no difference in efficiency between the two methods. The rate of reoperation in the surgical group was 21%, and it was 52% for PLDD, which is higher than expected. Although the rate of recurrence in the PLDD group was relatively high, we can conclude that surgical discectomy was avoided in 48% of patients over a two-year period (19). A randomized controlled trial compared the efficacy of PLDD and conventional microdiscectomy. The study was conducted on 115 patients who had lumbar radicular pain caused by an intervertebral disc hernia no larger than one third of the spinal canal. The Roland-Morris Disability Questionnaire showed that, at 8 and 52 weeks, PLDD is equivalent to conventional surgery. Recovery was expected to occur faster with PLDD. The rate of reoperation was significantly lower with conventional microdiscectomy (38% vs. 16%). At one-year follow-up, PLDD proved to be equivalent to conventional microdiscectomy (20). A study comparing 500 microdiscectomies and 500 PLDDs was performed. In the microdiscectomy group, 428 patients (85.6%) had good or excellent results, as opposed to 419 in the PLDD group (83.8%). The complication rate in the microdiscectomy group was 2.2% (11 patients), while there were no complications in the PLDD group (21). The author of a different study, Dr Choy, presented the results of PLDD over 17 years; this study included 1,275 patients and 2,400 performed PLDDs (neck, thoracic and lumbar segments). The success rate according to MacNab criteria was 89%. The complication rate (infectious discitis) was 0.4%, and there were no cases of nerve root injury or spinal cord injury (22).

Although extrusion was previously considered as a contraindication, Choy et al presented the results of a study that included 21 patients with disc extrusion without sequestration. Eighteen patients experienced significant reductions in pain and, in some cases, a reversal of neurological deficits (23).

A group of authors performed a systematic review and meta-analysis to compare the complication rates of different discectomy methods. The methods that were compared were open discectomy/microdiscectomy (OD/MD) with microendoscopic discectomy (MED), percutaneous endoscopic lumbar discectomy (PELD) and percutaneous laser disc decompression (PLDD) and tubular discectomy. They found 17 randomized controlled trials and 20 cohort studies that met their criteria. Meta-analysis of RCTs showed that the overall complication rates for OD/MD, MED, PELD, PLDD and tubular discectomies were 16.8%, 16.2%, 21.2%, 5.8%, 8.4% and 25.8%, respectively. Reoperation rates were 8.4%, 4.7%, 6.7%, 23.2% and 11.7%, respectively. Meta-analysis of cohort studies showed that overall complication rates were 7.6%, 6.2%, 9.1%, 3.5% and 11.6%, respectively. Reoperation rates were 5.5%, 0.8%, 9.4%, 3.2% and 3.7%, respectively (24). Patel and Singh, in a retrospective study conducted on 65 patients treated with PLDD, reported that the preprocedure VAS score was 7.6/10 and at 2-week, 6-week, 3-6 month intervals, it was 3.7/10, 4.3/10, and 4.1/10, respectively (25). Proper patient selection for PLDD treatment has short-term and long-term benefits.

Conclusion

Percutaneous laser disc decompression is a minimally invasive procedure, with a low complication rate, high success rate, and rapid recovery. Proper selection of patients with lumbar radicular pain caused by herniated intervertebral disc produces good results and may delay surgery or, in some cases, be an alternative to surgery.

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Original article

Validity of Neuroimaging in Juvenile Headaches

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Abstract

Aim: The objectives of this study are to evaluate the incidence of headache considering the type of headache, to define the types of headaches, to determine the difference in the status of diagnostic scanning in children with headaches, to establish a correlation between the psychological profile of the child and the incidence of headache, and to establish a correlation between socio-demographic characteristics and the type of headache.

Patients and Methods: The study included 139 patients with headache symptoms up to the age of 18, hospitalized at the Pediatric Clinic of the University Hospital Center Osijek from 1/1/2017 to 31/12/2018. The data included demographic data, diagnosis, environmental factors, EEG findings, neuroimaging data processing and other indicated medical tests.

Results: A headache usually occurs between the ages of 12 and 18 (69.8%). It is more common in girls (70.5%). The common localizations are frontal and occipital. Altered standard EEG findings were reported in 26.7% of patients. Additional neuroradiological processing (brain MRI) was indicated in 98 patients (70.5%), with changes found in 56 patients (57.1%). Psychological assessment indicated that patients with functional headaches predominantly suffer from anxiety, emotional instability and somatization, while patients with organic headaches reported high stress levels (82%).

Conclusion: Headaches occur more frequently in pubescent girls. The most common concomitant symptoms include nausea and vomiting, while the most common localization is frontal. Patients also report emotional instability, cognitive deficits and somatization, as well as high stress levels. Headache as a result of psychological tension is the most common diagnosis in the observed group of patients.

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Introduction

A headache in childhood is not a rare symptom, regardless of whether the headache background is organic or functional. According to the experience of numerous authors, childhood headache is most commonly of organic origin (1). The headache is determined by localization (frontal, occipital, temporal), pain type (dull, pulsating pain), frequency, duration and associated symptoms and signs (2).

The incidence of headaches depends on age and gender, as well as on the type or category of headache. The prevalence of headaches of different types at the age of 7 is 31 51%, increasing to 57-82% at the age of 15 (3). In school children, the most common type are primary headaches, which are defined as headaches that are not associated with an underlying disorder (Group 1-4, according to the classification of the International Headache Society (IHS Classification)) and classified as migraines or tension-type headaches (TTH). The most common causes of secondary headaches are viral respiratory infections (29-39%) and mild head injuries (4, 5). Researchers have found that the incidence of migraine (except for migraine with aura) is similar in girls and boys, while tension-type headaches occur more often in girls, and that gender has some effect on IHS criteria for migraine, but it has almost no effect on those for tension-type headaches (6). Risk factors for headaches or chronic headaches are as follows: dysfunctional family situation, regular alcohol consumption, caffeine intake, smoking, low levels of physical activity, physical or emotional abuse, bullying, misconduct at school, and insufficient leisure time (4).

Each child with a headache requires an individual approach. The assessment of a child suffering from a headache begins with a thorough physical examination, including blood pressure measurement, a neurological examination and a meticulous medical history (3). If a child with recurrent headaches has a normal neurological status during the examination, it is not necessary to routinely (urgently) perform a neuroradiological assessment (brain CT or MRI). A neuroradiological assessment is certainly indicated for determining the cause of secondary headaches. Cerebrospinal fluid analysis is needed in febrile immunocompromised children with a headache due to suspected meningitis. It is not necessary to routinely perform electroencephalography (EEG) in the evaluation of children with headaches. EEG is indicated if epileptic seizure or a secondary headache (caused by a tumor, head trauma, encephalitis, intracranial hemorrhage or ischemia) is suspected. However, changes in EEG are in principle neither specific nor pathognomonic for specific causes of secondary headaches (7).

The purpose of this study was to examine the types of headaches, the most common symptoms of headaches and their comorbidities, and to emphasize psychological examination in addition to the usual neurological treatment.

Patients and Methods

The study was conducted at the Pediatric Clinic of the University Hospital Center Osijek. The study included 139 pediatric patients diagnosed with headaches, hospitalized and treated between 1 January 2017 and 31 December 2018.

Data from the medical records and information system of the Pediatric Clinic included: age, sex, headache diagnosis, perinatal risk factors and psychomotor development of the child, child's admission status (clinical and neurological status), family history, data on the child's education and/or kindergarten attendance, child's psychological profile, EEG findings, neuroimaging processing, and other indicated tests.

Ethical approval for the study was obtained from Ethical Committee Faculty of Medicine University of Osijek and approval to conduct study was obtained from Clinical Hospital Centre Osijek.

Statistical analysis

The data collected were processed using statistical software R (version 3.3.2, www.r-project.org). Categorical data are descriptively represented by absolute and relative frequencies. Differences between categorical variables were examined using the $\chi 2$ test and the binominal test (23, 24). The level of statistical significance was determined as p < 0.05.

Results

The total number of hospitalized patients over the two observed years was 5554, of which 139 had headaches. Thus, the prevalence of hospitalization for patients with headaches was 2.5% with a 95% confidence interval of 2.11% to 2.95%. During the two observed years, there were no significant differences in the number of hospitalized patients (p = 0.799). In 2017, the number of hospitalized patients with headaches was 71, and in 2018, it was 68. A significant difference was found in the number of hospitalizations based on gender (p < 0.001). There was a significantly higher proportion of hospitalized female patients (70.5 %) in comparison to male patients (29.5%). When it comes to age, the highest prevalence of hospitalized patients was between the ages of 12 and 18 (69.78 %), followed by those between the ages of 5 and 12 (26.62 %), while only five patients were under the age of 5 (Table 1).

Table 1. Patients' demographic data

		n (%)	p*
Year	2017	71 (51.08)	0.799
	2018	68 (48.92)	
Gender	Male	41 (29.5)	< 0.001
	Female	98 (70.5)	
Age group	≤ 5	5 (3.6)	< 0.001
	5 to 12	37 (26.62)	
	12 to 18	97 (69.78)	

*χ^2 test

There was a significantly higher proportion of newly diagnosed headaches (59 %), p = 0.034. In

regard to the number of hospitalizations, a significant number of patients were hospitalized more than once (58.7%). Functional headaches were reported in 41.01% of cases and organic headaches with comorbidities in 58.99% of cases (p = 0.034) (Table 2).

Table 2. Related characteristics of hospitalized patients with headaches

		n (%)	p*
Headache	Newly diagnosed	82 (58.99)	0.034
	Controlled	57 (41.01)	
Number of hospitalizations	1	57 (41.3)	0.041
	> 2	81 (58.7)	
Headache type	Functional	57 (41.01)	0.034
	Organic	82 (58.99)	

*χ^2 test

The highest number of patients complained of frontal headaches (40.29%), followed by occipital (17.27 %), parietal (13.67 %) and temporal (10.79 %) headaches. A lower number of patients suffered from headaches with multiple localizations: frontotemporal – 4.32 %, parieto-occipital – 2.16 % and diffuse – 3.6 % (Table 3).

Table 3. Headache localization

	n (%)	p*
Frontal	56 (40.29)	< 0.001
Parietal	19 (13.67)	
Occipital	24 (17.27)	
Temporal	15 (10.79)	
Frontotemporal	6 (4.32)	
Parieto-occipital	3 (2.16)	
Diffuse	5 (3.6)	

*χ^2 test

A statistically significant difference was found in the frequency of concomitant symptoms. Nausea and vomiting (44.85 %) are reported most frequently, followed by dizziness (33.09 %), loss

of consciousness (25.74 %), blurred vision (25 %), weakness and fatigue (17.65 %), tingling in hands (11.51 %). Tics are rarely reported as a concomitant symptom of headache (0.74 %) (Table 4).

Table 4. Concomitant symptoms of headaches

	n (%)	p*
Nausea and vomiting	61 (44.85)	< 0.001
Dizziness	45 (33.09)	
Loss of consciousness	35 (25.74)	
Blurred vision	34 (25)	
Febrility	6 (4.41)	
Tingling sensation in hands	16 (11.51)	
Absence seizure (petit mal)	5 (3.68)	
Photophobia	13 (9.56)	
Hand tremors	4 (2.94)	
Weakness and fatigue	24 (17.65)	
Tics	1 (0.74)	
Non-epileptic seizure	13 (9.56)	

*χ² test

There were 80 % of patients with normal laboratory findings (p = 0.003). There were 26.72 % of patients with altered standard EEG, as well as 31.76 % of patients who underwent partially sleep-deprived EEG. 5 0% of patients who underwent MR angiography of the brain had altered findings. 36.67 % of patients who underwent VEP testing had altered findings as well. There were 22.88 % of patients with altered ophthalmic findings and 46.43 % with altered ORL findings. Of all the patients who underwent an ECG, only one patient had altered findings, as well as pathologically changed continuous blood pressure monitoring. Two out of five patients had altered lumbar puncture findings and two out of 11 patients who underwent an initial CT scan reported changes (Table 5).

Table 5. Test results

		n (%)	p*
Laboratory data	Normal	20 (80)	0.003
	Altered	5 (20)	
EEG, standard	Normal	96 (73.28)	< 0.001
	Altered	35 (26.72)	
EEG, partially sleep-deprived	Normal	58 (68.24)	< 0.001
	Altered	27 (31.76)	
Brain MRI	Normal	42 (42.86)	0.669
	Altered	56 (57.14)	
MR angiography	Normal	3 (50)	> 0.999
	Altered	3 (50)	
VEP	Normal	19 (63.33)	0.144
	Altered	11 (36.67)	
Ophthalmic findings	Normal	91 (77.12)	< 0.001
	Altered	27 (22.88)	
ORL examination	Normal	15 (53.57)	0.706
	Altered	13 (46.43)	
ECG	Normal	34 (71.14)	< 0.001
	Altered	1 (2.86)	
Pressure Holter	Normal	3 (75)	0.317
	Altered	1 (25)	
Lumbar puncture	Normal	3 (60)	0.655
	Altered	2 (40)	
Head CT	Normal	9 (81.82)	0.035
	Altered	2 (18.18)	

*χ2 test

Statistically significant differences were found in the incidence of headache types and particular categories of psychological disorders (p = 0.024). Significantly more disorders were reported in terms of emotional instability (15.15 %), cognitive deficits (15.15 %) and somatization (18.18 %) in functional headaches, while significantly higher levels of stress and similar conditions were

observed with organic headaches (82 %) (Table 6).

Table 6. Psychological disorder and headache type

	Functional	Organic	p*
	n (%)		
Emotional instability	5 (15.15)	4 (8)	0.024
Cognitive deficits	5 (15.15)	3 (6)	
Stress	17 (51.52)	41 (82)	
Somatization	6 (18.18)	2 (4)	

*χ2 test

Extremely difficult psychological conditions in children required a psychiatric consultative examination. 10.79 % of patients underwent psychiatric examination as part of headache treatment (p < 0.001) (Table 7).

Table 7. Reasons according to consultative psychiatric examination

	n (%)	p*
Extremely high depression level	1 (6.67)	0.395
In therapy because of suicidal thoughts and self-harm	2 (13.33)	
Pronounced symptoms of somatization disorders and anxiety-related disorders	1 (6.67)	
Organic origin of headache excluded; in psychiatric therapy until further notice	1 (6.67)	
Somatization headaches	2 (13.33)	
Outpatient treatment started	5 (33.33)	
In therapy because of bullying at school	1 (6.67)	
Identified PTSD symptoms due to loss of parent(s)	1 (6.67)	
Multiple hospitalizations	1 (6.67)	

*χ2 test

A significant difference was found in the proportion of final headache diagnoses. Headaches as a consequence of psychological tension (26.62 %) account for the largest proportion (Table 8).

Table 8. Definitive headache diagnosis

	n (%)	p
Head trauma	8 (5.76)	< 0.001
SAH	2 (1.44)	
Tumor	0 (0)	
Sinusitis	13 (9.35)	
Acute infection	13 (9.35)	
Inflammatory CNS disease	1 (0.72)	
Cessation of AET intake	2 (1.44)	
Consequence of psychological tension	37 (26.62)	
Papillary edema of the optic nerve	5 (3.5)	

*χ2 test

Discussion

Headache is the most common neurological symptom and one of the most common childhood pains (8). According to the literature, the estimated incidence of recurrent headaches in children is about 80 %, with 10 % of them experiencing headaches more than 5 days a month, which negatively affects both the child and family. The most common types of childhood headaches are migraines and tension-type headaches. Studies have shown that learning disabilities have a high prevalence in children with primary headaches, especially migraines (8, 9).

This study found that headache symptoms were more common in female subjects, as was the case in the study by Wilcox et al. (10). Adolescent girls are more prone to developing headaches than boys, and consequently, they are at a higher risk of migraine or other primary headaches in young adulthood (11). The number of newly diagnosed headaches in 2017 was 71 (51.0 %), and in 2018, it was 68 (48.92 %). The number of hospitalizations indicates that a significantly high number of patients was hospitalized on more than one occasion (58.7 %).

Despite the high prevalence of headaches in the pediatric population, the characteristics of adolescent headaches remain unclear. Several studies have examined the age and gender-related differences in migraines and a few reported differences between children and adults, as well as between younger and older children. The onset of migraines in younger children is often in the form of cyclic vomiting or abdominal migraines, and is not accompanied by a headache, while in older children, more complicated migraines are accompanied by focal neurological symptoms. Due to the effect of hormones on the onset of migraines, migraines are 50% more likely to stop in males after adolescence (10, 12, 13).

The transition from childhood to adolescence is a sensitive and critical period for neurodevelopment, especially in the context of neurological disorders such as migraine (10). The mean age of onset of puberty is about 10 in girls

and about 12 in boys (2). In this study, headaches were more common in puberty, in children over the age of 12, in 69.78 % of cases. Other studies confirm that migraine occurs in 3% to 10 % of children at puberty, increases with age and spontaneously withdraws after puberty in half of the children, but if it begins during adolescence, it is more likely to persist throughout adulthood (10, 12).

In this study, children complained of 7 different headache localizations. The most common localization was frontal, with 40.29 % of the patients reporting it. Somewhat less common were occipital 17.27 % and parietal 13.67 % headaches. An eight-year epidemiological study of unilateral headaches reported that unilateral primary headaches are more common in short-term headaches (<4 hours), but long-lasting headaches, including migraine, tension-type headache and new daily persistent headache, can also occur with pain on one side (14).

