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HEMOKOREKTORI ZASNOVANI NA PERFLUOROKARBONSKIM GASNO-TRANSPORTNIM EMULZIJAMA KOJE SE DODAJU U KRV

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ABSTRACT

Hemocorrectors based on perfluorocarbon gas-transport blood-substituting emulsions are complex multiphase systems used in the biomedical field as multifunctional drugs, in particular, as gas-transport substitutes for a blood donor. The aim of this review was to discuss their physicochemical and medico-biological properties. A number of preparations from both Russian and foreign manufacturers based on chemically inert perfluorocarbon blood-substituting emulsions of a nano-size level as hemocorrectors with a gas transport function are shown. The analysis of the effect of perfluorocarbon emulsion on the blood gas transport indicators showed that perfluorocarbon particles in the bloodstream will significantly improve the conditions of gas exchange in tissues. The most important issue is the concentration of perfluorocarbon blood-substituting emulsions. The perfluorocarbon emulsions can be considered as a means of correcting the gas transport properties of blood, increasing the reserve capacity of blood cells-red blood cells to deliver oxygen to the tissues. Taking into account all facts about perfluorocarbon hemocorrectors, it can be concluded that they can be used as universal nanocarriers for the transdermal delivery of oxygen and biologically active compounds in various fields of biomedicine and cosmetology.

Keywords: *hemocorrectors, perfluorocarbon gas-transport blood-substituting emulsions, biological characteristic, medical characteristic.*

SAŽETAK

Hemokorektori zasnovani na perfluorokarbonskim gasno transportnim emulzijama koje se dodaju u krv su složeni multifazni sistemi koji se koriste u biomedicinskoj oblasti kao multifunkcionalni lekovi, posebno kao gasno transportni supstituti za donore krvi. Cilj ovog rada je da razmotri njihove fizičko-hemijske i mediko-biološke osobine. Jedan broj preparata od ruskih i stranih proizvođača zasnovanih na hemijski inertnim perfluorokarbonskim emulzijama koje se dodaju u krv na nivou nano-veličine kao hemokorektori sa gasno transportnom funkcijom su prikazani. Analiza dejstva perfluorokarbonske emulzije na gasno transportne indikatore krvi je pokazala da će perfluorokarbonske čestice u krvotoku značajno poboljšati uslove razmene gasova u tkivima. Najvažnija stvar je koncentracija perfluorokarbonskih emulzija koje se dodaju u krv. Perfluorokarbonske emulzije se mogu smatrati sredstvom korigovanja gasno transportnih svojstava krvi, povećavajući kapacitet rezervi krvnih ćelija-crvenih krvnih zrnaca da dopremaju kiseonik do tkiva. Uzimajući u obzir sve činjenice o perfluorokarbonskim hemokorektorima, može se zaključiti, da se mogu koristiti kao uiverzalni nanonosači za transdermalno dopremanje kiseonika i biološko aktivna jedinjenja u različitim oblastima biomedicine i kozmetologije.

Ključne reči: *hemokorektori, perfluorokarbonske gasno transportne emulzije koje se dodaju u krv, biološke karakteristike, medicinske karakteristike.*

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INTRODUCTION

Hemocorrectors based on the perfluorocarbon gas-transport blood-substituting emulsions are complex multiphase systems of colloid chemistry that are successfully used in the medical and biological field as multifunctional drugs, in particular, as gas-transport substitutes for the donor blood.

Perfluorocarbon gas transport hemocorrectors are widely used in the treatment of various forms of anemia and blood loss, intravascular hemolysis and anaerobic infections, carbon monoxide poisoning and gas exchange disorders in the lungs, where it is necessary to restore the function of O_2 transport.

Indications for the clinical use of the perfluorocarbon blood-substituting emulsions such as Perftoran are broadly used for all types of shock; metabolic and gas exchange disorders; circulatory disorders in multiple injuries; anti-ischemic protection of organs; use for hemodilution, in the absence of the donor blood and red blood cell mass, etc.

The perfluorocarbon emulsions are a multicomponent heterogeneous substance in which many processes occur simultaneously: the thermal motion of particles, molecular diffusion, clumping, and settling. The perfluorocarbon emulsion is an unstable system in which the dispersion of the system changes both as a result of coalescence and coagulation, and according to the laws of Ostwald ripening period.

Researchers of many research centers are trying to create aggregative and sedimentation-resistant hemocorrectors, in terms of the colloid-chemical properties of perfluoroemulsions, while maintaining their high medical and biological effectiveness. Several attempts have been made to search for and synthesize the proper perfluoro compounds and their emulsifiers with help of whom it would be possible to accomplish this task.

In Russia, this problem was partially solved (1-4) in domestic drugs using a binary mixture of two organofluorine compounds: perfluorodecalin and perfluoromethylcyclohexylpiperidine, stabilized by a block copolymer of ethylene oxide and propylene to an average size of gas carrier particles ("artificial red blood cell") from 30 to 150 nanometers inthe artificial plasma with electrolytes, followed by sterilization by dynamic ultrafiltration (Table 1).

In Japan, as the author points out (5-7), the emulsification of a binary mixture of perfluorodecalin and perfluorotropylamine (PFD/PFTPA) was carried out with a mixture of phospholipids and pluronic F-68, which did not allow for full sterilization of the Japanese blood-substituting emulsion Fluosol-DA 20%. Since the point of turbidity of pluronic f-68 is approximately at a temperature of 115°C, above which its effectiveness as an emulsifier decreases sharply.

In the US, the question was solved (6, 7) by using also a known emulsifier of phospholipid of an egg protein, which improved the stability of the US blood-substituting emulsion of Oxygent binary mixture of perftoroktilbromid and perftordioctilbromid (PFOB/PFDB) during sterilization - autoclaving, but with a partial loss of medico-biological efficiency (e.g.: rheology, hemodynamic properties, etc.).

THE QUESTION OF ADEQUATE CONCENTRATION AND OXYGEN CAPACITY

The most important issue is the concentration of perfluorocarbon blood-substituting emulsions. The opinions of scientists are very contradictory.

So, all American perfluorocarbon emulsions of the second generation that are undergoing clinical trials, such as Oxigent, Oxyfluor, Oxycyte, are very concentrated emulsions. The American researchers seem to assume that a high concentration of the gas carrier - perfluorocarbon - can bring the artificial hemocorrector closer to the level of oxygen capacity of the donor blood (~20 vol.%). Thus, the American drug Oxygent has an oxygen capacity of 24 vol.% (at pO_2=760 mmHg), but the efficiency of oxygen return (or dynamic oxygen capacity) in their emulsions, due to their high viscosity, is very low (0,77 vol.%). It is likely that in the future, created highly concentrated hemocorrectors with an oxygen capacity similar to that of the donor blood will be unpromising and not safe due to frequent allergic reactions (Table 2).

Our calculations show that if a 20% perfluorocarbon emulsion injected into the body at a dose of 10 ml/kg dissolves only 0,5% of O_2, and the remaining O_2 is carried by red blood cells - 98,2% and plasma - 1,3%, then an increase in the concentration of perfluorocarbons in the emulsion even twice (up to 40%) will lead to an increase in oxygen dissolution to only 1% (at the same dose), but the viscosity of the blood-substituting emulsion will significantly increase. Obviously, this is not effective, and a further increase in the concentration or dose of the emulsion will not lead to the desired effect (Table 3).

In our opinion, the most promising emulsion of the second generation is likely to be the low concentrated (1-10%) proksanola-fluorocarbon emulsion submicron level (nanoemulsion) type of FTRORANs (FTORemulsion-III). At first glance, the ability to gas-dissolve the low-concentrated perfluorocarbon emulsions is not large enough. So, 1% emulsion of Ftoran-1 dissolves oxygen only in 2,6-2,7 vol.% (at pO_2 =760 mmHg), but the gas exchange surface characteristic of 1 L emulsion will be about 600 m^2, which is slightly less than the surface of 1 L of red blood cells – 700 m^2.

Table 1. Compositions and physico-chemical properties of perfluorocarbon blood-substituting preparations-gas-transport hemocorrectors FTOROSAN – PERFTORAN – FTOREmulsion-III, created in the USSR-Russia in the period 1979-2009.

	I drug Beloyartsev F.F. and Co.		II drug Vorobyev S.I. and Co.	III drug Vorobyev S.I. and Co.
Name	FTOROSAN 1979-1983	FTOROSAN PERFTORAN 1983-1985	PERFTORAN 1986-1997	FTOR emulsion-III 1999-2009
Components	Clinical trial during 1983-1985		Approved for the usage	Runs the registration
Chemical components (g)*:*				
Perfluorodecalin	15,2	15,2	13,0	13,0
Perfluoromethylcyclogesilpiperidin	7,6	7,6	6,5	6,5
Proxanol 168 (Mw - 8000 D)	3,8	3,8	-	-
Proxanol 268 (Mw - 13000 D)	-	-	4,0	4,0
Sodium cloride	0,6	0,597	0,6	0,6
Kalium chloride	0,04	0,387	0,039	0,039
Magnesium chloride	0,02	0,02	0,019	0,019
Sodium hydrocarbonate	0,15	0,15	0,065	0,065
Sodium bicarbonate	-	0,014	0,02	0,02
Calcium chloride	0,03	0,028	-	-
Glucose	0,18	0,2	0,2	0,2
Albumin	3,0	3,0	-	-
H_2O (ml)	до 100	до 100	до 100	до 100
Physical, chemical and biological features:				
Average partial size (mkm)	0,10-0,15	0,10-0,15	0,03-0,15	0,03-0,10
Dispersion: particals > 0,2 мкм	+	+	+	-
Sterilisation of the drug	-	-	-	+
Viscosity (sP)	3,5	3,0	2,5	2,5
Number of side effects (%)	~30%	~30%	~15-20%	~6-7%

Table 2. Gas transport properties of the Russian and American blood-substituting emulsions of the second generation [3, 4, 5, 6].

Features	Blood	Emulsion II generation (Russia)[3,4]		Emulsion II generation (USA)[5,6]	
		FTOR-emulsion-III 20	FTOR-emulsion-III 10	Oxigent AF0104	Oxigent AF0143
Concentration of perfluororganic compounds	Hct-45%	20%	10%	90%	90%
Concentration of O_2 (relatively in pO_2=760 mm Hg), volume %	20	7	4	24	24
Viscosity, cP	5	2,5	1,7	31	55
Dynamic oxygen capacity (O_2 / viscosity), volume %	4	2,8	2,35	0,77	0,43

Table 3. Gas transfer and oxygen dissolution with intravenous administration of 20% perfluorocarbon emulsion type FTORemulsion-III at a dose of 10 ml / kg [3, 4].

Components	Dissolution of O_2 (%)	Const of Crog's diffusion for O_2	Time of oxygenation (msec)	Gas exchange surface (m^2)
Erythrocytes	98,2	-	200 – 250	3500
Plasma	1,29	$5{,}3 \cdot 10^{-5}$	-	-
Emulsion of perfluorocarbon	0,5*	$4{,}4 \cdot 10^{-4}$**	14 - 26***	8400****

*) increased mass transfer of O2 due to additional physically dissolved O2 with1/3 in perfluorocarbons;
**) increased O2 mass transfer due to accelerated diffusion in perfluorocarbons;
***) increased O2 mass transfer due to high oxygenation rate in perfluorocarbons;
****) increased O2 mass transfer due to larger gas exchange in perfluorocarbons.

With a decrease in the oxygen capacity in the low-concentration blood-substituting emulsions of the Ftoran-1 type, the total gas diffusion rate can be successfully compensated by increasing the gas exchange surface where gas diffusion occurs, i.e., the total diffusion rate can be maintained by increasing the dispersion of the perfluorocarbon phase itself. The higher the dispersion, the larger the surface of phase's division and, consequently, the larger the gas exchange area.

There are two quantitative characteristics of emulsions: the maximum volume of the emulsion (Vmax) and the maximum surface of the emulsion (Smax). The maximum volume of the emulsion (Vmax) is not an objective characteristic. A more objective value is the maximum surface of the emulsion (Smax), which is determined by the number of submicron particles and depends on the dispersion of the emulsion – this is the maximum gas exchange surface through which the gases are diffused. In other words, by dispersing and breaking up 1 gram of perfluorocarbons to the average nanoparticle sizes of 30-80 nm, a huge phase division can be obtained, i.e., the gas exchange area of 60 m2. (for example, 1 pill of activated carbon has the surface area of 125 m2).

The analysis of the effect of perfluorocarbon emulsion on the blood gas transport indicators showed that perfluorocarbon particles in the bloodstream will significantly improve the conditions of gas exchange in tissues by the following indicators: increasing the arterial-venous difference in O2 tension; changes in the conditions of oxygenation and deoxygenation of red blood cells; increasing the flow of oxygen from the blood to the tissues (in proportion to the gas voltage gradient), due to the increased solubility of gases in the perfluoroorganic phase, creating an additional reservoir for gases in the plasma or a damper that creates an additional flow for oxygen while it is being consumed. The perfluorocarbon emulsions can be considered as a means of correcting the gas transport properties of blood, increasing the reserve capacity of blood cells-red blood cells to deliver oxygen to the tissues (10, 11).

All of the above-mentioned effects of perfluorocarbons can and should appear already in the presence of a relatively low concentration of perfluorocarbons in the bloodstream. It can be argued, the author continues (10), that low concentrations of perfluorocarbons in the blood (up to 3-4 vol.% or 1,5-2,0 %) can have a disaggregating effect on red

blood cells, thereby improving the fluidity and delivery of gases by red blood cells.

ADVERSE REACTIONS OF PERFLUOROCARBON EMULSIONS

Other positive characteristics of the perfluorocarbon hemocorrectors - low-concentrated proxanol-fluorocarbon nano-emulsions of the second generation are also certain. However, with all the positive properties of perfluorocarbon emulsions of the first and second generation, both proxanol and phospholipid, it is impossible not to point out their reaction, associated with both the activation of the blood plasma complement system and the activation of the macrophage activity on the introduction of actual particles of the perfluoro-emulsion. The adverse reactions have been shown to be mainly associated with large particles of the emulsion, both on proxanol and phospholipids, and with the toxicity of the emulsifier-pluronic F-68. These reactions are similar to the flu-like symptoms - pain in the sacrum, redness of the face, fever, sometimes manifestations of the anaphylactoid reactions. Similar manifestations are observed in the lipid emulsions for parenteral nutrition (without the perfluoro compounds). They are typical for many preparations containing particle dispersion with the adsorptive surface (3, 4).

However, as shown by our early studies (3), the number of reactions and their degree in the perfluorocarbon emulsions are different. Thus, the reactogenicity of the FTOREmulsion-III emulsion (formerly called Perftoran-plus) is ~ 6,6%, Perftoran ~ 15-20%, the Fluosol-DA emulsion 20% is apparently even higher than ~30%. The Oxygent emulsion also has side effects comparable in quantity to Fluosol-DA 20%.

Our studies (3, 8) on the complement-activating effect of the American drug did not reveal the activation of the complement system by the Oxigent emulsion (AF0104), which underlines correctness of the assumption that the side reactions of phospholipid-fluorocarbon emulsions are associated with another system - reticulo-endothelial, with the activation of the macrophage activity. At the same time, the level of activation of the complement system of the Oxygent emulsion (AF0104), determined by the value of the neutropenic index in blood plasma, did not exceed the control values.

It should be noted that the domestic emulsifier proxanol-268, the analogue of which is pluronic F-68, activated the complement system in foreign researchers, in our experiments, it did not cause a similar reaction. This seems to be due to the physical and chemical structure of the foreign emulsifier and as our early experiments (3, 8) showed, to its toxicity.

It is known that the molecular weight of pluronic F-68 was 8300 D, its main hydrophobic block 21%, while the toxicity of this emulsifier, studied in mice (LD50), was high and amounted to 9.4 g/kg. On the contrary, the domestic proxanol 268, which was used in our emulsions, was less toxic. This emulsifier had the molecular weight of 7200 D and its main hydrophobic block-popr was -19%, the toxicity in LD50 mice was 2 times lower than the foreign analogue, and it was 20 g/kg. It is the possible activation of the complement system by pluronic F-68 that led many researchers to change the synthetic emulsifier to the animal - phospholipid one. However, as clinical studies have shown, this did not completely solve the problem. Phospholipids, according to the American researchers, do not activate the complement system of blood plasma, but the phospholipid-fluorocarbon emulsions, nevertheless, have reactogenicity. And this reactogenicity, as already noted, in our opinion, is associated with another reticuloendothelial system with phagocytosis (3-5).

All these factors were carefully taken into the account when creating new domestic blood-substituting emulsions of the FTORANs series. Therefore, there was no replacement of proxanol with phospholipids, given that the phospholipid-fluorocarbon emulsions also showed negative characteristics.

The problem was that, after determining the system responsible for the occurrence of adverse reactions on the proxanol-fluorocarbon emulsions, and this, as already mentioned above, is the blood plasma complement system, it was necessary to eliminate or minimize the reasons for turning on the complement system. It turned out that the factor triggering the complement-activating effect of the emulsion is the dispersed composition of the emulsion particles, i.e. the reactogenicity of the drug increases as the total and average particle diameter increases, as well as the number of coarse particles in the emulsion.

SORPTION ACTIVITY AND REACTOGENICITYOF PERFLUOROCARBON EMULSIONS

Our study has shown (12) that from 20 to 35% of immunoglobulin G (IgG) are sorbed on the surface of the emulsion, and the dispersion of the obtained perfluoro-emulsion particles plays an important role in this process. Thus, the coarse-gained emulsion with an average particle size of 0.5 microns sorbs up to 35% IgG on its surface, while the microfine emulsion with a diameter of 50 nm (0,05 microns) sorbs about 20% IgG. It is the sorption of IgG, as the most “reactogenic” immunoglobulin, that causes an increased activation of the blood plasma complement system. So, apparently, the sorption of a large amount of IgG on the surface of the perfluoro coarsed emulsion, compared with the fine emulsion, may be associated with less stable surface active layer consisting of a block copolymer of ethylene oxide and propylene, on the particles of the coarse emulsion. Since the sorption of IgG occurs, apparently, on the surface of the hydrophobic part of the perfluorocarbon drops, the IgG molecule will be able to shift the proxanol (emulsifier) molecule in a less strong and rather “loose” surface-active layer of the emulsifier of a coarse perfluoroalsifier. The

surface-active layer of proxanol in the finely dispersed perfluoroacid emulsion is denser and more uniform; the surface of perfluorocarbons themselves is completely covered by a mono- or bilayer of proxanol. Therefore, the sorption activity of a fine emulsion concerning IgG sorption is less expressed.

To reduce the reactogenicity, it was supposed to create a strong, stable surface-active layer (surface-mechanical barrier) on the surface of the emulsion particles. The stable surface-active layer on the particles can be obtained under certain homogenization regimes, when the submicron-sized particles are created in the mass (about 99%). In this case, the coarse particles with a “loose” less strong surface-active layer are further eliminated by dynamic ultrafiltration. This significantly reduces the most “attractive” sorption surface for “reactogenic proteins” of various molecular weights, mainly responsible for the complement.

As our further research (4) showed, this led to positive results. The reactogenicity of the blood-substituting emulsions of the FTORemulsion III type with a strong surfactant layer in clinical trials did not exceed 7%.

The nano-dispersion emulsions, in the process of long and effective homogenization, have been shown to acquire aggregate stability of the dispersed phase due to the dense proxanol layer on the surface of the oil drop in the perfluoro compound, which consists of tightly intertwined hydrophilic chains – a structural and mechanical barrier-proxanol, which has certain structural and rheological properties. The use of modern high-tech technologies that provide the production of monodisperse nanoparticles with a narrow dispersion distribution a dense and homogeneous surface-active layer of proxanol, are factors that largely determine the properties of perfluoroemulsions. In addition, the active cleaning of emulsifiers and perfluorocarbons and sterilization of the perfluoroacid emulsion by dynamic ultrafiltration allow to improve the functional activity and significantly reduce the reactogenicity of perfluorocarbon gas-transport hemocorrectors.

CONCLUSION

In conclusion, it is necessary to note the main medical and biological characteristics of the gas-transport hemocorrectors -perfluorocarbon blood-substitutingemulsions: gas-transport, sorption, membrane stabilization, detoxification, anti-is-chemic, cardioprotective, ophthalmoprotective, immuno-tropic, antioxidant, diuretic, anti-toxic, radio-and-chemopro-tective, anti-burn, wound healing, nanoscale,etc. However, perfluorocarbon hemocorrectors can be used as universal nanocarriers for the transdermal delivery of oxygen and bio-logically active compounds in various fields of biomedicine and cosmetology.

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CONFLICT OF INTEREST

Authors declare no conflict of interest.

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ATTITUDES OF STUDENTS FROM THE HIGH MEDICAL COLLEGE OF PROFESSIONAL STUDIES AND NURSES TOWARDS PEOPLE SUFFERING FROM DEMENTIA

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STAVOVI STUDENATA VISOKE ZDRAVSTVENE ŠKOLE I MEDICINSKIH SESTARA PREMA LJUDIMA KOJI PATE OD DEMENCIJE

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ABSTRACT

Dementia is characterized by a progressive decrease in cognitive functions, and the term includes different etiologies. Cognitive decline includes loss of memory and deterioration in executive functions, such as planning and organizing skills, sufficient to influence social activities. The aim of this study was to examine and compare the attitudes (knowledge, emotions and behaviour) of students at the High Medical College of Professional Studies and nurses towards people suffering from dementia. The study was designed as a qualitative study with the use of a questionnaire. The Dementia Attitudes Scale (DAS) was used in our study. A total of 283 respondents answered the survey: 56.25% were students, and 43.75% were nurses. The internal consistency of the DAS was found to be good with a Cronbach's α of 0.792. In the overall score for attitudes, a significant difference was found between students (100.47±10.91) and nurses (95.51±16.10). The students had a better score regarding questions describing their behaviour towards these individuals (p<0.001) and emotions for these patients (p<0.001). For knowledge, there was no difference between the two groups of subjects (p=0.901). Regarding the overall score, attitudes of students and nurses towards people with dementia were positive. This research suggested that the training of senior team members who then had dementia expertise was a key component in developing attitudes and improving care practices and outcomes for these patients. Continuous education of all medical staff who have contact with people who suffer from dementia is important.

Keywords: *attitude, dementia, DAS scale, nurses, students of High medical college of the professional studies*

SAŽETAK

Demencija je termin koji se odnosi na različite etiologije koje karakteriše progresivno smanjenje kognitivnih funkcija. Kognitivni pad uključuje gubitak pamćenja i pogoršanje izvršnih funkcija, kao što su planiranje i organizovanje veština koje su neophodne u društvenim aktivnostima. Cilj ovog istraživanja bio je ispitati i uporediti stavove studenata Visoke zdravstvene škole strukovnih studija i medicinskih sestara (znanje, emocije i ponašanje). Studija je osmišljena kao kvalitativna studija uz upotrebu pitanja. U našoj studiji korišćena je skala stavova o demenciji (DAS). Ukupno 283 ispitanika odgovorilo je na anketu: 56,25% su bili studenti, a 43,75% su bile medicinske sestre. Utvrđena unutrašnja koezistentnost za DAS skalu je dobra, sa Cronbach's α od 0,792. U ukupnom skoru stavova utvrđena je značajna razlika između studenata 100.47 ± 10.91 i medicinskih sestara 95.51 ± 16.10. Studenti su imali bolji rezulatat u vezi pitanja koja opisuju svoje ponašanje prema ovim osobama (p<0,001) i emocijama za ove paciente (p<0,001). Što se tiče znanja, nije bilo razlike između ove dve grupe ispitanika (p=0,901). Stavovi studenata i medicinskih sestara prema osobama sa demencijom su pozitivni u ukupnom rezulatatu. Ovo istraživanje sugeriše da su stariji članovi tima sa stručnim područjem za demenciju ključna komponenta za razvoj stavova i poboljšanje prakse i ishoda sa tim pacijentima, kao i kontinuirano obrazovanje svih medicinskih sestara koje imaju kontakt sa ljudima koji pate od demencije.

Ključne reči: *stav, demencija, DAS skala, medicinske sestre, studenti Visoke zdravstvene škole strukovnih studija*

ABBREVIATIONS

WHO - World Health Organization
PWD - people with dementia
DAS - Dementia Attitudes Scale
ADRD - Alzheimer's disease and related dementia

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INRODUCTION

Dementia is a progressive decrease in cognitive functions and the term refers to different etiologies. Cognitive decline includes loss of memory and deterioration in executive functions, such as planning and organizing skills, sufficient to influence social activities. Alzheimer's disease is the most common type of dementia, accounting for 50–75% of all dementia cases (1-3). According to WHO recent documents, dementia affects more than 4% of people over 65 years, and the total number of people with dementia (PWD) is currently estimated at 35.6 million worldwide (4). Globally, every ninth person older than 65 years has been diagnosed with Alzheimer's disease, and this number increases with years of life such that every fourth person over 65 years suffers from some form of dementia (3, 5-7). Of the total number of PWD, 58% live in countries with medium and low gross national incomes, including the Republic of Serbia. According to the 2011 census, there are 7.2 million people in Serbia, of whom 17.4% are older than 65 (8). There is no registry of patients with dementia in Serbia. According to the estimates of the Alzheimer's Association, approximately 13% of people older than 65 are suffering from Alzheimer's disease (9). It is estimated that only 4% of the diagnosed patients receive adequate therapy (9). The National Guide for Alzheimer's Disease from 2013 emphasizes the importance of early recognition, diagnosis, treatment and prevention of this disease, precisely because of the increase in the number of people aged 65 years and older according to the previous census, with age as the most significant known risk factor for the development of dementia (5).

The high global prevalence, economic impact of dementia on families, caregivers and the community, as well as the related stigma and social exclusion, represent a significant public health challenge (3, 4, 6, 7).

The attitude towards the elderly varies depending on their health; therefore, some old people can be seen in a negative context (10). Many authors consider that additional education of the population, especially future health workers, is needed to address this specific problem (1, 11, 12). Early recognition and improved access to PWD has far-reaching consequences on their acceptance and the organization of adequate health care (1, 10).

Attitudes represent positive or negative evaluations of people, ideas or objects. There are three components of attitudes: there is an emotional component (emotional reactions), a cognitive component (knowledge and belief) and the component of behaviour (it consists of procedures, visible behaviour with intent and motivation with action). Attitudes towards dementia contain three basic components: knowledge, behaviour and an emotional component (1).