This study found that the most common side effects of headache were nausea and vomiting, which were reported by 61 subjects (44.85 %). Antonaci et al. reported that recurrent primary headaches can have a significant impact on the quality of life of children and adolescents due to the unpredictability of attacks and the presence of concomitant symptoms such as nausea, vomiting, photo- and/or phonophobia (5).

The most important diagnostic tools in the correct diagnosis of headache type are meticulous medical history, clear medical history and clinical examination. One of the key components is the distinction between primary and secondary headaches (15). Each patient admitted to the Pediatric Clinic with a headache, in addition to providing a detailed medical history and undergoing clinical and neurological examination, underwent complete neuroradiological assessment, ophthalmic and otorhinolaryngologic (ORL) examination. In this study, 57.14 % of patients had altered magnetic resonance imaging (brain MRI). The majority of the changes found were pineal gland cyst, vascular and gliotic lesions, and small demyelinating lesions. Altered standard EEG findings were present in 26.72 % of patients,

while 31.76 % of patients who underwent partially sleep-deprived EEG had altered findings as well. There were 6 (4.32 %) patients with clinical indications for additional neuroradiological processing (MR angiography), of which 3 (50 %) had pathological findings of the cerebral blood vessels. Based on advanced neurological processing, the indication for VEP was found in 30 (21.58 %) patients, 11 of whom (36.67 %) had pathological findings. There were 28 (20.14 %) patients with an indication for additional otorhinolaryngologic evaluation, 13 of whom (46.43 %) were diagnosed with sinusitis. Invasive neurological treatment, which included lumbar puncture, was indicated in 5 (3.59 %) patients, and after the procedure, the findings were altered in 2 (40 %) patients, 1 of whom was diagnosed with an inflammatory disease of the central nervous system. 11 (7.91 %) patients had an urgent indication for computed tomography of the brain (CT), 2 of whom (18.18 %) presented with abnormalities (intracranial hemorrhage).

All patients admitted to the Pediatric Clinic with anamnestic headache data were also subjected to psychological examination. Findings thereof were extremely important in defining some of the psychological difficulties, as well as environmental factors and living conditions of each child. More recent studies, such as this one, have emphasized the importance of recognizing psychosocial components in a child with a headache for elucidating the etiology thereof, but also in order to be able to access therapeutic procedures based on adequate psychological analysis. Events that greatly affect the incidence and type of headache include a range of family situations such as family conflicts and social problems, parental (un)employment, and economic hardships (16, 17).

Psychiatric disorders, especially depression, neurosis and anxiety, show significant

comorbidity with migraine and are more common among people with chronic migraine compared with episodic migraine (18). In the group of children with functional headaches, more disorders were observed in terms of emotional instability (15.15%), cognitive deficits (15.15 %) and somatization (18.18 %), while in regard to organic headaches, there was a significantly higher level of stress and similar conditions (82 %). Of all the definitive diagnoses of headaches, headache as a result of psychological tension is the most common diagnosis (26.62 %).

Conclusion

This study aimed to research the incidence and frequency of headaches, to examine the correlation between comorbidity and the type of headache, and to examine whether there is a correlation between the psychological profiles of children at the time of onset of symptoms and the type of headache. This study confirmed that the highest proportion of definitive diagnoses of headaches were headaches that result from psychological tension. A smaller proportion are secondary headaches caused by inflammation of the central nervous system, sinusitis, trauma and subarachnoid hemorrhage. By preserving the mental health of children, we can prevent somatization, which is most common in puberty.

Disclosure

Competing interests. None to declare.

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[1]

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Statistical expertise: Serdarušić I, Pušeljić S, Tomac V, Romić M

Original article

Characteristics of Thyroid Disease in Pediatric Population

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Abstract

Aim: In the last decade, an increase in the incidence of autoimmune thyroid disease in children has been observed. The aim of the study was to determine the incidence of thyroid disease in the pediatric population treated at the Pediatric Clinic in Osijek; to determine if there was a rise in thyroid disease incidence in pediatric population in the observed period; and to determine whether there was any association of certain factors with an increased incidence of thyroid disease.

Methods: This study was a two-year retrospective study in two separately observed one-year periods (2010 and 2017). All patients with thyroid endocrinological disorders treated at the Pediatric Clinic of the University Hospital Centre Osijek were included in the study. Their medical records were used to obtain data. Differences between variables were tested by the $\chi 2$ test, Fisher's exact test and Mann-Whitney U test. The level of statistical significance was accepted for $p < 0.05$.

Results: There was a 2.4-fold increase in the approximate incidence of thyroid disease, 9.7-fold higher approximate incidence of autoimmune thyroiditis, 9.75-fold higher approximate incidence of unspecified hypothyroidism and a triple increase in the approximate incidence of non-toxic goiter. Other unspecified thyroid diseases are also statistically significantly on the rise. An analysis of factors such as anamnestic features, age, gender, comorbidities and other relevant data could not explain the increased incidence of thyroid disease.

Conclusion: The incidence of thyroid disease has increased considerably in the observed years, following world trends. Efforts should be taken to clarify the mechanisms and factors involved in causing thyroid diseases in the pediatric population.

(Tomic A, Puseljic S, Tomac V. Characteristics of Thyroid Disease in Pediatric Population. SEEMEDJ 2020; 4(2); 77-87)

Introduction

The normal thyroid secretes thyroid hormones - triiodothyronine (T3) and thyroxine (T4), which are crucial for normal growth and development, body temperature and regulation of energy levels. The basic functions of the thyroid hormones are regulation of oxidation in cells, stimulation of oxidative processes in which heat is generated, stimulation of protein synthesis, stimulation of growth and differentiation and acceleration of the metabolism of carbohydrates, fats and vitamins (1).

Proper thyroid function is essential for early neurocognitive development, growth and development during childhood and adolescence (2). Optimal concentration of thyroid hormones is crucial, since a prolonged hypothyroid condition can result in a variety of disorders. Hypofunction of the thyroid gland occurring before the age of 2 usually leads to delayed maturation of the skeleton and the development of intellectual disability (1). On the other hand, hypothyroidism occurring after a period crucial for neurocognitive development can lead to a slowdown in linear growth and maturation of the skeleton, with no effect on cognitive development and obesity development (3, 4). Furthermore, hypothyroidism diagnosed in the late prepubertal years, which usually happens due to Hashimoto's thyroiditis, can delay the onset of puberty or lead to premature isosexual pseudo-puberty (development of breasts and internal reproductive organs in girls and increased testicular volume without adrenarche in boys). Later in adolescence and young adulthood, hypothyroidism can lead to menstrual disorders, infertility and miscarriages (5, 6). Several dozens of diseases have an autoimmune origin and such diseases affect 5-7% of the world's population (7). Thyroid disorders are one of the most common endocrinological problems in children and adolescents. Autoimmune thyroid disease is estimated to be the most common organospecific autoimmune disease, affecting up to 5% of the general population and 0.3 to 9.6% of the pediatric and adolescent population (8-10).

Epidemiological studies have suggested an increase in the incidence of autoimmune diseases in Western societies over the last few decades (11). The rise in the incidence of autoimmune diseases raises the question of the factors that perpetuate it. Given a decreasing frequency of infections in Western countries, with an increase in the incidence of allergic disorders and neoplasms, which is accompanied by an increase in the incidence of autoimmune diseases, a hygiene hypothesis has been put forward (12). Concordance of autoimmune disease in identical twins is 12-67%, which also highlights the potential role of epigenetics in disease development (13). Over the last few decades, there have been significant changes in nutrition, the environment and exposure to pollution, environmental infections and stress management. Another study highlights the significance of tobacco smoking, psychological distress, iodine intake, intrauterine development, bacterial and viral infections and medications such as interferon (14). Therefore, with the previously established contribution of genetics, increasing attention is shifted on environmental factors and the lifestyle in Western countries (15). The link between the causative factors of autoimmune disease – genetics and the environment – has not yet been fully elucidated. Although much progress has been made in understanding the pathogenesis of autoimmune diseases, many questions remain unanswered.

Numerous studies conducted both in clinical settings and in experimental models have shown that changes in posttranslational histone modifications and DNA methylation – two major epigenetic mechanisms – could potentially cause immune tolerance breakdown and the development of autoimmune diseases (16, 17). Therefore, autoimmune diseases actually reflect the complex interactions between gene variation and the environment (18). In most cases, the origin of the stimuli leading to epigenetic changes in patients with autoimmune diseases remains undefined, but may include different external (e.g. diet, sun exposure, chemicals, environment, medicines) and internal (e.g. aging, stress, sex hormones) stimuli. X-linked genes are typically unmethylated (active) in males, while females have one methylated

and one unmethylated gene. Several molecules encoded on the X chromosome have also been found to play a significant role in the development of autoimmune diseases (7). An epigenetic link between diet and autoimmune diseases has also been observed, since certain foods provide donors of methyl groups (methionine, choline) and cofactors (folic acid, vitamin B12 and pyrodoxal phosphate), which are essential for DNA and histone methylation (19). In addition to nutrition, ultraviolet radiation has been shown to stimulate epigenetic changes involving the hypermethylation of numerous promoter genes, some of which have an immunosuppressive effect (20). Furthermore, studies conducted on twins have shown that, although identical twins are epigenetically minimally different at a young age, they happen to show numerous differences in DNA methylation and histone acetylation later in life (21, 22). Taken together, epigenetics has become increasingly important in the study of the etiopathogenesis of the entire spectrum of diseases, especially autoimmune disorders, and any new insights will be of great importance for a better understanding of this growing health problem.

The objectives of present study were to determine the incidence of thyroid disease in the pediatric population treated at the Pediatric Clinic in Osijek; to determine if there was a rise of thyroid disease incidence in pediatric population in 2010 and 2017; and to determine whether there was any association of certain factors, such as anamnestic features, gender, comorbidities and other relevant factors, with an increased incidence of thyroid disease in childhood for a given period.

Patients and Methods

All pediatric patients treated at the Pediatric Clinic of the University Hospital Centre Osijek within the periods from January 1st 2010 to December 31st 2010 and from January 1st 2017 to December 31st 2017 who were monitored for thyroid disorders were included in the study. Their medical records were used to obtain data. The data collected from medical records included age, sex, diagnoses of thyroid disease, status of thyroid hormones, presence/absence of thyroid gland specific anti-bodies,

comorbidities, components of physical status and family history data. During a follow-up period, the same pediatric specialist and pediatric endocrinology subspecialist monitored and guided the patients. The criteria for inclusion of the subjects in the research were as follows: - Patient under the age of 18 treated at the Pediatric Endocrinology Outpatient Clinic in the periods from January 1st 2010 to December 31st 2010 and from January 1st 2017 to December 31st 2017; Patient has one of the following diagnoses of thyroid disease (Revision 10 of the International Classification of Diseases and Related Health Problems): congenital hypothyroidism E03.0 / E03.1, other unspecified hypothyroidism E03.9, non-toxic goiter E04, hyperthyroidism / thyrotoxicosis E05, autoimmune thyroiditis E06.3, unspecified thyroid disorder E07.9, postprocedural hypothyroidism E89.0, malignant thyroid neoplasm C73, and observation Z03. Based on the data of the total population in the Republic of Croatia, the incidence of new pediatric thyroid disease cases was estimated. Since there were no subjects over 14 years of age (specifically, in both years, the oldest ones were 12 years old), the data from the population register for ages 0-14 were used (24). Due to the dynamics of population change and the inability to present current population values, the approximation of the population incidence was expressed at 95% confidence intervals.

The study was approved by the Ethics Committee of the Faculty of Medicine Osijek.

Statistical analysis

Categorical data are represented by absolute and relative frequencies. Numerical data are described by the median and the limits of the interquartile range. Category variables differences were tested by the Hi-square test and, if necessary, by the Fisher's exact test.

The normality of the distribution of numerical variables was tested by the Shapiro-Wilk test. Differences of the numerical variables between the two independent groups were tested with the Mann-Whitney U test. All P-values are two-sided. The significance level was set to Alpha = 0.05. MedCalc Statistical Software version 18.2.1

(MedCalc Software bvba, Ostend, Belgium; http://www.medcalc.org; 2018) and SPSS (IBM Corp. Released 2013. IBM SPSS Statistics for Windows) were used for statistical analysis (Version 21.0. Armonk, NY: IBM Corp.).

Results

Categorical data are represented by absolute and relative frequencies. Numerical data are described by the median and the limits of the interquartile range. Category variables differences were tested by the Hi-square test and, if necessary, by the Fisher's exact test.

The normality of the distribution of numerical variables was tested by the Shapiro-Wilk test. Differences of the numerical variables between the two independent groups were tested with the Mann-Whitney U test. All P-values are two-sided. The significance level was set to Alpha = 0.05. MedCalc Statistical Software version 18.2.1 (MedCalc Software bvba, Ostend, Belgium; http://www.medcalc.org; 2018) and SPSS (IBM Corp. Released 2013. IBM SPSS Statistics for Windows) were used for statistical analysis (Version 21.0. Armonk, NY: IBM Corp.).

Table 1. Incidence of thyroid diseases by diagnosis during 2010 and 2017

	2010 Newly diagnosed	Incidence	95% C.I	2017 Newly diagnosed	Incidence	95% C.I	P
Autoimmune thyroiditis	9	11.65	5-33	88	113.91	91-26	<0.001
Congenital hypothyroidism	1	1.29	0-3	2	2.59	0-31	>0.999
Unspecified hypothyroidism	4	5.18	1-41	39	50.48	35-88	<0.001
Hyperthyroidism	1	1.29	0-3	1	1.29	1-41	>0.999
Non-toxic goiter	15	19.42	10-87	45	58.25	42-46	<0.001
Other thyroid diseases	0	0.00	0-0	18	23.30	13-81	<0.001

*Incidence per 100,000 inhabitants, C.I. – confidence interval

Table 1 presents the incidence of thyroid diseases by diagnosis during 2010 and 2017 The number of newly diagnosed cases of autoimmune thyroiditis in 2010 was 9, accounting for the incidence of 11.65 per 100,000 inhabitants aged 0-14, and in 2017, the number of newly diagnosed cases was 88, accounting for the incidence of 113 newly diagnosed children per 100,000 inhabitants. Such a 9.7-fold increase in incidence was found to be statistically significant (p <0.001). Also, there was a 9.75-fold increase in the incidence of unspecified hypothyroidism during the years mentioned. In 2010, the incidence of unspecified hypothyroidism was 5.18, and in 2017, 50.48 per 100,000 inhabitants (p <0.001). A threefold

increase in the incidence of non-toxic goiter was detected, with an incidence of 19.42 newly diagnosed children in 2010, compared to 58.25 newly diagnosed children per 100,000 inhabitants aged 0-14 (p <0.001). Other unspecified thyroid diseases are also on a statistically significant rise – no cases were reported in 2010, and in 2017, the incidence was 23.3 new cases per 100,000 inhabitants aged 0-14.

Table 2 presents distribution of patients in regard of nutritional status. There were no significant differences in relation to malnutrition, or normal nutrition and overweight/obesity among subjects examined in 2010 and 2017.

Table 2. Distribution of subjects in relation to nutrition status

Nutrition	Number (%) of subjects			P*
	2010	2017	Overall	
Malnourished	3 (5)	4 (2)	7 (3)	
Normal weight	36 (60)	117 (56)	153 (57)	
Overweight	12 (20)	33 (16)	45 (17)	0.20
Obese	9 (15)	54 (26)	63 (24)	
Overall	60 (100)	208 (100)	268 (100)	

*Hi-square test

Also, no significant differences were observed between two populations studied in terms of specific aspects of physical examination (i.e. characteristics of the skin, eyes, presence of palpitations, appetite, body mass and stool and urination, Table 3).

Table 3. Subjects according to specific aspects of physical examination

	Number (%) children			P*
	2010	2017	Total	
Skin				
Regular moisture and warmth, without trembling	67 (93)	186 (94)	253 (94)	0.77
Thyroid symptomatology	5 (7)	11 (6)	16 (6)	
Total	72 (100)	197 (100)	269 (100)	
Eyes				
Normal	69 (99)	196 (100)	265 (99.6)	0.26
Pathology	1 (1)	0	1 (0.4)	
Total	70 (100)	196 (100)	266 (100)	
Palpation				
Regular	46 (68)	132 (61)	178 (63)	0.39
Enlarged	22 (32)	83 (39)	105 (37)	
Total	68 (100)	215 (100)	283 (100)	
Appetite				
Regular	68 (94)	151 (92)	219 (92)	0.39
Changed	4 (6)	14 (8)	18 (8)	
Total	72 (100)	165 (100)	237 (100)	
Body mass				
Constant	71 (99)	161 (98)	232 (98)	>0.99
Fluctuating	1 (1)	4 (2)	5 (2)	
Total	72 (100)	165 (100)	237 (100)	
Stool				
Regular	59 (89.4)	159 (94.6)	217 (93.1)	>0.99
Irregular	7 (10.6)	9 (5.4)	11 (4.7)	
Total	66 (100)	167 (100)	233 (100)	
Urination - regular	72 (100)	165 (100)	237 (100)	-

*Fisher's exact test

Presence of family history of thyroid diseases was significantly more frequent in data collected in 2017; however, no difference was observed in terms of appearance in paternal, maternal or kin side (Table 4). Also, comorbidities were equally

distributed between two studied groups of patients (Table 5).