In the Republic of Serbia, to date, attitudes towards patients with dementia have not been examined. According to previous international research, it is particularly important to examine the attitudes of young people who are preparing for a future profession as a health worker, as well as the attitudes of already educated healthcare workers towards people suffering from dementia. From the obtained results, a new strategy could be developed with the aim of better treatment of the sick and empowering families who are nurturing PWD.

The main aim of this study was to examine and compare the attitudes (knowledge, emotional and behaviour) of students from the High Medical School of Professional Studies and nurses towards people suffering from dementia.

MATERIALS AND METHODS

This study was designed as a qualitative study with the use of a questionnaire. The study sample included 283 subjects from two research groups. The first group consisted of students from the High Medical College of Professional Studies in the 2016/17 academic year, and the other group consisted of employed nurses who worked in different departments at the same hospital.

The study was conducted enrolling the first- and third-year students at the High Medical College of Professional Studies in Belgrade and enrolling nurses who worked at the Departments of Surgery, Neurology, Haematology and Geriatrics during March 2017. Participation in the study was voluntary and anonymity was assured.

Instrument

The questionnaire on socio-demographic status provided data on sex, age, education and length of employment. Attitudes towards dementia were examined with the Dementia Attitudes Scale (DAS) (11). This scale, which assessed attitudes towards patients with Alzheimer's disease and related dementia, contains 20 questions. Of the 20 items from the whole questionnaire, fourteen items in the scale had a positive polarity, and six items (2, 6, 8, 9, 16 and 17) had negative polarities, and they were transferred to the positive polarities prior to analysis. The Likert scale ranged from 1 (completely disagree) to 7 (completely agree). When administering the DAS, the lowest score was 20, the neutral score was 80, and the highest score was 140. The items concerned: knowledge of dementia-10 items, emotions against dementia-6 items and behaviour towards the dementia patients - 4 items.

Statistical analysis

Statistical data analysis was performed with IBM SPSS 20 software. Continuous variables are presented as the mean ± standard deviation, and the categorical variables are presented as the percentage of the frequency of each category. To test the differences in the mean values of continuous variables, the Student's t test or the Mann-Whitney U test and Kruskal-Wallis test were used, depending on whether there was a normal distribution or not, which was tested with the Kolmogorov-Smirnov test. To check the significance of the differences for the categorical variables, a chi-square test was used, and the Yates's continuity correction was used for a 2 x 2 contingency table. All analyses were evaluated to the level of significance $p<0.05$. The results were tabulated after processing. For the DAS scale, Kronbach's alpha coefficient was calculated, which assessed the internal consistency.

RESULTS AND DISCUSSION

A total of 283 respondents answered the survey: 159 (56.25%) were students at the High Medical College of Professional Studies, and 124 (43.75%) were qualified nurses. The average age in the group of students was 21.28 ± 2.71, while the average age in the group of nurses was 40.13 ± 9.99. A significant difference was found between the first- and third-year students regarding age ($p<0.001$), while there was no significant difference between nurses versus age relative to the type of department where they worked ($p=0.059$) (Table 1). Most students were female (93.7%), and 92.7% of the nurses were female.

Table 1. Socio-demographic characteristics of respondents

		Age (M±SD)	p value	Gender; n (%)		p value
				Male	Female	
Students	1st year	20.11±2.25	p<0.001*	4	72	p=0.839**
	3rd year	22.39±2.65		6	76	
	Total	21.28±2.71		10 (6.3)	148 (93.7)	
Nurses	Geriatrics	43.09±9.41	p=0.059#	3	40	p=0.539**
	Haematology	39.92±9.55		2	12	
	Surgery	37.15±10.50		2	47	
	Neurology	40.78±8.79		2	16	
	Total	40.13±9.99		9 (7.3)	115 (92.7)	

*- Mann-Whitney U test; #- Kruskal-Wallis test;
**- Chi-squared test

The internal consistency for DAS results was found to be good with a Cronbach's α of 0.792.

Our study showed that in 14 of 20 DAS items, a significant difference was found in the distribution of responses between these two groups of respondents.

The mean range for students was 3.34-6.10, and in nurses, it was 3.24-5.58. A statistically significant difference between attitudes of students and nurses was found for the following questions: 1, 2, 4, 5, 6, 7, 8, 10, 12, 13, 14, 15, 16, 19, and 20 (Table 2).

Table 2. DAS descriptive statistics for nurses and students

Questions from 1-20		Nurses		Students		p value*
		Mean	Std. Deviation	Mean	Std. Deviation	
1.	It is rewarding to work with people who have ADRD*	3.77	1.79	4.33	1.54	.003
2.	I am afraid of people with ADRD	5.58	1.45	6.10	1.21	.000
3.	People with ADRD can be creative	4.83	1.60	5.20	1.31	.087
4.	I feel confident around people with ADRD	3.24	1.71	3.51	1.34	.041
5.	I am comfortable touching people with ADRD	4.80	2.01	5.21	2.08	.023
6.	I feel uncomfortable being around people with ADRD	5.25	1.68	5.91	1.39	.000
7.	Every person with ADRD has different needs	5.43	1.42	5.99	1.30	.000
8.	I am not very familiar with ADRD	4.85	1.73	3.94	1.63	.000
9.	I would avoid an agitated person with ADRD	4.51	1.90	5.01	1.28	.081
10.	People with ADRD like having familiar things nearby	5.55	1.43	5.21	1.39	.016
11.	It is important to know the past history of people with ADRD	5.16	1.57	5.26	1.33	.913
12.	It is possible to enjoy interacting with people with ADRD	4.44	1.60	5.25	1.25	.000

Questions from 1-20		Nurses		Students		p value*
		Mean	Std. Deviation	Mean	Std. Deviation	
13.	I feel relaxed around people with ADRD	3.90	1.70	4.35	1.10	.006
14.	People with ADRD can enjoy life	4.70	1.54	5.23	1.38	.002
15.	People with ADRD can feel when others are kind to them	5.42	1.42	5.84	1.19	.004
16.	I feel frustrated because I do not know how to help people with ADRD	4.40	1.72	4.33	1.41	.596
17.	I cannot imagine taking care of someone with ADRD	5.01	1.67	5.31	1.42	.173
18.	I admire the coping skills of people with ADRD	4.76	1.61	5.08	1.30	.169
19.	We can do a lot now to improve the lives of people with ADRD	5.55	1.35	6.06	0.99	.001
20.	Difficult behaviours may be a form of communication for people with ADRD	4.37	1.63	3.34	1.38	.000

*Mann-Whitney U test
*ADRD - Alzheimer's disease and related dementia

Table 3 presents the distribution of the total score for responses on attitudes of students (100.47±10.91) and nurses (95.51±16.10) towards patients with dementia.

The students had a better score regarding questions describing their behaviour towards these individuals ($p<0.001$) and emotions for these patients ($p<0.001$).

Table 3. Distribution of the total score for responses on attitudes towards patients with dementia

Score (Number of question)	Nurses	Students	p value*
Behaviour (1, 5, 9, 12)	17.52±4.45	19.81±3.72	p<0.001
Emotion (2, 4, 6, 13, 16, 18)	27.13±5.23	29.27±3.84	p<0.001
Knowledge (3, 7, 8, 10, 11, 14, 15, 17, 19, 20)	50.86±9.00	51.39±6.03	p=0.901
Total	95.51±16.10	100.47±10.91	p=0.004

*- Mann-Whitney U test

DISCUSSION

Many studies have been conducted to examine the attitudes towards dementia due to the great obstacle that dementia brings for people who work or live with such patients (12, 15-20). These studies have identified significant factors and divided them into: emotional factors, behavioural factors and factors related to knowledge of dementia (11-17). Our study is the first with this theme in our country.

Nurses in our study indicated that they have knowledge about dementia patients that may produce an optimal relationship with these patients. Conversely, students had a more positive attitude than nurses when working with dementia patients.

In our country, a higher level of knowledge for nurses was related to a positive attitude towards dementia, which is in keeping with the results of a study conducted in Korea in 2015 (17). In this Korean study, those who had more knowledge of dementia had positive attitudes (attitudes of students in the health-related department towards major dementia vs. a non-health-related department; 19.10±5.30 vs. 21.41±6.55, respectively). Additionally, the results obtained for students in our research were independent of the knowledge they possessed, while the research conducted at the University of Malta (16) found students who underwent training to work with dementia patients had a more positive attitude than those who did not (DAS score: 105.56±13.46; 101.32±13.10; p=0.009; Dementia training, Yes, No, respectively). Similar results were found by Garrie, who showed that attitudes of students changed significantly after receiving an education about dementia (18), because intervention and increased knowledge about dementia

significantly increased the average score on this statement (3.64±1.36 *vs*. 4.72±2.15; preintervention *vs*. postintervention, respectively). However, in our study, a less positive attitude was found in the group of nurses. This result was probably due to the extremely poor conditions of work. In a survey conducted in Colombia, examination of the mental health of medical staff involved in the care of people suffering from dementia showed that they have only a slightly higher level of depression compared to the control group but not a higher level of stress and health problems (21).

In students, attitudes can indicate a lack of experience in working with these patients; hence, the largest percentage of students in our research had a neutral attitude. Providing adequate nursing care for patients with dementia is difficult and requires special skills, attitudes and knowledge (15). Additional support for people who work with dementia patients can increase their mental strength, and may also reduce anxiety and stress (13).

Nurses in our study showed a lack of support for patients with dementia and had less positive attitudes than students. It is necessary to use some of the experimental learning models, as already shown in the USA and Europe, to help nurses during their work to have additional methods, experiences and opportunities to take care of these patients, which would produce more positive attitudes (18, 19, 22).

When asked if it was rewarding to work with people with ADRD, nurses had an average score (3.77 SD±1.789) that was lower than the average score for students (4.33 SD ± 1.545) in our research. Nurses, although having more contact with dementia sufferers, do not experience this job as rewarding. We can suggest reasons, such as being overburdened with work, dissatisfaction, and insufficient support, while the neutral attitude of students can be the result of lack of knowledge or contact with people suffering from dementia. These reasons were not supported in previous studies and are different from the results of the research study in North Korea and Malta (16, 17). In a survey conducted in Croatia in which the attitudes of medical and non-medical staff were examined, it was found that those who had less knowledge had a less positive attitude and vice versa (12).

Because nurses are the primary caregivers of these patients, their attitudes and professional experience can affect the quality of care they provide (23). Problems encountered by nurses with long term nursing of patients with dementia could be overcome by improving their communication skills, which need to be constantly renewed and updated throughout their entire career. Work organization in hospital nursing should be individual patient-oriented, rather than according to work assignments, since every patient with Alzheimer's disease is unique(24).

The aim of the National Dementia Strategy is to improve the quality of care for people living with dementia in general hospitals through leadership that addresses quality improvements in dementia care, defined care pathways and the use of liaison mental health teams. Additionally, the importance of education and training is to reduce the stigma associated with dementia and to raise awareness in healthcare workers (25).

The overall score of the DAS scale between students of the High Medical College of Professional Studies and nurses showed a significant difference, from 100.47±10.91 to 95.51±16.10 (p=0.004). Similar results were found in research (11) in which the total score was 98,64±12,82. The students had a more positive attitude towards the behavioural and emotional components towards dementia patients compared to nurses. When subcategories were evaluated, students had a better score on questions describing their behaviour towards these individuals (p<0.001), as well as emotional questions, or questions on empathy for these patients (p<0.001). Regarding knowledge, there was no difference between these two groups (p=0.901). The attitude of nurses differed from the positive or neutral attitudes that were published from research on the attitudes of nurses from nursing homes in central Sweden (15). The Croatian study showed a clear difference between respondents with medical and non-medical knowledge in favour of medical staff who had significantly better knowledge, i.e., 4.25 versus 3.78 on DAS score (13). It also showed that the overall score improved in older students compared to the youngest students in the first year due to the acquired knowledge during the studies. In a study conducted with nursing students in Malta, their attitudes were compared with American psychology students, and it was concluded that clinical experience in working with people with dementia produced a more positive attitude (17). In another study in India, pre-med students had a lower total score (95.0±1.47) than students in our study (26). A study on non-medical staff in England showed that younger people have a more positive attitude towards dementia sufferers than those who had experience with dementia. Thus, our research supported younger people having a more positive attitude, but it disagreed with the fact that those with experience with the illness have less positive attitudes towards the patients (27).

It is necessary to build more positive attitudes with medical workers to provide adequate patient care with dementia (19, 23). By removing barriers that make it difficult for staff to provide care for PWD with organized support from experts in the respective specialties, attitudes of nurses can be improved. The students from the High Medical College of Professional studies are able to provide more experience and time with communication that focuses on people and not tasks, which provides better attitudes towards care and acceptance and emotion towards people with dementia.

CONCLUSIONS

The findings of this study indicated that efforts are required to maintain the positive attitudes of medical staff and future medical workers towards patients who have dementia. Nursing staff members need to maintain the dignity of the patient, to learn and recognize the needs of the patients and to act in the most professional way with a patient. Additionally, nurses need the support of society to achieve adequate care for patients and to preserve their dignity and the dignity of their profession.

This research suggested that such strategies as raising dementia awareness alone will not improve care or outcomes for patients with dementia. Instead, senior team members with dementia expertise are key components for developing attitudes and improving care practices and outcomes in these patients, as well as continuous education of all medical staff that have contact with people who suffer from dementia.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this paper.

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There was no relevant financial interest in this article.

ETHICAL APPROVAL

The Dementia Attitudes Scale (DAS) used in our study was approved for use by Melissa O'Connor, a foreign author, using a double-translation method. The study was carried out after obtaining a decision from the Ethics Committee of the High Medical College of Professional studies (Decision number 140 of February 20, 2017) and the Clinical Hospital Centre (Decision number 1256/1 of February 27, 2017).

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MAGNESIUM IN IDIOPATHIC MITRAL VALVE PROLAPSE

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UPOTREBA MAGNEZIJUMA KOD IDIOPATSKOG PROLAPSA MITRALNOG ZALISKA

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ABSTRACT

The aim of our research was to increase the effectiveness of the therapy administered to the patients with idiopathic mitral valve prolapse by pharmacological correction of magnesium deficiency. 79 patients (23 females and 56 males with average years of age 35.7±4.3) with undifferentiated connective tissue dysplasia and mitral valve prolapse of the 1st and 2nd degree were examined. The control group consisted of 20 healthy individuals, comparable by sex and age. A test by the UNESCO Institute for Microelements was used for the preliminary diagnostics of magnesium deficiency. Daily ECG monitoring with heart rate variability analysis, echodopplercardiography with the assessment of left ventricular diastolic function and determination of magnesium concentration in blood serum were performed. For the demonstration of autonomic dysfunction "the test for detection of the signs of vegetative changes" was used (10). For the assessment of situational and personal anxiety an "anxiety test" by Ch. D. Spielberg and Y. L. Hanin (25, 26) was used. The succeeding study was performed after 6 months. It was found that complex therapy with magnesium orotate in patients with idiopathic mitral valve prolapse helps to reduce the frequency of clinical manifestations of neurovegetative disturbances in the majority of examined patients contributing to harmonization of the autonomic nervous system function. It has a favorable effect on dysplastic changes and the state of bioelectrical activity of the heart, as well as correction of the psychoemotional state.

Keywords: *valvular disease, magnesium deficiency, treatment*

SAŽETAK

Cilj našeg istraživanja bio je povećanje efikasnosti terapije koja se primenjuje kod pacijenata sa idiopatskim prolapsom mitralnog zaliska farmakološkom korekcijom nedostatka magnezijuma. Ispitivano je 79 pacijenata (23 žene i 56 muškaraca, prosečne starosne dobi 35,7 ± 4,3 godina) sa nediferenciranom displazijom vezivnog tkiva i prolapsom mitralnog zaliska I i II stepena. Kontrolnu grupu činilo je 20 zdravih pojedinaca, uporedivih po polu i starosti. Test UNESCO Instituta za mikroelemente korišćen je za preliminarnu dijagnostiku nedostatka magnezijuma. Vršeni su dnevni EKG monitoring sa analizom varijabilnosti srčanog ritma, ehokardiografija sa procenom dijastoličke funkcije leve komore i određivanje koncentracije magnezijuma u serumu. Za demonstraciju autonomne disfunkcije korišćen je „test za otkrivanje znakova vegetativnih promena" (10). Za procenu situacione i lične anksioznosti korišćen je Ch. D. Spilbergov i Y. L. Haninov „test anksioznosti" (25, 26). Naredna studija izvršena je nakon 6 meseci. Utvrđeno je da složena terapija magnezijum orotatom kod pacijenata sa idiopatskim prolapsom mitralnog zaliska pomaže da se smanji učestalost kliničkih manifestacija neurovegetativnih poremećaja kod većine pregledanih pacijenata koja doprinosi usklađivanju funkcije autonomnog nervnog sistema. Terapija povoljno utiče na displastične promene i stanje bioelektrične aktivnosti srca, kao i na psihoemocionalno stanje.

Ključne reči: *oboljenje valvule, manjak magnezijuma, lečenje*

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INTRODUCTION

Mitral valve prolapse (MVP) or mitral valve prolapse syndrome is considered to be one of the most common cardiac valve anomalies. The results of population-based studies on prevalence of MVP are inconsistent. In the Framingham Heart Study (1) the prevalence of MVP syndrome in the population of 26 to 84 years of age (average age 56.7±1.5) was 2.4% with no differences in sex and age. The maximum prevalence of this pathology (17-38%) was observed in women (twice as often as men) and young people (2). Moreover, severe mitral regurgitation was observed in males with MVP over 50 years old more often than in young women with this pathology. It was demonstrated that the frequency of MVP detection with young people (18-27 years old) starts from 4.3% to 8.1% and increases with athletes up to 11-18% (2, 3).

At present, there is no universal terminology and classification of MVP. It is generally accepted to classify MVP by etiology as primary (idiopathic, congenital) and secondary one (4). Primary MVP should be considered in the context of genetically determined mesenchymal anomaly and respectively within the nosological frame of undifferentiated connective tissue dysplasia (CDT). The conventionality of the term "primary" or "idiopathic" in connection to MVP should be mentioned. Pathogenetically, it is associated with a specific cause – a congenital generalized defect of connective tissue. In addition, mitral valves in differentiated hereditary syndromes and undifferentiated CDT, differing etiologically, are virtually identical in pathogenesis. Secondary MVP is found in ischemic heart disease, chronic rheumatic heart disease, myocarditis, hypertrophic cardiomyopathy, congenital heart disease, etc.

MVP may appear as a clinically mild "phenomenon of echocardiography", as clinically significant complication occur in 2-4% of cases, and almost in the absolute case (95-100%) in the presence of myxomatous degeneration of the valves, i.e. in MVP syndrome (5). Sudden death is a rare complication of MVP, occurring in less than 2% of patients with MVP during prolonged follow-up with an annual mortality of less than 1% (6). In most cases, sudden cardiac death in MVP is of arrhythmogenic genesis and is caused by the occurrence of idiopathic ventricular tachycardia (fibrillation) or in the QT prolonged interval syndrome (7). The risk factors for sudden cardiac death in patients with MVP are the presence of severe mitral regurgitation and LV (left ventricle) systolic dysfunction.

Particular importance in the development of CTD is the deficiency of magnesium, which leads to disruption of the formation of connective tissue structures of the supporting and trophic carcass of the heart. This causes chaotic distribution of collagen fibers, disturbance of collagen synthesis and its biodegradation. Thus, in conditions of magnesium deficiency, fibroblasts produce incomplete collagen of the mitral valve flaps (8). On the other hand, magnesium deficiency leads to an increase in the total activity of matrix metalloproteinases and more aggressive degradation of collagen fibers, which also worsens mechanical resistance of connective tissue (9). We should not forget that the disturbance of the structure and function of connective tissue in MVP affects not only the chordal and valvular apparatus of the valve, but also the connective tissue stroma of the myocardium. In some cases, this leads to the disruption of the synchronicity of contractions of both separate muscle fiber groups and the whole myocardium, and possibly it leads to a decrease in its inotropic reserve, remodeling and, as a result, to the manifestation of heart failure signs. The problems of the relevant treatment and methods of correction of idiopathic MVP remain poorly studied. In connection to this, our goal was to increase the effectiveness of the therapy for the patients with idiopathic prolapse of the mitral valve via pharmacological correction of magnesium deficiency.

MATERIALS AND METHODS

The study involved 79 patients of 18-40 years of age with MVP and phenotypic signs of undifferentiated CTD (Table 1). According to echodopplercardiography, MVP of the 1st degree was diagnosed in 46 patients and MVP of the 2nd degree in 33 patients. Mitral regurgitation was not found in 12 patients. It should be mentioned that 37 patients had mitral regurgitation of the 1st degree and 30 patients had mitral regurgitation of the 2nd degree.

The initial examination was made by applying a specially prepared test including a detailed collection of complaints, anamnesis and physical examination for phenotypic signs of undifferentiated CTD.

Inclusion criteria: males and females ≥18 years old; presence of idiopathic mitral valve prolapse; informed patient consent to participate in the study.

Table 1. Clinical characteristics of the examined patients

Index	General group (n=79)
Average age, years	35.7±4.3
Males/Females	56/23
MVP I degree/II degree	46/33
Mitral regurgitation	
I degree/II degree	37/30
Smoking, n (%)	34
Family history of early cardiovascular events, n (%)	21
BMI (body mass index), kg/m²	24.3±1.6
Height, cm	171.5±1.8

Exclusion criteria: foci of chronic infection, congenital or acquired heart disease, degenerative-inflammatory myocardial lesions, hemodynamic disturbances, thyroid gland pathology, coronary heart disease, arterial hypertension, concomitant diseases of internal organs, differentiated forms of

CTD (Marfan syndrome, etc.); patients younger than 18 years of age.

The control group consisted of 20 healthy individuals without CTD, comparable by sex and age (10 males and 10 females, average age 35.3±4.6) without morphofunctional features of the heart structure according to echocardiography study.

All examined patients underwent standard clinical, biochemical and instrumental studies. For the detection of autonomic dysfunction "the test for detection of the signs of vegetative changes" was used (10). The sum of the scores, if equal to or more than 15, suggested the presence of autonomic dysfunction.

Daily ECG monitoring was performed with the "CARDIOSENS" equipment ("XAI-Medica", Ukraine). Cardiac arrhythmias and conduction disorders, coronary insufficiency and heart rate variability (HRV) were assessed (11). The registration and automated processing of ECG signals were made by the calculation of the parameters of time and spectral analysis, as well as by the indexes obtained on their basis and suggested by P.M. Baevsky (12). For the analysis of vegetative regulation, the following parameters were used:

TI is the tension index of regulatory systems (TI=AMo/2×BP×Mo), where Mo (mode) is the most frequent value of RR, AMo (mode amplitude) is the number of cardio intervals corresponding to the mode range (in %); VR (variation range) is the difference between the maximum and minimum values of RR;

SDNN is a standard deviation in the duration of normal intervals R-R; pNN 50 is the percentage of all analyzed cardio intervals; RMSSD is the square root of mean squares of the difference between adjacent RR-intervals – activity index of parasympathetic link of vegetative regulation. The higher is the value of RMSSD, the more active is the link of parasympathetic regulation (12).

LF/HF is the index of the vagosympathetic interaction, – the ratio of high-frequency and low-frequency components of the heart rhythm. It indicates the change of vegetative balance to the sympathetic or parasympathetic division.

Structural and functional parameters of the left ventricle were assessed using echodopplercardiography (EchoCG) with the ultrasound scanner "Vivid 3" (Japan) and a 3.5MHz probe in prone position on the left side from the parasternal and apical four-chamber views.

The following indices of EchoCG were assessed: aortic diameter, aortic, mitral (MV), tricuspid valve opening amplitude, the hole area of all these valves. Morphometry and evaluation of mitral valve function were performed in M-mode in the standard position II; in the mode of two-dimensional echocardiography – in the parasternal projection of the LV long axis and the LV transverse axis at the level of the mitral valve; and in the apical four-chamber position. EchoCG showed that the sign of mitral valve prolapse was the displacement of the valve (s) to the left atrial cavity by more than 3mm. The systolic deflection of one or both valves of the MV (mitral valve) in the LA (left atrium) in the parasternal longitudinal position by 3.0-5.9 mm is defined as I degree MVP, by 6-8.9 mm – II degree MVP and by more than 9 mm – III degree. Normal values of the MV anterior cusp length were taken as 21-24 mm and of the posterior one as 12-14 mm.

The degree of the severity of myxomatous degeneration was assessed on the ground of the thickness of the MV leaflet during the diastole phase in the middle part outside the chord zone, creating a false impression of its thickening. Common standards for leaflet thickness are 2-4 mm; an increase by more than 5 mm indicates a pathological change (myxomatosis, etc.) (4); by less than 5 mm – non-classical MVP and by 5 mm or more – classical MVP.

The morphology of the valve apparatus, as well as the presence and extent of regurgitation were evaluated. During the assessment of the degree of regurgitation on the mitral valve, the LA depth, the area of mitral regurgitation, the percentage ratio of the jet area, and the LA area were taken into account (13).

LV dimensions and volumes, LV stroke volume and ejection fraction, the thickness of the LV posterior wall and the interventricular septum were also measured during EchoCG examination. Disturbances in the LV local contractility were outlined by the recommendations of the American Society of Echocardiography. The type of LV architectonics was determined by the following parameters: myocardial mass, myocardial index, relative wall thickness, and sphericity index. It was mandatory to determine the size of the LA, RV (right ventricle) and RA (right atrium) cavities, pericardial condition and pressure in the pulmonary artery. The character and flow rate on the valves in systole and diastole pressure gradient were determined with Doppler echocardiography. The diastolic function was assessed by transmittal flow in the pulse-wave Doppler mode, as well as by the analysis of the motion of the fibrous ring of the mitral valve by the method of tissue Doppler imaging. In the Doppler study, LV diastolic function was evaluated according to the time of isovolume relaxation, the deceleration time for the early LV diastolic filling (DT), the maximum rate of LV early filling (peak E), the maximum rate of the atrial systole A and the E/A ratio.

For the assessment of situational and personal anxiety, an "anxiety test" by Ch. D. Spielberg (1973), adapted by Y. L. Hanin (containing 40 questions), was used. The result was assessed as follows: up to 30 points – low anxiety, 31-45 points – moderate anxiety, 46 points and more – high anxiety.