Table 4. Subjects by family history and years observed

Family history	Number (%) children			P*
	2010	**2017**	**Total**	
Family history				
Positive	23 (44)	107 (67)	130 (61)	**0.004[‡]**
Negative	29 (56)	53 (33)	82 (39)	
Total	52 (100)	160 (100)	212 (100)	
Positive family history				
Maternal side	16 (73)	64 (65)	80 (61)	
Paternal side	2 (9)	19 (19)	21 (16)	0.06
Combined (brother/sister/maternal/paternal)	4 (18)	15 (16)	19 (23)	
Total	23 (100)	107 (100)	130 (100)	

*Fisher's exact test; [†]χ2 test

Table 5. Distribution according to comorbidities regarding the years observed

	Number (%) of subjects			P*
	2010	**2017**	**Total**	
Obesity	1 (3.6)	22 (12.5)	23 (11.27)	0.213
Polycystic ovary syndrome/ dysmenorrhea	0 (0)	4 (2.3)	4 (1.96)	>0.999
Haematological irregularities (↓Trc, anaemia)	0 (0)	4 (2.3)	4 (1.96)	>0.999
Skin disorders (acne, psoriasis, vitiligo, atopy)	0 (0)	7 (4)	7 (3.43)	0.597
Respiratory diseases of allergic etiology (Allerg.rhinitis/bronchitis/asthma)	1 (3.6)	4 (2.3)	5 (2.45)	0.526
JRA, polyarthralgia, Sy Raynaud	1 (3.6)	6 (3.4)	7 (3.43)	>0.999
Benign nodus	0 (0)	6 (3.4)	6 (2.94)	>0.999
Hypertension, heartbeat irregularities/palpitations	0 (0)	8 (4.5)	8 (3.92)	0.602
Celiac disease	0 (0)	5 (2.8)	5 (2.45)	>0.999
Gastritis, GERB	0 (0)	2 (1.1)	2 (0.98)	>0.999
Trembling, vertigo, headache	1 (3.6)	11 (6.3)	12 (5.88)	>0.999
Epilepsy and epileptic convulsions	1 (3.6)	1 (0.6)	2 (0.98)	0.256
Chromosomopathies (Sy Down, Sy Turner, Sy DiGeorge)	0 (0)	5 (2.8)	5 (2.45)	>0.999
Hypotrophy, retarded growth	0 (0)	6 (3.4)	6 (2.94)	>0.999
Accelerated growth, pubertas praecox	2 (7.1)	4 (2.3)	6 (2.94)	0.192
Hyperlipidaemia	1 (3.6)	5 (2.8)	6 (2.94)	0.593

*Fisher's exact test

Discussion

Based on the data of the total population in the Republic of Croatia, the incidence of newly diagnosed children with thyroid disease was estimated. Since no newly diagnosed subjects were over 14 years of age (specifically, in both years, the oldest newly diagnosed subject was 12 years old), the data from the population register for ages 0-14 were used (24). Due to the peculiarity of the pediatric population of eastern Croatia, patients from Osijek-Baranja and Vukovar-Srijem counties gravitate mostly to the University Hospital Centre Osijek. For this reason, the 2010 census data for Osijek-Baranja County and Vukovar-Srijem County were used to approximate the incidence of newly diagnosed subjects, which totalled to 77,257 children aged 0-14 (Osijek-Baranja: 46,806 children, Vukovar-Srijem: 30,451 children). Due to the dynamics of population change and the inability to present current population values, the approximation of the population incidence was expressed at 95% confidence intervals. Our results suggest that the total number of newly diagnosed cases of thyroid disease has increased significantly. A similar trend in the incidence of thyroid disease has been reported in other studies (25). Looking at the incidence of thyroid disease by years, there is a statistically significant increase in the incidence of autoimmune thyroiditis, unspecified hypothyroidism, non-toxic goiter, and other unspecified thyroid diseases. The increase in the incidence of thyroid disease, especially autoimmune thyroid disease, is a global trend, present especially in Western countries, where the decrease of infectious diseases incidence is also being observed (11, 26). This research confirms that a similar trend can be observed in subjects. Furthermore, probably mostly due to the existence of screening for congenital hypothyroidism during the early neonatal period, figures regarding this diagnosis have not changed significantly over the years, and fortunately remained low.

So far, numerous studies on gender and autoimmunity have concluded that thyroid diseases, such as autoimmune thyroiditis and goiter, are more common in women, as confirmed by this research. This pattern has been partially elucidated by the influence of female sex hormones and by X inactivation, but remains to be fully explained (27). In present study, subjects were analyzed regarding the nutrition status expressed by body mass index. Patients with chromosomopathies (Down Syndrome, DiGeorge Syndrome, Turner Syndrome) were excluded, since their growth and development do not follow the usual patterns. Subjects were classified into 4 groups depending on the centile - malnourished, normal weight, overweight and obese subjects. The study showed that the majority of subjects in both observed years had normal body weight (57%), 3% were malnourished, while 41% of subjects were overweight and obese. In both observed years, a large proportion of childre n were overweight and obese. In a 2010 study conducted on healthy Croatian school-age children, it was found that 69.9% of children were normal weight and 30.1% were overweight and obese (28). Taking into account the results of our study on the population of children suffering from thyroid disease, it is obvious that there is a slightly higher proportion of overweight and obese children (by 10.9%) among children with thyroid disorders than the population of healthy children, while the share of children with normal body weight was slightly lower (12.9%). Although statistically insignificant, it could suggest a higher incidence of thyroid disease among children of a higher body mass index, as explained by the interaction of metabolically active adipose tissue and leptin and the thyroid-stimulating hormone (29). Specifically, adipose tissue plays a role in the pathophysiology of thyroid disorders. Fat cells produce leptin, which is why they are considered to be an active endocrine organ. Leptin is a potential link between the TSH and body mass index; it acts as a neuroendocrine regulator of the hypothalamus-pituitary-thyroid axis by regulating TSH gene expression in certain brain regions. Vice versa, the TSH stimulates leptin secretion in adipose tissue. Leptin also promotes the conversion of T4 to T3 (30). In obese children, elevated TSH and T4 levels have become a common finding. Since these values appear to

result from an elevated body mass index, it might be possible to normalize thyroid hormone levels by correcting or losing extra body weight. However, the incidence of thyroid antibodies is low in these patients, suggesting that adiposity via the previously explained association with leptin affects TSH levels, but not the thyroid antibody levels (31, 32). Obesity has become a growing public health problem. At the same time, it also appears to be a perpetuating factor in the increased incidence of thyroid disease.

Other physical status data were also taken from the subjects' medical records to determine if there were significant changes between these variables in 2010 and 2017. By processing the data collected, it was concluded that there was no statistically significant difference between the variables observed in the years compared by which we could explain the increased incidence of thyroid disease.

In addition to all other information, one of the characteristics of thyroid disease is certainly family clustering and a positive family history. A positive family history of thyroid disease in 2010 was present in 44% of the subjects, while in 2017, this number increased statistically significantly to 67%. Given the invariability of the genetic component of the disease, the increase in the number of subjects with a positive family history could be explained by the greater awareness of patients with thyroid disease and cognition of similar diseases within their own families, but also by the indirectly increased incidence of thyroid disease in both the adult and pediatric populations (26).

The most common comorbidities include obesity, diabetes mellitus type 1, cystic ovarian syndrome and dysmenorrhea, skin changes, respiratory diseases of allergic etiology, hypertension, palpitations, celiac disease, gastritis and GERD, chromosomopathies, praecox puberty and accelerated growth, which, according to available literature, have been previously linked to thyroid disease and thyroid hormone imbalance (33-42). However, the difference in the incidence of these comorbidities in the observed years was not found to be statistically significant. Such persistent co-occurence might suggest that there is a pathophysiological link between some of these diseases and autoimmune thyroid disease.

Based on the research conducted and the analysis of the data obtained, several conclusions can be drawn. The incidence of thyroid disease in general, autoimmune thyroiditis, unspecified hypothyroidism and non-toxic goiter increased multiple times in the observed period. Other unspecified thyroid diseases are also on the statistically significant rise. During the observed period, there was a statistically significant increase in the number of diseased female children compared to the male children in each year separately, but the ratio of the diseased did not change significantly during the observed years. Also, there were no significant differences in comorbidities in the subjects compared to the years observed, which could explain the increased incidence. The consistency of a positive family history in the direction of thyroid disease was confirmed, with the prevalence of a positive family history along the maternal line.

Conclusion

In conclusion, it is evident that autoimmune diseases in general are a local and global issue that will appear even more frequently in the future. With the aim of successful treatment and potential prevention, additional efforts are needed to determine the exact incidence and to identify potential risk factors for the development of these diseases.

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Original article

Early Vital Indicators of Newborns Born After Medically Assisted Reproduction

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Abstract

Aim: In this case-control study we wanted to compare the perinatal outcome of naturally conceived newborns to those born after in vitro fertilization (IVF).

Methods: At the University Hospital Centre Osijek, in the period from January 2014 to December 2016, we analysed 120 in vitro fertilization pregnancies and compared to 120 natural conception pregnancies. Characteristics of mothers, birth method, perinatal outcome, and vital characteristics of newborns were analysed.

Results: IVF-ET pregnancies included 70.0% singleton pregnancies, 28.3% twin and 1.7% triplet pregnancies, while all naturally conceived pregnancies were singletons. When pregnancies were compared between IVF-ET and naturally conceived groups, the following characteristics of mothers were established: age 34.83 (5.8):30.39 (3.9); previous pregnancy 13.3%:56.7%; complications in pregnancy 50.0%:25.0%; Caesarean section (CS) 69.2%:35.8%. All of these were statistically significant (p < 0.001). Comparison of newborns between those two groups established the following: prematurity 39.5% vs. 12.7%; lowest BW 2,114 vs. 3,000 grams; lowest GA 22.29 vs. 28.71 weeks. In the IVF-ET group, 5.7% of newborns had a gestational age of 22-25 weeks, but there were no newborns of that gestational age among the naturally conceived newborns. These were also statistically significant differences (p < 0.001). Apgar score was 10 for both groups, but the difference was in the interquartile range, the values of which were lower in the IVF-ET group.

Conclusion: In vitro fertilization pregnancies are high risk due to the characteristics of both the mother and the infant and, as such, require special attention and care.

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Introduction

The first baby girl born through medically assisted fertilization (MAF) was born back in 1978, and nowadays children that have been born through implementation of this technique make up 1.7% to 4.0% of the population in developed countries [1, 2]. There are only a few fields in medicine that have progressed as rapidly as MAF, which, today, represents the last resort for infertile couples. Clinical guidelines indicate that the availability of a high-quality in vitro fertilization (IVF) and embryo transfer (ET) programme can significantly contribute to the birth rate [3]. After four decades of experience in the field of MAF, there are now relevant studies being conducted in China, Australia, Great Britain and Belgium, which clearly show that children born after MAF differ from naturally conceived children [4-7] in terms of perinatal outcome and vital characteristics. Although available studies differ in methodology, they all include a comparison of pregnancies conceived after MAF (number of foetuses, complications in pregnancy) and of perinatal outcomes (gestational age, birth weight) after naturally conceived pregnancies [4-11]. Those studies have shown that newborns born after IVF-ET are more sensitive during the perinatal period in comparison to newborns born after naturally conceived pregnancies. Such results are to be expected because the characteristics of women who undergo MAF differ from those of women from the general population (higher age, lower parity, comorbidity, infertility or subfertility). It has been demonstrated that MAF methods have contributed to a greater number of multiple pregnancies, primarily twin pregnancies, but also triplet and quadruplet pregnancies [13].

Studies conducted in Croatia are consistent with the results of the above-mentioned research, and they likewise show that there are significantly more multiple pregnancies in the IVF group than with naturally conceived pregnancies [8, 14]. Newborns from the IVF group are born with lower birth weight (BW) and gestational age (GA), and they stay in the intensive care unit (ICU) longer after birth. By

researching available literature, we observed that there is a 30-40% higher risk of congenital malformations after the IVF-ET procedure, and this population had a higher incidence of perinatal mortality [5, 8, 10].

Perinatal outcomes of newborns born after MAF also vary in regard to the different methods of MAF. A pilot study conducted in Vienna from 2003 to 2009 in a tertiary health care centre showed a poorer perinatal outcome (lower BW, lower GA, lower Apgar score) among children who were conceived using the ICSI method, compared to children who were conceived after IVF-ET treatment [15].

The association of IVF with neurodevelopmental disorders has been demonstrated in numerous published studies, which showed that children born after IVF have an increased risk of developing cerebral palsy, as well as various disorders resulting from erroneous genomic imprinting [4, 5, 16]. In exploring the relationship between IVF and autism spectrum disorders (ASD), we found conflicting studies. A Swedish study, which was conducted over a period of 25 years, showed that the IVF procedure was not associated with a higher risk of ASD, but with a significantly higher risk of developing mental retardation. On the other hand, meta-analysis by Liu et al. showed that MAF was associated with a higher percentage of ASD [17, 18].

In accordance with these findings, it is justified to claim that the perinatal outcome of IVF cannot be equal to the outcome after natural conception, bearing in mind the diseases and conditions which led to the need for assisted fertilization; likewise, pregnancies after MAF are high-risk pregnancies, whether they are singleton or multiple. The aim of the study was thus to examine the difference in the vital characteristics of newborns born after medically assisted fertilization in comparison with natural conception, as well as characteristics and the course of pregnancy in women who conceived through medically assisted fertilization and those who conceived naturally.

Material and Methods

The study was designed as a three-year retrospective case-control study and was conducted in the Gynaecology Clinic and neonatal intensive care unit (NICU) of the University Hospital Centre Osijek. The study included all infants who were born from pregnancies through assisted reproduction and the first following infant born after natural conception at the University Hospital Centre Osijek from 1 January 2014 to 31 December 2016. Ethical approval for this study was given by the Ethics Committee of the Faculty of Medicine.

General data on the mother (age, occupation, marital status), parity data (number of births, number of miscarriages, accompanying comorbidities, pregnancy and comorbidities during pregnancy) and data on the characteristics of newborns (sex, length, Apgar score) were collected.

Statistical analysis

The data were analysed using statistical procedures for testing differences and correlations, using the statistical program SPSS 17.0. The mean values of the continuous variables are expressed by the arithmetic mean and standard deviation for normally distributed variables and the median and interquartile range for variables that are not normally distributed.

The Mann-Whitney U test for nonparametric analysis was used to determine the differences between the two independent samples. The $\chi 2$ test was used to determine the differences between the proportions between the two independent samples. Statistical significance was accepted for $p < 0.05$.

Results

A total of 240 births were analysed, of which 120 were pregnancies after IVF-ET, while the control group included pregnancies where birth occurred immediately after the observed IVF birth, likewise a total of 120 births. Since there were multidisciplinary pregnancies among the observed pregnancies, a total of 277 subjects were included in the study.

A total of 240 mothers was divided into two groups – the control group, or 120 mothers that conceived naturally, and the IVF group, or 120 mothers that conceived after IVF-ET treatment. Mothers in the IVF group are older than those in the control group (arithmetic mean ((SD) of age 34.83 (5.8) vs. 30.39 (3.9)), $p < 0.001$. According to the level of education, mothers from the IVF group have a higher level of education (academic degree), while mothers from the control group predominantly have a low or medium level of education (secondary school degree, primary school or no education), which is a statistically significant difference, $p < 0.001$ (Figure 1).

Figure 1. Level of education of mothers

		Control group n = 120 (%)	IVF group n = 120 (%)	p* (χ² test)
Level of education	High or higher (academic degree)	29 (24.2)	66 (55.0)	< 0.001
	Low or medium (secondary school, primary school, no education)	91 (75.8)	54 (45.0)	

Figure 2. Previous pregnancies and miscarriages by groups

	IVF n = 120 (%)	Control group n = 120 (%)	p* (χ2 test)

Pregnancies				
	Yes	16 (13.3)	68 (56.7)	
				< 0.001
	No	104 (86.7)	52 (43.3)	
Miscarriages	No	95 (79.2)	96 (80.0)	
				> 0.999
	Yes	25 (20.8)	24 (20.0)	

A significantly higher portion of mothers in the IVF group had not been pregnant before (p < 0.001; $^*\chi 2$ test), as shown in Figure 2. Comparison of the previous number of miscarriages by group did not show a statistically significant difference between the groups, $\chi 2$ test (p > 0.999) (Figure 2).

Of the total number of mothers (240), 150 (62.5%) mothers experienced no complications during pregnancy (HELLP syndrome, bleeding, use of tocolytics, etc.). However, group distribution shows that more complications during pregnancy were experienced in the IVF group

(60% of mothers with complications are from the IVF group).

Statistically significant differences were found for the groups in regard to the method of delivery, $\chi 2$ test (p < 0.001). Vaginal delivery was the predominant way of giving birth within the control group, whereas pregnancies within the IVF group were mostly completed by Caesarean section, as shown in Figure 3. The highest portion of pregnancies in this study were singleton pregnancies, followed by twin pregnancies. Triplets were found in two pregnancies. All multiple pregnancies were from the IVF group, as shown in Figure 4.