For the preliminary diagnostics of magnesium deficiency, a test by Trace Element Institute for UNESCO was used. The test results were read as follows: 0-9 points – no magnesium deficiency, 10-19 points – risk group for magnesium

deficiency, 20-29 points – moderate magnesium deficiency, 30-39 points – magnesium deficiency, 40-56 points – significant magnesium deficiency (14). The concentration of magnesium in blood serum was evaluated with the automatic biochemical analyzer "Humalyzer 2000" (Germany, the range of normal oscillations is 0.85-1.2mmol/l).

After initial screening, the patients were randomly divided into 2 groups: 39 patients (group 1) received complex therapy including β-adrenoblocker and magnesium orotate 500 mg 3 times a day for 6 months. The second group included 40 people who received monotherapy with a β-blocker. Its administration was preconditioned by the presence of clinical signs of an increase of the sympathetic nervous system tone (cardialgia, palpitations, irregular heart function, dyspnea, etc.) in all examined patients. These groups of patients with MVP were comparable by age, sex and the presence of magnesium exchange disorders. The follow-up study was performed after 6 months of observation.

The effectiveness of the therapy in each patient was assessed as clinically significant with a decrease of the severity (in points) of the analyzed parameters by 50% or more from the baseline.

Statistical processing of the results was made with the program Statistica 6.0. For the quantitative indices measured on an interval scale the mean value, standard deviation and mean error were calculated. For "qualitative" and "ordinal" indices, the index detection frequency in percents and the frequency of registration of different index rank score respectively were defined. The Student's t-test was used in the analysis of the inter-group index differences. In case of the indices measured at the nominal scale, the reliability of the differences in the frequency of index detection in two compared groups was assessed by the Student's t-test and the Fisher transform, linear correlation coefficients and rank correlations were calculated. The reliability of the relationship between the indices measured on a nominal or rank scale was further evaluated using contingency tables – with the calculation of several modifications of the Pearson chi-square test and Cramer's conjugacy coefficients. Differences in mean values and correlations were considered reliable at a significance value of $p<0.05$.

RESULTS AND DISCUSSION

Undifferentiated CTD is characterized by the polymorphism of anomalies of disembriogenesis ("stigma"), which are represented in a phenotype with different frequencies. During the analysis of external phenotypic features of the patients with MVP the most informative were CTD markers, shown in Table 2.

It has been previously observed that different clinical symptoms in MVP patients also depend on magnesium deficiency (3, 14). The study of some aspects of magnesium exchange and its influence on the dynamics of the MVP course has been of great importance. In the analysis of clinical signs of magnesium deficiency it was observed that in the patients of groups I and II, moderate deficiency of magnesium was diagnosed in 74.4% and 70%, the risk of magnesium deficiency development was recorded in 15.4% and 20%, and the signs of magnesium deficiency were absent in 10.2 % and 10%, respectively. Consequently, the majority of patients with MVP of 1st and 2nd degree exhibited clinical signs of magnesium deficiency of varying severity . Differences were statistically significant comparing to the control group – $p<0.01$. During the evaluation of magnesium concentration in blood serum, hypomagnesemia was diagnosed in 82% (32 patients) of group I and 80% (32 patients) of group II. Consequently, the values of serum magnesium were within normal limits in 18% (7 patients) in group I and in 20% (8 patients) in group II.

Table 2. Prevalence of external phenotypic markers in patients with idiopathic MVP (n=79)

Phenotypic markers	Detection frequency,%
Ectomorphy	67.0
Hypotrophy	54.4
Radial-lacunar type of the iris	54.4
The predominance of the length of the 4th hand digit over the length of the 2nd one	50.6
Varicose veins of the lower extremities, developed in adolescence	46.8
Scoliosis	41.8
Chest deformation	39.2
The predominance of the length of the 2nd toe above the length of the 1st one	32.9
Curved little fingers	29.1
Platypodia	29.1
Protruding ears	25.3

The concentration of magnesium in blood serum is the most commonly used marker of magnesium exchange in the body (15). However, the level of magnesium in the serum provides only proximate information about the presence or absence of magnesium deficiency. Hypomagnesemia clearly indicates magnesium deficiency, but its absence does not exclude significant magnesium deficiency in tissues. The concentration of magnesium in the blood serum is not associated with the content of this trace element in other biomaterials (16).

It is expected that in the pathogenesis of diverse clinical symptoms and signs in patients with primary MVP, a leading role is played by the disturbances in the function of the autonomic nervous system with an increase of the sympathetic tone. The predominance of adrenergic effects in MVP is associated both with an increase in the sensitivity of adrenoreceptors to stimulation and with an increase of their total number (17). Changes in vegetative homeostasis are so common in patients with primary MVP that most researchers consider

it an obligate manifestation of this pathology (2, 3). The manifestations of autonomic dysfunction were observed in 75 (94.9%) patients (Table 3).

Table 3. Prevalence of manifestations of autonomic dysfunction in patients with idiopathic MVP (n=79)

Symptoms	Detection frequency,%
Heartache	94.9
Heart palpitations and disturbances	73.4
Headache	74.7
Dizziness	73.4
Hyperventilation syndrome	63.3
Dysfunction of the gastrointestinal tract	45.6
Raynaud's syndrome	37.9
Disturbances of thermoregulation	27.8
Syncope	15.2

At baseline, the examined patients with MVP showed average score on the "test for the signs of vegetative changes" of 45.9±2.1 points whereas the scores of healthy individuals reached 12.3±2.3 ($p<0.001$) points. The study of vegetative homeostasis during the analysis of HRV (heart rate variability) showed the prevalence of simpathicotonia in 70.9% of the examined patients. The data obtained confirm the significant contribution of the autonomic nervous system disorders to the structure of the main clinical manifestations of idiopathic MVP.

The autonomic nervous system is currently being considered to play an important role in the emergence of various heart rhythm disorders (2). It is known that the parasympathetic link inhibits negative adrenergic effects on the heart (18). Decreased vagal activity and/or increased sympathetic activity may lead to the development of prognostically unfavorable cardiac rhythm disorders (19).

Analysis of HRV parameters at the baseline allowed us to diagnose the presence of vegetative disorders in the examined patients with MVP.

At baseline, in patients with MVP, the mode amplitude twice exceeded the results of healthy individuals, the stress index –was increased 3.5 times (all $p<0.001$), and the variation range was reduced by 1.4 times ($p<0.05$) indicating the prevalence of sympathetic activity in the autonomic nervous system. In addition, compared with the control group, a significant decrease in the total heart rhythm variability (SDNN) by 1.3 times and a decrease of the parasympathetic component of cardiac rhythm regulation (RMSSD) by 1.3 times (all $p<0.001$) were observed in patients with MVP. The predominance of sympathetic influences over vagal ones in patients with idiopathic MVP probably indicates an initially high level of adrenergic stimuli in this pathology.

One of the common symptoms of CTD is arrhythmic syndrome. Pathogenetic factors of cardiac arrhythmias are myxomatous degeneration of the conduction system of the heart and valves, especially the posterior one, as well as mitral regurgitation. In the genesis of supraventricular arrhythmias, a special emphasis is placed on the stimulation of the subendocardial areas of the left atrium with regurgitating blood stream, leading to the development of the foci of ectopic excitation. Atrial fibrillation usually develops in patients with atriomegaly caused by hemodynamically significant mitral regurgitation. Among the causes of ventricular rhythm disturbances, hypersympathicotonia, i.e. an abnormal traction of papillary muscles, (20) is taken into consideration.

The existence of a causal relationship between ventricular and atrial arrhythmias and intracellular magnesium content has been established (14). It is expected that hypomagnesemia may contribute to the development of hypokalemia (21). In this case, the membrane resting potential is increased, the processes of depolarization and repolarization are interrupted and the cell excitability decreases. The conductivity of electric impulse slows down contributing to the development of arrhythmias (14, 21). In addition, intracellular magnesium deficiency increases the activity of the sinus node, reduces absolute refractoriness and extends a relative one [22].

Detection frequency of various types of cardiac rhythm and conduction disturbances in the examined patients according to daily ECG monitoring is presented in Table 4.

The inclusion of magnesium orotate in complex therapy for 6 months in patients with idiopathic MVP of the 1st and 2nd degree led to a significant increase of magnesium content in blood serum from 0.61±0.02mmol/l to 0.97±0.03mmol/l ($p<0.001$). Moreover, magnesium concentration in blood serum in patients of group I did not differ significantly from the control group, which apparently indicates the compensation of magnesium deficiency in the studied patient population. At the same time, in the patients of group II no significant changes in magnesium content of blood serum were observed after therapy.

After the treatment, there was a significant ($p<0.05$) decrease in the frequency of clinical manifestations of neurovegetative disorders in the majority of the patients examined. Assessing the effect of magnesium therapy on symptomatology and the severity of all clinical manifestations in patients with MVP, it is necessary to emphasize the significant improvement of the general state of patients and reduction in the frequency and severity of all clinical syndromes and symptoms of the disease.

Table 4. Changes in the findings of daily ECG monitoring on treatment

Indicators	Group II (n=39)		Group II (n=40)	
	Initially	After treatment	Initially	After treatment
Heart rate, bpm	83.6±3.6	68.8±2.3**	82.7±3.2	73.2 ± 2.3*
Supraventricular arrythmia	32.8±10.7	8.9±4.9*	34.6±10.3	27.3±4.6
Ventricular arrhythmia	198±13.8	26±11.6**	179±11.3	44±10.5**
Paroxysmal supraventricular tachycardia,%	7.7	0	12.5	7.5
Blockade of the right leg of the bundle of His,%	30.7	30.7	30.0	30.0
Syndrome of early repolarization of ventricles,%	33.3	0*	35	35

* – statistical significance in comparison to the original data, $p<0.05$; ** – $p<0.001$.

At the same time, the most significant was the dynamics of asthenic complaints ($p<0.05$) of cardialgia, palpitations, cardiac disruptions, headaches, dizziness; tolerability of moderate physical activity ($p<0.05$) in comparison with the patients of group II also improved. Clinically significant decrease in the severity of vegetative dystonia syndrome was observed in 69.2% of patients during the course of magnesium orotate and in 47.5% in the comparison group ($p<0.05$).

Analysis of the effectiveness of drug therapy in the patients with autonomic dysfunction showed positive dynamics of clinical status. The data on A.M. Wayne (10) self-evaluation scale of general state (16) showed that the sum of scores in group I decreased from 45.9±2.4 to 16.8±2.1 points ($p<0.001$) and from 45.8±2.2 to 29.8±2.1 ($p<0.001$) points in group II. The reduction of the total score by 50% after the treatment was considered a positive result. The sum of scores decreased in group I by 63.4% and in group II by 34.9% (all $p<0.001$) indicating a significant decrease of vegetative signs during the administration of complex therapy with magnesium orotate.

After the treatment the patients of both groups showed a decrease in the rate of sympathetic activity. Thus, the stress index in group I decreased at 67.7% ($p<0.001$) and in group II – at 47.3% ($p<0.001$); the mode amplitude – by 33.6% ($p<0.001$) and 16.5% ($p<0.01$); the variation range increased by 64.3% ($p<0.01$) and 38.4% ($p<0.01$), respectively, indicating an improvement in vegetative tone in the patients of group II and reactivation of vegetative balance in group I. In patients with MVP, who additionally received complex therapy with magnesium orotate, a significantly better result was observed in comparison with the experimental group on the stress index (by 62.7%, $p<0.001$) and the variation range (by 21.6%, $p<0.05$). Thus, in the group which additionally received magnesium orotate, harmonization of the function of the autonomic nervous system was observed.

General heart rate variability (SDNN) and parasympathetic regulation of the cardiovascular system (RMSSD) increased simultaneously. In particular, the SDNN index, representing the overall effect of the autonomic regulation of blood circulation, increased in the patients of group I by 27.3% ($p<0.01$), and in group II – by 8.8% ($p>0.05$).. The RMSSD index indicating the activity of the parasympathetic link of vegetative regulation in group I increased by 27.7% ($p <0.01$) and in group II by 8.47% ($p>0.05$).. pNN 50 index showing the degree of the prevalence of the parasympathetic link in group I increased by 27.1% ($p<0.01$) and in group II – by 11.7% ($p>0.05$) (Table 4). Thus, in the patients with arterial hypertension and autonomic dysfunction, the combined therapy with magnesium orotate both led to a more significant increase in general rhythm variability and decreased activity of the sympathetic part of the autonomic nervous system and reactivation of the vegetative balance.

The indices of the structural-functional state of the left ventricle are presented in Table 5.

The analysis of the parameters of intracardiac hemodynamics (Echo-CG findings) showed that in patients of group I there was a significant decrease in the size of the left atrium in comparison with the initial data by 6.6% (all $p<0.05$). In the patients of both groups there was a slight increase in the end-systolic and end-diastolic size of the left ventricle,which was marginally more significant in the comparison group. That corresponded to a slight increase in the stroke volume and ejection fraction of the left ventricle in group I patients, whereas in contrast in group II patients the stroke volume did not change and the left ventricular ejection fraction even slightly decreased but stayed within the normal range. The data obtained reflect the favorable effect of magnesium orotate on dysplastic changes, which conforms to the previously obtained results (23).

Table 5. Dynamics of the structural-geometric condition of LV in the examined patients during treatment

Index	Group I (n=39)		Group II (n=40)	
	initially	6 months	initially	6 months
LA (left atrial diameter), cm	3.51±0.07	3.28±0.05*	3.52±0.06	3.46±0.07
ESD (end-systolic dimension), cm	3.34±0.06	3.35±0.03	3.36±0.04	3.37±0.03
EDD (end-diastolic dimension), cm	4.72±0.05	4.74±0.03	4.74±0.06	4.76±0.04
ESV (end-systolic volume), ml	40.3±1.2	41.3±1.3	40.7±1.3	42.5±1.3
EDV (end-diastolic volume), ml	112.0±1.5	113.5±1.7	112.4±1.6	113.9±1.6
SV (stroke volume), ml	71.9±1.4	73.5±1.2	71.7±1.2	71.6±1.3
EF (ejection fraction), %	63.3±0.7	64.7±0.6	63.5±0.8	62.6±0.6
E/A (peak early filling (E-wave) and late diastolic filling (A-wave) velocities)	0.96±0.06	1.22±0.03*	0.97±0.06	1.08±0.04
Degree of mitral regurgitation	1.08±0.13	0.62±0.11*	1.09±0.16	1.03±0.11

* – statistical significance compared with the initial data $p < 0.05$.

The analysis of the diastolic function characteristics showed that after the treatment,the rate of LV early diastolic filling (peak E) increased by 14.9% ($p<0.05$) in group I and by 7.8% ($p<0.05$) in group II. The maximum rate of atrial systole A after the end of the treatment in group I decreased by 9.6% ($p<0.05$) and by 7.1% ($p<0.05$) in the patients of group II. As a result of the observed changes in these velocity streams in group I patients, the E/A peaks significantly increased by 27.1% ($p<0.05$), indicating improvement in left ventricular relaxation and an increase of the blood volume accepted in the first phase of diastole. At the same time, the increase in the ratio of E/A by 11.5% ($p<0.05$) exceeded the results of the comparison group, where the changes of this index became a tendency. Additionally, a significant ($p<0.05$) decrease in the degree of mitral regurgitation was observed in group I patients (Table 6). In general, the data obtained are the results of the improvement of the connective tissue diffusivity and its architectonics, determining the improvement of its elasticity and extensibility.

After the treatment the patients of group I (Table 5) experienced a significant reduction in the heart rate, in the number of ventricular extrasystoles and supraventricular extrasystole. The antiarrhythmic activity of magnesium orotate is apparently preconditioned by its component magnesium, a natural calcium antagonist, which has a membrane-stabilizing effect, prevents a loss of potassium by the cell, reduces the dispersion of QT interval and also weakens the sympathetic effect on the heart (24).

At baseline the patients with MVP showed an increase in anxiety levels on the scale by Ch. D. Spielberg and Y. L. Hanin, (25, 26) which is explained by the peculiarities of patients' response to the emergence of the disease and associated psychological changes, as well as premorbid features of the patients' personalities. Thus, the degree of reactive and personal anxiety was respectively (49.2±2.3) and (48.8±2.6) in group I and (48.2±2.4) and (47.7±2.6) in group II. High and moderate levels of reactive and personal anxiety were distinct for the majority of patients with MVP (Table 6).

In the patients of group II with low, moderate and high levels of reactive and personal anxiety at baseline no significant changes were observed after the treatment. At the same time, the patients of group I showed a significant decrease in the level of reactive anxiety by 36.8% ($p<0.001$) and personal anxiety by 38.6% ($p<0.001$). Moreover, magnesium orotate proved to be the most effective in the group with high and medium anxiety level, as indicated by the shift of 64.1% and 69.2% in patients reaching a lowlevel of reactive and personal anxiety respectively. At the same time, in the patients with a low degree of anxiety no significant changes were observed at baseline. The difference in the change of the levels of situational and personal anxiety in groups I and II was found to be statistically significant (39.1%, $p<0.001$ and 46.6%, $p<0.001$ respectively). Consequently, after the complex treatment with magnesium orotate the patients with MVP showed positive change of situational and personal anxiety, which indicated an improvement in the psychoemotional state of the patients.

Table 6. Changes of indices of reactive and personal anxiety in patients with idiopathic MVP (M ± m)

Index	Level	Group	Initially	After treatment
Reactive anxiety	low	I	28.2±1.3 (n=2)	28.1±1.2 (n=25)
		II	28.4±1.3 (n=2)	28.5±1.4 (n=3)
	moderate	I	42.6±2.5 (n=16)	33.9±2.3* (n=11)
		II	40.8±2.6 (n=17)	37.2±2.4 (n=18)
	high	I	56.3±2.9 (n=21)	46.1±2.7* (n=3)
		II	56.1±2.5 (n=21)	51.4±2.3 (n=19)
Personal anxiety	low	I	28.5±1.5 (n=2)	28.1±1.4 (n=27)
		II	28.6±1.6 (n=2)	28.2±1.3 (n=3)
	moderate	I	42.8±2.6 (n=17)	33.6±2.4* (n=9)
		II	42.1±2.7 (n=19)	39.3±2.3 (n=21)
	high	I	55.9±2.8 (n=20)	45.6±2.6* (n=3)
		II	55.3±2.7 (n=19)	53.1±2.3 (n=16)

* – statistical significance in comparison with the original data, p <0.05.

CONCLUSION

1. The majority of patients with idiopathic MVP of 1st and 2nd degree have clinical signs of magnesium deficiency of different severity and hypomagnesemia associated with the disturbances of autonomic regulation of the cardiovascular system in the form of relative increase of sympathetic influences and weakening of parasympathetic ones. Patients with MVP showed a decrease in the level of psychological health, manifested as a growth of the number of people with high and moderate levels of reactive and personal anxiety.
2. Complex therapy with magnesium orotate for 6 months in the patients with idiopathic MVP of 1st and 2nd degree led to the improvement of clinical symptoms and signs, to a decrease in the severity of the vegetative dystonia syndrome, toa decrease in the degree of mitral regurgitation and inthe size of the left atrium, as well as to the improvement in the diastolic function of the left ventricle along with the replenishment of magnesium deficiency according to the content of this trace element in the blood serum.
3. Administration of the combined therapy with magnesium orotate in the patients with MVP and autonomic dysfunction led to an improvement in HRV parameters and state of bioelectrical activity of the heart, as well as to a decrease in the level of reactive anxiety, which enables an increase of functional capacities of the body based on the improvement of the psychoemotional state.

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THE IMPACT OF DIFFERENT ANTIEMETICS ON THE NAUSEA IN EARLY POSTOPERATIVE PERIOD AFTER LAPAROSCOPIC HOLECYSTECTOMY

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UTICAJ RAZLIČITIH ANTIEMETIKA NA NASTANAK MUČNINE U RANOM POSTOPERATIVNOM PERIODU NAKON LAPAROSKOPSKE HOLECISTEKTOMIJE

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ABSTRACT

Postoperative nausea and vomiting (PONV) is a patient-important outcome; patients often rate PONV as worse than postoperative pain. This clinical study was aimed to asses the efficiency of standard aniemetics administration separately or in combination in prevention of PONV in patients who underwent to the same surgical procedure - laparoscopic cholecystectomy. Also, this article could provide a novel information about the best choice for prevention and treatment of PONV. This study included 87 patients divided into four groups according to the postoperative phramacological treatment: First group was control group without treatment, Second group was group of patients with ondasetron treatment in postoperative period, Third group was group of patients with ondasetron+dexamethasone treatment, and fourth group was group of patients with dexamethasone treatment in postoperative period for nausea. PONV was distributed in Ondansetron+Dexamethasone group in the lowest percent (4.5%), which means that this conbination of antiemetics was very effective. Than, Dexamethasone group was in relation with low incidence of PONV (14.3%), and after that were Ondansetron and Control groups. Also smokers and males has lower incidence of PONV, especially in combination with Ondansetron+Dexamethasone treatment.

The incidence of PONV is lower in male smokers patients who were underwent to combination of two antiemetics, ondansetron and dexamethasone compared to monotherapy and female nonsmokers. Preventive strategies for PONV must include risk stratification followed by prophylactic approach and also testing the newer antiemetics. Because of the high incidence of postoperative nausea and vomiting as a patient-important outcome, the preventive strategies should be considered as serious condition which requires multimodal approach.

***Keywords**: Postoperative nausea and vomiting, antiemetics, Laparoscopic cholecystectomy.*

SAŽETAK

Postoperativna mučnina i povraćanje (PONV) je za pacijenta važan ishod; pacijenti često PONV ocenjuju gorim od postoperativnog bola. Ova klinička studija imala je za cilj da proceni efikasnost standardne primene anemetike odvojeno ili u kombinaciji u prevenciji PONV-a kod pacijenata koji su bili podvrgnuti istom hirurškom zahvatu - laparoskopskoj holecistektomiji. Takođe, ovaj članak pruža nove informacije o najboljem izboru za prevenciju i lečenje PONV-a. Ovo istraživanje je obuhvatilo 87 pacijenata podeljenih u četiri grupe prema postoperativnom framakološkom tretmanu: prva grupa je bila kontrolna grupa bez lečenja; druga grupa je bila grupa pacijenata sa tretmanom ondasetrona u postoperativnom periodu; treća grupa je bila grupa pacijenata sa lečenjem tadasetron + deksametazon, a četvrta grupa je bila grupa pacijenata sa lečenjem deksametazonom u postoperativnom periodu za mučninu. PONV je raspodeljen u grupi Ondansetron + Deksametazon u najnižem procentu (4,5%), što znači da je ova kombinacija antiemetika bila veoma efikasna. Zatim je grupa deksametazona bila u vezi sa niskom učestalošću PONV-a (14,3%), a posle toga su bile Ondansetron i kontrolne grupe. Takođe, pušači i muškarci imali su nižu učestalost PONV-a, posebno u kombinaciji sa lečenjem Ondansetron + deksametazonom.

Učestalost PONV-a manja je kod pacijenata pušačima koji su bili podvrgnuti kombinaciji dva antiemetika, ondansetrona i deksametazona, u poređenju sa monoterapijom i ženama koje su nepušači. Preventivne strategije za PONV moraju uključivati stratifikaciju rizika praćenu profilaktičkim pristupom, a takođe i testiranje novijih antiemetika. Zbog velike učestalosti postoperativne mučnine i povraćanja, kao važnog ishoda za pacijenta, preventivne strategije treba shvatiti kao ozbiljno stanje koje zahteva multimodalni pristup.

***Ključne reči:** Postoperativna mučnina i povraćanje, antiemetičari, laparoskopska holecistektomija.*

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INTRODUCTION

Postoperative nausea and vomiting (PONV) is a patient-important outcome; patients often rate PONV as worse than postoperative pain (1-3). PONV usually resolves or is treated without sequelae but may require unanticipated hospital admission and delay recovery room discharge (2, 3). In addition, vomiting or retching can result in wound dehiscence, esophageal rupture, aspiration, dehydration, increased intracranial pressure, and pneumothorax (4, 5).

The term PONV is typically used to describe nausea and/or vomiting or retching in the post-anesthesia care unit (PACU) or in the immediate 24 postoperative hours. Post-discharge nausea and vomiting (PDNV) refers to symptoms that occur after discharge for outpatient procedures (5-7).

The incidence of PONV varies with patient factors, anesthetic choices, and possibly the type of surgery (8-11). Patients factors which could induce PONV are patients with other diseases such as renal disease, history of PONV or motion sickness, age and chemotherapy-induced nausea and vomiting (10, 12).

On the second place are anesthetic factors which are usually associated with PONV such as anesthetic technique (11), volatile anesthetics and intravenous anesthetics (13), duration of anesthesia and opioid administration (14). Most of these drug factors are known and very rare today because by reducing the this known factors, we reduced the risk for PONV.

Previous study mentioned that type of surgery could be a very significant risk factor for PONV and studies of the effect of the type of surgery on the incidence of PONV have reported conflicting results (15-17). Literature data suggested that cholecystectomy, gynecologic, and laparoscopic procedures are associated with modestly increased risk of PONV compared with other general surgical procedures (16-19).

Laparoscopic cholecystectomy is the most commonly minimally invasive surgical procedure (11). The indication for this procedure is usually cholecystitis with or without stone. An incision is made on the navel, so the Hasson trocar or Verres needle is inserted through a small aperture. Insufflator is inserted into the abdominal cavity of CO2. In this way, the individual abdominal organs are separated from each other and the creator in which the organs can be seen and worked on with a special optical instrument. A special camera records and displays the image on the monitor. Complications include injuries to blood vessels, bile ducts, intestines and stomach. Direct postoperative complication is the onset of pain and vomiting, due to high pressure in the abdomen during interventions and due to instrument manipulation near the stomach.

In prevention of PONV there are well known strategies in clinical practice which are based on risk assessment and preventive evaluation of patinets characteristics, evaluating the individual patients's risk and evidence-based interventions when PONV occurs (15, 19). One of the used intervention is administration of aniemetics, where the usually are administried scopolamine patch, dexamethasone in dose of 4-8 mg intravenously after induction or ondansetron in dose of 4 mg intravenously at the end of the surgery (17). If nausea and vomitting occur in the post-anaesthesia care unit, administration of some other drug class of antiemetic could be effective, such as prochlorperazine in dose of 5-10 mg or droperidol in dose of 0.625 mg (18). Other procedures for prevention and reducing the PONV risk include adequate hydration, intravenous dextrose solution administration and multimodal postoperative pain control (19).

This clinical study was aimed to asses the efficiency of standard aniemetics administration separately or in combination in prevention of PONV in patients who underwent to the same surgical procedure - laparoscopic cholecystectomy. Also, this article could provide a novel information about the best choice for for prevention and treatment of PONV.