Figure 3. Method of delivery

	IVF group n = 120 (%)	Control group n = 120 (%)	p* (χ^2 test)
Vaginal	37 (30.8%)	77 (64.2%)	
CS	83 (69.2%)	43 (35.8%)	< 0.001

Figure 4. Distribution by number of offspring between groups

	IVF group n = 120	Control group n = 120	p* (χ^2 test)
Singletons	84 (70.0%)	120 (100%)	
Twins	34 (28.3%)	0 (0.0%)	< 0.001
Triplets	2 (1.7%)	0 (0.0%)	

When comparing these two groups based on the sex of the newborn, there were no

statistically significant differences. Statistically significant differences were found for every numerical variable (birth weight, gestational age, Apgar score) except for the mean value of pH (p = 0.747). Mean gestational age in the IVF group was 35.57 weeks (22.29 – 37.57), and it was 38.14 (28.71 – 39.29) weeks in the control group. The shortest duration of pregnancy in the control group was 28.71 weeks, and it was 22.29 weeks in the IVF group (Figure 5). The distribution of newborns by gestational age within the groups shpwed that two children were born between the 22nd and 25th week of pregnancy in the IVF group (p < 0.001; Fisher's exact test), while there were no births within that period in the control group (Figure 6).

Figure 5. Duration of pregnancy (in weeks)

	Number of mothers	Arithmetic mean (SD)	Minimum	Maximum
Control group	120	38.92 (2.06)	28.71	42.14
IVF group	120	36.22 (4.39)	22.29	40.86

(data are shown as arithmetic mean and standard deviation (SD))

Figure 6. Distribution of newborns by gestational age

Gestational age (weeks)	IVF group Number (%)	Control group Number (%)
22-25	9 (5.7)	0 (0.0)
26-29	10 (6.4)	1 (0.8)
30-33	12 (7.6)	3 (2.5)
34-36	31 (19.7)	10 (8.4)
37+	95 (60.5)	105 (88.2)

There were 12% of births in control group which were premature (N =12), while 39% of total births in IVF group were preterm births (N = 62). The high proportion of multiple pregnancies within the IVF group resulted in a significantly higher percentage of prematurity in that group, (p < 0.001; χ2 test, ‡Mann-Whitney U test, data not shown). Likewise, the birth weight of the newborns in the IVF group was lower than of those in the control group, as was expected. The lowest birth weight in the IVF group was 2114 grams, and the highest was 3330 grams. In the control group, the lowest birth weight was 3000 grams, and the highest 3785 grams; these values were significantly different between the groups. In spite of the same median of the average Apgar score, which equals 10 in both groups, the difference is in the interquartile range, which is shifted to lower values (9.5-10) in the IVF group (data not shown).

Figure 7. Birth weight distribution of newborns

	Median (25.00-75.00)	
	IVF group (range)	Control group (range)
Birth weight (grams)	2880 (2114 - 3330)	3350 (3000 - 3785)

Discussion

Research regarding the vital characteristics of newborns born after in vitro fertilization has not been conducted so far at the University Hospital Centre Osijek, even though medically assisted fertilization has been performed in this institution in accordance with the highest standards for over a decade.

Since our search of the literature did not produce any studies that compare the socioeconomic characteristics of the mother, such as the mother's level of education and marital status, we included these parameters in our study. The results were as expected: there is a significantly higher percentage (55.0%) of mothers with a high or higher level of education in the IVF group, while in the control group this percentage is 24.20%. Such a result can be explained by an increase in the number of women in the academic community, their commitment to their careers and professional development and, consequently, the postponed maternity. This result can also be directly related to the mothers' age – the average age of IVF mothers was 34.88, while it was 30.39 in the control group. This result is consistent with the result obtained in a study that included the same parameter, but which also linked singleton pregnancy with higher maternal age, which was not analysed in our study [19]. By observing the mothers' marital status, we found that there is a higher percentage of mothers who are married that conceived by IVF, compared to the control group. This result did not surprise us, given that the goal of most married couples is to have children, which means that they have to treat infertility if it exists. In the IVF group, 110 (91.70%) mothers were married, while 97 (80.80%) mothers were married in the control group.

As expected, the IVF group contains 104 (86.7%) mothers that had not been pregnant previously, which is statistically significant when compared to the control group, where the observed pregnancy was the first pregnancy for 52 (43.30%) mothers. This brings us back to the definition of medically assisted reproduction, which states that this method is the last resort for treating infertility [3]. Even though we could assume that mothers from the IVF group have a higher incidence of miscarriages, there is no statistically significant difference in this parameter between the two groups.

Out of a total of 240 respondents, 150 of them (62.50%) experienced no complications during pregnancy, but when analysed each group individually, 60 (50.0%) of the IVF mothers experienced complications during pregnancy, while that number was 30 (25.0%) in the control group. This result is not surprising, since the mothers who conceived through IVF could not conceive naturally, which is also why they have a more complicated pregnancy.

Out of the total number of pregnancies (240), 126 (52.50%) were finished with the Caesarean section. If we divide that number according to group, we get a statistically significant difference (p < 0.001). 69.2% of Caesarean sections were performed in the IVF group, while vaginal birth occurred in 30.80% of women. In contrast, in the control group, 35.80% of pregnancies were completed with the Caesarean section and 64.20% of women experienced vaginal birth. The obtained number can be directly related to the number of newborns, where we also found a statistically

significant difference (p < 0.001). Out of 120 pregnancies in each group, 28.30% of them in the IVF group were twin pregnancies, while 1.70% of them were triplet pregnancies. In the control group, all pregnancies were singleton pregnancies. The fact that all multiple pregnancies were in the IVF group justifies the prevalence of Caesarean section as the method of delivery, since multiple pregnancy is one of the indications for Caesarean section. Our results from this study are similar to those obtained in studies that also compared these parameters [8, 19]. Taking into account the data from the previous section, shorter duration of pregnancies in the IVF group in comparison with the control group is justified. The shortest pregnancy in the IVF group lasted 22.29 weeks, while the shortest one in the control group lasted 28.71 weeks. If we put those numbers in the context of perinatal outcomes, we get a statistically significant difference for a newborn child. There is a higher incidence of premature infants in the IVF group than in the general population. When duration of pregnancies was expressed in weeks and analysed both groups, one could observe that there are no newborns born between the 22nd and 25th week in the control group, and only one newborn born between the 26th and 29th week. Most children are born "at full term", i.e. 88.20% from the 37th week onwards. In the IVF group, the situation is very different. 5.70% (9) of the newborns were born in the critical period between 22 and 25 weeks, and "only" 60.50% from the 37th week onwards. It is clear that all of the above parameters also affect the birth characteristics of newborns in both groups. Median birth weight

in the control group was 3,350 grams, with a higher lowest birth weight than the one in the IVF group. In the IVF group, median birth weight was 2,880 grams, with the very low 2,114 grams as the lowest value. The highest birth weight in the IVF group was lower than the average birth weight in the control group, which is not significantly different from data in other studies [8, 19, 20].

Conclusion

With the direct link between medically assisted reproduction and low birth weight, as with gestational age in this study and high maternal age, we can conclude that IVF-conceived pregnancies are high-risk pregnancies and should as such receive special attention and care. Since medically assisted reproduction is more common now than it was before, it is the right time to see early vital indicators and collect them to compare with later data. This study thus opens a lot of possibilities and indicates the need for long-term prospective follow-up of these respondents, in order to obtain more information and findings about the outcomes of children born after medically assisted fertilization.

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[1]

Original article

The Impact of Physical Activity and Sports on Academic Achievement of Students in Primary and Secondary Schools in Osijek-Baranja County, Croatia

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Abstract

Introduction: The study aimed to examine the prevalence of sports outside of regular school classes among primary and secondary school students. The secondary aim was to study the correlation of physical activity, students' socioeconomic status and parents' level of education with students' educational outcomes.

Material and Methods: Data were collected from medical records of the Croatian Institute for Health Insurance in the Osijek-Baranja County and through regular physical examination of students in the fifth and eighth grade of primary school and the first grade of secondary school.

Results: Over a 5-year period, 120 boys and 141 girls were examined; 66.28% of the students were involved in sports activities in the fifth grade, 49.04% were involved in sports in the eighth grade, and in the first grade, 43.68% of students were involved in sports. No statistically significant differences were observed in the seventh and eighth grade of primary school (p = 0.076) and in the first grade of secondary school (p = 0.057). Students in the seventh and eighth grade who played sports had slightly higher grades (4.45 ± 0.68) compared to those who did not participate in sports activities (4.3 ± 0.69). Similar results were obtained for students who were involved in sports in the first grade of secondary school, who had achieved slightly better results in the seventh grade (4.41 ± 0.69) compared to those who did not participate in sports activities (4.41 ± 0,69).

Conclusion: The results of this study suggest that physical activity in children could be associated with better school performance, which may have implications for sports having positive health benefits in both childhood and adulthood.

(Vučić A, Bilić-Kirin V. The Impact of Physical Activity and Sports on Academic Achievement of Students in Primary and Secondary Schools in Osijek-Baranja County, Croatia. SEEMEDJ 2020; 4(2); 97-107)

Introduction

The link between exercise and children's mental functioning has not, until recently, been systematically researched (1). Exercise in childhood is associated with greater cognitive control, memory and academic achievement (2-6,1,7-9). Growing evidence suggests that cognitive and academic differences due to exercise may have an underlying cerebral biological foundation. Specifically, children that are more active have greater brain structural volume in the hippocampus and dorsal striatum, two subcortical regions important for memory and learning, as well as more efficient brain activation patterns measured by magnetic resonance imaging (MRI) and event-related potential (ERP) during surveillance and interference tasks over lower-ability peers (3-6,1,7,10-12). It is believed that exercise increases both cerebral blood flow and alertness, accelerates the development of brain-derived neurotrophic factor related to neuronal growth and plasticity, as well as stimulates angiogenesis and neurogenesis in the hippocampus. Besides, research has shown that there might be indications of vascularization and irregular growth in the prefrontal cortex in brain regions related to executive functions (13-17).

Exercise could improve other biological outcomes such as bone density, arterial wall elasticity and general mental health (18,19). Despite the many benefits of physical activity and exercise, obesity in children is a growing problem in developed countries. In 2011, 31.8% of school children were found to be overweight and 7.9% of children in Europe were obese (20).

Childhood obesity has been shown to increase the risk of chronic diseases in adulthood, such as cardiovascular disease, type 2 diabetes, certain types of cancer, and osteoarthritis (21,22). Important adverse effects on growth, blood pressure, lipids, and glucose metabolism, as well as respiratory problems such as asthma and obstructive sleep apnoea have also been reported. Childhood obesity is also considered to be an important risk factor for early adulthood myocardial infarction (23-27). Based on the abundance of positive effects, physical activity and exercise, as well as playing sports in school, may be beneficial to children; in contrast to obesity, physical activity has a potential positive correlation with academic achievement (28,29).

Material and Methods

For this study, data were obtained from medical records of the Croatian Institute for Health Insurance in the Osijek-Baranja County in the period between 2013 and 2018. Data were collected through regular longitudinal physical examinations conducted in the fifth and eighth grade of primary school and in the first grade of secondary school. The study sample included 120 boys and 141 girls from Osijek and Baranja. Additional data collected included participation in sports activities throughout the fifth and eighth grade of primary school and the first grade of secondary school, academic achievement at the end of the fourth, seventh and eighth grade of primary school, form of education continued after primary school (in regard to vocational and grammar school education programmes). Physical characteristics, which include body mass index (BMI) and the locomotor system, were also reported. Demographic data containing socioeconomic factors, such as the place of residence, parents' professional qualifications and their age, were likewise collected. Written parental consent was obtained before conducting this study. The study was approved by the Ethics Committee of the Institute of Public Health for the Osijek-Baranja County.

Statistical analysis

The data were statistically analysed using the Statistica software (StatSoft, Oklahoma, USA, version 11). Categorical variables are presented descriptively, using absolute and relative frequencies, and numerical variables are presented using arithmetic mean and standard deviation. Distribution was tested for normality using the Kolmogorov-Smirnov test. Differences between categorical variables were examined using the $\chi 2$ test, and among numerical variables, one-way and two-way repeated

measurements ANOVA was used. P-levels lower than 0.05 were considered statistically significant.

Results

The study included 120 boys and 141 girls (p = 0.194), 59% of them living in a city and 41% living in the countryside. Considering the parents' level of education, 59% of mothers and 62.45% of fathers completed secondary education, 28.35% of mothers and 27.2% of fathers are university graduates, while 12.64% of mothers and 10.34% of fathers finished primary school. 54.02% of students enrolled in a vocational school and 45.98% went to a grammar school (Table 1).

Table 1. General information about the respondents

		N (%)		p
Gender	Male	120 (45.98)		0.194[*]
	Female	141 (54.02)		
Respondents' secondary school	Vocational school	141 (54.02)		0.194[*]
	Grammar school	120 (45.98)		
Involved in sports	5th grade of primary school	173 (66.28)	< 0.001[*]	
	8th grade of primary school	128 (49.04)	0.757[*]	
	1st grade of secondary school	114 (43.68)	0.041[*]	
Academic achievement, 4th grade of primary school	4th grade of primary school	4.7 ± 0.5	< 0.001[†]	
	7th grade of primary school	4.38 ± 0.69		
	8th grade of primary school	4.48 ± 0.6		

*χ2 test, †repeated measures ANOVA, data are expressed and arithmetic mean and s.d. − standard deviation

When observing academic achievement, the grade point average in the fourth grade of primary school was 4.7 ± 0.5, in the seventh grade it was 4.38 ± 0.69, and in the eighth grade it was 4.48 ± 0.6. In the fifth grade of primary school, 66.28% of students were involved in sports, 49.04% were involved in sports activities in the eighth grade, and 43.68% were involved in sports in the first grade of secondary school.

Statistically significant differences were found in BMI percentiles (p < 0.001). In the fifth grade of primary school, the average BMI percentile was 65.6 ± 28.74, in the eighth grade, it was 59.48 ± 27.55, and in the first grade of secondary school, it was 59.43 ± 28.23.

An increase in the incidence of scoliosis is observed in older students. The lowest percentage of scoliosis (4.6%) was observed in the fifth grade of primary school, while twice as many eighth grade students (9.2%) had scoliosis; however, the highest number was observed in the first grade of secondary school (12.25%). In contrast, the students' posture improved inversely. Fifth and eighth grade students had an overall poor posture (20.31%), while an improvement was observed in the first grade of secondary school, where the numbers decreased (17.24%).

Observing the students' feet, an increase of about 12% was observed in the proportion of normal findings with increasing age of the students (p = 0.008). In the fifth grade of primary

school, the proportion of regular findings was 60.77%, in the eighth grade it was 60.92%, and in the first grade of secondary school it was 72.03% (Table 2).

Table 2. The physical status of the spine and feet

		5th grade of primary school	8th grade of primary school	1st grade of secondary school	
			N (%)		p*
Physical findings of the spine	**Proper Kyphosis**	196 (75.1)	181 (69.35)	176 (67.43)	< 0.001
		0 (0)	3 (1.15)	8 (3.07)	
	Scoliosis	12 (4.6)	24 (9.2)	32 (12.26)	
	Poor posture	53 (20.31)	53 (20.31)	45 (17.24)	
Normal findings, feet status		158 (60.77)	159 (60.92)	188 (72.03)	0.008

*χ2 test

No statistically significant interactions were observed between academic achievement and sports. The highest achievement in all cases was in the fourth grade of primary school, followed by a decline in achievement and then an increase in the eighth grade of primary school (Figure 1). Statistically significant values were not recorded in the seventh grade, but were present in the eighth grade (p = 0.076) and in the first grade of secondary school (p = 0.057). Students who were involved in sports in the eighth grade of primary school had a slightly higher achievement rate in the seventh grade (4.45 ± 0.68) compared to those who did not play sports (4.3 ± 0.69). Similar results were found for students who played sports in the first grade of secondary school, who had a better performance in the seventh grade (4.41 ± 0.69) compared to those who did not play sports (4.41 ± 0.69) (Table 3)..

Table 3. The correlation between academic achievement and sports

		Achievement in the 4th grade	p**	Achievement in the 7th grade	p**	Achievement in the 8th grade	p**	p*
Playing sports in the 5th grade	No	4.64 ± 0.53	0.112	4.32 ± 0.64	0.493	4.47 ± 0.57	0.509	0.401
	Yes	4.74 ± 0.48		4.4 ± 0.72		4.48 ± 0.62		
Playing sports in the 8th grade	No	4.68 ± 0.53	0.447	4.3 ± 0.69	0.076	4.41 ± 0.62	0.452	0.16
	Yes	4.73 ± 0.46		4.45 ± 0.68		4.54 ± 0.57		
Playing sports in the 1st grade of secondary school	No	4.69 ± 0.51	0.859	4.35 ± 0.69	0.057	4.45 ± 0.6	0.424	0.923
	Yes	4.73 ± 0.48		4.41 ± 0.69		4.51 ± 0.6		

* two-way ANOVA, * Student's t-test

Figure 1. presents the relationship between GPA and sports activities at three different points in time. No statistically significant differences in interactions were found (p = 0.437). There are indications that students who were involved in sports throughout their education had slightly higher academic achievement, although the results are not statistically significant.