PATIENTS AND METHOD

Design of study

This clinical opservational study was designed to evaluate patients in early postoperative period up to 24 hours after laparoscopic holecystectomy. All patients were observed before and after surgical intervention in Clinical Center Podgorica in Montenegro. All procedures were performed according to the Helsinski declaration and with Good Clinical Practice guidelines. All prcedures also were approved with guidelines of local institutional ethical committee.

Patients and Protocol of study

This study included 87 patients divided into four groups according to the postoperative phramacological treatment: First group was control group without treatment, Second group was group of patients with ondasetron treatment in postoperative period, Third group was group of patients with ondasetron+dexamethasone treatment, and fourth group was group of patients with dexamethasone treatment in postoperative period for nausea. Inclusion criteria were adult patinets with good general health, negative anamnesis regarding the previous PONV history and known socioepidemiological data.

Before inclusion in study, all patients were underwent internal and anesthesiology examination before surgical intervention, After 24 hours from the surgical procedure, we observed incidence and presence of nausea or vomitting in each patient.

Data analyses

Results are presented as frequency and distribution in percent (%). All data are presented in form of Tables. Analyses was done in SPSS version 22.0 statistical software.

RESULTS

Demographic and anamnestic data of study population

In study population female were present in 45.5% and male in 54.5% in Dexamethasone group, in 36.4% were present male gender and in 63.6% female in Ondansetron+Dexamethasone group, while the males were present in 34.8% and 33.3% in Ondansetron and Control group, as well as females in 65.2% and 66.7% in the same groups (Table 1). Also, female and male gender was differently distributed in groups with PONV, with predominantly present females in Control and Ondansetron groups with PONV (71.4% and 100%) (Table 2).

Regarding the distribution of the blood antigen in relation to group of patients, the most frequently was O antigen in all groups, than A antigen (31.8-57.1%). Also, B and AB antigens were present in 9.1% to 21.7% in all groups (Table 1).

Table 1. Distribution of gender, blood antigen and smokers in study population

All patients	**Groups**			
Gender	Dexamethasone	Ondansetron + Dexamethasone	Ondansetron	Control
Male	12 (54.5%)	8 (36.4%)	8 (34.8%)	7 (33.3%)
Female	10 (45.5%)	14 (63.6%)	15 (65.2%)	14 (66.7%)
	Dexamethasone	Ondansetron+ Dexamethasone	Ondansetron	Control
A	7 (31.8%)	8 (34.6%)	7 (30.4%)	12 (57.1%)
O	11 (50%)	10 (45.5%)	8 (34.8%)	6 (28.6%)
B	2 (9.1%)	2 (9.1%)	5 (21.7%)	2 (9.5%)
AB	2 (9.1%)	2 (9.1%)	3 (13.0%)	1 (4.8%)
	Dexamethasone	Ondansetron+ Dexamethasone	Ondansetron	Control
Smokers	5 (22.7%)	11 (50%)	10 (43.5%)	6 (28.6%)
Non-Smokers	17 (77.3%)	11 (50%)	13 (56.5%)	15 (71.4%)

Results are expressed as frequency (%) in Dexamethasone, Ondansetron+Dexamethasone, Ondansetron and Control group

Table 2. Distribution of PONV in study population and gender and smokers in PONV group of patients

Patients with PONV	**Groups**			
Incidence of PONV	Dexamethasone	Ondansetron + Dexamethasone	Ondansetron	Control
PONV	3 (14.3%)	1 (4.5%)	5 (21.7%)	7 (33.3%)
Non-PONV	18 (85.7%)	21 (95.5%)	18 (78.3%)	14 (66.7%)
	Dexamethasone	Ondansetron+ Dexame-thasone	Ondansetron	Control
Male	1 (33.3%)	0 (0%)	0 (0%)	2 (28.6%)
Female	2 (66.7%)	1 (100%)	5 (100%)	5 (71.4%)
	Dexamethasone	Ondansetron+ Dexame-thasone	Ondansetron	Control
Smokers	1 (33.3%)	0 (0%)	2 (40%)	2 (28.6%)
Non-Smokers	2 (66.7%)	1 (100%)	3 (60%)	5 (71.4%)

Results are expressed as frequency (%) in Dexamethasone, Ondansetron+Dexamethasone, Ondansetron and Control group

Incidence of PONV in study population

Generally, incidence of PONV in study population was 18.4%, and 16 patients from 87 have PONV. The most frequently present PONV was in Control group (33.3%), than in Ondansetron group (21.7%), Dexamethasone group (14.3%) and Ondansetron+Dexamethasone group (4.5%) (Table 2).

Incidence of smokers in study population and in PONV patients

In Dexamethasone group smokers were present in 22.7%, in Ondansetron+Dexamethasone group in 50%, while in Ondansetron group were present in 43.5% and in 28.6% in Control group (Table 1). In relation to PONV, 33.3% were smokers in dexamethasone group with PONV and 40% in Ondansetron group of patients with PONV, while there were no patinets with PONV in group with PONV which was treated with Ondansetron+Dexamethasone (Table 2).

Influence of the different pharmacological treatment on PONV incidence in study population

PONV was distributed in Ondansetron+Dexamethasone group in the lowest percent (4.5%), which means that this conbination of antiemetics was very effective. Than, Dexamethasone group was in relation with low incidence of PONV (14.3%), and after that were Ondansetron and Control groups (Table 2). Also smokers and males has lower incidence of PONV, especially in combination with Ondansetron+Dexamethasone treatment (Table 2).

DISCUSSION

This clinical study was aimed to asses the efficiency of standard aniemetics administration separately or in combination in prevention of PONV in patients who underwent to the same surgical procedure - laparoscopic cholecystectomy. Also, this article could provide a novel information about the best choice for prevention and treatment of PONV.

A variety of antiemetics acting via different mechanisms are used for prevention and treatment of PONV. In general, clinicians choose among these agents based upon side effect profile, personal experience, cost, and formulary considerations (20-22). Previous study suggested that commonly used antiemetics reduce the risk of PONV by approximately 25 percent (23).

In our study, PONV was distributed in Ondansetron+Dexamethasone group in the lowest percent (4.5%), which means that this conbination of antiemetics was very effective. Than, Dexamethasone group was in relation with low incidence of PONV (14.3%), and after that were Ondansetron and Control groups (Table 2). Also smokers and

males has lower incidence of PONV, especially in combination with Ondansetron+Dexamethasone treatment (Table 2).

Well, according to our result it is clear that antiemetics are very effective procedure in reducing the PONV incidence after the surgery. But, beside that we know that there is some problems with the effects of antiemetics. The absolute benefit of an antiemetics depends on the degree of baseline risk, for example, for a female nonsmoker patient with a history of motion sickness who requires opioids in postoperative period some of antiemetics would reduce a risk for PONV for a bigger procent than in contrast (23, 24).

We have founded that In Dexamethasone group smokers were present in 22.7%, in Ondansetron+Dexamethasone group in 50%, while in Ondansetron group were present in 43.5% and in 28.6% in Control group (Table 1). In relation to PONV, 33.3% were smokers in dexamethasone group with PONV and 40% in Ondansetron group of patients with PONV, while there were no patinets with PONV in group with PONV which was treated with Ondansetron+Dexamethasone (Table 2).

Definitely, smoking and gender are very important risk factors for development of PONV. Maybe the effects of drugs that acts on different receptors are additive rather than synergistic, and each added drug results in reducing the PONV risk (25).

In our study the most effective was ondansetron in combination with dexamethasone. The first-generation serotonin antagonists (ie, ondansetron, granisetron, and, outside the United States, dolasetron, ramosetron, and tropisetron) are equally efficacious for PONV at equipotent doses (relative risk [RR] 0.76 versus placebo) (26). All of these drugs have potential to prolong the electrocardiogram intervals, particularly the QT interval, and should be avoided for patients at risk for QTc prolongation. These agents are administered as a single dose at the end of surgery. Ondansetron is available as an orally disintegrating film (ODF) and as an orally disintegrating tablet (ODT), which are as effective as IV ondansetron (27, 28). The ODT may be useful for postdischarge administration and may be administered to children over the age of five.

On the other hand, dexamethasone as a glucocorticoid is effective as ondansetron for prevention of PONV (27), and it is the most common used and studied corticosteroid (28). Dexamethasone may be beneficial because of a direct antiemetic effect and by reducing postoperative pain and the need for postoperative opioids. Lower doses of dexamethasone may be required for PONV prophylaxis than for pain relief. A meta-analysis of 60 randomized trials with 6700 patients found that a dose of 4 to 5 mg IV dexamethasone was as effective as 8 to 10 mg IV for reduction of PONV (29). In contrast, two meta-analyses of studies comparing lower-dose (<0.1 mg/kg IV) dexamethasone with higher doses (>0.1 mg/kg) found that higher doses were required for reduced opioid requirement (30, 31). A subsequent multicenter randomized trial including 1350 patients who underwent bowel surgery reported that a single postinduction dose of dexamethasone 8 mg IV reduced the incidence of PONV at 24 hours and the need for rescue antiemetic for up to 72 hours.

Beside all these facts, administration of antiemetics, especially of ondansetron and dexamethasone should be individualized, becase these drugs have also adverse effects such as wound infection, decreasing cell counts, bone loss and other. In these cases, there are some other very effective antiemetics such as phenothiazines, which are effective in postoperative PONV but limited by their sedation and extrapyramidal effects (32, 33).

Also, literature dana suggested that metoclopramide and midazolam could be effective in PONV reducing (33) but less than ondansetron. Because of the adverse effects such as hypotension, tachycardia as well as sedation and postoperative delirium induced by midazolam, administration of these drugs are limited (25, 31).

Future studies and current investigations are oriented to the examination of cannabinoids which may be effective in treatment of chemotherapy-induced nausea and vomiting, but there is some suggestions regarding the PONV prevention (30).

Our study examined the effects of antiemetics on early nausea and vomiting in postoperative period (first 24 hours after surgery) and we concluded that combination of two antiemetics in male smokers were the most effective. But, stil lis unknown what is the best treatment for postdischarge nausea and vomiting (within 48 hours of discharge). Literature dana suggested that in these periods, the opioids are the most effective choice for reducing the nausea and vomiting incidence (29), but these assumptions must be examined in the future clinical studies.

Limitation of this study is small number of patients who were included and examined, so the future research shoud be on large study population with widely included variables which could interfere with incidence of PONV.

CONCLUSION

The incidence of PONV is lower in male smokers patients who were underwent to combination of two antiemetics, ondansetron and dexamethasone compared to monotherapy and female non-smokers. Preventive strategies for PONV must include risk stratification followed by prophylactic approach and also testing the newer antiemetics. Because of the high incidence of postoperative nausea and vomiting as a patient-important outcome, the preventive strategies should be considered as serious condition which requires multimodal approach.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests.

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INHIBITORY POTENTIAL OF MURRAYA KOENIGII (L.) AND FICUS CARICA L. EXTRACTS AGAINST ALDOSE REDUCTASE (ALR), ADVANCED GLYCATION END PRODUCTS (AGES) FORMATION AND SORBITOL ACCUMULATION

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INHIBITORNI POTENCIJAL EKSTRAKTA MURRAYA KOENIGII (L.) I FICUS CARICA L. NA ALDOZA REDUKTAZU (ALR), STVARANJE NAPREDNIH GLIKACIONIH KRAJNJIH PROIZVODA (AGES) I AKUMULACIJU SORBITOLA

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ABSTRACT

Introduction: Murraya koenigii (L.) and Ficus carica L. are traditionally used plants with significant medicinal and nutritional values. Aim and Objective: The present study was focused on the evaluation of hydro-alcoholic and aqueous extracts of M. koenigii (L.) leaves [MKHA (M. koenigii (L.) hydro-alcoholic extract) and MKAQ (M .koenigii (L.) aqueous extract)] and dried fruits of F. carica L. [FCHA (F. carica L. hydro-alcoholic extract) and FCAQ (F. carica L. aqueous extract)] in the attenuation of markers of microvascular complications associated with diabetes mellitus which can be further used to investigate the pharmacological activity of these plants in treatment of diabetes and its complications. Material and Method: The attenuating effect of the extracts was evaluated by calculating the ALR1 enzyme inhibition in a kidney of Wistar rat, anti-glycation activity in bovine serum albumin (BSA) and erythrocyte sorbitol accumulation inhibition in heparinized human blood. Results: A significant inhibitory effect (IC_{50} 6.47µg/ml,7.26µg/ml,8.93 µg/ml and 9.66µg/ml) was observed with different concentrations of extracts (MKHA, MKAQ, FCHA and FCAQ) respectively, against ALR enzyme. After the 4th week of incubation, the inhibition of AGEs formation by MKHA, MKAQ, FCHA and FCAQ (500µg/ml) was found to be 82.58%, 78.58%, 74.39% and 69.56% respectively. MKHA, MKAQ, FCHA and FCAQ were found to exhibit significant inhibition against the accumulation of sorbitol in RBCs with IC_{50} 188.88 µg/ml, 247.74µg/ml, 291.94µg/ml and 345.34µg/ml, respectively. Conclusion: The administration of different concentrations of MKHA, MKAQ, FCHA and FCAQ significantly attenuated ALR, AGEs and sorbitol accumulation; hence, it can provide a basis for identification and development of new inhibitors of these biomarkers.

***Keywords**: ALR inhibition, AGEs formation, Sorbitol accumulation, Ficus carica, Murraya koenigii.*

SAŽETAK

Uvod: Murraya koenigii (L.) i Ficus carica L su tradicionalno korišćene biljke sa značajnom lekovitom i nutritivnom vrednošću. Cilj: Ova studija je fokusirana na proceni hidro-alkoholnih i vodenih ekstrakta listova M. koenigii (L.) [MKHA (M. koenigii (L.) hidro-alkoholni ekstrakt) i MKAQ (M .koenigii (L.) vodeni ekstrakt) i sušenih plodova F. carica L. [FCHA (F. carica L. hidro-alkoholni ekstrakt) i FCAQ (F. carica L. vodeni ekstrakt)] u slabljenju markera mikrovaskularnih kompikacija povezanih sa dijabetesom melitusom što se može dalje koristiti da bi se ispitalo farmakološko dejstvo ovih biljaka u lečenju dijabetesa i njegovih komplikacija. Materijali i metode: Ublažavajuće dejstvo ekstrakta je procenjeno proračunom inhibicije ALR1 enzima u bubregu Wistar pacova, antiglikacionog dejstva u goveđem serumskom albuminu (BSA) i inhibicije akumulacije sorbitola u heparinizovanoj humanoj krvi. Rezultati: Značajan inhibitorni efekat (IC_{50} 6.47µg/ml,7.26µg/ml,8.93 µg/ml i 9.66µg/ml) je primećen sa različitim koncentracijama ekstrakta (MKHA, MKAQ, FCHA i FCAQ) na ALR enzim. Posle četvrte nedelje inkubacije, inhibicija stvaranja AGEs pomoću MKHA, MKAQ, FCHA i FCAQ (500µg/ml) je bila 82.58%, 78.58%, 74.39% i 69.56%. MKHA, MKAQ, FCHA i FCAQ su pokazali značajnu inhibiciju na akumulaciju sorbitola u RBCs sa IC_{50} 188.88 µg/ml, 247.74µg/ml, 291.94µg/ml i 345.34µg/ml. Zaključak: Primena različitih koncentracija MKHA, MKAQ, FCHA i FCAQ značajno je oslabila ALR, AGEs i akumulaciju sorbitola, stoga, ona može da obezbedi osnovu za prepoznavanje i razvoj novih inhibitora ovih biomarkera.

***Ključne reči:** ALR inhibicija, stvaranje AGEs, akumulacija sorbitola, Ficus carica; Murraya koenigii.*

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INTRODUCTION

Diabetes mellitus (DM) is a chronic, progressive and metabolic disease resulting in chronic hyperglycemia as well as altered metabolism of carbohydrates, proteins and fats and a number of people affected by diabetes is expected to increase to 354 million by 2030 (1-3). Diabetes is also responsible for many short and long-term complications like diabetic neuropathy, retinopathy and nephropathy (2). Development of diabetic complications is triggered by hyperglycemia. Moreover, in chronic diabetes, there is an initiation of various metabolic pathways (hexosamine; mitogenactivated protein kinases (MAPKs); sorbitol-aldose reductase; protein kinase C; advanced glycation end products (AGEs), their receptors and nitric oxide synthase) (4-8). Formation of AGEs leads to damage of target cells (9) prompting receptor-mediated liberation of reactive oxygen species (ROS) (10). Aldehyde reductase (ALR), advanced glycation end products (AGEs) and sorbitol are prominent markers of diabetic complications. Reduction of aldehydes and carbonyls is caused due to activity of ALR, which is a key enzyme for aldehyde detoxification, osmotic regulation, and metabolism of catecholamines and steroids under normal physiological conditions (11). During glucose metabolism, a number of proteins or lipids are produced called as AGEs, which become glycated upon exposure to sugar, causing various diseases like diabetes, atherosclerosis, etc. (7). ALR also reduces glucose to a slow metabolizing alcoholic sugar (sorbitol) which produces a laxative effect by drawing water into the large intestine (12).

Interaction of AGEs and its receptors produces oxidative stress, which leads to the damage and vascular aging. During hyperglycemic conditions, the reduction of glucose to sorbitol is caused by the consumption of cofactor nicotinamide adenine dinucleotide phosphate (NADPH) by ALR. This overexploitation of NADPH affects other homeostatic mechanisms, such as the glutathione (GSH) production; an antioxidant enzyme, which further leads to oxidative stress. There is the accumulation of excessive sorbitol and extracellular glucose in several sensitive tissues such as eye lenses, retinal cells, peripheral nerves, renal cells, etc. which ultimately causes long-term complications. Hence, the therapeutic agents that can inhibit ALR, AGEs formation and sorbitol accumulation may have a therapeutic potential for treating diabetic complications (13,14).

***Murraya koenigii* (L.)** Sprengel, (Rutaceae), has been used in folk and traditional medicine for treating a traumatic injury, snake bite, rheumatism, as a stimulant, and in the management of diabetes mellitus (15). Various phytoconstituents (alkaloids and coumarin glycoside) present in the leaves of *M. koenigii* were found to possess antioxidant, antihyperlipidemic, antifungal, antibacterial, larvicidal, anti-carcinogenic, anti-hyperglycemic, anti-lipid peroxidative, and hypotensive activity (16). The various chemical constituents found in the leaves of *M. koenigii* are: Alkaloids: mahanine, koenine, koenigine, koenidine, girinimbiol, girinimibine, koenimbine, O-methyl murrayamine A, O-methyl mahanine, isomahanine, bismahanine, bispyrayafoline. Coumarin glycoside: scopotin, murrayanine. Essential oil: di- alpha phellandrene, D-pinene, D-sabinene, D-terpinol, dipentene and caryophyllene. 5, 8-dimethyl furanocoumarin, 1-al, 3[6', 6' dimethyl 5-hexene] carbazole, β-sitosterol. Phosphorus, iron, thiamine, riboflavin, niacin, vitamin c, carotene, oxalic acid and calcium (17-18).

***Ficus carica* Linn.** (Moraceae), is used in Ayurveda, homoeopathy and siddha system of medicine (19). The bark, leaves and fruits are traditionally used to treat different disorders such as respiratory diseases, gastrointestinal diseases, diabetes, skin diseases, ulcers, dysentery and haemorrhoids (20). *F. carica* has anti-inflammatory, cytotoxic, and anti-hyperlipidemic activities. Various phytoconstituents such as amino acids, phytosterols, anthocyanins, organic acid, hydrocarbons, aliphatic alcohols, volatile components, fatty acids, phenolic components, etc. have been isolated from different parts of *F. carica* (21, 22). Psoralen, bergapten, umbelliferone, *β*-sitosterol, campesterol, stigmasterol, fucosterol, fatty acids, 6-(2-methoxy-Z-vinyl)-7-methyl-pyranocoumarin, 9, 19-cycloarlane triterpenoid, 6-O-acyl-*β*-Dglucosyl-*β*-sitosterol,calotropenyl acetate, lupeol acetate and a few other classes of secondary metabolites (23).

Many active and potent synthetic ALR inhibitors and AGE inhibitors have been synthesized by many researchers but they did not pass clinical trials due to their poor pharmacokinetics, low efficacy and safety. Therefore, the focus has been shifted towards herbal drugs to explore new agents with better efficacy and lesser side effects (24-26). So, this study was designed to investigate the inhibitory activity of hydroalcoholic and aqueous extracts of *M. koenigii* and *F. carica* against ALR, AGEs and sorbitol accumulation.

MATERIALS AND METHODS

Plant material used

Fresh leaves of *M. koenigii* (L.) were collected in August - September from the herbal garden of Maharishi Markandeshwer College of Pharmacy (MMCP), Maharishi Markandeshwer (Deemed to be) University, Mullana Ambala, Haryana and dried fruits of *F. carica* L. were collected from a local market of Ambala, Haryana and authenticated by Dr. K. Madhava Chetty from the Department of Botany, Sri Vankateshwara University, Tirupati, India. The plant specimen voucher numbers 0384 and 1296 are present in the herbarium of the University for future reference.

Reagents used

Ethanol, NADPH (nicotinamide adenine dinucleotide phosphate), Glyceraldehyde, Bovine serum albumin (BSA), Aminoguanidine, Heparinized human blood, NADH (Nicotinamide adenine dinucleotide), Fructose, Ascorbic acid.

Preparation of Extracts

The fresh leaves of *M. koenigii* (L.) were shadedried and crushed to powder. 100 g of the powdered leaves were first

extracted with aqueous ethanol (40%) and then with water for 48 hours by maceration with the aid of an electric magnetic stirrer. Also, the fruits of *F.carica* L. were further dried and powdered. 100 g of the powdered fruits were extracted by maceration sequentially using hydro-alcohol i.e. aqueous ethanol (40%) and water for 48 hours by using an electric magnetic stirrer. The extracts were filtered and concentrated at the temperature of 40°C by using a rotary evaporator under the reduced pressure. The extraction yield was calculated and the crude extracts were dissolved in water and used for the assessment of various assays.

ALR1 enzyme inhibition

The fractional purification of ALR1 enzyme was carried out from the kidney of a rat (IAEC Protocol No.: MMCP/IAEC/24). The activity of ALR1 was calculated by checking the oxidation of NADPH and was assessed spectrophotometrically at 340 nm at 37 °C as an element of time. Glyceraldehyde was used as a substrate for the estimation of ALR1 enzyme inhibition.

The IC_{50} values were calculated by plotting the percent inhibition versus the inhibition concentration (27). The experiment was done in triplicate.

Anti-glycation activity

In-vitro anti-glycation activity of the extracts was estimated by measuring the capability of extracts in inhibiting fluorescence of bovine serum albumin (BSA) on a weekly basis up to four weeks, Matsuda et al. (28). Aminoguanidine was considered as a reference compound. The formation of AGEs was measured from the intensity of fluorescence at the excitation wavelength of 355 nm and emission wavelength of 460 nm. Elico-SLI74 Spectrofluorometer was employed for determination of the anti-glycation activity. The experiment was done in triplicate.

Erythrocyte sorbitol accumulation inhibition

The inhibition of accumulation of sorbitol in erythrocytes was estimated according to the method of Haraguchi et al. in which 5 ml of human blood (heparinized) was taken from a healthy male volunteer kept overnight fasting. Erythrocytes were isolated then from plasma by centrifugation (29). Elico-SLI74 Spectrofluorometer was used to measure the relative fluorescence due to NADH at the excitation wavelength of 366 nm and emission wavelength of 452 nm.

RESULTS

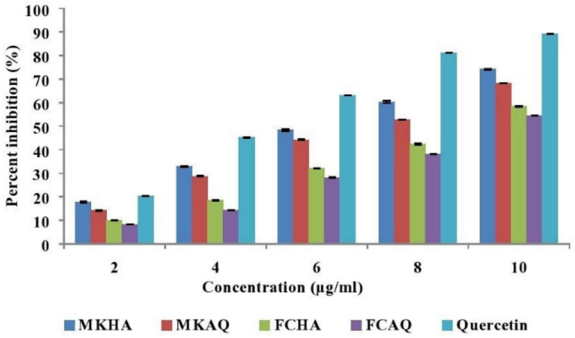
Extraction yield of the extracts:

The extraction yield of hydro-alcoholic extract of *M. koenigii* (MKHA) was found to be 24.45% and the extraction yield of aqueous extract of *M. koenigii* (MKAQ) was found to be 17.7% Whereas, the extraction yield of hydro-alcoholic extract of *F. carica* (FCHA) was found to be 34.57% ,the extraction yield of aqueous extract of *F. carica* (FCAQ) was found to be 28.42%.

Inhibition of ALR

ALR enzyme was isolated from the kidney of Wistar rat and the activity of ALR was monitored spectrophotometrically by measuring the oxidation of NADPH at 340 nm. Different concentrations (2, 4, 6, 8, 10 µg/ml) of MKHA, MKAQ, FCHA and FCAQ produced a good inhibitory effect against ALR enzyme with IC_{50} 6.47 µg/ml, 7.26 µg/ml, 8.93 µg/ml and 9.66 µg/ml respectively whereas, IC_{50} of quercetin (standard) was found to be 4.87 µg/ml. (Figure 1)

Figure 1. Inhibitory effects of MKHA, MKAQ, FCHA and FCAQ against ALR enzyme inhibition



Inhibition of AGEs

The production of AGEs was observed on a weekly basis by measuring the intensity of fluorescence of BSA-fructose solutions for 4 weeks and MKHA, MKAQ, FCHA and FCAQ (the concentration 25, 50, 100, 200, 400, 500 µg/ml) significantly attenuated the production of AGEs. After the 4th week of incubation, the inhibition of AGEs formation by aminoguanidine (standard) (500 µg/ml) was found to be 97.53 % whereas the percentage inhibition by MKHA, MKAQ, FCHA and FCAQ (500 µg/ml) was found to be 82.58%, 78.58 %, 74.39 % and 69.56 % of AGEs formations respectively (Figure 2, 3, 4 and 5).