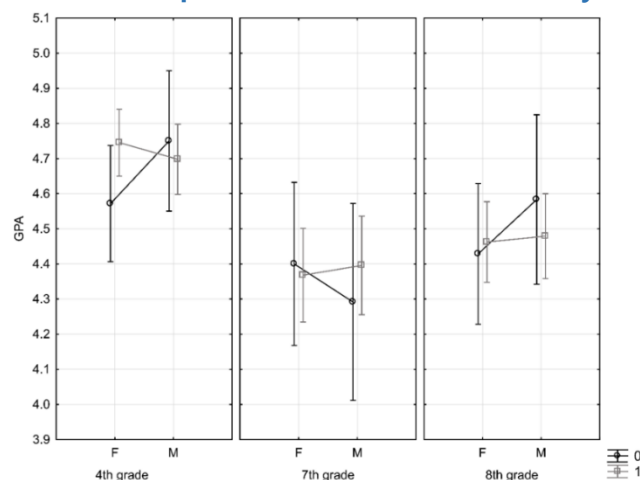**Figure 1. The relationship between GPA and sports activities at three different points in time**
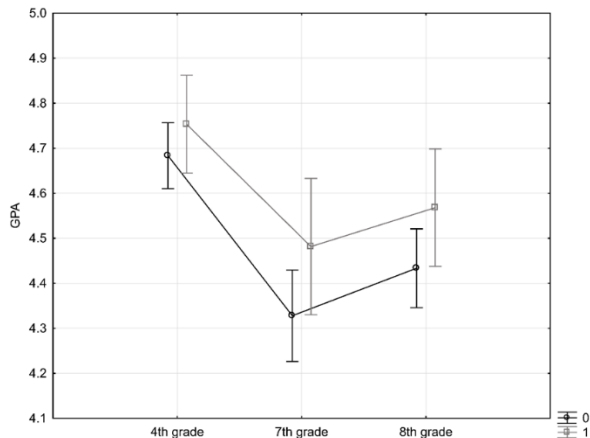
GPA – grade point average, 0 – no sports activities, 1 – sports activities during all school years, p = 0.437

Figure 2. presents GPA scores in children who were involved in sports for at least one school year. Statistically significant differences were found in the interactions (p = 0.048). In the fourth grade of primary school, girls who played sports had higher academic achievement than those who did not play sports, while this difference was not statistically significant in boys. In the seventh grade of primary school, the differences were not statistically significant, and there was considerable variety in the results of children who did not play sports compared to those who did. It is similar in the eighth grade of primary school. Girls who played sports had slightly better achievement than those who did not, but the results were not statistically significant.

Figure 2. GPA scores in children who were involved in sports for at least one school year

GPA – grade point average, 0 – no sports activities, 1 – sports activities during at least one school year, p = 0.048

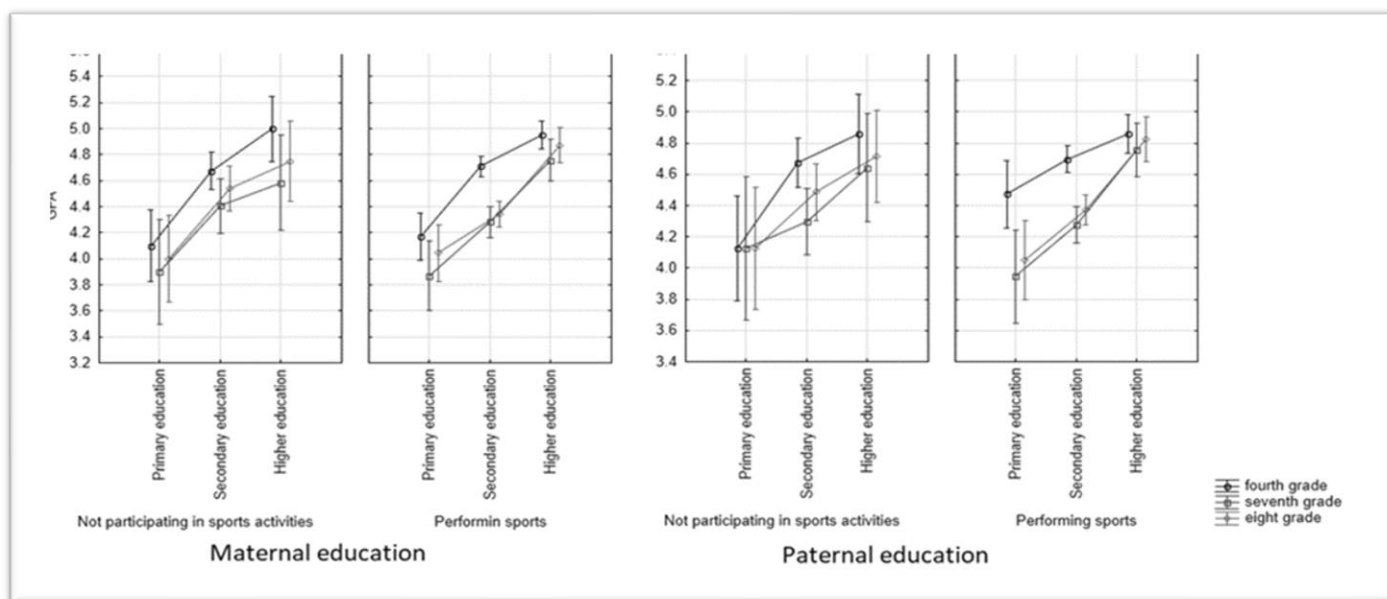**Figure 3. Impact of parents' level of education on academic achievement of students**

Figure 3 contains school outcomes in different grades, based on students' sports activities and their parents' professional qualifications. Based on the parents' levels of education, no differences were observed in the interactions between their professional qualifications and sports activities (p = 0.173 for maternal, and p = 0.109 for paternal education).

Discussion

The recommended daily amount of moderate to vigorous physical activity is 60 minutes (18,30). Only one third of young people worldwide are found to be sufficiently active (31). A population-based study found that more than 50% of children in Australia did not meet these recommendations (32-35). According to the National Health and Nutrition Examination Survey (NHANES), only 42% of children in America participate in the recommended daily amount of physical activity (36,35), while approximately 67% of children and young people in Canada do not participate in the recommended amounts of daily physical activity (37). A study from Japan, however, states that, according to a 2017 National Nationwide Survey conducted by the National Institute for Educational Policy Research, 67.3% of third-year secondary school students participated in extracurricular sports activities, and 83.7% of such students were involved in sports for 1 hour a day or more (38).

From the data collected in physical examinations in the Osijek and Baranja area, we could not determine how much daily activity was moderate to severe physical activity, but what we noted was that in the fifth grade of primary school, 66.28% of students were involved in sports, in the eighth grade it was 49.04% students, and in the first grade of secondary school, it was only 43.68% students. Although increasing physical activity and playing sports has been suggested as a way of improving children's health and academic achievement, there have often been contradictions between various studies regarding exercise, aerobic exercise and academic achievement. This may be due to a

lack of measurement parameters if only physical activity or exercise is measured, without the inclusion of other socioeconomic variables.

In this study, no statistically significant differences were observed between the interaction of academic achievements and sport. No statistically significant differences were recorded in the seventh grade, in the eighth grade and in the first grade of secondary school between children who played sports and those who did not. Children who played sports in the eighth grade of primary school had slightly higher achievement in the seventh grade in comparison with those who did not play sports. Similar results were found for students who were involved in sports in the first grade of secondary school, who had slightly better success in the seventh grade than those who did not play sports.

Results similar to ours were also obtained in the research by David M. Hansen et al. (39), who studied the linear and nonlinear relationship between physical activity and aerobic exercise with children's academic achievement. This research did not show a significant correlation between academic achievement and physical activity. Another study published in 2016 also found no significant correlation between moderate to vigorous physical activity and working memory or academic achievement when comparing children involved in activity measurement and those excluded by age, gender, ADHD, height, weight, BMI, puberty stage, exercise, overweight or obesity (40). However, there are many more studies that show a positive correlation between academic achievement and sports.

In a study conducted in Finland on children from the age of 12 (n = 1,723, 49% boys) and 15 (n = 2,445, 48% boys) until they were 40 years old, Jaana T. Kari et al. (41) confirmed the longitudinal correlation between physical activity and educational outcome, but also that physical activity in adolescence may not only predict academic achievement during compulsory primary education, but may likewise improve educational outcomes later in life.

In South Korea, students who were more physically active were generally found to have higher academic achievement. In addition, their final exam scores in English, mathematics and science were significantly correlated with the PAPS (Physical Activity Promotion System) scores (42). Extensive research has also been conducted in Australia to measure cardiovascular endurance, muscle strength, strength and academic achievement at school. They also found a positive correlation between physical activity and academic achievement, which is inconsistent with our results (43). The same goes for a study of the California Department of Education, which observed aerobic capacity, body composition, strength, and flexibility in addition to California Standard Tests for assessing language, the arts, and mathematics success as academic identifiers (44).

Other similar research on this topic was conducted by Castelli et al. (8). They measured aerobic performance, flexibility, muscle strength and found that exercise had a positive effect on mathematics achievement and overall academic achievement, while Stevens et al. (45) found a positive correlation of physical activity with mathematics and reading success achieved among boys and girls.

Sometimes different results are observed between boys and girls. In Japanese adolescents, a hybrid approach reported a positive effect of exercise on boys' academic achievement, but was observed as not statistically significant among girls (46). If we compare these same differences in our research, we can see that in the fourth grade of primary school, girls who played sports had higher academic achievement than those who did not play sports, while this difference was not statistically significant in boys. In the seventh grade of primary school, the differences were not statistically significant, and there was greater variability in the results of children who did not play sports compared to those who did. This is similarly observed in the eighth grade of primary school. Girls who played sports had slightly better academic achievement than those who did not; however, the results were not statistically significant.

In relation to sports and other physical activity, obesity is becoming a growing problem in school children. Corresponding to this thesis, it has been investigated how weight can affect academic achievement, but the evidence itself is not conclusive. Luis B. Sardinha et al. (47) noted that cardiorespiratory exercise and weight were independently and collectively linked to academic performance of seventh grade students. One study found that in Turkey, among children aged 10-19 in urban areas, one in five were obese; the incidence of obesity among children in urban areas was thus twice as high as in children from rural areas (48). Such data indicate a significant need for research to improve our understanding of the factors contributing to the high prevalence of childhood obesity, as well as for the development of potential urban intervention strategies (49).

Our study involved 120 boys and 141 girls (p = 0.194), where 59% of children live in the city and 41% live in the countryside. It has been confirmed that academic success may be correlated with the parents' level of education, as well as with socioeconomic status (41). Looking at the professional qualifications of parents, we observed that 59% of mothers and 62.45% of fathers completed secondary education, 28.35% of mothers and 27.2% of fathers are university graduates, while 12.64% of mothers and 10.34% of fathers finished primary school. In our study, based on the data about education after primary school, we found that 54.02% of students enrolled in a vocational school and 45.98% in a grammar school. Parents' level of education lower than secondary school qualifications negatively affected academic achievement in boys and girls in a study conducted in Japan, which observed the students' academic success (46).

Conclusion

Today, more and more studies are being conducted on this topic and similar topics, and each of these studies highlights the positive effects of physical activity in children. Nonetheless, the results are mostly devastating in the sense that fewer and fewer children are engaged in physical activity and sports. What is even more worrying is that there has been an increase in health problems in children. Although our data did not show a significant statistical correlation between sports activities

and academic achievement, research alone could contribute to a better understanding of the subject, as well as to the creation of positive habits that influence the reduction of overweight and obesity, and thus also the diseases associated with it. The disadvantages of this research are that it was not possible to determine the exact amount of physical activity in children involved in sports or the intensity of exercise. Since it has been proven that physical activity improves the quality of life, it is necessary to involve local communities in organising sports activities that would be easily accessible to children and parents, which would increase the number of interested children and adults. It is important to make society aware of the fact that sports and sports activities are not reserved exclusively for top athletes, but that they are an important aspect of child development and adult recreation for persons who care about their physical and mental health.

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Original article

Pathohistological Diagnosis of Adrenal Tumors: Experience of a Single Center

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Abstract

Introduction: To investigate adrenal tumors of patients operated on at the University Hospital Center Osijek from 2016 to 2019 for the purpose of examining the location and histopathological findings of the tumors and determining whether there is a difference related to that in terms of the age and sex of the subjects.

Subjects and Methods: This was a cross-sectional study with historical data analysis. The subjects were patients of both sexes (N=23) diagnosed with adrenal tumors (N=23) who underwent surgery at the University Hospital Center Osijek in the period from 2016 to 2019. The archive was used in the University Hospital Center Osijek.

Results: Adrenal tumors were more often unilateral than bilateral. No differences were found in the localization of tumors of the right and left adrenal glands. Benign tumors were more common than malignant tumors and were the most common adenoma. Seventeen women underwent surgery and were 10 years older on average (61.6 years). There were no significant differences regarding tumor occurrence with respect to the patients' age. Benign tumors were more common in women, but the difference was not statistically significant. Regarding histopathological findings, women were more likely to have adenoma (N=10) and hyperplasia (N=4), while men had other benign tumors (N=3) and metastatic tumors (N=1). However, the difference was not statistically significant.

Conclusion: In patients who have undergone surgery for adrenal tumors, there were more unilateral tumors compared to bilateral ones, but there was no difference in tumor localization (left and right). Benign tumors were more common, the most common of which were adenomas. There were no significant differences with respect to age and sex.

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Introduction

The adrenal glands are bilateral glands located above each kidney, which typically weigh about 5 grams, but may be much larger in patients with chronic disease due to increased stimulation. The right adrenal gland is pyramid-shaped, while the left is crescent-shaped. The adrenal cortex in adults accounts for about 90% of the weight of the glands and there are three zones of the cortex: the glomerulosa zone, the fasciculata zone, and the reticularis zone. The neoplasms of the adrenal cortex can be benign (usually adenomas), malignant (cancers) and metastatic tumors. Furthermore, adrenal tumors can be hormone-active and inactive. Both functional and non-functional tumors occur more frequently in women than in men between the ages of 40 and 60 (1). Adenomas of the adrenal cortex are the most commonly benign lesions of the adrenal glands and the most common lesions in general (2). Adrenocortical cancers are extremely rare, with an incidence rate of one to two cases per million people per year, and as many as 15% of adrenal cancers are detected by chance. They have a bimodal distribution, that is, they appear in the first and fourth decade of life and are estimated to be functional in as many as 80% of cases, resulting in a characteristic clinical picture and in 55-60% of cases involving the female sex (3). The adrenal tumors (adenomas and hyperplasia) are round, of well-circumscribed margins and a homogeneous structure. These tumors have visible separation from the surrounding tissues and do not branch into surrounding structures.

This study investigates the localization and pathohistological findings of adrenal neoplasms, as well as differences in terms of age and sex in subjects with adrenal tumors who underwent surgery at the University Hospital Center Osijek from 2016 to 2019.

Patients and Methods

The study involved an analysis of the medical records on patients diagnosed with adrenal tumors who underwent surgery at the Department of Abdominal Surgery of the University Hospital Center Osijek from 2016 to 2019, which are kept by the Department of Abdominal Surgery of the University Hospital Center Osijek and the Clinical Institute of Pathology and Forensic Medicine. The data collected comprised: the patients' age and sex, diagnosis, tumor localization, pathohistological findings and the degree of pathohistological differentiation according to Weiss criteria. Adrenal tumor samples were analyzed morphologically using an Olympus® CX40 microscope on existing archival histological slides stained with the standard hematoxylin and eosin stain. The subjects were patients of both sexes diagnosed with adrenal tumors who underwent surgery at the University Hospital Center Osijek in the period from 2016 to 2019. The sample consisted of a total of 23 patients (N = 23). Samples of adrenal tumors were examined histologically at the Clinical Institute of Pathology and Forensic Medicine, University Hospital Center Osijek, which determined the degree of pathohistological differentiation according to Weiss criteria. The study used the medical records on patients diagnosed with adrenal tumors who were operated on at the Department of Abdominal Surgery of the University Hospital Center Osijek from 2016 to 2019, which are kept by the Department of Abdominal Surgery of the University Hospital Center Osijek and the Clinical Institute of Pathology and Forensic Medicine. The analysis was performed on existing archival histological slides using standard (hematoxylin and eosin) staining (N=23). Approval of the institutional ethical committee for this study was obtained from Faculty of Medicine Osijek.

Statistical analysis

In statistical analysis three tests were conducted. Fisher's Exact test, ANOVA test and Student's T-test. Fisher's Exact Test was used for statistical analysis of unilateral or bilateral adrenal neoplasms, malignancy between left or right neoplasms, pathological differences on adrenal neoplasms between sexes, localization of adrenal neoplasms between sexes. ANOVA

test was used for statistical analysis for pathological diagnosis and age. Student's T-test was used for statistical analysis for age and sex and for age and size of adrenal neoplasms. P<0.05 was set as a level of significance.

Results

Patients who have undergone surgery on for adrenal tumors at the University Hospital Center Osijek from 2016 to 2019 are shown. During this period, 23 patients were surgically treated. Tumors were more often unilateral (N = 18) than bilateral (N = 5). No differences were found between tumor localization in the left (N = 9) and right (N = 9) adrenal glands. In this study, it was observed that benign neoplasms had a higher incidence (N = 22) than malignant neoplasms (N = 1). Adrenal adenomas (N = 12) and hyperplasia (N = 4) have the highest incidence of benign neoplasms, while only metastatic renal cancer was observed in malignant neoplasms (N = 1). The average age of the subjects was 58.9 (SD 11.6). The average age of men was 51.2 (SD 16.6). The average age of women was 61.6 (SD 8.3). It was observed that tumors occur in women at a later age, but the difference was not statistically significant (Student's T-test, p = 0.189). The mean age of patients with unilateral tumors was 59.1 (SD 12.7) and with bilateral tumors 58.4 (SD 7.16). There was no statistically significant difference in adrenal tumor distribution in respect to age (data not shown) (Student's T-test, p = 0.914). The average age of patients with tumors of the left adrenal gland was 63.6 (SD 9.96) and of the right adrenal gland 54.6 (SD 14.13), which was similar (ANOVA, p = 0.267). The average age of individuals with adenoma was 60.3 (SD 9.17), with hyperplasia 61.5 (SD 6.14) and with other benign tumors 54.7 (SD 18.77). There was no statistically significant difference in respect to age and PHD of the tumor (ANOVA, p = 0.777). Benign tumors were more common in women, but the difference was not statistically significant (Fisher's Exact Test, p = 0.261). According to the histopathological findings of adrenal tumors, women had more frequent adenomas and hyperplasia, while benign tumors and metastases were more frequent in men, but the

difference was not statistically significant (Fisher's Exact Test, p = 0.110).

The average tumor size was 4.87 cm (SD 1.95 cm). The average tumor size in men was 5.77 cm (SD 2.42). The average tumor size in women was 4.55 cm (SD 1.73), which was similar between men and women. The average adenoma size was 4.098 cm (SD 1.22), hyperplasia 4.18 (SD 0.96), other benign tumors 7.22 (SD 1.91).

Patients with unilateral tumors were slightly older than patients with bilateral tumors. The patients with tumors of the left adrenal gland were slightly older than the patients with tumors of the right adrenal gland. The patients with adenoma and adrenal hyperplasia were older than patients with other benign adrenal changes. There was no correlation between age and histopathological diagnosis of the tumor (data not shown).

Women with adrenal tumors who were surgically treated were older than men. Men averaged 51.2 years of age (SD 16.6), while women averaged 61.6 years of age (SD 8.3), but the difference in age was not statistically significant.

Discussion

The study is a review of archival medical records kept by the Department of Abdominal Surgery of the University Hospital Center Osijek and the Clinical Institute of Pathology and Forensic Medicine from 2016 to 2019. The data were collected for 23 patients diagnosed with adrenal tumors who were operated on at the Department of Abdominal Surgery of the University Hospital Center Osijek. The data collected were analyzed to examine the existence of differences in localization (single-sided localization, left-right localization) and histopathological findings of tumors between subjects of both sexes. In our study, it was observed that unilateral neoplasms are more common than bilateral ones, which is consistent with the results of other studies (1,4,10,11).

Data on tumor localization vary considerably in different studies and their samples. Mantero et al. and Kasperlik-Zaluska et

al. showed in their studies that neoplasms in the right adrenal gland were more common, unlike Bovio et al., who observed that tumors were more common in the left adrenal gland (3,5,6). In our study, neoplasms of the right and left adrenal glands were equally common, which is in agreement with a similar study by Casola et al. (7).

In our study, obtained data suggest that benign adrenal neoplasms are the most common neoplasms (Figure 3), which is in agreement with a study by Szolar et al. and Cawood et al. (8.9). More than half of pathohistological diagnoses were adenomas, which is in line with other studies that cite adenomas as the most common pathohistological diagnosis (8,9). Metastases are the most common malignant adrenal lesions and the second most common adrenal lesions in general (after adenoma) (2). The most common cancers that metastasize to the adrenal gland are cancers of the breast, lung, stomach, kidney and melanoma (10). In the case of multiple metastases, treatment is performed by methods other than solitary metastases only in the adrenal glands that are treated surgically (5). According to Angeli A et al., women are more commonly affected by adrenal tumors (1,2), which, in addition to the statistical significance, is shown by the results of this study based on the data from the University Hospital Center Osijek.

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The risk of malignancy increases in proportion to the size of the tumor (12,13). The best ratio of sensitivity to specificity is the tumor diameter of 4 cm (5).

Considering the limitations due to a small sample size used in our study, the value of this research is that it demonstrates our center's experience with histopathological findings of adrenal tumors. Monitoring needs to be continued in order to obtain data over a longer period.

Adrenal tumors were more often unilateral than bilateral, with equal incidence of the tumors of the right and left side, more often benign, most often adenomas. There was no significant difference with respect to age. Women had adenomas and hyperplasia more frequently, while other benign tumors and metastatic tumors were more frequent in men, but the difference was not statistically significant.

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Original article

The Histopathological Findings of Operated Tumors of the Parathyroid Glands and Patient Data: A Single Centre Experience

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Abstract

Introduction: Parathyroid proliferative disorders include adenoma, hyperplasia and carcinoma. Adenoma and hyperplasia are more commonly found in women, while carcinoma, which is very rare, is equally common in both sexes. The aim of this study was to analyze parathyroid tumors location and histopathology and to compare differences between the sexes. Tumors were surgically removed at the University Hospital Centre Osijek between 2016 and 2019.

Patients and Methods: Patients of both sexes who underwent parathyroidectomy for parathyroid tumor at the University Hospital Centre Osijek between 2016 and 2019 were included in the study. Parathyroid tumor samples were histologically analyzed, and their size and histopathology were noted. Existing documentation on patients with parathyroid tumor was used. Analyses were done on archived histologic material stained with hematoxylin and eosin.

Results: Overall, 19 samples of parathyroid tumor were included in this study. Tumors of the parathyroid glands were most commonly localized on the lower left parathyroid (8 cases, 42%), and least commonly on the upper right parathyroid (2 cases, 11%). The most common disorder was adenoma (11 cases, 58%), followed by hyperplasia, while no cases of cancer were diagnosed. Women underwent tumor operations more frequently than men (17 versus 2 cases). There was no correlation between histopathology of parathyroid tumor and sex (Fisher's exact test, p = 1) or between tumor location and sex (Fisher's exact test, p = 1).

Conclusion: The most common locations of tumors of the parathyroid glands were the inferior glands; the most common location was the left inferior parathyroid gland and the most common disorder was adenoma. Women were operated on more frequently than men.

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Introduction

Parathyroid glands are 4 nodular structures located behind every pole of the thyroid gland. They are separated from thyroid gland tissue by a fibrous capsule (1). Their size is mostly around 5 x 3 x 1 mm, and they weigh up to 50 mg (2). On rare occasions, the number of parathyroid glands can vary from 1 to 12 parathyroid glands (3). Due to embryonic development, inferior parathyroid glands are most frequently located ectopically. Common ectopic locations are within the thymus, the mediastinum and the thyroid gland, but they are also rarely found within the vagus nerve, the pharynx and the esophagus (4). The main function of parathyroid glands is calcium homeostasis. Parathyroid hormone increases serum calcium by increasing renal tubular reabsorption of calcium, bone resorption and by indirectly increasing vitamin D levels, which leads to increased intestinal calcium absorption. Parathyroid hormone excretion is regulated by ionized calcium levels, extracellular phosphate levels and vitamin D (5).

Chief cells, adipocytes and oxyphil cells make up the parathyroid gland parenchyma. Chief cells produce and secrete parathyroid hormone (6). Oxyphil cell function is not fully understood. They are considered to secrete parathyroid hormone in secondary parathyroid hyperplasia (7). Hematoxylin and eosin stain is used to observe parathyroid gland structure, while immunochemistry is used to identify molecular markers (8).

Parathyroid gland dysfunction can have different causes. Increased secretion of parathyroid hormone from at least one parathyroid gland leads to primary hyperparathyroidism. It is mostly caused by a single adenoma, but can be caused by multiple adenoma, hypertrophy of all 4 parathyroid glands, and rarely by carcinoma (9). Secondary hyperparathyroidism is a condition of parathyroid hormone oversecretion in response to abnormally low calcium in the blood due to other pathological processes, such as kidney failure, gastrointestinal malabsorption, or vitamin D deficiency. Parathyroid hyperplasia caused by prolonged hypocalcemia, which is seen mostly in chronic kidney failure, is defined as tertiary hyperparathyroidism (10, 11). Hypoparathyroidism is a rare disorder that occurs after damage to or removal of parathyroid glands, for example during thyroidectomy (12). Pseudohypoparathyroidism occurs when the body, and especially the kidneys, develops resistance to the parathyroid hormone (13).

Parathyroid proliferative disorders include adenoma, hyperplasia and carcinoma. These usually manifest as primary hyperparathyroidism (2). Parathyroid adenoma usually occurs between the ages of 50 and 70. Microadenomas usually do not have a capsule. Larger adenomas have a thin fibrous capsule and can have cystic degeneration in the tumor. Their average weight is one gram. Macroscopically, the adenoma is smooth, soft and reddish-brown in color, in contrast to the yellow-brown of a normal parathyroid gland (2). There may be normal parenchyma of a parathyroid gland around the edge. Histologically, there is a population of enlarged cells, glandular architecture with secretions, atrophy or compression of parathyroid gland tissue (14, 15). Parathyroid hyperplasia usually occurs as a result of calcium deficiency, mostly due to chronic kidney disease. All 4 parathyroid glands are enlarged. In certain cases where glands are unevenly enlarged, they may be mistaken for adenoma. Resection will not resolve the problem in such case (2). Histologically, there is parenchymal fat accumulation and nodular distribution of chief and oxyphil cells (14, 15).

Parathyroid carcinoma is mostly seen in middle-aged patients. Risk factors include hereditary syndromes such as MEN 1 and MEN 2 (multiple endocrine neoplasia type 1 and type 2), familial isolated hyperparathyroidism, neck radiation and chronic kidney disease. Parathyroid hormone and calcium are more increased than in adenoma. In addition to the usual hypercalcemia symptoms such as nausea, vomiting, ulcers, pancreatitis, depression and languor, there are also special symptoms of the disease, such as osteoporosis, subperiosteal

bone resorption and pathological fractures. Using ultrasound, carcinoma is identified as a mass, usually in inferior poles of the thyroid, with a size greater than 3 cm (16, 17). Histologically, carcinomas look very similar to adenomas, which is why it is difficult to diagnose the disease with only histological analysis. Histological features of parathyroid carcinoma are thick fibrous capsule, adherence to surrounding tissue, vascular and capsular invasion, necrosis and increased mitotic activity (18). Immunohistochemical methods help differentiate carcinoma from adenoma. The only unequivocal criterion for carcinoma are metastases (16).

Ultrasound and technetium Tc 99m sestamibi scintigraphy scan are most commonly used for localization of parathyroid tumor. SPECT (single-photon emission computed tomography) allows a 3-dimensional image. Scintigraphy and SPECT combined have up to 98% sensitivity for detection of parathyroid adenoma (2). Detection of parathyroid glands by imaging methods is of no importance for primary hyperparathyroidism confirmation. Other localization techniques include MRI (magnetic resonance imaging), CT (computerized tomography) and venous parathyroid hormone sampling (19).

Parathyroidectomy is the only definitive treatment of primary hyperparathyroidism. Observation and pharmacological therapy are less effective and less cost-effective than surgery, even in asymptomatic patients (19 - 21). According to the Guidelines of the American Association of Endocrine Surgeons, parathyroidectomy is indicated in all patients with symptomatic primary hyperparathyroidism; when the serum calcium level is more than 1 mg/dL (0,25 mmol/L) above normal, regardless of whether objective symptoms are present or absent; when there is objective evidence of renal involvement, including silent nephrolithiasis, nephrocalcinosis, hypercalciuria, or impaired renal function; in patients with primary hyperparathyroidism and osteoporosis; when primary hyperparathyroidism is diagnosed in patients aged 50 or younger; in patients with parathyroid cancer; in patients who are unable or

unwilling to comply with observation protocols; in patients with neurocognitive and/or neuropsychiatric symptoms that are attributable to primary hyperparathyroidism. Many different surgical methods to streamline parathyroidectomy and reduce the risk of complications have been developed; they are collectively termed minimally invasive parathyroidectomy (MIP). The most commonly used auxiliary method that significantly increases surgery success is intraoperative parathyroid hormone measurement (IPM). If, during minimally invasive parathyroidectomy, intraoperative parathyroid hormone measurement indicates residual hypersecreting tissue, conversion to bilateral exploration (BE) is necessary. Minimally invasive parathyroidectomy has the most success with solitary adenoma (19). Preventive measures and routine monitoring of serum calcium, creatinine and bone density are recommended in asymptomatic patients that do not meet the criteria for surgery (22).

The aim of this cross-sectional study with historical records was to investigate parathyroid tumors that were surgically removed at the University Hospital Centre Osijek between January 2016 and March 2019. The aim was also to review tumor location and histopathology and to compare differences between sexes.

Patients and Methods

Patients of both sexes who underwent parathyroidectomy for parathyroid tumor at the University Hospital Centre Osijek between January 2016 and March 2019 were included in the study. Data were obtained for 19 patients. In the Clinical Department of Pathology and Forensic Medicine, parathyroid tumor samples were histologically analyzed; their size and level of histopathological tumor differentiation were noted. Existing documentation on patients with parathyroid tumor of the Clinical Department of Pathology and Forensic Medicine and the Department of Maxillofacial Surgery was used. Analysis was done on archived histologic material stained with hematoxylin and eosin. Approval of the institutional ethical committee

for this study was obtained from Faculty of Medicine Osijek.

Statistical analysis

Statistical analysis was done using MedCalc Statistical Software version 18.9. Normal distribution of variables was tested using the Kolmogorov-Smirnov test. Comparisons between nominal variables due to the small number of samples were made using Fisher's exact test. Alfa = 0.05 was set as the level of statistical significance.

Results

Data were obtained for 19 patients diagnosed with parathyroid tumor. In regard to histopathology, the studied sample included only adenoma and hyperplasia.

Women underwent parathyroid tumor operations more often than men. Of total of 19 samples, there were 2 (11%) male samples, and 17 (89%) female samples.

Table 1. Parathyroid tumor location in both sexes

	Right inferior parathyroid gland	Left inferior parathyroid gland	Right superior parathyroid gland	Left superior parathyroid gland	
Female	5 (26%)	8 (42%)	2 (11%)	2 (11%)	p = 1 *
Male	1 (5%)	0	0	1 (5%)	
Total	6 (31%)	8 (42%)	2 (11%)	3 (16%)	

* Fisher's exact test

Parathyroid tumor location was observed. Table 1 shows that parathyroid tumors were predominantly located on inferior parathyroid glands (14 cases, 74%) in comparison with superior parathyroid glands (5 cases, 26%). The most common location of the tumor was the left inferior parathyroid (8 cases, 42%). There were 6 cases (31%) of right inferior, 3 cases (16%) of left superior and 2 cases (11%) of right superior parathyroid gland involvement.

Of the 2 obtained samples of parathyroid tumor in men, one was located on the right inferior and one on the left superior parathyroid gland. In regard to histopathology, one sample was of hyperplasia, and the other was of parathyroid adenoma.

Table 2. Distribution of parathyroid gland tumor in both sexes by histopathological diagnosis

	Adenoma	Hyperplasia	Undetermined disorders of parathyroid tissue	
Female	10 (53%)	3 (16%)	4 (21%)	p = 1 *
Male	1 (5%)	1 (5%)	0	
Total	11 (58%)	4 (21%)	4 (21%)	

* Fisher's exact test

In our research, we examined the distribution of parathyroid tumor by histopathological diagnosis. The most common parathyroid tumor was adenoma. In regard to histopathology, 11 samples (58%) were determined as adenoma, 4 samples (21%) as hyperplasia and 4 samples

(21%) as undetermined disorders of parathyroid tissue (Table 2). In four cases termed as "undetermined disorders of parathyroid tissue", due to damaged samples, it was not clear whether the tissue histopathology matched adenoma or hyperplasia.

Although women exhibited more adenoma compared to hyperplasia than men, there is no correlation between histopathology of parathyroid tumor and sex (Fisher's exact test, p = 1). Likewise, even though there were more inferior parathyroid tumors in regard to superior in women when compared to men, there is also no correlation between tumor location and sex (Fisher's exact test, p = 1). Statistical analysis is not valid because the male data sample was small.

Discussion

Parathyroid tumors include adenoma, hyperplasia and carcinoma. They most often present as primary hyperparathyroidism. Parathyroidectomy is the only definitive treatment. From 1925, when Felix Mandl performed the first successful parathyroidectomy in Vienna, many surgical techniques have been developed that have become less and less invasive (23). Minimally invasive parathyroidectomy is a relatively new technique that is used increasingly commonly in parathyroid adenoma treatment. Its success depends on imaging of the tumor. Ultrasound, technetium Tc 99m sestamibi scintigraphy scan, and intraoperative parathyroid hormone measurement can aid the success of the operation. The most common parathyroid tumor locations are inferior parathyroid glands, with frequency of occurrence from 70 to 86.7% (24 - 26). In our study, tumor was likewise most commonly located on inferior parathyroid glands. According to our study, the most common parathyroid tumor location was the left inferior parathyroid gland, which was also the case in El-Hady's and Usta's studies (24, 25). Minimally invasive parathyroidectomy decreases the risk of postoperative hypocalcemia, the length of procedure, the length of hospital stay and overall treatment cost (27). Primary hyperparathyroidism is more often found in women (10). In the conducted study, women were also more affected by parathyroid tumors.