Figure 2. Inhibitory effect of MKHA against AGEs inhibition

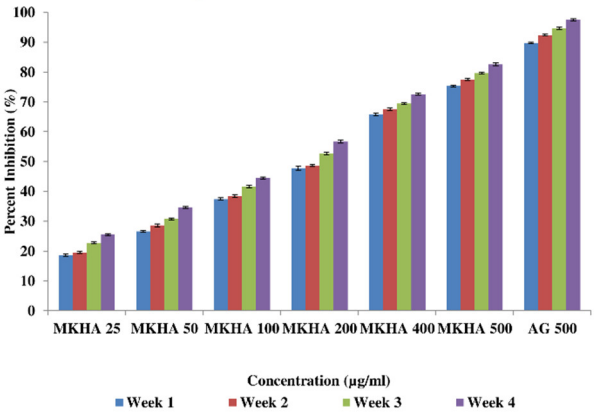


Figure 3. Inhibitory effect of MKAQ against AGEs inhibition

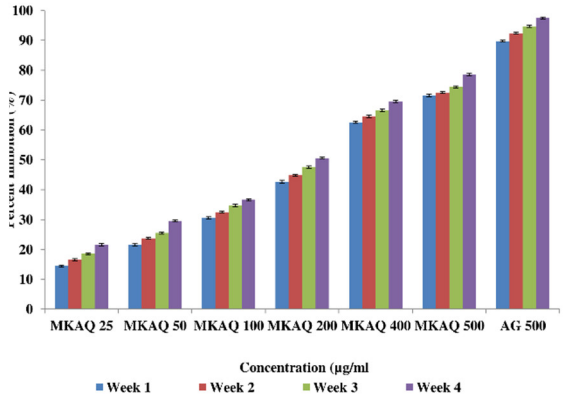


Figure 4. Inhibitory effect of FCHA against AGEs inhibition

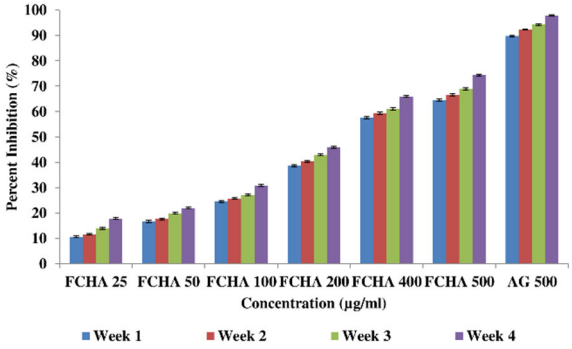
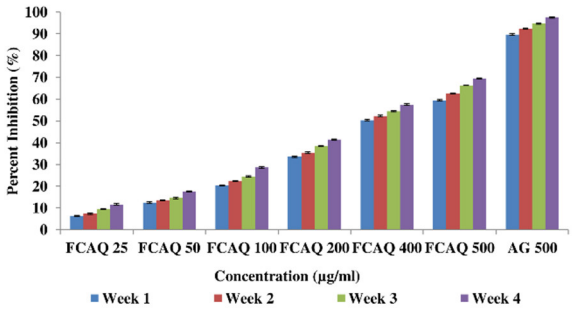


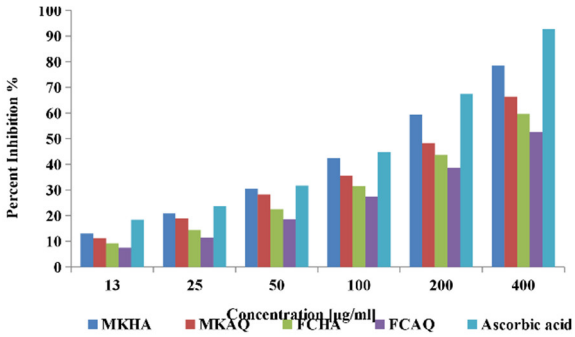
Figure 5. Inhibitory effect of FCAQ against AGEs inhibition



Erythrocyte sorbitol accumulation inhibition

In the present study, different concentrations (12.5, 25, 50, 100, 200, 400, 500 µg/ml) of MKHA, MKAQ, FCHA and FCAQ were prepared and their effect was measured against the accumulation of sorbitol in terms of relative fluorescence due to NADH. Different concentrations of MKHA, MKAQ, FCHA and FCAQ were found to exhibit the significant inhibition of accumulation of sorbitol in erythrocytes with an IC_{50} 188.88 µg/ml, 247.74 µg/ml, 291.94 µg/ml and 345.34 µg/ml respectively while as IC_{50} of ascorbic acid was found to be 150.06 µg/ml [Figure 6].

Figure 6. Inhibitory effects of MKHA, MKAQ, FCHA and FCAQ against Sorbitol accumulation



DISSCUSION

The reduction of aldehydes and carbonyls is caused due to the activity of ALR, which is a key enzyme for aldehyde detoxification, osmotic regulation, and metabolism of catecholamines and steroids under the normal physiological conditions (11). ALR has a low affinity for glucose therefore; its metabolism is not affected under the normal physiological conditions (14, 30). During the chronic hyperglycemic conditions, hexokinase enzyme that is responsible for glucose phosphorylation for the production of energy is saturated and the surplus of glucose enters the polyol pathway resulting in the reduction of glucose to sorbitol. During hyperglycemia, ALR consumes cofactor NADPH for reducing glucose to sorbitol. This over-exploitation of NADPH affects other homeostatic mechanisms, such as the glutathione (GSH) production; an antioxidant enzyme, which further leads to oxidative stress (13).

Amadori products (by the reaction of proteins/lipids with reducing sugars) via Mallard reaction are formed because of non-enzymatic glycation due to chronic hyperglycemia. These products are further transformed to cross-linking AGEs like pentosidine and crosslines, having a specific fluorescence. Due to the production of excessive AGEs, the receptors of AGEs (RAGEs) are also overexpressed, which further leads to pathogenesis of diabetic complications. Several studies have confirmed the vital role of inhibition of AGEs leading to the attenuation of diabetic complications (31-34), therefore, the inhibition of AGEs can be proposed as a good therapeutic option to lag the progression of diabetic complications.

ALR also reduces glucose to a slow metabolizing alcoholic sugar (sorbitol) which produces a laxative effect by drawing water into the large intestine (12). The excessive sorbitol formation during the polyol pathway and extracellular glucose gets accumulated in several sensitive tissues such as eye lenses, retinal cells, peripheral nerves, renal cells, etc. which ultimately causes long-term complications (30, 13).

MKHA has produced better inhibitory effect against ALR enzyme with IC_{50} 6.47 µg/ml than MKAQ which produced IC_{50} 7.26 µg/ml and FCHA produced IC_{50} 8.93 µg/ml and

FCAQ produced IC_{50} 9.66 µg/ml, respectively in comparison to quercetin (standard) i.e. 4.87 µg/ml. The higher activity of MKHA as compared to MKAQ, and that of FCHA as compared to FCAQ may be due to the additive effect of higher concentration of different flavonoids present in the hydro-alcoholic extract than the aqueous extracts (38, 39). Several researchers have studied the potential of flavonoids against ALR enzyme that encouraged our results (14,35,36,37). In the present study, the formation of AGEs was observed weekly by measuring the fluorescence intensity of bovine serum albumin (BSA)-fructose solutions for subsequent four weeks and MKHA, MKAQ, FCHA and FCAQ were found to have an inhibitory effect against the formation of AGEs. After the 4th week of incubation, the inhibition of AGEs formation by aminoguanidine (standard, 500 µg/ml) was 97.53 % whereas MKHA, MKAQ, FCHA and FCAQ (500 µg/ml) inhibited 82.58%, 78.58 %, 74.39 % and 69.56 % of AGEs formations respectively. In the present study, different concentrations of MKHA, MKAQ, FCHA and FCAQ (12.5, 25, 50, 100, 200, 400, 500 µg/ml) were prepared and their effect was measured against sorbitol accumulation in terms of relative fluorescence due to NADH. The different concentrations of MKHA, MKAQ, FCHA and FCAQ were found to exhibit a significant inhibitory effect on the accumulation of sorbitol in red blood cells with an IC_{50} 188.88 µg/ml, 247.74 µg/ml, 291.94 µg/ml and 345.34 µg/ml respectively which was comparable to Ascorbic acid i.e. 183.08 µg/ml.

CONCLUSION

This study has revealed the inhibitory potential of hydro-alcoholic and aqueous extracts of *M. koenigii* (L.) and *F. carica* L. against ALR enzyme activation, AGEs formation and sorbitol accumulation. Thus, these extracts of *M. koenigii* (L.) and *F. carica* L. may have a beneficial role to delay the progression of diabetic complications.

CONFLICT OF INTEREST

The authors have no conflict of interest.

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THE HEALTH STATE OF WOMEN IN SERBIA IN THE PERIOD 2006-2016

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ZDRAVSTVENO STANJE ŽENA U SRBIJI U PERIODU 2006-2016.

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ABSTRACT

Women's health is of particular importance because of the large and specific sensitivity of this population group and the fact that women are consistently concerned about their own health, but also about the health of their children, parents and other family members. The aim of this study was to considering the health of women in Serbia in the period from 2006 to 2016, in order to highlight the priority problems, which would serve to create preventive programs and measures aimed at improving the health of this population group. Chronic non-communicable diseases (malignancies, acute coronary syndrome, diabetes mellitus, etc) are the leading causes of morbidity and mortality, and one of the main reasons for the use of health care in the population of women in our country in the period from 2006 to 2016. How it comes to preventable diseases, actions in the field of health promotion should be directed at preventing or modifying risk factors that are responsible for the occurrence of these diseases, as well as the creation of national strategies for the prevention and control, monitoring the performance of screening and other preventive programs, planning and organization of health care. By promoting healthy lifestyles, empowering women and their active participation in the community can have significant positive effects on raising the health potential of this population.

Keywords: *women, women's health, chronic non-communicable diseases, neoplasms, diabetes mellitus*

SAŽETAK

Zdravlje žena od posebne je važnosti zbog velike osetljivosti ove populacione grupe i zbog činjenice da žene brinu o sopstvenom zdravlju, ali i o zdravlju svoje dece, roditelja i ostalih članova porodice. Cilj ovog istraživanja je sagledavanje trenda kretanja nejčešćih bolesti kod žena u Srbiji u periodu od 2006-2016. godine, kako bi se izdvojili prioritetni problemi, koji bi poslužili za kreiranje preventivnih programa u cilju unapređenja zdravlja žena. Hronične nezarazne bolesti (maligniteti, akutni koronarni sindrom, diabetes melitus, druge) su vodeći uzroci obolevanja i umiranja, i jedan od glavnih razloga korišćenja zdravstvene zaštite u populaciji žena u Srbiji u periodu od 2006-2016. godine. Kako se radi o preventivnim bolestima, akcije u oblasti promocije zdravlja treba usmeriti ka sprečavanju i modifikaciji faktora rizika koji su odgovorni za pojavu ovih bolesti, kao i stvaranje nacionalnih strategija za prevenciju i kontrolu, praćenje performansi skrininga i drugih preventivnih programa, planiranja i organizacije zdravstvene zaštite. Promovisanjem zdravih stilova života, osnaživanje žena i njihovo aktivno učešće u zajednici može imati značajne pozitivne efekte na podizanje zdravstvenog potencijala ove populacije.

Ključne reči: *žene, zdravlje žena, hronične nezarazne bolesti, neoplazme, diabetes melitus*

INTRODUCTION

Women's health is of particular importance because of the large and specific sensitivity of this population group and the fact that women are consistently concerned about their own health, but also about the health of their children, parents and other family members (1).

The health status of women results from a complex interaction of genetic, biological, physiological, medical and social factors (2, 3).

Identification and analysis of women's health, early diagnosis and timely, adequate, effective treatment and qual-

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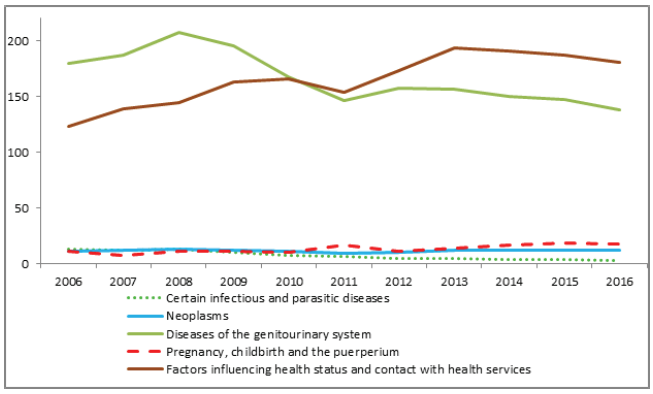
Table 1. Indicators of securing, burdening and using primary health care of women in Serbia 2007-2016.

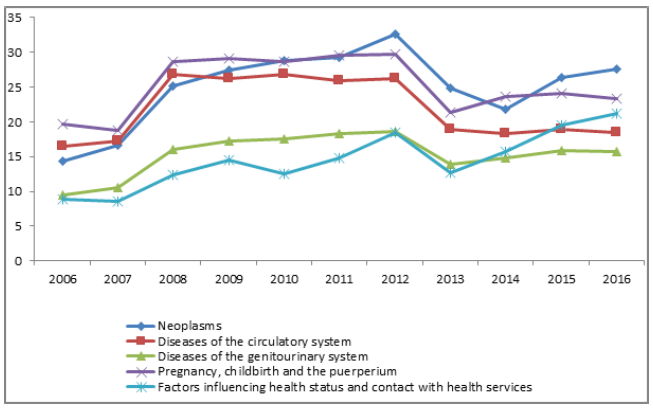
Year	Number of doctors	Number of nurses with secondary education	Ratio of nurses to physicians	Average annual number of visit per physicians	Average annual number of visits per one woman 15+	Ratio of first and total number visits to doctor	Number of women 15+ per doctor in primary health care of women health services
2007	542	883	1.6	3801.8	0.64	43.67	5972
2008	558	878	1.6	3916.0	0.68	64.29	5785
2009	578	892	1.5	3782.9	0.68	45.20	5569
2010	588	850	1.4	3428.3	0.63	43.84	5458
2011	586	815	1.4	3368.8	0.62	43.30	5459
2012	575	821	1.4	3259.1	0.59	44.99	5548
2013	576	794	1.4	3329.3	0.60	45.49	5515
2014	565	775	1.4	3374.3	0.60	46.17	5996
2015	568	758	1.3	3246.0	0.59	45.31	5535
2016	567	766	1.4	3389.8	0.61	47.51	5516

ity management of health and quality of life are crucial factors for reducing the serious consequences of women's disease, their families and the community as a whole. Women's health can be improved by promoting the active and adequate programs, which means creating conditions that support well-being and allowing women to maintain the healthy and integrated lifestyle, both before/in the illness and after illness (4, 5).

The aim of this study was to considering the health of women in Serbia in the period from 2006 to 2016, in order to highlight the priority problems, which would serve to create preventive programs and measures aimed at improving the health of this population group.



Graph 1. The most commonly diagnosed diseases, conditions and injuries in primary health care women in Serbia, 2006-2016.



Graph 2. The most common causes of hospitalization by groups of disease, women, Serbia, 2006-2016.

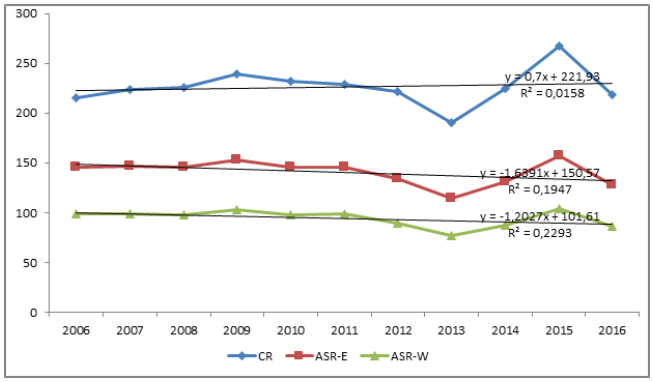
METHOD

The study was designed as a descriptive epidemiological study. Analysis was made on the basis of the Health Statistical Year-book from Institute of Public Health of Serbia „Dr Milan Jovanovic Batut" for the period 2006 to 2016. All the data of interest were presented and analyzed by adequate mathematical-statistical methods appropriate for the data type. Standardized rates of incidence and mortality were shown. Linear trend and regression analysis were used to analyze the trend of incidence and mortality. All statistical calculations were performed using commercial, standard software package SPSS, version 20.0 (The Statistical Package for Social Sciences software (SPSS Inc, version 20.0, Chicago, IL)).

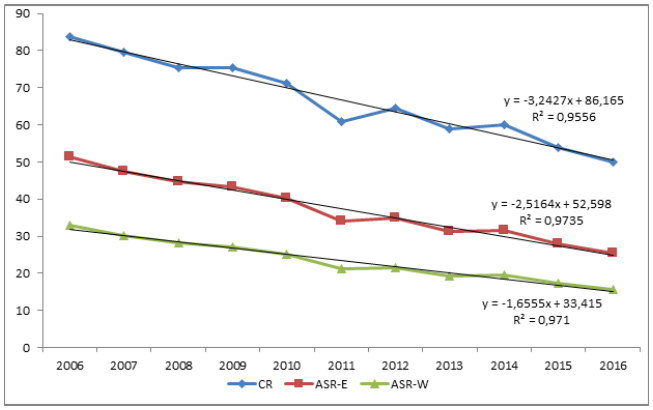
RESULTS

According to the data of the Statistical Office of the Republic of Serbia, on the territory of Serbia in the period 2006-2016 the number of women ranged from 3 807 871 in 2006 and 3 620 692 in 2016.

At the primary level, women's primary care in the Republic of Serbia in 2016 was provided by 567 doctors, assuring 1 doctor per 5996 women aged 15 and over. The average annual visit to the doctor per woman was 1.4, while the percentage of participation of the first in the total number of visits to the doctor amounted to 45.31 in 2016 (Table 1).



Graph 3. Standardized incidence rates of acute coronary syndrome per 100.000 of population, women, Serbia, 2006-2016.
Legend:
CR: Crude Rates
ASR-E: A Standardized Rates-Europe
ASR-W: A Standardized Rates-World



Graph 4. Standardized mortality rates of acute coronary syndrome per 100.000 of population, women, Serbia, 2006-2016.

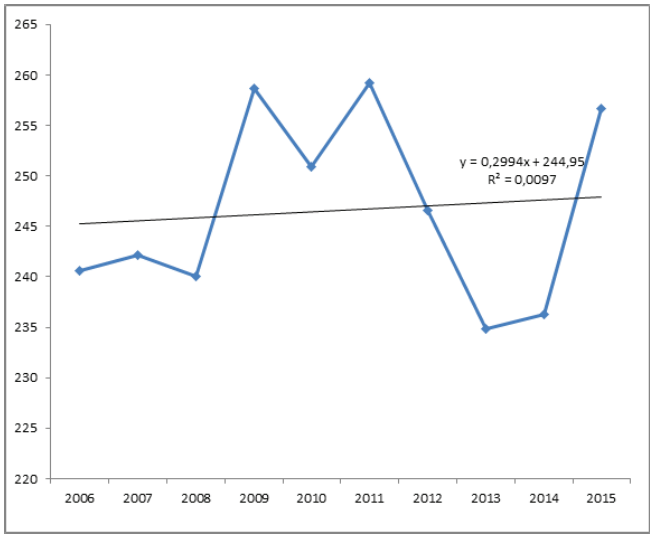
The most common groups of diseases registered in primary health care of women in Serbia in the period 2006-2016 were diseases of the genitourinary system, certain infectious and parasitic diseases, neoplasms, and pregnancy, childbirth and the puerperium (Graph 1).

The most common causes of hospitalization in females in Serbia, in the observed ten-year period were neoplasms, diseases of the circulatory system, diseases of genitourinary system, pregnancy childbirth and the puerperium (Graph 2).

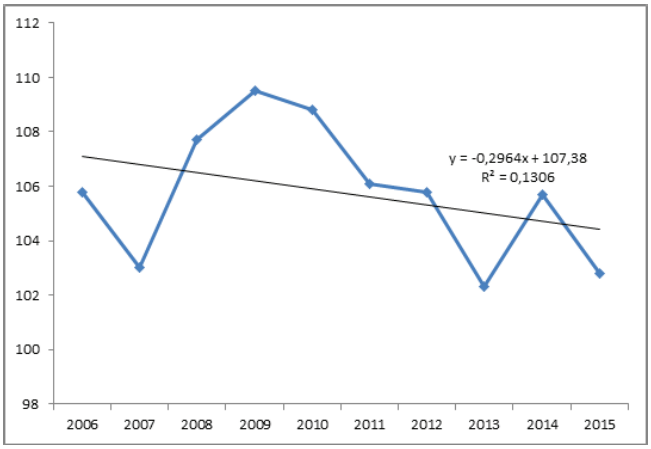
Chronic non-communicable diseases (diseases of the heart and blood vessels, malignant tumors and diabetes mellitus) are the most common causes of morbidity and mortality in the female population of Serbia in the period 2006-2016 years.

In the heart and blood vessel diseases, the most common disorders are ischemic heart diseases. Among ischemic heart diseases, by frequency at the leading position, both in morbidity and mortality, there is an acute coronary syndrome that includes myocardial infarction and unstable angina pectoris. In spite of dominant position in the pathology of the female population of Serbia, acute coronary syndrome records the trend of falling standardized rates of incidence and mortality in the observed period. The average standard incidence rate of acute coronary syndrome in women was 88.6/100.000. The linear trend of incidence rates shows a negative trend (y=0.797x+85.05). The average standardized mortality rate from acute coronary syndrome in women was 20.8/100.000 showing a significant trend in mortality reduction (y=-2.386x+31.58) (Graph 3, 4).

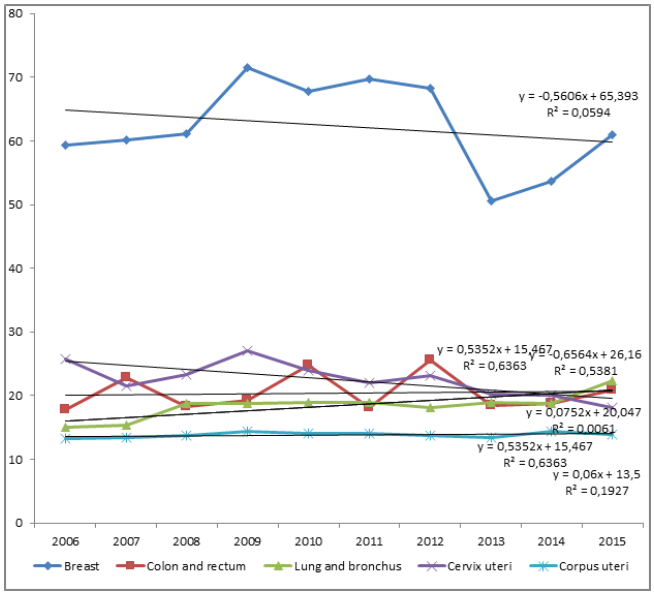
When it comes to malignant neoplasms, the average standardized incidence rate of all localized malignant neoplasms was 246.6/100.000, and the average standardized mortality rate of all localized malignant neoplasms was 105.7/100.000. In the 10-year period, standardized incidence rates of malignant tumors show a trend of growth (y=0.299x+244.95), while standardized mortality rates of malignant tumors show a downward trend (y=-0.296x+107.38) (Graph 5, 6).



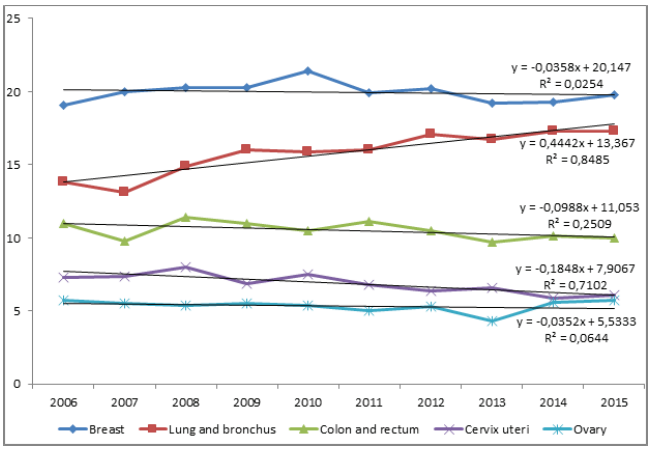
Graph 5. Standardized cancer incidence rates per 100.000 of population, by all sites women, Serbia, 2006-2015.



Graph 6. Standardized cancer mortality rates per 100.000 of population, by all sites women, Serbia, 2006-2015.



Graph 7. Standardized cancer incidence rates per 100.000 of population by leading primary sites, women, Serbia, 2006-2015.



Graph 8. Standardized cancer mortality rates per 100.000 of population by leading primary sites, women, Serbia, 2006-2015.

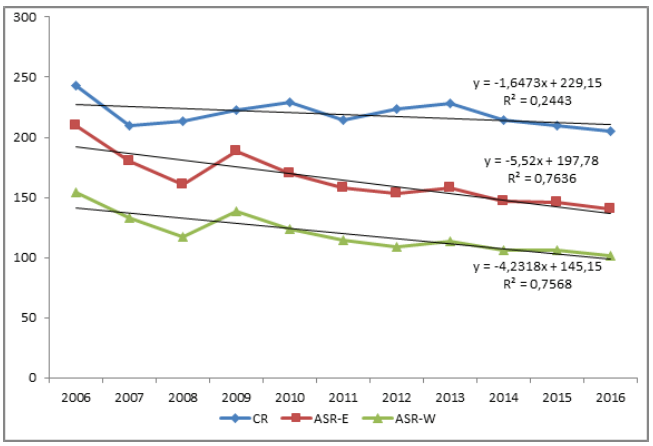
If we observe the leading localization of malignant tumors in women, the most standardized incidence rates in 2016 have been reported for breast cancer 61 per 100.000 women, lung and bronchus 22.4 per 100.000, colon and rectum 20.9 per 100.000, cervical cancer 18.1 per 100.000 and uterine cancer of 13.9 per 100.000 women (Graph 7).

The most common localizations of malignant tumors in women, are, at the same time, the most common causes of deaths from malignant diseases. There was an increase in standardized mortality rates among all the leading localization of malignant tumors, except for colon - rectal cancer. Due to organized screening and early detection of cervical cancer, in mortality from this disease was observed downward trend in the observed period (Graph 8).

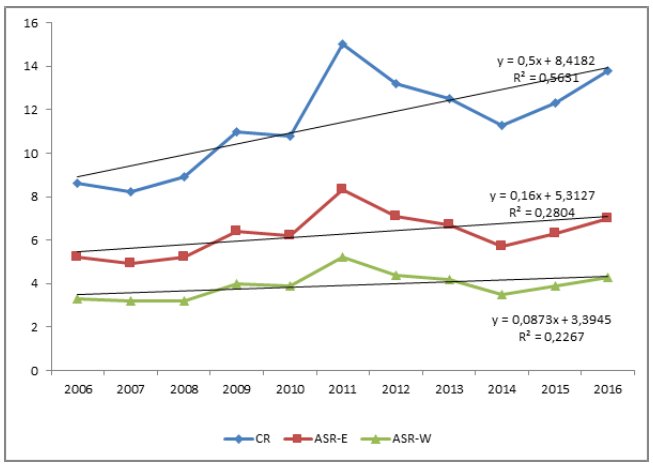
The one of the most common diseases in women was diabetes mellitus type 2, which as the growing public health problem in Serbia showed an increase in mortality rates in our observed period. At the same period, the incidence rate is decreasing (Graph 9, 10).

DISCUSSION

Special attention is paid to the health care of women in all organized health care systems due to the exceptional sensitivity of this population group to the effect of socio-economic conditions and other environmental factors. Women's health protection is an integral part of the health care system that is provided to the entire population (1, 2). In Serbia, at the primary level, the health care of women is provided by the health care services of women, general medicine and occupational medicine. Women's health protection is not always adequate in terms of organization, accessibility and working methods, where this problem is more pronounced in rural areas where health care is being implemented in a reduced amount and content compared to that offered in urban areas (1, 2). At least 35% of women in developing countries still do not have prenatal care, almost 50% are without professional help, and 70% do not have postpartum protection. On the contrary, healthcare is almost complete in developed countries (97% of women perform at least one antenatal visit, 99% are provided with professional help, and 90% have at least one postpartum visit) similar to ours (1, 2).



Graph 9. Standardized incidence rates of type 2 diabetes per 100.000 of population, women, Serbia, 2006-2016.



Graph 10. Standardized mortality rates of type 2 diabetes per 100.000 of population, women, Serbia, 2006-2016.

Feminization of the population carries a different burden on diseases and causes of death in the total population. Although mortality rates in males exceed those of women in all age categories, women tend to report poorer health. Women report health problems more often, but these conditions tend to be less serious and less lethal than those that men are more likely to suffer. Women on average live longer were creating the gap that is reflected in inequalities in health between men and women. Women's lifetime is accompanied by an increase in morbidity, and women's longevity contributes to their health disability. Structural gender inequalities in resource allocation, such as income, education, health care, nutrition and political voice, are strongly associated with poor health status and reduced well-being. Very often, this structural gender discrimination of women in many other areas has an indirect impact on the health status of women. For example, since women in many developing countries are unlikely to be part of an official labor market, they often do not have access to occupational safety and social protection, including access to health care (3, 4).

Data from many studies show that women report poorer health status because they face more obligations and higher demands within their social activities. Although women receive more social assistance, they have lower levels of experienced control and self-esteem. Therefore, women report lower levels of health as a result of the different roles that the gender are taking, differently perceiving symptoms and pathological processes, which often lead to an overestimation of morbidity in women. In line with the changes in the framework of women and men jobs in private and social life in relation to the past few decades, it is clear that there is a steady upward trend in the employment of women, more equal distribution of jobs to the gender. Although women generally have better health behaviors than men and are more interested in monitoring their health and their families' health, the health status of women in many countries is unsatisfactory. Violation of women's health can lead to serious consequences both on the personal, interpersonal and social level. By promoting healthy lifestyles, empowering women and their active participation in the community can have significant positive effects on raising the health potential of this population group (5).

Chronic non-communicable diseases are the leading causes of morbidity and mortality in the world. They account for two-thirds of all deaths in the world, and almost 80% of all deaths in developing countries. Diseases such as cardiovascular disease, diabetes mellitus, and malignant neoplasm were once considered to be a disease of wealth; they are now common even in low- and middle-income countries. The epidemiological transition that has led to the shift of disease patterns over time to the extent that infectious and parasitic diseases are gradually decreasing is a result of industrialization, urbanization, economic development and globalization of the market. All this has led to changes in nutrition and lifestyle that have a significant impact on the health status of the population of women, especially in developing countries. Non-communicable diseases are reaching epidemic proportions worldwide. They have become an important public health problem in many countries of the world, with significant prevalence in people of both gender (6, 7). The results of our research show that chronic non-communicable diseases occupy a significant place in illness and mortality in women in Serbia in the last 10 years, which is associated with negative socio-economic trends. Other studies also support the fact that the representation of chronic non-communicable diseases is high in the population of women in many countries of the world (8, 9, 10, 11). Worldwide, cervical and breast cancer is one of the most common causes of the morbidity and mortality of cancer in women (12, 13). High standardized rates of incidence and mortality are found in East, West and South Africa, South Central Asia, South America, Malaysia and Central Africa (14). Although incidence and mortality has been reduced in high-resource countries, the incidence of these diseases remains high in developing countries and low-resource countries due to lack of awareness and difficulties in carrying out the screening program (15, 16).

Regular screening is the most effective way to success in the reducing of the incidence and mortality from cervical cancer and breast. The most of developed countries use organized screening model in their health systems. However, the status of implementation is uneven (17, 18, 19).

Diabetes mellitus is a major public health problem that is approaching epidemic proportions globally (20). The global prevalence of diabetes is estimated to increase by about 2.2% per year. An estimated 46% of cases are undiagnosed (21). Each year 7 million people develop Diabetes and the most dramatic increases in type 2 Diabetes have occurred in populations where there have been rapid and major changes in lifestyle, demonstrating the important role played by lifestyle factors and the potential for reversing the global epidemic. The number of people with diabetes is increasing in both developed and developing countries, affluent and deprived populations, and people of all ages (22), which is also indicated by the data of our research.

Health and social policy and systems encounter the difficulties to ensure chronic diseases management. The measures like integrated care models and self-management programmers are seen as potential solutions (23, 24).

CONCLUSION

Chronic non-communicable diseases are the leading causes of woman morbidity and mortality, and the one of the main reasons for the use of health care in the population of women in our country. How it comes to preventable diseases, actions in the field of health promotion should be directed at preventing and modifying risk factors that are responsible for the occurrence of these diseases, as well as the creation of national strategies for the prevention and control, monitoring performance of screening and other preventive programs, planning, organization of health care and put the patients in the center of health system.

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BOTULINUM TOXIN A TREATMENT OF DELAYED FACIAL PALSY IN A RANDOMIZED TRIAL

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TRETMAN ODLOŽENE PARALIZE FACIJALISA BOTULINOM TOKSINOM A-RANDOMIZIRANA STUDIJA

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ABSTRACT

Introduction: Delayed facial palsy (DFP) is a common complication appearing ≥3 days after neurosurgery. In cases where glucocorticoids are contraindicated, other treatments are needed. Methods: The efficacy of BoNT-A injections was evaluated in patients with DFP after vestibular schwannoma resection. Patients received: Group I, BoNT-A (40–50 IU); Group II, prednisolone (1 mg/kg per day, 5–7 days); Group III, glucocorticoids with BoNT-A; Group IV, refused treatment. Functional efficacy was assessed. Results: Among 75 patients, pretreatment facial nerve dysfunction was mild, moderate, and moderate-to-severe in 48.0%, 33.3%, and 18.7%, respectively. One month post-treatment initiation, Group III had a significantly higher rate of facial symmetry normalization versus Groups II and IV ($P < 0.05$). After 3 months, complete recovery of facial nerve function was significantly higher in Groups I–III versus Group IV ($P < 0.05$). Conclusion: BoNT-A injections may be recommended for DFP treatment to attenuate facial asymmetry and improve functional recovery.

Keywords: *delayed facial palsy, botulinum toxin type A, house–brackmann scale, rehabilitation.*

SAŽETAK

Uvod: Odložena paraliza facijalnog nerva (OPF) je česta komplikacija koja se javlja ≥ 3 dana nakon neurohirurške intervencije. U slučajevima kada su glukokortikoidi kontraindikovani, potrebni su drugi tretmani. Metode: Efikasnost injekcija BOTX-A procenjena je kod pacijenata sa DFP nakon resekcije vestibularnog švanoma. Pacijenti su bili podeljeni na: I grupu, BOTX-A (40-50 IU); II grupa, prednizolon (1 mg/kg dnevno, 5-7 dana); III grupa, kombinovana primena glukokortikoida sa BOTX-A; IV grupa, odbila je lečenje. Procenjena je funkcionalna efikasnost svakog tretmana. Rezultati: Među 75 pacijenata, disfunkcija facijalnog nerva pre tretmana bila je blaga, umerena i umerena do teška kod 48,0%, 33,3% i 18,7%. Jednomesečno nakon započinjanja tretmana, III grupa je imala značajno veću stopu normalizacije simetrije lica u odnosu na II i IV grupu ($P <0,05$). Posle 3 meseca, potpuni oporavak funkcije facijalnog nerva bio je značajno veći u I, II i III grupi u odnosu na IV ($P <0,05$). Zaključak: Injekcije BOTX-A mogu se preporučiti za lečenje OPF-om radi ublažavanja asimetrije lica i poboljšanja funkcionalnog oporavka.

Ključne reči: *odložena paraliza lica, botulinum toksin tip A, house-brackamannova skala, rehabilitacija.*

ABBREVIATIONS

BoNT A - botulinum toxin type
ACGI - Clinical Global Impression
DFP - delayed facial palsy
FDI - Facial Disability Index
GC - glucocorticoid
HB - House–Brackmann [scale]
PF - Physical Function [subscale of the Facial Disability Scale]
SD - standard deviation
SWBF - Social/Well-Being Function [subscale of the Facial Disability Scale]
VSR - vestibular schwannoma resection

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INTRODUCTION

Facial nerve injury after cerebellopontine angle surgery, especially after vestibular schwannoma resection, remains a challenging problem that significantly decreases the quality of life of patients in the postoperative period (1).

Depending on the time of deterioration of facial nerve function, one can identify facial nerve palsy with early (immediately after the surgery) and late (delayed) onset (2-4). Delayed facial palsy (DFP) is less widely studied in scientific literature compared to acute facial nerve palsy. DFP is characterized by spontaneous palsy of mimic muscles in patients with intact facial nerve function during the early postoperative period [4].

According to current research data, the prevalence of DFP after vestibular schwannoma resection is 4.8–41% (3, 4), with a mean prevalence of 19% (2). There is significant variability in the definition of 'delayed facial neuropathy', making it difficult to compare published research data. Some authors diagnose DFP in all patients with any degree of facial nerve function deterioration emerging after the initial postoperative assessment (5), or between the initial postoperative assessment and follow-up assessment 3–5 days later (6). Others only consider patients with facial nerve function deterioration emerging several days after surgery (2, 4).

In contrast to idiopathic facial palsy (Bell's palsy), which is primarily associated with infection and inflammation, ischemia, or compression [7, 8], DFP pathophysiology in the postoperative period remains unclear. Deterioration of facial nerve function may be a result of several factors including perineural edema, vascular spasm, and reactivation of viral infection [6, 9-11]. The most probable causes are vascular spasm and local perineural edema (which are more pronounced compared to idiopathic neuropathy because of the close anatomic relationship between the acoustic-facial group of nerves and tumor, resulting in surgical injury during tumor resection), developing during the first few days after the intervention and described as postoperative (12, 13).

The etiology of DFP is largely unknown, but there is evidence to suggest that it may be related to viral reactivation or the surgical approach. The research literature indicates an elevation of antibody titer in patients who develop DFP after surgery to herpes simplex virus types 1 and 2 and also to the varicella-zoster virus (9, 11). Nevertheless, current preoperative algorithms (14) do not support routine prevention of viral infection reactivation. Current research focusing on the association of the surgical approach to the resection of vestibular schwannomas and the development of DFP has provided inconclusive results. Several small studies have failed to demonstrate a correlation between different surgical approaches and the development of DFP (3, 5). At the same time, some studies have shown a 2- to 3-fold increase of risk of deterioration of facial nerve function in the case of the retrosigmoid approach to vestibular schwannoma (2, 15). Some authors suggest that bone dust dispersion, resulting from insufficient irrigation in the posterior cranial fossa during grinding of the petrous pyramid and internal auditory canal during the translabyrinthine and retrosigmoid approaches, may lead to increased local inflammatory reaction and development of DFP (2). It is assumed that prolonged decompression of facial nerves in the translabyrinthine approach decreases the risk of postoperative neural edema (2). However, the choice of a surgical approach is rarely based on current research results, presuming that DFP treatment in > 85% of cases results in restoration of facial nerve function to grade I–II on the House–Brackmann (HB) facial paralysis scale (2, 16).

Large tumor size (Koos stage III–IV), tumor structure, and the relationship of the nerve with the tumor have been assessed as risk factors for DFP (2, 6). A large volume of resection is likely to be associated with more pronounced edema of surrounding tissues, and viral infection reactivation may also occur in the postoperative period (6).

At present, a standard of care for DFP is lacking, because the etiology of the condition is not fully understood. Many researchers have tried glucocorticoid (GC) therapy (17). However, the broad spectrum of adverse effects associated with GC treatment limits its use and has necessitated the search for additional highly effective and safe treatment strategies (18). Botulinum toxin type A (BoNT-A) has demonstrated efficacy in the treatment of acute facial nerve injury after neurosurgical interventions, which allows it to be considered as a therapeutic option for DFP (19).

This study was performed to evaluate the efficacy and safety of BoNT-A therapy in patients with DFP after vestibular schwannoma resection, including patients with medical contraindications for GC therapy.

METHODS

2.1. Study design

This single-center, open-label, rater-blinded, randomized study recruited patients with DFP developing ≥ 3 days after vestibular schwannoma resection in the Burdenko National Scientific and Practical Center for Neurosurgery (Moscow, Russia) from 2012 to 2017. The study period was 3 months.

Primary exclusion criteria included facial nerve paresis of nonsurgical etiology and complete rupture of the facial nerve during surgical resection of the tumor and noncompliance with the treatment regimen (including control visits).

The study protocol was approved by the local Ethical Committee of the N.N. Burdenko Research Institute of Neurosurgery, Moscow, Russia. All procedures complied with the standards established by the Declaration of Helsinki and written, informed consent was gathered from all patients.

Patients were assigned to 4 treatment groups as described below; those assigned to Groups II or III were randomized in a 1:1 ratio. Group I included patients who did not receive GC

therapy because of contraindications to GC treatment, and thus received injections of incobotulinumtoxinA (Xeomin®, Merz Pharmaceuticals GmbH, Frankfurt am Main, Germany) into mimic muscles on the intact side to decrease elevated muscle tone (which determines the level of traction of mimic muscles towards the intact side) and provide relative facial symmetry. Patients in this group received a single cycle of consecutive injections into mimic muscles on the intact side: m. frontalis (3 U per 2 injection points), m. corrugator supercilii (2–4 U per 2 injection points), m. levator labii superioris alaeque nasi (2 U), m. zygomaticus minor and major (2 U), m. levator labii superioris (2 U), m. levator anguli oris (2 U), m. orbicularis oris (1 injection point above the upper lip—1 U), m. depressor labii inferioris (1–2 U), m. mentalis (2 U) and m. platysma (4–5 U per 2–4 injection points). The total dose of incobotulinumtoxinA was 40–50 U per patient and the total number of injection points varied between 10 and 15.

Patients in Group II received oral prednisolone tablets soon after the development of facial palsy, 1 mg/kg/day, once daily for 5–7 days with a subsequent gradual tapering off and discontinuation over 3–4 days. Patients in Group III received GC treatment (as per Group II) combined with BoNT-A injections similar to Group I. Patients in Group IV refused any treatment and only visited the clinic for dynamic evaluation.

Koos classification was used for grading tumor size: (i) Koos Grade I: tumor involves only the internal auditory canal; extracranial diameter 1–10 mm; purely intracanalicular tumor limited to the internal auditory canal only; (ii) Koos Grade II: tumor causes widening of the internal auditory canal and extends into the cerebellopontine angle; diameter reaches 11–20 mm; < 2 cm extracanalicular/cerebellopontine angle extension without brainstem compression; (iii) Koos Grade III: tumor reaches brainstem, but does not cause compression; diameter reaches 21–30 mm; extracanalicular/cerebellopontine angle extension > 2 cm, with no brainstem compression; (iv) Koos Grade IV: tumor causes brainstem compression; diameter reaches > 30 mm; extracanalicular/cerebellopontine angle extension with any degree of brainstem compression.

2.2. Efficacy assessments

Treatment efficacy was assessed clinically. HB scale assessments were performed by an independent researcher who was blinded to the treatment administered to each patient. The severity of facial palsy was assessed using the HB scale at baseline, 1 month, and 3 months after treatment initiation (Grade 1: normal facial function; Grade 2: mild dysfunction; Grade 3: moderate weakness; Grade 4: moderate-to-severe weakness; Grade 5: severe weakness). At the end of the study, patients completed a Clinical Global Impression (CGI) scale. At baseline, 1 month and 3 months after treatment initiation, patients also completed the Facial Disability Index (FDI) self-assessment scale, which assesses physical function and social wellbeing on a combined scale from 0 (worst) to 200 (best).

2.3. Statistical analysis

Changes in mean HB scale scores were analyzed using 1-sample t-tests with no replacement of missing data. Comparison between groups for functional improvement was performed by student's t-test. A P-value of < 0.05 was considered significant. All statistical analyses were performed using SAS version 8.2 or later (SAS Institute, Cary, NC, USA).

2.4. Safety assessments

Safety assessments included the monitoring of adverse events based on patient-reported events at follow-up visits.

RESULTS

3.1. Patient baseline characteristics

Seventy-five patients, 31–64 years of age (mean age 48.47 ± 7.15 years), were recruited and assigned to treatment groups as follows: (i) Group I: 20 patients: 11 men (55.0%) and 9 women (45.0%), including patients who did not receive GC therapy because of the presence of gastric or duodenal ulcer (n = 5; 25.0%), severe chronic heart failure (n = 5; 25.0%), type 2 diabetes mellitus (n = 4; 20.0%) and stage III hypertension (n = 8; 40.0%); (ii) Group II: 16 patients: 7 men (43.8%) and 9 women (56.2%); (iii) Group III: 18 patients: 10 men (55.6%) and 8 women (44.4%); (iv) Group IV: 21 patients: 9 men (42.9%) and 12 women (57.1%).

During the study, an analysis was performed on the topographic or anatomic aspects of the facial nerve and tumor size in patients with DFP. In 43 patients (57.3%), tumor size was 21–30 mm; tumor sizes < 20 mm and > 30 mm were identified in 29 patients (38.7%) and 3 patients (4.0%), respectively. In 61 patients (81.3%), the facial nerve was situated along the anterior-inferior surface of the tumor and, in 54 patients (72.0%), it was difficult to separate the facial nerve from the surface of the tumor due to arachnoid commissures; in 32 patients (42.7%), the nerve could not be visualized as a result of significant thinning or stretching. In 48 patients (64.0%), oral tumor expansion was observed predominantly and, in most cases (54 patients, 72.0%), the tumor had an unclear border and a soft membrane. In 40 patients (53.3%), radical tumor resection was performed, which was accompanied by more active surgical manipulations near facial nerve structures and a higher degree of traumatization (Table 1).

DFP developed typically after ≥ 11 days (up to 23 days) after surgery (n = 40; 53.3%) and less often on Days 6–10 (n = 24; 32.0%) or Days 3–5 post-operation (n = 13; 17.3%) (Table 1). No differences between the studied groups in topographic or anatomic aspects of the facial nerve, tumor size, or time to postoperative development of mimic muscle palsy were observed.

Table 1. Baseline demographic and clinical characteristics

	Group I (n = 20)	Group II (n = 16)	Group III (n = 18)	Group IV (n = 21)
Mean ± SD age, years	50.3 ± 8.3	49.6 ± 7.5	52.3 ± 7.9	45.0 ± 7.3
Female, n (%)	9 (45.0)	9 (56.2)	8 (44.4)	12 (57.1)
Male, n (%)	11 (55.0)	7 (43.8)	10 (55.6)	9 (42.9)
Topographic and anatomic aspects of the facial nerve, n (%)				
Facial nerve location on anterior-inferior surface of tumor	15 (75.0)	15 (93.8)	14 (77.8)	17 (81.0)
Visual control of facial nerve location is impossible	9 (45.0)	7 (43.8)	8 (44.4)	8 (38.1)
Arachnoid commissures of the tumor with facial nerve	14 (70.0)	11 (68.8)	14 (77.8)	15 (71.4)
Oral direction of tumor growth relative to a normal axis of facial nerve	12 (60.0)	10 (62.5)	12 (66.7)	14 (66.7)
Surface of arachnoid dissection is unclear or absent	14 (70.0)	11 (68.8)	13 (72.2)	16 (76.2)
Radical tumor resection	10 (50.0)	9 (56.3)	10 (55.6)	11 (52.4)
Tumor size, n (%)				
11–20 mm (Koos Grade II)	7 (35.0)	7 (43.8)	7 (38.9)	8 (38.1)
21–30 mm (Koos Grade III)	12 (60.0)	9 (56.3)	10 (55.6)	12 (57.1)
> 30 mm (Koos Grade IV)	1 (5.0)	–	1 (5.6)	1 (4.8)
Time to development of mimic muscle palsy after surgical treatment, n (%)				
3–5 days	4 (20.0)	3 (18.8)	2 (11.1)	4 (19.0)
6–10 days	6 (30.0)	5 (31.3)	6 (33.3)	7 (33.3)
> 11 days	10 (50.0)	8 (50.0)	10 (55.6)	10 (47.6)

BoNT A - botulinum toxin type A,
GC - glucocorticoid,
SD - standard deviation,
Group I (n = 20) received BoNT-A injections,
Group II (n = 16) received oral prednisolone tablets,
Group III (n = 18) received combined GC and BoNT-A injections,
Group IV (n = 21) did not receive any treatment.

3.2. Efficacy after treatment

Before the start of treatment, facial nerve function HB scale score was 2 points in 36 patients (48.0%), 3 points in 25 patients (33.3%), and 4 points in 14 patients (18.7%). There was an improvement in facial nerve function in all groups 1 month after the start of treatment; however, a significantly higher rate of facial symmetry normalization was observed in patients receiving GC therapy combined with BoNT-A injections (Group III) compared to Groups II and IV ($P < 0.05$). Low-grade facial nerve dysfunction in Group III was observed in a significantly lower percentage of patients, compared to other groups ($P < 0.05$).

After 3 months of treatment, a significantly higher rate of complete recovery of facial symmetry on the HB scale score was observed in all groups on active treatment (Groups I–III), compared to Group IV ($P < 0.05$). A trend toward complete facial symmetry restoration was observed in patients receiving GC therapy combined with BoNT-A injections compared with BoNT-A alone or prednisolone, although the difference did not reach statistical significance (Table 2).

Table 2. Changes in facial asymmetry HB scale score

Group	Follow-up period	HB scale score				
		Grade 1 n (%)	Grade 2 n (%)	Grade 3 n (%)	Grade 4 n (%)	Mean (SD)
I (n = 20)	Baseline	–	9 (45.0)	7 (35.0)	4 (20.0)	2.75 (0.79)
	1 month post-treatment	12 (60.0)	5 (25.0)	3 (15.0)	–	1.55 (0.76)*
	3 months post-treatment	15 (75.0)	3 (15.0)	2 (10.0)	–	1.35 (0.67)*
II (n = 16)	Baseline	–	8 (50.0)	5 (31.3)	3 (18.7)	2.69 (0.79)
	1 month post-treatment	9 (56.2)	5 (31.3)	2 (12.5)	–	1.56 (0.73)†
	3 months post-treatment	14 (87.5)	2 (12.5)	–	–	1.13 (0.34)†
III (n = 18)	Baseline	–	8 (44.4)	6 (33.3)	4 (22.3)	2.78 (0.81)
	1 month post-treatment	16 (88.9)	2 (11.1)	–	–	1.11 (0.32)‡
	3 months post-treatment	17 (94.4)	1 (5.6)	–	–	1.01 (0.24)‡
IV (n = 21)	Baseline	–	11 (52.4)	7 (33.3)	3 (14.3)	2.62 (0.74)
	1 month post-treatment	6 (28.6)	6 (28.6)	6 (28.6)	3 (14.3)	2.29 (1.06)
	3 month post-treatment	8 (38.1)	5 (23.8)	5 (23.8)	3 (14.3)	2.14 (1.11)

BoNT A - botulinum toxin type A, GC - glucocorticoid, HB - House–Brackmann

The severity of facial palsy was assessed as follows: Grade 1, normal facial function; Grade 2, mild dysfunction; Grade 3, moderate weakness; Grade 4, moderate-to-severe weakness; Grade 5, severe weakness. Assessments were performed at baseline, 1 month, and 3 months after treatment initiation by an independent assessor who was blinded to the administered treatments.

Group I (n = 20) received BoNT-A injections; Group II (n = 16) received oral prednisolone tablets; Group III (n = 18) received combined GC and BoNT-A injections; Group IV (n = 21) did not receive any treatment.

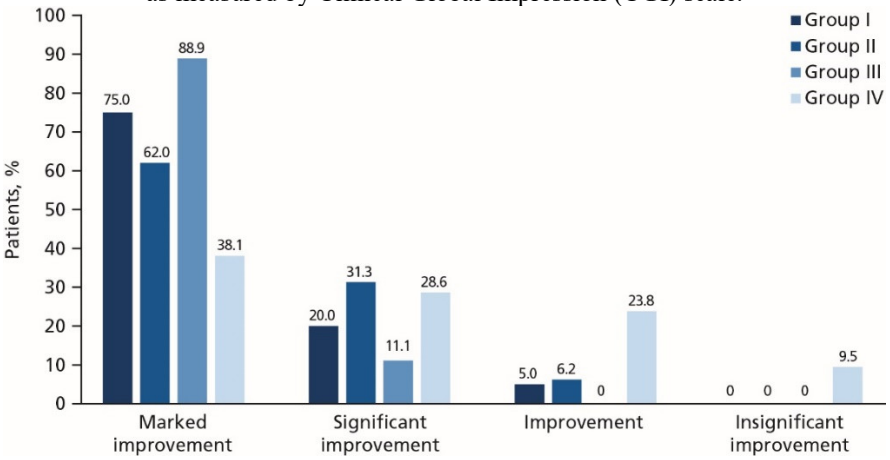
* $P < 0.05$ for Group I versus Group IV (two-tailed probability).

† $P < 0.05$—statistically significant difference between Groups II and IV.

‡ $P < 0.001$—statistically significant difference between Groups III and IV.