The most common parathyroid gland tumor is adenoma (80-85%), which is followed by hyperplasia (15%), and carcinoma, which is extremely rare (2). In our study, a similar distribution was seen, but there were no cases of carcinoma. Depending on the literature, carcinoma represents from under 1% to 5% of cases of parathyroid tumor (28-30). Diagnosing carcinoma is not simple. There are no special clinical manifestations and histology is ambiguous. Clinical manifestations can be more extreme than with other causes of primary hyperparathyroidism (16). Atypical parathyroid adenoma or parathyroid neoplasm of uncertain malignant potential is a special histological entity that does not meet all the criteria for carcinoma. Its course is more benign, it is less aggressive than parathyroid carcinoma, and it has a greater 5-year survival rate (31, 32). In the same period, three patients had parathyroidectomy for multi-gland hyperplasia due to secondary hyperparathyroidism (results not shown). All three of them had chronic kidney failure and were on a hemodialysis program.

In the conducted study, primary hyperparathyroidism was the most common clinical diagnosis, followed by secondary hyperparathyroidism. Primary hyperparathyroidism occurs in over 2% of the population older than 55 (33). It is usually caused by solitary adenoma, but can also be caused by multiple adenomas, hypertrophy and carcinoma. Risk factors include neck radiation, lithium intake or hereditary factors such as MEN 1 and MEN 2 syndromes (8). It manifests as hypercalcemia with increased or normal level of parathyroid hormone. Patients usually have no symptoms, but in symptomatic patients, it manifests as fatigue, weakness, depression, gastroesophageal reflux and bone pain. In the advanced stage, it can manifest as skeletal disease, nephrolithiasis and cardiovascular disease (10).

In secondary hyperparathyroidism, there is normal parathyroid hormone secretion as a response to chronically low calcium levels. Low calcium levels are caused by malabsorption, vitamin D deficiency, kidney failure and taking certain medication, such as thiazide diuretics. Serum calcium level is low or normal, and parathyroid hormone level is normal. In tertiary hyperparathyroidism, after an initial stimulus,

117

parathyroid glands become autonomous, and parathyroid hormone levels and calcium levels resemble primary hyperparathyroidism. Clinical symptoms of secondary hyperparathyroidism depend on the underlying cause (8, 34).

Our study did not show a correlation between sex and tumor location, or between sex and type of tumor. To our knowledge, no study has so far analyzed the correlation between sex and tumor location. Only 2 male samples were analyzed, which was not sufficient for making an adequate statistical analysis. Corresponding to the correlation between sex and tumor location, it is known that adenoma is found 3 times more often in women than in men (18, 35). In the study by Cvasciuc (36), patients with parathyroid adenoma were predominantly female, and there was a similar female-to-male ratio in regard to adenoma and hyperplasia. Carcinoma affects both sexes equally commonly (16).

It is important to note that these are data collected from patients who were subjected to parathyroidectomy at our University Hospital Centre. Not all patients with primary or secondary hyperparathyroidism are operated on, so this study can in no way present the prevalence of hyperparathyroidism in the area gravitating to the University Hospital Centre in Osijek. The sizes of tumors in samples from this area were 0.7-2.2 cm (results not shown).

The main limitation of the study was its small sample, which is probably the reason why there were no cases of carcinoma. If the sample were larger, carcinoma frequency could have been assessed. The aim of this study was to review the possible connection between sex and parathyroid tumor histopathology or location, but only 2 male samples were obtained, which was not sufficient for making a statistical analysis.

To conclude, the study shows that women are operated on for tumors of the parathyroid gland more often, which most commonly manifest as adenomas. Parathyroid tumors were more frequently located in inferior parathyroid glands, and the most common location was the left inferior parathyroid gland.

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Original article

Croatian Translation and Linguistic Adaptation of the Short Assessment of Health Literacy for Spanish Adults (SAHLSA-50)

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Abstract

Introduction: Population needs health literacy (HL) in order to understand health-related topics. Short Assessment of Health Literacy for Spanish Adults (SAHLSA-50) could be a high-quality tool for assessing HL in adults. Unlike questionnaires in the English language, which are not easily modifiable for Croatian, translation of SAHLSA-50 from Spanish into Croatian could be an appropriate choice. There are no similar questionnaires to assess HL in Croatian to date.

Aim: To translate SAHLSA-50 into Croatian using a validated procedure and to adapt it linguistically, so that it could be used in the Croatian-speaking area to measure HL in the Croatian population.

Material and Methods: SAHLSA was developed for the Spanish-speaking population by Lee et al. It consists of 50 items and is based on multiple choice questions. A score between 0 and 37 suggests that the respondent has inadequate HL. After the protocolized translation process (translation/back-translation), the final version of the Croatian translation of SAHLSA-50 was produced, after we introduced several changes that do not represent a direct translation of the Spanish items. Most of the changes were made because of a cultural difference in common use or in the meaning of the term.

Conclusion: SAHLSA-50 was translated and adapted into Croatian using the standard translation/back-translation procedure. The Croatian version will now be available as an important part of epidemiological and clinical studies, as a useful tool for evaluating the levels of HL in the Croatian-speaking population. Validation of the translated Croatian version in practice would be the next step.

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Introduction

Health literacy (HL) is a combination of skills, including the ability to perform basic reading and numerical tasks required to function in a healthcare environment (1). The definition used by the Institute of Medicine states that it is the degree to which individuals have the capacity to obtain, process and understand basic health information and services needed to make appropriate health decisions (2). HL has become an important and fruitful area of research, mostly because of the relationship of limited HL with patients' knowledge, health behaviors, health outcomes and medical costs (3). Today, there are multiple different assessment tools for assessing HL (4, 5). Short Assessment of Health Literacy for Spanish Adults (SAHLSA-50) is an HL assessment tool containing 50 items designed to assess Spanish-speaking adults' ability to read and understand common medical terms (6). SAHLSA-50 was originally developed and validated in Spanish. It is an easy tool for researching HL in the general population (6). It has been validated for a Spanish-speaking country – Chile, and it was recognized as a useful tool for assessing HL in the adult Chilean population. Its use and incorporation into local research can be especially recommended in areas of education and health promotion (7). While the interest in HL was until recently mainly concentrated in the United States and Canada, it has become more internationalized over the past decade (8). Although the European Union produced less than a third of the global research on HL between 1991 and 2005 (9, 10), the importance of the issue has been increasingly recognized in European health policies. As a case in point, HL is explicitly mentioned as an area of priority action in the European Commission's Health Strategy 2008-2013 (11). We chose SAHLSA-50 as appropriate for the Croatian language because of its structure, which is intended for a language that is written phonetically (Spanish), unlike the English language. To the authors' knowledge, there are no similar HL questionnaires in the Croatian language to date.

Material and Methods

SAHLSA-50

SAHLSA-50 was developed for the Spanish-speaking population by Lee et al. (6), based on the Rapid Estimates of Adult Literacy in Medicine (REALM), known as the most easily administered tool for assessing HL in English. SAHLSA-50 consists of 50 items and is based on multiple choice questions (specifically three choices). Each item consists of a stem medical term and for each term, a respondent chooses between the two offered, potentially related terms, of which only one is right. The right term is called a key term and is in some way related to the stem medical term (synonym or descriptor). The other term is a distractor, which has nothing in common with the stem medical term (or at least in comparison with the key term). The third choice is "I do not know", which is an option for avoiding guessing the answer. For each correct related term chosen, the respondent is given a point. A score between 0 and 37 suggests that the respondent has inadequate HL (5). The authors of the original questionnaire approved the translation into Croatian language.

Procedure

Our translation was modeled after Francisco et al. (12) and steps taken followed those described by Wild et al. (13); they are shown in a flowchart (Figure 1). The first step included the first forward translation from Spanish into Croatian by two of the authors of this paper (two students of medicine with passive knowledge of the Spanish language) and one Spanish speaking physician, all Croats, with the help of a professor of the Spanish language (a native speaker of Croatian). In the second step, corrections were made by another professor of the Spanish language (a native speaker of Croatian). Back-translation was done by a fluent bilingual native speaker of Spanish living in Croatia, who is also a Spanish language teacher by profession. Finally, the translation team made the harmonization, including the modification for Croatian language.

The final version of the Croatian-language version of SAHLSA-50 was the result

Results

Table 1 shows SAHLSA-50 versions during the translation process, from the original version to the final translated version in Croatian. The final version of the Croatian SAHLSA-50 is available in Supplement 1. The majority of the items was translated directly and without modification. Several items underwent modification during the translation process. Most of the changes were done due to cultural or linguistic differences or divergence in meaning. For example, in item 10, "vigilance" ("budnost") in Croatian is better associated with "coffee" ("kava") than with the original "energy" ("energija"). In item 17, "small egg" ("jajašce") has been replaced by ovulation ("ovulacija"), because it was less confusing, since in Croatian an "egg" colloquially also means a "testicle". Another change was done because the authors wanted to achieve concordance with more common Croatian expressions in terms of the word category that is typically used. Thus, the adjective in item 22, "happy" ("sretan"), was replaced with "happiness" ("sreća"), which is a noun, and in item 23, the words "to lose", which are a verb in Croatian ("izgubiti"), replaced the word "loss", which is a noun in Croatian. Regarding miscarriage, it is more common to say that someone lost the baby ("izgubili bebu" in Croatian) and not that they experienced a "loss" ("izgubiti" vs "gubitak" in the record). Another difference was introduced in item 6, where we changed "meals", a plural noun ("obroci"), into a singular noun – "meal" ("jelo"), which is more common and less confusing ("obroci" has an additional different meaning besides "meals"). Through empirical medical knowledge it was concluded that in item 20, the word "irritation" ("iritacija") in Croatian usually relates to "inflammation" ("upala") and not "pain" ("bol"), as was the case in the original Spanish version. The final change was made with the word "convulsions" in item 49, which does not have a singular meaning in Croatian, while the word "epilepsija", meaning "epilepsy" (related to "convulsions"), is more common and does have

a recognizable related Croatian description in the word "padavica" (the term for a grand mal seizure characterized by falling down – as "pad" in "padavica"). The procedure of translation and back-translation went smoothly, without major differences between the attitudes, proposals or final solutions of the members of the translation team. The translation team agreed on the final version without lingering objections.

Discussion

We made the Croatian version of SAHLSA-50 in order to enable HL research in Croatia. The translation was made using standard validated translation procedures (12-14). Since the Spanish questionnaire was found to be more suitable than the English one, it was quite easy to translate the items. Croatian and Spanish have a simple way of pronouncing written text, where only one sound corresponds to each letter, and vice versa. Thus, being literate in these two languages (Spanish and Croatian) is supposed to be comparably difficult (or easy), unlike English, where each word is pronounced in a certain way, depending on many complicated rules and exceptions. However, we had to make several changes of certain Spanish words, since we wanted the questionnaire to be more appropriate for Croats in terms of recognizable content and category of words used. Similar changes in wording or scoring are commonly done to ensure cultural adaptation (15). We hope that the next step – the validation of the Croatian version of SAHLSA-50 in practice – will confirm the adequacy of our choice. The next step after this report is validation of the translated questionnaire in the Croatian-speaking population.

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	Spanish original			Forward translation			Corrections			Back translation		
1.	PRÓSTATA	glándula	circulación	PROSTATA	Žlijezda	Cirkulacija	PROSTATA	Žlijezda	Cirkulacija	LA PRÓSTATA	La glándula	La circulación
2.	EMPLEO	trabajo	educación	ZAPOSLENJE	Posao	Obrazovanje	ZAPOSLENJE	Posao	Obrazovanje	EL EMPLEO	El negocio	La educación
3.	MENSTRUAL	mensual	diario	MENSTRUALAN	Mjesečno	Dnevno	MENSTRUACIJSKO	Mjesečno	Dnevno	MENSTRUAL	Mensual	Diario
4.	GRIPE	sano	enfermo	GRIPA	Zdrav	Bolestan	GRIPA	Zdrav	Bolestan	LA INFLUENZA	Saludable	Enfermo
5.	AVISAR	medir	decir	UPOZORITI	Mjeriti	Reći	UPOZORITI	Mjeriti	Reći	ADVERTIR	Medir	Decir
6.	COMIDAS	cena	paseo	OBROCI	Večera	Šetnja	JELO	Večera	Šetnja	LA COMIDA	La cena	El paseo
7.	ALCOHOLISMO	adicción	recreo	ALKOHOLIZAM	Ovisnost	Rekreacija, odmor	ALKOHOLIZAM	Ovisnost	Rekreacija	EL ALCOHOLISMO	La adicción	La recreación
8.	GRASA	naranja	manteca	MAST, MASNOĆA	Naranča	Maslac, mast	MASNOĆA	Naranča	Maslac	LA GRASA	La naranja	La mantequilla
9.	ASMA	respirar	piel	ASTMA	Disati	Koža	ASTMA	Disati	Koža	LA ASMA	Respirar	La piel
10.	CAFEÍNA	energía	agua	KOFEIN	Energija	Voda	KOFEIN	Budnost	Voda	LA CAFEÍNA	La vigilancia	Agua
11.	OSTEOPOROSIS	hueso	músculo	OSTEOPOROZA	Kost	Mišić	OSTEOPOROZA	Kost	Mišić	OSTEOPOROSIS	El hueso	El músculo
12.	DEPRESIÓN	apetito	sentimientos	DEPRESIJA	Tek	Osjećaji	DEPRESIJA	Tek	Osjećaji	LA DEPRESIÓN	El apetito	Los sentimientos
13.	ESTREÑIMIENTO	bloqueado	suelto	OPSTIPACIJA	Zatvor, blokiran	Rastresit, nekompletan	KONSTIPACIJA	Zatvor	Proljev	EL ESTREÑIMIENTO	El estreñimiento	La diarrea
14.	EMBARAZO	parto	niñez	TRUDNOĆA	Porođaj	Djetinjstvo	TRUDNOĆA	Porođaj	Djetinjstvo	EL EMBARAZO	El parto	La infancia
15.	INCESTO	familia	vecinos	INCEST	Obitelj	Susjedi	INCEST	Obitelj	Susjed	EL INCESTO	La familia	El vecino
16.	PASTILLA	tableta	galleta	PILULA	Tableta	Dvopek, kolačić	PILULA	Tableta	Kolačić	LA PÍLDORA	La tableta	El pastelito
17.	TESTÍCULO	óvulo	esperma	TESTIS	Jajašce	Sperma	TESTIS	Ovulacija	Sperma	EL TESTÍCULO	La ovulación	El semen
18.	RECTAL	regadera	inodoro	REKTALAN	Kanta za polijevanje	WC, dezodorans	REKTALNO	Tuš	WC	RECTAL	La ducha	El inodoro
19.	OJO	oír	ver	OKO	Čuti	Vidjeti	OKO	Sluh	Vid	EL OJO	El oído	La vista

20.	IRRITACIÓN	rígido	adolorido	IRITACIJA	Ukočen	Bolan	IRITACIJA	Ukočeno	Bolno	LA IRRITACIÓN	Tenso	Doliente
21.	ABNORMAL	diferente	similar	ABNORMALNO	Drukčije	Slično	ABNORMALNO	Drukčije	Slično	ABNORMAL	Diferente	Similar
22.	ESTRÉS	preocupación	feliz	STRES	Zabrinutost	Sretan	STRES	Zabrinutost	Sreća	EL ESTRÉS	La preocupación	La felicidad
23.	ABORTO ESPONTÁNEO	pérdida	matrimonio	SPONTANI POBAČAJ	Gubitak	Brak	SPONTANI POBAČAJ	Izgubiti	Brak	EL ABORTO ESPONTÁNEO	Perder	El matrimonio
24.	ICTERICIA	amarillo	blanco	IKTERUS, ŽUTICA	Žut	Bijel	IKTERUS	Žuto	Bijelo	LA ICTERICIA	Amarillo	Blanco
25.	PAPANICOLAOU	prueba	vacuna	PAPANICOLAU	Test	Kozice	PAPANICOLAU	Test	Cjepivo	PAPANICOLAOU	La prueba	La vacuna
26.	IMPÉTIGO	pelo	piel	OSIP	Dlaka	Koža	OSIP	Dlaka	Koža	LA ERUPCIÓN	El cabello	La piel
27.	INDICADO	instrucción	decisión	INDICIRAN, OZNAČEN	Uputa	Odluka	NAZNAKA	Uputa	Odluka	LA INDICACIÓN	Instrucciones	La decisión
28.	ATAQUE	herida	sano	NAPAD	Ozljeda	Zdrav	NAPAD	Ozlijeđen	Zdrav	EL ATAQUE	Lesionado	Sano
29.	MENOPAUSIA	señoras	niñas	MENOPAUZA	Gospođe	Djeca	MENOPAUZA	Gospođe	Djevojke	LA MENOPAUSIA	Señoras	Chicas
30.	APÉNDICE	rascar	dolor	SLIJEPO CRIJEVO	Grepsti	Bol	SLIJEPO CRIJEVO	Ogrebotina	Bol	EL APÉNDICE	El rasguño	El dolor
31.	COMPORTAMIENTO	pensamiento	conducta	PONAŠANJE	Misao	Vladanje	PONAŠANJE	Misao	Vladanje	EL COMPORTAMIENTO	El pensamiento	La conducta
32.	NUTRICIÓN	saludable	gaseosa	PREHRANA	Zdrav	Gazirano piće	PREHRANA	Zdravlje	Gazirano piće	LA NUTRICIÓN	La salud	Las bebidas carbonatadas
33.	DIABETES	azúcar	sal	DIJABETES	Šećer	Sol	DIJABETES	Šećer	Sol	LA DIABETES	El azúcar	El sal
34.	SÍFILIS	anticonceptivo	condón	SIFILIS	Kontraceptivi	Kondom	SIFILIS	Kontraceptivi	Kondom	LA SÍFILIS	Anticonceptivos	El condón
35.	INFLAMATORIO	hinchazón	sudor	UPALJEN	Oteklina	Znoj	UPALA	Oteklina	Znoj	LA INFLAMACIÓN	El hinchazón	El sudor
36.	HEMORROIDES	venas	corazón	HEMOROIDI	Vene	Srce	HEMOROIDI	Vene	Srce	LAS HEMORROIDES	Las venas	El corazón
37.	HERPES	aire	sexo	HERPES	Zrak	Seks, spol	HERPES	Zrak	Seks	HERPES	El aire	El sexo