Most patients in Groups I–III were satisfied with the results of treatment and reported notable improvement of mimic muscle function as rated by CGI scale score. Some patients in Group IV reported an improvement, but the substantial improvement rate in that group was significantly lower than in Groups I–III ($P < 0.05$) (Fig. 1).

Figure 1. Improvement in facial symmetry score during treatment as measured by Clinical Global Impression (CGI) scale.



Patients completed the CGI assessment at the end of the study (3 months after treatment initiation). Group I (n = 20) received botulinum toxin type A (BoNT-A) injections; Group II (n = 16) received oral prednisolone tablets; Group III (n = 18) received combined glucocorticoid and BoNT-A injections; Group IV (n = 21) did not receive any treatment.

One month after the start of treatment, there was a significant improvement in physical and social functioning scores on the FDI scale in all groups; however, the mean (SD) FDI score in the Physical Function (PF) and Social/Well-Being Function (SWBF) subscales in Group III was higher than that in other groups ($P < 0.05$) (Table 3). Three months after the start of treatment, the mean (SD) FDI score in the PF and SWBF subscales in Group IV was lower than in other groups ($P < 0.05$) (Table 3).

Table 3. FDI scale scores.

Follow-up period	FDI subscale score, mean (SD)	Group I	Group II	Group III	Group IV
Baseline	PF	39.0 (10.9)	39.3 (9.4)	38.1 (11.9)	34.5 (11.4)
	SWBF	62.2 (9.0)	61.6 (6.5)	63.6 (7.9)	62.9 (5.9)
1 month post-treatment	PF	77.3 (8.0)	78.3 (6.7)	83.3 (5.9)[*,†]	66.9 (12.6)[‡,§,ǀ]
	SWBF	83.0 (6.1)	86.4 (7.2)	92.0 (4.9)[*,†]	79.0 (11.3)[ǀ]
3 months post-treatment	PF	91.5 (3.3)	89.7 (5.8)	91.4 (2.3)	75.2 (9.4)[‡,§,ǀ]
	SWBF	90.0 (5.6)	90.9 (6.1)	92.9 (2.6)	77.9 (11.4)[‡,§,ǀ]

BoNT-A - botulinum toxin type A, **FDI** - Facial Disability Index,
GC – glucocorticoid, **PF** - Physical Function,
SWBF - Social/Well-Being Function, **SD** - standard deviation

FDI self-assessment by patients rated PF and SWBF at baseline, 1 month, and 3 months after treatment initiation on a combined scale from 0 (worst) to 200 (best).

Group I (n = 20) received BoNT-A injections; Group II (n = 16) received oral prednisolone tablets; Group III (n = 18) received combined GC and BoNT-A injections; Group IV (n = 21) did not receive any treatment.

[*] $P < 0.05$ for Group I versus Group III.
[†] $P < 0.05$ for Group II versus Group III.
[‡] $P < 0.01$ for Group I versus Group IV.
[§] $P < 0.05$ for Group II versus Group IV.
[ǀ] $P < 0.05$ for Group III versus Group IV.
All two-tailed probability.

3.3. Safety

Adverse events associated with BoNT-A treatment were observed in 2 patients (10.0%) in Group I, including eyebrow ptosis (1 patient, 5.0%) and difficulty speaking as a result of mimic muscle weakness (1 patient, 5.0%), which resolved within 1 month. In Group III, 1 patient (5.6%) reported pain at the injection points. All adverse symptoms resolved without treatment and did not require intervention.

Treatment with GCs resulted in elevated blood pressure (135/90 mm Hg) in Group II after 2 days of treatment (1 patient, 6.25%) and elevated blood glucose on Day 9 of GC treatment during the dose-reduction period (1 patient, 6.25%). Patients in Group III reported epigastric discomfort (1 patient, 5.6%) and elevated blood glucose on Day 8 of GC treatment during the dose-reduction period (1 patient, 5.6%). In Groups II and III, elevated blood glucose resulted in GC treatment discontinuation, while other adverse symptoms resolved without treatment and did not require intervention.

DISCUSSION

The results reported in this study show that active treatment of DFP can result in significantly improved recovery compared with no treatment. After 1 month, BoNT-A injections combined with GC therapy showed significantly improved facial symmetry (HB scale score) compared with prednisolone, and improved physical and social functioning (FDI scale score) compared with all other groups. After 3 months, all active treatment groups showed improved HB, FDI and CGI scale scores compared with those receiving no treatment.

Large tumor size has been investigated as a potential factor for increased risk of DFP development after vestibular schwannoma resection [2, 6]. In the study reported here, 61.3% of patients had tumors of Koos Grade 3–4 and all had tumors of Koos Grade ≥ 2.

A retrospective cohort study demonstrated that patients who developed DFP were more likely to have undergone total resection compared with those without DFP (83% and 71%, respectively; odds ratio = 2.03, 95% confidence interval = 1.00–4.18, $P = 0.05$) [2]. In our study, total resection had been performed in 53.3% of patients with DFP.

Suitable therapy offers a favorable prognosis for functional recovery of DFP. One month after the development of facial nerve palsy, a complete or almost complete recovery can be generally achieved when timely and adequate treatment is given. Our results agree with published global literature: most patients can achieve full recovery of function [3, 4, 16].

The use of GC therapy is justified both in Bell's palsy [7, 8] and in facial nerve palsy developing as a result of surgical treatment of vestibular schwannoma to decrease inflammation and edema, but GC use may be limited by contraindications.

BoNT-A therapy is an effective method for the correction of mimic muscle tone, which is used in facial neuropathies of different origin [19, 20]. The unilateral decrease of mimic muscle tone results in a significant increase of antagonistic muscle traction on the intact side, which leads to a constant stretching of paretic muscles [19, 21]. Based on our previous research in patients with facial nerve palsy developing after neurosurgical operations, we postulated that BoNT-A injections on the intact side—as in patients with acute facial nerve palsy—provided prolonged relaxation of the mimic muscles on the hyperactive intact side in patients with DFP. This leads to improved facial symmetry at rest and during movement of expression, resulting in more rapid recovery of function in the weak muscles on the injured side. Muscle recovery is due to a decrease of muscle hyperactivity on the intact side and decrease of traumatization by traction and stretching of paretic muscles on the injured side [21]. During the current study, we performed BoNT-A injections, with or without GC therapy, in patients with DFP, demonstrating its efficacy in improving the facial symmetry and the interaction of mimic muscles on both the intact and injured sides. The combined use of BoNT-A and GC therapy promoted more rapid recovery of facial symmetry, compared to other treatment modalities.

The small sample size and lack of placebo control are the main limitations to our study. Also, the observers were not blinded to the treatments that patients had received. Future research could also offer a comparison of the effects of BoNT-A in patients with acute facial palsy.

CONCLUSION

This study was the first to demonstrate the efficacy and safety of BoNT-A injections in patients with DFP developing after vestibular schwannoma resection. BoNT-A therapy demonstrated results comparable to GC therapy (standard treatment). BoNT-A injections may be recommended as a treatment option for patients with DFP to increase facial symmetry and improve functional recovery in cases of limitation or contraindication for GC therapy. GC therapy in combination with BoNT-A injections promotes more rapid recovery of facial nerve function.

CONFLICT OF INTEREST AND SOURCES OF FUNDING

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DATA SHARING

The authors confirm that the data supporting the findings of this study are available within the article.

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OXID COMPARATIVE ANALYSIS OF THE SIGNIFICANCE OF BISAP AND MEWS SCORE FOR AN EARLY ASSESSMENT OF ILLNESS SEVERITY AND TREATMENT OUTCOME OF ACUTE PANCREATITIS

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UPOREDNA ANALIZA ZNAČAJA BISAP I MEWS SKORA ZA RANU PROCENU TEŽINE BOLESTI I ISHODA LEČENJA AKUTNOG PANKREATITISA

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ABSTRACT

The aim of this study was to determine the significance of the use of the BISAP score, which is specific for patients with AP, in relation to the application of the MEWS score that is important for assessing the condition of critically ill patients in intensive care units, but is not specific for patients with AP. The research was conducted as a cohort prospective study and included patients of both sexes, older than 18 and diagnosed with AP. BISAP and MEWS score were monitored at least at four time points: on admission to the hospital (zero), 48 hours, 72 hours and 7 days after admission to the hospital.

High levels of discrimination between patients with fatal outcome and cured patients are determined in both cases, with discrimination at MEWS being somewhat higher than BISAP score. The $BISAP_0$ had the best discrimination for BISAP score, AUROC (0.807) and also $MEWS_0$ for MEWS score, AUROC (0.899). In our research, the highest sensitivity was shown by $BISAP_{7d}$ (92.1%) and $MEWS_{48}$ (88.1%), and a high specificity of 87.5% had BISAP score, 48h, 72h and MEWS score at all four points of measurement.

BISAP score has a better prognostic value in relation to the form of pancreatitis, the development of complications and the outcome. However, the calculation of the MEWS score is based on monitoring the basic vital parameters so that its application is much simpler and does not require additional costs.

***Keywords**: pancreatitis acute, BISAP, MEWS.*

SAŽETAK

Cilj ovog rada bio je da se utvrdi značaj primene BISAP skora koji je specifičan za bolesnike sa AP u odnosu na primenu MEWS skora koji je važan za procenu stanja kritično obolelih bolesnika u JIM, ali nije specifičan za bolesnike sa AP.

Istraživanje je sprovedeno kao kohortna prospektivna studija u koju su uključeni bolesnici oba pola, stariji od 18 godina kod kojih je postavljena dijagnoza AP. BISAP MEWS skor su praćeni u najmanje 4 vremenske tačke: na prijemu-nultog dana, 48 i 72 sata i 7 dana nakon prijema u bolnicu.

Visoki stepen diskriminacije između pacijenata sa smrtnim ishodom i pacijenata koji su preživeli je utvrđen kod oba skora, pri čemu je diskriminacija kod MEWS-a nešto viših vrednosti u odnosu na BISAP. Za BISAP skor najbolju diskriminaciju daje $BISAP_0$, AUROC (0.807), a kod MEWS skora, $MEWS_0$, AUROC (0.899). U našem istraživanju, najveću senzitivnost su pokazali $BISAP_{7d}$ (92.1%) i $MEWS_{48}$ (88.1%), a visoku specifičnost 87,5% imali su BISAP skor, nultog dana, 48h, 72h i MEWS skor u sve četiri tačke merenja.

BISAP skor ima bolju prognostičku vrednost u odnosu na formu pankreatitisa, razvoj komplikacija i konačni ishod. Međutim, izračunavanje MEWS skora se zasniva na praćenju osnovnih vitalnih parametara tako da je njegova primena znatno jednostavnija i ne zahteva nikakve dodatne troškove.

***Ključne reči:** akutni pankreatitis, BISAP skor, MEWS skor.*

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INTRODUCTION

Acute pancreatitis (AP) is an inflammatory condition of the pancreas that can cause local injury, systemic inflammatory response syndrome, and organ failure (1).

On acute inflammatory process in the pancreas, the organism responds with an adaptive response which is marked as Systemic Inflammatory Response Syndrome – SIRS (2). In the initial phase, SIRS is characterized by increased immune function, hyperdynamic and hypermetabolic processes. The aim of these processes is to eliminate the causative agent, localize the process and provide sufficient amount of oxygen and nutritional substances necessary for tissue reparation and immune cell function. In parallel with the release of inflammatory mediators from different type of cells (monocytes, endothelial cells), they release anti-inflammatory mediators and lead to compensatory anti-inflammatory syndrome - CARS. At the same time, pro-coagulation and anti-coagulation systems are activated. Ideally, CARS and SIRS function to provide defense against an invasion of the pathogen. However, if the inflammatory response prevails, progressive endothelial damage, microcirculation vasodilatation and micro thrombi will occur. These changes occur in selected areas of all organs and lead to a progressive damage of their function and to multiple organ disfunction syndrome – MODS, which often has lethal outcome. About 30-50 % of patients who develop severe form of AP in the first 72 hours, despite the use of all available treatment options, die due to MODS development (2).The presence of SIRS during the early phase, in the first 24h, has high sensitivity for prediction of organ failure, but it does not have adequate specificity for severe forms of the disease (3). The sooner the AP is diagnosed and intensive treatment begins, there is greater chance to achieve reversal of organic insufficiency which significantly reduces the morbidity and mortality in these patients (4).

AP can be diagnosed if at least two of the following three conditions are met: abdominal pain, triple increase in pancreatic amylase levels in relation to the upper reference limit, positive CT findings, rarely magnetic resonance findings or transabdominal ultrasonography findings (5-9). The first symptom of the disease is most commonly acute development of persistent, intense epigastric pain propagating towards the back and is often accompanied by nausea and vomiting. However, this is not sufficient to set the diagnosis because these symptoms are non-specific and may indicate to a series of other illnesses. Setting the diagnosis significantly contributes to the increase in pancreatic enzymes (amylase/lipase) to values that are at least three times higher than the upper limit of the reference values. The increase in these enzymes usually occurs in the first 24 hours after the onset of pain (2). If severe abdominal pain (with or without irradiation in the back) indicates AP, but serum amylase/lipase values are not increased at least three times in relation to the reference values, it is necessary to do computerized tomography in order to confirm the diagnosis (4, 9).

In patients already diagnosed with AP, treatment, the prediction of complications and treatment outcome depend on an early assessment of the illness severity. According to the currently valid recommendations (1), the AP can be: a mild AP in which the fatal outcome is extremely rare, moderately severe AP that is characterized with transient insufficiency of an organ system and is associated with relatively low mortality and severe AP that is characterized by persistent organ failure and high mortality which is 36-50% according to various authors (10, 11, 12).

Laboratory analysis, diagnostic imaging and scoring systems can be used to predict the severity and outcome of the disease. Most score systems primarily provide early identification of organ failure considering that this is one of the most important predictors of the treatment outcome in patients with severe forms of AP (13).

Scoring systems specific to AP are: Ranson score, Pancreas score (Glasgow - Imrie Criteria for Severity of Acute Pancreatitis), BISAP (Bedside index of severity in acute pancreatitis), HAPS (Harmless Acute Pancreatitis Score), modified CTSI (Modified Computed Tomography Severity Index), Hong Kong criteria. There are some of the score systems which are used in intensive care units (ICU) for assessment of critically ill patients, but are not specific for AP: APACHE (Acute Physiology and Chronic Health Evaluation), SOFA (Sequential Organ Failure Assessment), MEWS (Modified Early Warning Score), etc. In our research, we decided to compare the significance of calculating BISAP and MEWS score.

BISAP score was defined in 2008 by Wu and co. for the assessment of illness severity and the prognosis of the risk of intra-hospital mortality in patients with AP. Patient with a score of 0-2 have <2% chance of lethal outcome. Patients with a score of 3-4 have >15% chance of death, and a score of 5 predicts mortality of 22%.

MEWS score is derived from the EWS score which was first applied in the UK (14). EWS score initially included 5 parameters, but later the diuresis measurement was added so that the EWS score was changed to the modified EWS or MEWS. Monitoring of this score is especially important for patients in the early postoperative period, as with all critically ill patients. The maximum score is 14. The value of the score ≥4 indicates that patient condition is getting worse. The value of the score >5 indicates that the chance of fatal outcome is about 30%.

The aim of this study is to determine the significance of the use of the BISAP score, which is specific for patients with AP, in relation to the application of the MEWS score that is important for assessing the condition of critically ill patients in intensive care units, but is not specific for patients with AP.

Based on this research, it will be possible to evaluate which score system is simpler and more objective for

assessing the condition of patients with AP, and which score system at different stages of treatment of patients with AP has the highest calibration and discrimination power, best shows the relationship between the predicted and the realized mortality rate.

MATERIAL AND METHODS

The research was conducted as a cohort prospective study in the period from 01/01/2016 until 31/12/2017 at the Clinical Hospital Center (KBC) Bezanijska kosa in Belgrade. It was approved by the competent Ethics Committee and the patients or their relatives signed an information form on the study. The study included patients of both sexes, older than 18 and diagnosed with AP. The study excluded pregnant women and patients who were translated into ICU KBC Bezanijska kosa from other hospitals after more than 48 hours since the onset of the disease. The parameters that were necessary for calculating the BISAP and MEWS scores are given in Table 1 and 2. For the calculation of these scoring systems, we used the calculators that can be found online on the site: www.mdcalc.com.

Table 1. Calculating BISAP score

Parameters	**Parameter value**	**Score**
B lood urea nitrogen	BUN>25 mg/dL (8.92 mmol/L)	1
I mpaired mental status	Impaired mental status: Disorientation, lethargy, coma or stupor	1
S IRS	Systemic inflammatory response syndrome ≥ 2 SIRS criteria	1
A ge	age > 60years	1
P leural effusion	pleural effusion present	1

Table 2. Calculating MEWS score

	3	2	1	0	1	2	3
AVPU Score	Unresponsive	Confused or agitated		Alert	Reactive to voice	Reactive to pain	Unresponsive
Respiratory rate	<8			8-20	21-30	31-35	>35
Heart rate	<40		40-50	51-100	101-110	11-130	>130
Systolic BP	<70	70-80	81-100	101-200		201-220	>220
Temperature	<34	34-35	35,1-36,0	36,1-37,9	38,0-38,5	38,6-40,0	>40
Pulse oksimetry	<85%	<90%					
Urin output		<20ml/2h or anuria 4 hours after admission	20-50ml/2h or anuria 4 hours after admission	>50ml/2h			

For BISAP score calculating it was needed to determine if the patient meets SIRS criteria based on having any of the following parameters: body temperature <36°C or >38°C, heart rate > 90/min, respiratory rate >20/min or $PaCO_2$<32mm Hg, WBC < 4000/mL or > 12000/mL or >10% immature (band) forms (15). If a patient has ≥2 parameters, it can be said that the patient meets SIRS criteria and gets 1 point for SIRS variable in BISAP score.

Scoring was conducted at several time points: on admission to the hospital (zero), 48 hours, 72 hours and 7 days after admission to the hospital. In all patients, after receiving hospitalization, a X- ray image of the lungs was performed and in the course of further treatment at the request of the physician. All obtained data were included in the forms specially prepared for this research. For all patients, the number of days spent in the hospital was recorded and for the patients who were accommodated in the ICU, the number of days spent in the ICU was recorded, and for those who needed respiratory support, the number of days spent on mechanical ventilation was recorded. Treatment complications (pleural effusion, sepsis, septic shock) and final outcome were also reported: hospital release or death.

All data obtained were statistically processed on a personal computer using standard statistical procedures and

purpose-built programs. The following methods of statistical processing were used: tabular and graphical representation of results, statistical testing using Student's t-test, χ^2 (hi-square), Mann-Whitney, Kruskal Wallis test, ANOVA procedure in variance analysis and Spearman coefficient of correlation of rank. Significance testing was carried out at $p < 0.05$, which is necessary and sufficient in the medical scientific and research work to make relevant conclusions. The IBM SPSS Statistics 22 statistical software package was used in statistical analysis.

RESULTS

Demographic and general characteristics of patients are shown in Table 3. The study included 50 patients, of whom 8 (16%) did not survive. The frequency of the full structure of subjects, the etiology, the frequency of patients being treated, and the incidence of necrosis did not significantly differ in survival according to statistics.

Table 3. Demographic and clinical characteristics of the respondents towards the final outcome (survivors/non-survivors)

	Total	Survivors	Non-survivors	p
Total number n (%)	50 (100.0)	42 (84%)	8 (16%)	
Sex				
Male, n(%)	26 (52.0)	22 (52.4)	4 (50.0)	0.902
Female, n(%)	24 (48.0)	20 (47.6)	4 (50.0)	
Age Group				
36-45, n(%)	6 (12.0)	6 (14.3)	0 (0)	
46-55, n(%)	7 (14.0)	5 (11.9)	2 (25.0)	
56-65, n(%)	8 (16.0)	8 (19.0)	0 (0)	
66-75, n(%)	21 (42.0)	17 (40.5)	4 (50.0)	
> 76, n(%)	8 (16.0)	6 (14.3)	2 (25.0)	
Etiology				
Gallstone	30 (60.0)	26 (61.9)	4 (50.0)	
Hyperlipidemia	8 (16.0)	6 (14.3)	2 (25.0)	
Alcohol	4 (8.0)	4 (9.5)	0 (0)	
Idiopathic	8 (16.0)	6 (14.3)	2 (25.0)	
Necrosis				
Yes, n(%)	24 (48.0)	20 (47.6)	4 (50.0)	0.902
No, n(%)	26 (52.0)	22 (52.4)	4 (50.0)	
Complications				
Without complication, n(%)	22 (44.0)	22 (52.4)	0 (0)	
Pleural effusion, n(%)	18 (36.0)	16 (38.1)	2 (25.0)	
Sepsis, Septic shock, n(%)	10 (20.0)	4 (9.5)	6 (75.0)	
Duration of MV, median (range)	0 (0-20)	0 (0-2)	4.5 (0-20)	<0.001*
Length of stay in ICU, median (range)	2 (0-20)	1.0 (0-7)	7.0 (0-20)	0.018*
Length of stay in hospital,median (range)	15 (6-62)	12.5 (6-62)	17.5 (9-27)	0.183
Severyt of AP				
Mild, n(%)	20 (40.0)	18 (42.9)	2 (25.0)	
Moderate, n(%)	20 (40.0)	19 (45.2)	1 (12.5)	
Severe, n(%)	10 (20.0)	5 (11.9)	5 (62.5)	

The incidence of sepsis and septic shock among the deceased is 75% and in the survivors 9.5%. Sepsis and septic shock are statistically more common in the group of non-surviving patients compared to a group of surviving patients (hi-square = 18.006, $p < 0.001$). The severe form of pancreatitis is statistically more common in patients with fatal outcome (hi-square = 10.752, $p = 0.001$).

The duration of MV as well as the length of stay in the ICU were statistically significantly higher in patients who died ($U = 45.0$, $p < 0.001$), ($U = 80.0$, $p = 0.018$). However, the length of stay in the hospital did not differ statistically between these two groups of subjects ($U = 118.0$, $p = 0.183$).

Subjects with a fatal outcome are significantly more common in severe forms of pancreatitis (hi-square = 10.752, p = 0.001).

In patients with fatal outcome, the values of the applied score systems were higher.

BISAP-5 and MEWS score > 5 with a predicted mortality rate of 22% and 30% monitored at all 4 time points are statistically more common in patients who did not survive (Table 4).

Table 4. The ratio of BISAP and MEWS scores in relation to the final outcome (survivors/non-survivors)

	Outcome		
	Total	Survivor	Non-survivors
Total number (predicted mortality rate %)	50 (100.0)	42 (84%)	8 (16%)
BISAP $_0$			
0-2 (<2%), n(%)	29 (58.0)	29 (69.0)	0 (0)
3-4 (>15%), n(%)	17 (34.0)	11 (26.2)	6 (75.0)
5 (22%), n(%)	4 (8.0)	2 (4.8)	2 (25.0)
BISAP $_{48}$			
0-2 (<2%), n(%)	29 (58.0)	29 (69.0)	0 (0)
3-4 (>15%), n(%)	11 (22.0)	9 (21.4)	2 (25.0)
5 (22%), n(%)	10 (20.0)	4 (9.5)	6 (75.0)
BISAP $_{72}$			
0-2 (<2%), n(%)	34 (68.0)	34 (81.0)	0 (0)
3-4 (>15%), n(%)	8 (16.0)	4 (9.5)	4 (50.0)
5 (22%), n(%)	8 (16.0)	4 (9.5)	4 (50.0)
BISAP $_{7d}$			
0-2 (<2%), n(%)	32 (69.9)	30 (78.9)	2 (25.0)
3-4 (>15%), n(%)	8 (17.4)	6 (15.8)	2 (25.0)
5 (22%), n(%)	6 (13.0)	2 (5.3)	4 (50.0)
MEWS $_0$			
0-2 (<7.9%), n(%)	30 (60.0)	30 (71.4)	0 (0)
3-4 (>12.7%), n(%)	12 (24.0)	10(23.8)	2 (25.0)
>5 (30%), n(%)	8 (16.0)	2 (4.8)	6 (75.0)
MEWS $_{48}$			
0-2 (<7.9%), n(%)	36 (72.0)	36 (85.7)	0 (0)
3-4 (>12.7%), n(%)	2 (4.0)	2 (4.8)	0 (0)
>5 (30%), n(%)	12 (24.0)	4 (9.5)	8 (100.0)
MEWS $_{72}$			
0-2 (<7.9%), n(%)	30 (60.0)	30 (71.4)	0 (0)
3-4 (>12.7%), n(%)	10 (20.0)	8 (19.0)	2 (25.0)
>5 (30%), n(%)	10 (20.0)	4 (9.5)	6 (75.0)
MEWS $_{7d}$			
0-2 (<7.9%), n(%)	30 (65.2)	30 (78.9)	0 (0)
3-4 (>12.7%), n(%)	10 (21.7)	6 (15.8)	4 (50.0)
>5 (30%), n(%)	6 (13.0)	2 (5.3)	4 (50.0)

However, the BISAP score determined at admission to the hospital - $BISAP_0$ even with lower values of 3-4 (with a predicted mortality rate of> 15%) was statistically more common in patients who did not survive, which is not the case with the MEWS score, likewise determined at the admission to the hospital - MEWS0. $BISAP_{72}$ value of 0-2 (<2%) was statistically more common in patients who survived (hi-square = 16.689, p <0.001)

The value of $BISAP_0$ and $MEWS_0$ has no relevance in relation to the form of pancreatitis. BISAP and MEWS value >5 determined for 48, 72 hours and 7 days after admission >5 (22% and 30%) is significantly more common in severe AP form (Table 5).