38.	ALÉRGICO	resistencia	reacción	ALERGIČAN	Otpornost	Reakcija	ALERGIJA	Otpornost	Reakcija	LA ALERGIA	La resistencia	La reacción
39.	RIŇON	orina	fiebre	BUBREG	Urin	Groznica	BUBREG	Mokraća	Vrućica	EL RIŇON	La orina	La fiebre
40.	CALORÍAS	alimentos	vitaminas	KALORIJE	Hrana	Vitamini	KALORIJE	Hrana	Vitamini	LAS CALORÍAS	La comida	Las vitaminas
41.	MEDICAMENTO	instrumento	tratamiento	LIJEK	Instrument	Terapija	LIJEK	Instrument	Terapija	LA MEDICINA	El instrumento	La terapia
42.	ANEMIA	sangre	nervio	ANEMIJA	Krv	Živac	ANEMIJA	Krv	Živac	LA ANEMIA	La sangre	El nervio
43.	INTESTINOS	digestión	sudor	CRIJEVA	Probava	Znoj	CRIJEVA	Probava	Znoj	LOS INTESTINOS	La digestión	El sudor
44.	POTASIO	mineral	proteína	KALIJ	Mineral	Protein	KALIJ	Mineral	Protein	EL POTASIO	El mineral	La proteína
45.	COLITIS	intestino	vejiga	KOLITIS	Crijevo	Mokraćni mjehur	KOLITIS	Crijeva	Mjehur	EL COLITIS	Los intestinos	La vejiga
46.	OBESIDAD	peso	altura	PRETILOST	Težina	Visina	PRETILOST	Težina	Visina	LA OBESIDAD	El peso	La altura
47.	HEPATITIS	pulmón	hígado	HEPATITIS	Pluća	Jetra	HEPATITIS	Pluća	Jetra	LA HEPATITIS	Los pulmones	El hígado
48.	VESÍCULA BILIAR	arteria	órgano	ŽUČNI MJEHUR	Arterija	Organ	ŽUČNI MJEHUR	Arterija	Organ	LA VESÍCULA BILIAR	La arteria	El órgano
49.	CONVULSIONES	mareado	tranquilo	GRČEVI, KONVULZIJE	Bolestan od morske bolesti	Miran	EPILEPSIJA	Padavica	Smirenost	LA EPILEPSIA	La epilepsia	La calma
50.	ARTRITIS	estómago	articulación	ARTRITIS	Želudac	Zglob	ARTRITIS	Želudac	Zglob	LA ARTRITIS	El estómago	La articulación

Conclusion

The translation of SAHLSA-50 from Spanish into Croatian was done using the standard validated procedure. The terms were modified in only several items, in order to adapt the Croatian version to the most similar sense in Croatian. The Croatian version of SAHLSA-50 is to be used in epidemiological and clinical studies as a useful tool for evaluating the levels of HL in the population. It should simplify future communication on both professional and non-professional sides.

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Original article

A Study of Patient Satisfaction With Healthcare in Zenica-Doboj Canton

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Abstract

Aim: Patient satisfaction and patient evaluation of healthcare can be seen as important results of provided care, as they reflect the level to which the patient's subjective and objective needs have been met. The aim was to evaluate patient satisfaction with healthcare and compare the results for 2017 with the results from 2011.

Methods: The study was conducted in the territory of Zenica-Doboj Canton in May 2011 and in October 2017. A questionnaire was filled out by 2,008 examinees in 2011 and by 2,000 examinees in 2017 outside healthcare institutions (in the street), using the EUROPEP questionnaire as a research instrument. The average age of the respondents was 38.4. The participants in the study were 52 % male and 48 % female. Student's T-test was used to compare the results between the two samples.

Results: A statistically significant difference was observed between the mean patient satisfaction in 2011 (3.19 ± 0.3, min. = 2.6, max. = 3.83, P = 0.00032) and the mean patient satisfaction in 2017 (3.47 ± 0.17, min. = 3.14, max. = 3.94, P = 0.000647), t(23) = 3.75. Increase in patient satisfaction in 2017 is evident compared to their satisfaction in 2011.

Conclusion: Surveying the satisfaction of healthcare recipients should be a common method of work as it gives the patients the impression that their opinion is valued, while at the same time it indicates to healthcare staff that their attitudes may need to be changed, their knowledge expanded and the organization of work improved, if they want to provide services of improved quality.

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Introduction

The patient is the one to be questioned whether the provided health care has helped to improve their health or quality of life. It is not only the results of healthcare that matter in terms of improved health; it is also important how the healthcare is provided: access to care, organization of services, staff education and their communication with the patient are all relevant factors. There is increased awareness among persons with health insurance policies and healthcare staff that patients can and must have a central role in defining optimal care and improved quality of healthcare. Engagement of patients in the process of improving healthcare is not only desirable; it is also a social, economic and technical necessity (1).

New concepts, such as the central role of patients, strengthening of patients, patients as partners and joint decisions, demonstrate an emancipation of patients. Engagement of patients is not only important from the ethical perspective. It is important to investigate not only the expectations, needs and priorities of patients within general healthcare practices, but also to gather information about the experiences of patients provided with care. Most patients can provide more than an opinion and assessment of care and treatment they received from a doctor or other healthcare staff. Such information may be very important. It may help the practitioners select those aspects of care where improvement is necessary. On the other hand, patient satisfaction and their evaluation of care may be observed as important results of provided care, since they reflect the level to which their subjective and objective needs are met. This cannot be observed as the only relevant result, as sometimes the patients have unrealistic demands; however, patient satisfaction can generally be seen as an important addition to other types of measures (health status, quality of life or costs) required to evaluate the quality of general medical practice (2).

Information about the patient's evaluation of care is mainly collected through (written) questionnaires filled out by patients who go to see a doctor or get the questionnaire by mail. In principle, patient surveys are among the most popular methods of assuring quality in a healthcare system.

Material and Methods

The EUROPEP instrument has been created to provide feedback to general practitioners / family doctors, general practitioner's offices as well as organizations of patients/healthcare recipients. It is a standardized instrument for evaluating patient satisfaction with healthcare. For this study, the EUROPEP questionnaire was translated into Bosnian (3).

The study was conducted in the territory of Zenica-Doboj Canton in May 2011 and in October 2017. The questionnaire was filled out by 2,008 examinees in 2011 and by 2,000 examinees in 2017 outside healthcare institutions (in the street). Student's T-test was used to compare the results between the two samples.

Results

The study was conducted in the territory of Zenica-Doboj Canton for the purpose of evaluating satisfaction with healthcare. The EUROPEP methodology (23 questions with a 5-point answering scale), which was developed by an international consortium of researchers and general practitioners in 1995, was used. The research also used a modified form with questions relevant for our field of work (length of time spent waiting for an examination, evaluation of staff actions during the stay in a medical institution, payment of services and medication, and reason for payment). The research was conducted in May 2011 and in October 2017. The survey was performed on a sample of 2,000 respondents outside medical institutions (in the street) in order to get as many objective answers as possible. The aim of the research was to obtain results for 2017 and compare them with those from 2011 (4,5).

The average age of the respondents was 38.4 (age range 12-83). The study included 51.95% of

male participants and 48.05% of female participants. The respondents' education structure is shown in Table 1, indicating that the highest number of respondents had secondary school education. Figure 1 shows the distribution of respondents by municipalities.

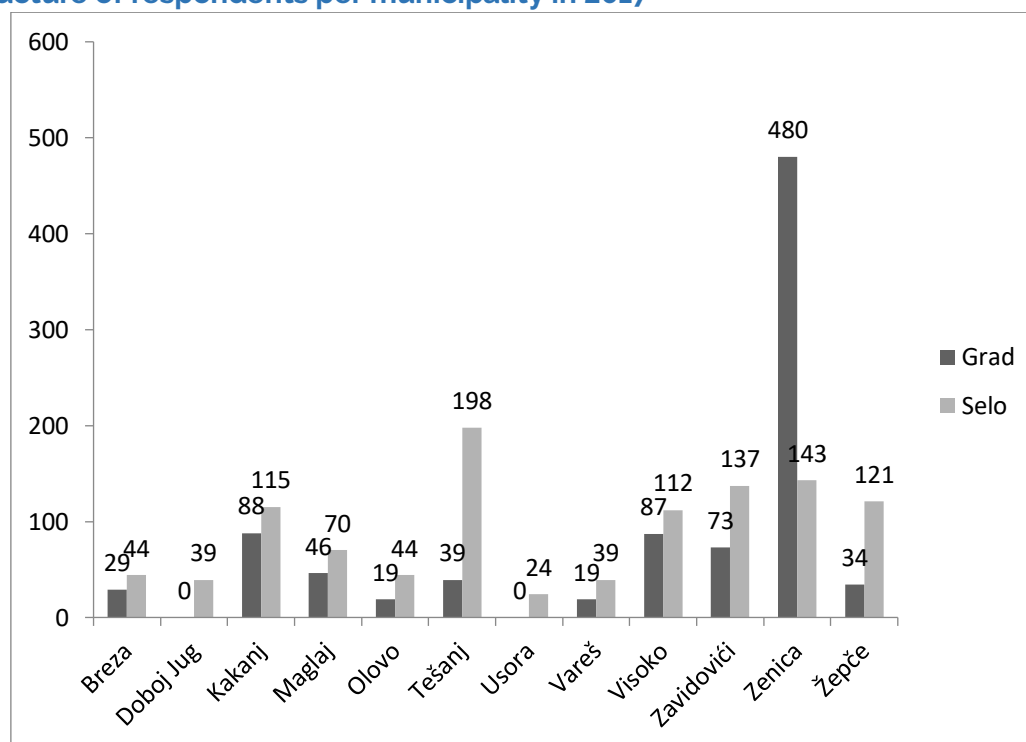**Figure 1. Structure of respondents per municipality in 2017**

Table 1. Level of respondents' education in 2017

Level of education	No.
	10
Without primary school	13
Primary school	257
Secondary school	1.230
College	250
University	240
Total	2.000

Table 2 provides a classification of the respondents from Zenica-Doboj Canton, showing their occupation in numbers and percentages. According to the occupation classification, the highest number of respondents were employed in the public sector (23.55%).

Table 2. Respondents' occupation in 2017

Occupation	No.	%
Unknown	16	0.8
		15.7
Student	315	5
Unemployed	221	11.05
Private entrepreneur	92	4.6
Employed in the public sector	268	13.4
Employed in the private sector	471	23.5
Pensioner	204	10.2
Farmer	76	3.8
Freelance (artist)	43	2,15
		12.9
Housewife	259	5
Other	35	1.75
	2.00	
Total	0	100

By comparing the results of the average grade for questions in Table 3 for the period 2011–2017, we obtained the following results: by using an independent samples T-test, comparison of the results for 2011 and 2017 was made for patient satisfaction in regard to provided services and

treatment by medical staff. A statistically significant difference was noted between the mean patient satisfaction in 2011 (3.19 ± 0.3, min = 2.6, max = 3.83, p = 0.00032) and the mean patient satisfaction in 2017 (3.47 ± 0.17, min = 3.14, max = 3.94, p = 0.000647), t(23) = 3.75. The difference

between mean values per group (mean difference of 0.41) was small (eta squared = 0.0468).

Table 3. Average grade per EUROPEP question (on a scale of 1 to 5 for each question)

Average grade per EUROPEP question	
Do you think that the doctor spends sufficient time with you?	3.33
Does the doctor show any interest in your problem?	3.41
Do you feel better when you tell the doctor about your problem?	3.46
Does the doctor involve you in making a decision about your treatment?	3.36
Does the doctor listen to you carefully while you are presenting your problems?	3.52
Does the doctor provide you with all information about your diseases?	3.42
Does the doctor try hard to relieve your symptoms as soon as possible?	3.47
Does the doctor help you feel better and return to your everyday work?	3.49
Does the doctor perform a physical examination?	3.75
Does the doctor perform a detailed physical examination?	3.51
Does the doctor work on the prevention of various diseases?	3.46
Does the doctor explain why you need to undergo additional tests and analyses?	3.44
Does the doctor provide explanation about your symptoms and disease?	3.47
Does the doctor help with your emotional problems related to your health condition?	3.30
Does the doctor explain to you why it is important to comply with his/her instructions?	3.46
Does the doctor explain what he/she is doing during the examination?	3.43
Does the doctor explain what you can expect at a specialist examination in hospital?	3.36
Are you assisted by other medical staff (nurse at the clinic)?	3.56
Can you make an appointment with the doctor?	3.80
Is it easy to make a phone call to the doctor?	3.31
Can you seek advice from the doctor by phone?	3.14
Do you wait long in the waiting room?	3.46
Does the doctor respond fast in emergency situations?	3.94

Discussion

By comparing surveys conducted in Bosnia and Herzegovina and other countries from the region, we reached the following conclusions: a

survey conducted at the Healthcare Centre of Zenica in 2013 showed that patient satisfaction with general practice offices was statistically most significant with regard to making appointments with practitioners at a time

suitable for the patients (p = 16.28), the possibility of making a phone call to the clinic (p = 32.55), and the length of time spent in the waiting room (p = 30.42). Obtained data confirmed a high level of patient satisfaction with units of general practice in primary healthcare. The EUROPEP questionnaire seems to be a useful tool for reviewing patient satisfaction with medical services (6).

In comparison to this study, in Bulgaria, 58.7% of respondents on average rated the level of care received as excellent. The waiting time in the waiting room was the most poorly rated item (33.8%). The item "keeping patients' records and data confidential" was the most highly rated item (88.8%). Patients were less satisfied with "providing quick services for urgent health problems" (78.5% excellent or good) and "getting an appointment to suit them" (76.2% excellent or good) (7).

In 2016, a cross-sectional study was performed among visitors of the Emergency Medical Service (EMS) of Gorenjska, Slovenia. It took into account the waiting time, which is considered to be associated with the success of the EMS organizational model. The EUROPEP questionnaire was used for rating the degree of patient satisfaction. Results showed a score higher than 4. Patients were least satisfied with the length of time spent waiting for an examination. Research results confirmed that the effectiveness of the EMS organizational model had an impact on the degree of patient satisfaction (8).

This study showed that most respondents had a positive opinion about the behavior of GPs, emphasizing their gentleness during medical examinations (83%), respect for privacy (82%), as well as a benevolent attitude towards patients (77%). However, despite the positive assessment of their behavior, in the opinion of 52% of the respondents, doctors did not offer them any preventive examinations, and in many cases (43%) did not provide information about a healthy lifestyle. A third of the patients (32%) was not informed about the side effects of medication by a doctor. The results showed that fewer than half of the GPs were interested in the psychosocial

sphere of their patients. Only 27% of the respondents received their doctor's help in regard to dealing with fears about their health. An even lower percentage of respondents stated that their doctor expressed interest in their personal (23%) or material (23%) situation, while 35% of patients received questions from their doctors about family members (9).

In the research conducted in Zenica-Doboj Canton in 2015, the questionnaire was made on the basis of the EUROPEP standardized questionnaire regarding patient satisfaction with healthcare. The older population evaluated secondary and tertiary healthcare practices better, they needed more time to reach health facilities, and they waited longer to receive healthcare services in primary healthcare practice (10).

Research conducted across Turkey's 81 provinces over the period 2010-2012, using the European Patients Evaluate General/Family Practice (EUROPEP), showed that the Family Medicine Model significantly improved patient satisfaction across a range of dimensions (11-12). This study has shown great similarity with the results of this research, showing evident increase in patient satisfaction. Numerous studies conducted in the area of the Federation of Bosnia and Herzegovina have shown significant improvement and increase in patient satisfaction with healthcare (6, 13-17).

Conclusion

As expected, the analysis of data obtained in the study of patient satisfaction in the area of Zenica-Doboj Canton shows that there was a significant increase in satisfaction in 2017 in comparison with 2011. Surveying the satisfaction of healthcare recipients should be a common method of work as it gives the patients the impression that their opinion is important and serves as an indication for medical staff in terms of required changes of attitudes, expansion of knowledge and organization of work.

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