Table 5. Relation of BISAP and MEWS in relation to form of AP

	Severity of AP		
	Mild	Moderate	Severe
Total number n (predicted mortality rate %)	20 (40.0)	20 (40.0)	10 (20.0)
BISAP $_0$			
0-2 (<2%), n(%)	13 (65.0)	12 (60.0)	4 (40.0)
3-4 (>15%), n(%)	6 (30.0)	7 (35.0)	4 (40.0)
5 (22%), n(%)	1 (5.0)	1 (5.0)	2 (20.0)
BISAP $_{48}$			
0-2 (<2%), n(%)	13 (65.0)	14 (70.0)	2 (20.0)
3-4 (>15%), n(%)	5 (25.0)	3 (15.0)	3 (30.0)
5 (22%), n(%)	2 (10.0)	3 (15.0)	5 (50.0)
BISAP $_{72}$			
0-2 (<2%), n(%)	14 (70.0)	17 (85.0)	3 (30.0)
3-4 (>15%), n(%)	5 (25.0)	0 (0)	3 (30.0)
5 (22%), n(%)	1 (5.0)	3 (15.0)	4 (40.0)
BISAP $_{7d}$			
0-2 (<2%), n(%)	13 (72.2)	15 (83.3)	4 (40.0)
3-4 (>15%), n(%)	5 (27.8)	1 (5.6)	2 (20.0)
5 (22%), n(%)	0 (0)	2 (11.1)	4 (40.0)
MEWS $_0$			
0-2 (<7.9%), n(%)	14 (70.0)	12 (60.0)	4 (40.0)
3-4 (>12.7%), n(%)	4 (20.0)	6 (30.0)	2 (20.0)
>5 (30%), n(%)	2 (10.0)	2 (10.0)	4 (40.0)
MEWS $_{48}$			
0-2 (<7.9%), n(%)	16 (80.0)	16 (80.0)	4 (40.0)
3-4 (>12.7%), n(%)	1 (5.0)	1 (5.0)	0 (0)
>5 (30%), n(%)	3 (15.0)	3 (15.0)	6 (60.0)
MEWS $_{72}$			
0-2 (<7.9%), n(%)	12 (60.0)	15 (75.0)	3 (30.0)
3-4 (>12.7%), n(%)	6 (30.0)	2 (10.0)	2 (20.0)
>5 (30%), n(%)	2 (10.0)	3 (15.0)	5 (50.0)
MEWS $_{7d}$			
0-2 (<7.9%), n(%)	13 (72.2)	14 (77.8)	3 (30.0)
3-4 (>12.7%), n(%)	5 (27.8)	2 (11.1)	3 (30.0)
>5 (30%), n(%)	0 (0)	2 (11.1)	4 (40.0)

The BISAP-5 and MEWS score of >5 (22% and 30%), determined per day, was most common in sepsis and septic shock. On the other hand, the BISAP and MEWS 0-2 values (with a predicted mortality rate <2% and <7.9%) are more frequent in patients who did not have complications (Table 6).

Table 6. BISAP and MEWS ratio in relation to complications.

	Complications		
	Without complica-tions	Pleural effusion	Sepsis, septic shock
Total number n (%) (predicted mortality rate %)	20 (40.0)	20 (40.0)	10 (20.0)
BISAP $_0$			
0-2 (<2%), n(%)	19 (86.4)	10 (55.6)	0 (0)
3-4 (>15%), n(%)	3 (13.6)	8 (44.4)	6 (60.0)
5 (22%), n(%)	0 (0)	0 (0)	4 (40.)

	Complications		
	Without complications	Pleural effusion	Sepsis, septic shock
BISAP $_{48}$			
0-2 (<2%), n(%)	17 (77.3)	12 (66.7)	0 (0)
3-4 (>15%), n(%)	5 (22.7)	6 (33.3)	0 (0)
5 (22%), n(%)	0 (0)	0 (0)	10 (100.0)
BISAP $_{72}$			
0-2 (<2%), n(%)	18 (81.8)	16 (88.9)	0 (0)
3-4 (>15%), n(%)	4 (18.2)	2 (11.1)	2 (20.0)
5 (22%), n(%)	0 (0)	0 (0)	8 (80.0)
BISAP $_{7d}$			
0-2 (<2%), n(%)	15 (78.9)	17 (100.0)	0 (0)
3-4 (>15%), n(%)	4 (21.1)	0 (0)	4 (40.0)
5 (22%), n(%)	0 (0)	0 (0)	6 (60.0)
MEWS $_{0}$			
0-2 (<7.9%), n(%)	19 (86.4)	11 (61.1)	0 (0)
3-4 (>12.7%), n(%)	3 (13.6)	5 (27.8)	4 (40.0)
>5 (30%), n(%)	0 (0)	2 (11.1)	6 (60.0)
MEWS $_{48}$			
0-2 (<7.9%), n(%)	22 (100.0)	14 (77.8)	0 (0)
3-4 (>12.7%), n(%)	0 (0)	2 (11.1)	0 (0)
>5 (30%), n(%)	0 (0)	2 (11.1)	100 (100.0)
MEWS $_{72}$			
0-2 (<7.9%), n(%)	16 (72.7)	14 (77.8)	0 (0)
3-4 (>12.7%), n(%)	6 (27.3)	2 (11.1)	2 (20.0)
>5 (30%), n(%)	0 (0)	2 (11.1)	8 (80.0)
MEWS $_{7d}$			
0-2 (<7.9%), n(%)	17 (89.5)	13 (76.5)	0 (0)
3-4 (>12.7%), n(%)	2 (10.5)	4 (23.5)	4 (40.0)
>5 (30%), n(%)	0 (0)	0 (0)	6 (60.0)

There is a statistically significant positive correlation between CRP_0 and $BISAP_0$ ($r = 0.386$, $p = 0.006$). Between PCT_0 and both scoring systems there is statistically significant positive correlation - PCT_0 and BISAP $_0$ ($r = 0.537$, $p < 0.001$) and PCT_0 and MEWS $_0$ ($r = 0.490$, $p < 0.001$).

Between CRP_{48} and $BISAP_{48}$, there is statistically significant positive correlation ($r = 0.368$, $p = 0.008$). Between PCT_{48} and both scoring systems there is statistically significant positive correlation - PCT_{48} and BISAP $_{48}$ ($r = 0.682$, $p < 0.001$) and PCT_{48} and $MEWS_{48}$ ($r = 0.734$, $p < 0.001$).

Between CRP_{72} and $BISAP_{72}$ there is statistically significant association ($r = 0.291$, $p = 0.040$), while values of CRP_{72} and $MEWS_{72}$ are not statistically significantly related ($r = 0.241$, $p = 0.092$).

PCT_{72} values have a statistically significant association with $BISAP_{72}$ ($r = 0.572$, $p < 0.001$) as well as with $MEWS_{72}$ ($r = 0.474$, $p = 0.001$).

There is a statistically significant relationship between CRP_{7d} and $BISAP_{7d}$ values ($r = 0.406$, $p = 0.005$), while the values of CRP_{7d} and $MEWS_{7d}$ are not statistically significantly related ($r = 0.289$, $p = 0.051$), although this is close to statistical significance $p = 0.051$.

PCT_{7d} values have a statistically significant association with $BISAP_{7d}$ ($r = 0.830$, $p < 0.001$) as well as with $MEWS_{7d}$ ($r = 0.778$, $p < 0.001$).

Values of PCT determined by days have a statistically significant positive association with both BISAP and MEWS. However, the CRP determined by days is statistically significantly related to BISAP but not with the MEWS score (Table 7).

Between the severity of pancreatitis and duration of MV there is a statistically significant positive correlation ($r = 0.318$, $p = 0.024$) same as between the severity of pancreatitis and the length of stay in ICU ($r = 0.285$, $p = 0.044$) (Table 8). Conclusion: patient with more severe form of AP spent more days on MV and treatment lasted longer.

High levels of discrimination between patients with fatal outcome and cured patients are determined in both cases, with discrimination at MEWS being somewhat higher than BISAP score. For the BISAP score, $BISAP_0$, AUROC (0.807) is best discriminated, and at MEWS, $MEWS_0$, AUROC (0.899) (Table 9).

Table 7. Correlation between CRP, PCT, MEWS and BISAP per days.

Parameters	Scoring system	Number	r	p
CRP_0	$MEWS_0$	50	0.207	0.149
	$BISAP_0$	50	0.386	0.006*
PCT_0	$MEWS_0$	50	0.490	<0.001*
	$BISAP_0$	50	0.537	<0.001*
CRP_{48}	$MEWS_{48}$	50	0.190	0.187
	$BISAP_{48}$	50	0.368	0.008*
PCT_{48}	$MEWS_{48}$	50	0.734	<0.001*
	$BISAP_{48}$	50	0.682	<0.001*
CRP_{72}	$MEWS_{72}$	50	0.241	0.092
	$BISAP_{72}$	50	0.291	0.040*
PCT_{72}	$MEWS_{72}$	50	0.474	0.001*
	$BISAP_{72}$	50	0.572	<0.001*
CRP_{7d}	$MEWS_{7d}$	50	0.289	0.051
	$BISAP_{7d}$	50	0.406	0.005*
PCT_{7d}	$MEWS_{7d}$	50	0.778	<0.001*
	$BISAP_{7d}$	50	0.830	<0.001*

Table 8. Correlation between forms of pancreatitis and MV duration and duration of treatment in ICU.

Parameters	Correlation	Form of pancreatitis
Duration of MV	r	0.318
	p	0.024*
	n	50
Length of stay in the ICU	r	0.285
	p	0.044*
	n	50

Spearman's correlation coefficient (r) was calculated, and significant relationships were marked (*).

Table 9. Area under curve (AUROC) for evaluating the discrimination of the BISAP and MEWS

	AUROC	95%CI	Cut-off	Sensitivity (%)	Specificity (%)
$BISAP_0$	0.807	0.670-0.905	≤2.0	69.0	87.5
$BISAP_{48}$	0.789	0.650-0.891	≤2.0	69.0	87.5
$BISAP_{72}$	0.780	0.640-0.885	≤2.0	78.6	87.5
$BISAP_{7d}$	0.783	0.637-0.891	≤3.0	92.1	62.5
$MEWS_0$	0.899	0.780-0.966	≤3.0	83.3	87.5
$MEWS_{48}$	0.872	0.747-0.950	≤3.0	88.1	87.5
$MEWS_{72}$	0.854	0.726-0.938	≤3.0	83.3	87.5
$MEWS_{7d}$	0.867	0.734-0.949	≤3.0	86.8	87.5

DISSCUSION

Acute pancreatitis is an acute inflammatory process that can clinically be manifested from a mild form with localized inflammation to a severe form of the disease that affects distant organ systems (16). In the United States, AP is a leading cause of inpatient care among gastrointestinal conditions: >275,000 patients are hospitalized for AP annually, at an aggregate cost of >$2.6 billion per year. The incidence of AP ranges from 5 to 30 cases per 100,000, and there is evidence that the incidence has been rising in recent years (1) and fatal outcome occurs in 2 to 10% of patients with AP, depending on the severity of AP (17). In our study, mortality was 16%. Respondents with a fatal outcome are statistically more common in severe forms of pancreatitis. The incidence of severe form of pancreatitis in survivor patients is 12%, and in non-surviving patients is 62.5%. Of the total number of subjects with severe AP, the death rate was recorded in 50% (10/5).

As for the gender and age structure of our patients, it does not significantly affect the survival. Men were slightly more affected (52/48%) and most often belonged to a group of over 65 years of age. According to data from the literature men more frequently suffer from AP. In the prospect study of Toh and associates, the ratio was 1.3 and in Kumar 1.4 (18, 19). In the mentioned study of Kumar and associates from 2017, the respondents belonged to the younger age group, between 40 and 50 years old, while in the study of Toouli and associates the subjects were slightly older (40-60 years), which is similar to our data (20).

Early identification of patients who develop a severe form of pancreatitis would allow early onset of intensive treatment of such patients and a better outcome prognosis outcome (16). A large number of numerical scoring systems were designed back for several decades in order to anticipate the severity of AP and monitor this disease. The oldest - Ranson score was released in 1974 (21). After that, APACHE II score, BISAP and Pancreas score (Glasgow-Imrie Criteria for Severity of Acute Pancreatitis) were designed. Although none of these scores applied alone can predict with certainty the development of organic insufficiency in the AP, their importance is significant for the early identification of potentially severe forms of AP and early onset of intensive treatment. In our study, we compared the importance of the application of the BISAP score that is specific for patients with AP in relation to the application of the MEWS score that is important for assessing the condition of critically ill patients in ICU but not specific for patients with AP. In patients who did not survive, higher values of both score systems (BISAP-5 and MEWS> 5) were obtained, followed by hospital admission, followed by 48 h, 72 h and after 7 days after admission. The higher values of scoring systems predict a worse outcome. The MEWS ≥3 values on admission to hospital and in the next 2 days indicate the development of SIRS and poorer prognosis, which is the development of a severe form of AP (22).

However, the $BISAP_0$3-4 value (with a predicted mortality rate of> 15%) was statistically more common in patients who died, which is not the case with the MEWS values also determined at the admission. The significance of the scoring system for assessing the severity of AP and predicting the outcome of treatment for these patients is growing. The study of 2015 APACHE II is more important than other scoring systems or CRPs, although the differences are not statistically significant (23). In the paper of Joon Hyun in 2015, the values of AUROC for Ranson, BISAP, APACHE-II score and CRP_{24} were: 0.69 (95% CI: 0.62-0.76), 0.74 (95% CI: 0.66-0.80), 0.78 (95% CI: 0.70-0.84) and 0.68 (95% CI: 0.57-0.78). The AUROCA values in our study showed a high degree of discrimination between patients who did not survive and those who survived, with discrimination at MEWS slightly higher than BISAP score. The best disinfection for the BISAP score is $BISAP_0$ with AUROC 0.807 (95% CI: 0.670-0.905) and at MEWS of the peak $MEWS_0$ with AUROC 0.899 (95% CI: 0.780-0.966). The significance of BISAP score in relation to other scoring systems has been proven in earlier studies. Singh et al. have showed that BISAP is equivalent to APACHE II scoring in predicting mortality of patients with AP (24).

$BISAP_0$ and $MEWS_0$ have no relevance to AP weight. The BISAP-5 and MEWS score of 48, 72 hours, and 7 days after admission> 5 (22% and 30%) is significantly more common in severe pancreatitis. A recent study by Chinese authors who have been able to create an AP-based prediction model based on BISAP, MEWS and routine test indices is interesting. Multivariable logistic regression analysis showed that BISAP and serum Ca2 + are independent severity prediction factors for AP, and MEWS is not. However, the model that represents the combination of BISAP and serum Ca2 + is significantly better than their individual application in the assessment of the severity of AP. This model is simple and convenient for clinical use (25).

A group of English authors published a study in 2017 that included 629 patients with diagnosed AP (26). They compared EWS with other multifactorial scoring systems specific for pancreatitis and laboratory analysis in the first three days of hospitalization. Early Warning Score (EWS) has been shown to be highly statistically significant over all three days, compared to the form of pancreatitis and survival. It was also the best predictor of negative outcomes among all clinical and laboratory variables with AUROC values of 0.81, 0.84 and 0.83 for days 1, 2 and 3, respectively. It showed slightly more inferior in predicting the severity of pancreatitis compared to APACHE II. The multivariable logistic regression analysis showed that EWS and low lymphocytes are the dominant factors that are independently related both to the severity of pancreatitis and to the outcome. EWS ≥2 in all three days showed dominance. Univariate logistic regression analysis of all scoring systems determined in the first three days showed high significance both with the severity of pancreatitis and with an outcome, but without any dominance. In our research, only MEWS >5 proved to be statistically significant in relation to the form of pancreatitis and survival.

The incidence of sepsis and septic shock in our study with the deceased is 75% and 9.5% in survivors. Sepsis and septic shock are statistically more common in the group of non-surviving patients compared with a group of surviving patients. The severe form of pancreatitis is statistically more common in patients who did not survive.

Early onset of SIRS and MOF (multi-organ failure) during AP indicate a potentially serious illness and a bad prognosis (27). In fact, this means that morbidity and mortality at an early stage of AP are associated with systemic inflammatory response and persistent organic disorder, and not with local complications (23).

EWS, as we have said, represents an acute inflammatory response, and as such recognizes the severity of SIRS in AP. This is directly related to an increased risk of adverse outcome (28).

In our study, the value of BISAP-5 and MEWS> 5 (with a predicted mortality rate of 22% and 30%) determined by days was most common in sepsis and septic shock. While the values of the scoring system of 0-2 (with a predicted mortality rate <2% and <7.9%) were more frequent in patients who did not have complications.

Multi-organ insufficiency (MODS) is the most severe complication in the study of Suppiah and associates from St James's University Hospital (The Pancreatic Unit) with the highest rate of mortality. Other causes of death include cholangitis, pneumonia, pancreatic necrosis with cardiac failure and abscesses in psoas muscle. Interesting is the fact that MEWS in patients with abscess was the first 3 days of hospitalization was low, but then there was a development of pneumonia and rapid deterioration. In patients with MODS, the $MEWS_0$ value was 2 and then the condition worsened, the third day there was a development of respiratory distress syndrome (ARDS) that was pre-graded in MODS. The mortality was 4.2% (22) while in our study it was significantly higher and amounted to 16%.

EWS is used by many medical centers. In many countries, it is working to define specific, national scores (NEWS). In the UK, the use of NEWS enabled the prediction of cardiac arrest, admission to ICU and mortality (29).

In our study, patients who died on the MV spent an average of 4.5 days and in ICU an average of 7 days, which is a statistically significant difference compared to survivors. However, the length of stay in hospital does not differ statistically between these two groups of subjects. Between the severity of pancreatitis and duration of MV and length of stay in ICU, there is statistically significant positive correlation in the following way: the greater the severity of pancreatitis, the longer the MV and the treatment in ICU were.

PCT values determined per day have a statistically significant positive correlation with BISAP and MEWS scores. However, the CRP determined by days is statistically significantly related to BISAP scores, but not with MEWS. A large number of studies assessed the role of PCT, and compared it with other inflammatory markers, in assessing the severity of AP, the final outcome, and the development of infectious necrosis (30, 31, 32, 33).

A recent study by Kim et al. from 2013 concluded that the PCT of 0.5ng/ml has a sensitivity and a specificity of only 87% and 24%. BISAP score ≥ 2 has high sensitivity and specificity (79% and 89%). This means that the PCT value at the reception in patients with AP does not predict a precise progression of the disease as opposed to BISAP score that show a significantly better correlation. The modified Glasgow score ≥ 3 and APACHE II ≥ 7 show lower sensitivity and specificity, similar to PCT (34).

In our research, the highest sensitivity was shown by $BISAP_{7d}$ (92.1%) and $MEWS_{48}$ (88.1%), and a high specificity of 87.5% had BISAP score, 48h, 72h and MEWS score at all four points of measurement.

However, some studies have shown that PCT has a better statistical significance in assessing the severity of AP and the final outcome compared to clinical scoring systems. A two-year study of Nepalese authors published in 2017, made on 135 subjects diagnosed with AP, proves that the increased value of PCT serves as a promising simple biomarker predicting the severity of AP with better accuracy compared to other scoring systems (19). The PCT serum value showed a slightly higher accuracy (AUC: 0.887, CI: 0.825-0.948) compared to CRP (AUC: 0.717, CI: 0.628-0.8.7) in predicting the severity of AP. However, both parameters showed statistical significance in the assessment of the severity of AP ($p < 0.001$).

The role of CRP in severity assessment and the course of the disease has been investigated many times. C-reactive protein is one of the most important indication of inflammation. In patients with AP elevated CRP levels may indicate the existence of pancreatic necrosis. Plasma CRP values greater than 150 mg/L in the first 72 hours of the onset of the disease are correlated with the presence of necrosis with sensitivity and specificity greater than 80%. However, given that the peak rise of CRP is registered 36-72 h after admission, this test is not helpful in assessing the severity of the disease at the reception (5). This also explains our results according to which CRP is statistically related to BISAP, but not to MEWS score.

CONCLUSION

For the positive outcome of the treatment of patients with AP, it is crucial to early assess the severity of the condition of the patients and timely apply adequate therapy. For this purpose, a number of different scoring systems have been designed, some of which are specific for AP patients such as the BISAP score and some that are applicable to all critical illnesses such as, for example, MEWS score. In our study, we have shown that in both of these scoring systems, they are

simple to calculate and do not require the carrying out of expensive hematological, biochemical, radiological or other tests, so that their calculation does not increase the cost of treatment. The application of these scores is feasible in our conditions, but requires staff to be trained and, first and foremost, to keep the medical records properly.

BISAP score has a better prognostic value in relation to the form of pancreatitis, the development of complications and the final outcome. However, the calculation of the MEWS score is based on monitoring the basic vital parameters so that its application is much simpler and does not require additional costs.

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CHARACTERISTICS OF SPEECH AND VOICE AS PREDICTORS OF THE QUALITY OF COMMUNICATION IN ADULTS WITH HYPOKINETIC DYSARTHRIA

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KARAKTERISTIKE GOVORA I GLASA KAO PREDIKTORI KVALITETA KOMUNIKACIJE KOD ODRASLIH OSOBA SA HIPOKINETIČKOM DIZARTRIJOM

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ABSTRACT

Hypokinetic dysarthria is characterized by a speech that gradually becomes monotonous, poorly modulated, quiet and ultimately unintelligible. The goal of this research is to determine the acoustic characteristics of voice and speech in adults with hypokinetic dysarthria and the impact of the altered voice on the quality of communication. The sample consisted of 30 elderly respondents of both genders with Parkinson's disease and hypokinetic dysarthria. In order to conduct a spectral analysis, the voice of patients was recorded while they were reading phonetically balanced text. The respondents conducted a self-assessment of the degree of their own handicap caused by voice disorder and impact of the voice handicap by completing the Voice Handicap Index (VHI). Statistically significant differences were determined in the position of some formants in respondents compared to the values of formants in typical speakers for the following vowels: F1 of the vowel /I/ and F2 of the vowels /E/, /I/, /O/ and /U/. By examining the relation between the score achieved on the VHI instrument and the value of formants, the only statistically significant correlation was achieved between the formant F1 of the vowel /A/ and functional and emotional subscale. By regression analysis used to determine the predictor of the quality of communication, it was confirmed that F1 of the vowel /A/ has a statistically significant contribution to the explanation of the score achieved on functional and emotional subscale, by explaining 15% of the functional subscale (Beta=-0,393 (11,30 – 47,37)) and 10% of the emotional subscale (Beta=-0,363 (-0,052 – 0,000)).

Keywords: hypokinetic dysarthria, spectral analysis, quality of communication.

SAŽETAK

Hipokinetičku dizartriju karakteriše govor koji vremenom postaje monoton, slabo moduliran, tih i na kraju nerazumljiv. Cilj ovog istraživanja je utvrđivanje akustičkih karakteristika glasa i govora kod odraslih osoba sa hipokinetičkom dizartrijom i uticaja izmenjenog glasa na kvalitet komunikacije. Uzorak je činilo 30 odraslih ispitanika oba pola koji imaju Parkinsonovu bolest i hipokinetičku dizartriju. Kako bi se izvršila spektralna analiza glasa sniman je govor pacijenta tokom čitanja fonemski izbalansiranog teksta. Samoprocenu stepena sopstvenog hendikepa izazvanog poremećajem glasa i uticaja glasovnog oštećenja na kvalitet komunikacije ispitanici su vršili popunjava-njem Indeksa glasovnog oštećenja (VHI). Utvrđene su statistički značajne razlike u položaju pojedinih formanata kod ispitanika u odnosu na vrednosti formanata tipičnih govornika i to za sledeće glasove: F1 vokala /I/ i F2 vokala /E/, /I/, /O/ i /U/. Ispitivanjem veze između skora na VHI instrumentu i vrednosti formanata (F1 i F2) jedina statistički značajna pove-zanost ostvarena je između formanta F1 glasa A i funkcionalne i emocionalne supskale. Regresionom analizom korišćenom za utvrđivanje prediktora kvaliteta komunikacije potvrđeno je da F1 glasa A statistički značajno doprinosi objašnjenju skora dobijenom na funkcionalnoj i emocionalnoj supskali, objašnjavajući 15% funkcionalne supskale (Beta=-0,393 (11,30 – 47,37)) i 10% emocionalne supskale (Beta=-0,363 (-0,052 – 0,000)).

Ključne reči: *hipokinetička dizartrija, spektralna analiza, kvalitet komunikacije.*

ABBREVIATIONS

VHI - Voice Handicap Index
F1, F2 - the first and the second vowel formants

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INRODUCTION

Acquired dysarthria is a neurologically conditioned motor speech disorder caused by abnormalities in power, strength, range, speed, control, precision of movement and tonus of muscles which are involved in the realization of respiration, phonation, resonance, articulation and prosody during the speech production (1). As such, dysarthria represents a multidimensional disorder which can affect all above stated aspects of speech or just some components of the speech production process (2). Dysarthria includes many voice, speech and speech fluency related problems, what undermines the intelligibility of speech and affects the success of communication, as well as psychosocial functioning of an individual (3, 4).

Hypokinetic dysarthria is present in individuals with Parkinson's disease, and it is believed that voice is most often the first affected speech component (5). Also, the prevalence of voice disorder in this population is very high (6). The main symptoms of hypokinetic dysarthria are: irregular breathing in speech, inadequate articulation, slow, slurred speech ending with unintelligible murmur, short phonation in which a person is not able to pronounce multisyllabic words and shorter sentences within one expiration without interruptions, as well as a sobbing, monotonous voice (7).

Graphic segmentation of speech into the basic acoustic elements is performed by a spectrographic analysis. The speech segment is parsed into different frequencies by a series of electronic filters and the intensity of each frequency is calculated. Formants represent an increased concentration of sound energy at certain frequencies. Those areas of increased frequencies reflect the main points of resonance in the vocal tract. All vowels and some consonants have formants. In speech, vowels show two, and more often three formants. Formant pattern (particularly F1 and F2) enables us to distinguish vowels or to recognize that some vowel is "the same" when repeated, even when produced by different speakers. This paper presents the results of a spectral analysis of all vowels, that is, the positions of the first and the second vowel formants, obtained from the processing of the recorded voice of speakers from the sample. In this way, it is determined whether there is a difference in the position of formants F1 and F2 in individuals without speech impairment and those with hypokinetic dysarthria.

It often happens that voice disorders resulting from the same impairments can result in different handicaps. Therefore, it is necessary to have standardized instruments for self-assessment of the voice disorder, which will be included in the clinical assessment and will influence the process of determining an adequate treatment and evaluation of the success of treatment. An increased interest in the quality of life of patients with voice and speech disorders and understanding of the importance of a human voice in social inclusion generated the questionnaires for assessing the subjective experience about the consequences of the voice disorder. Voice Handicap Index – VHI (8) used in this research has several potential applications in the clinical practice of the speech-language pathology. It is most often used for the assessment of a patients' experience about the impact of their own voice disorder on daily activities and quality of communication. It is also used as an instrument for measuring the efficiency of the outcome of voice therapy, as well as the assessment of the severity of a voice problem in an individual with speech pathology of different etiology. In this research, the respondents expressed their opinions on the impact of the characteristics of their voice and speech on their physical, emotional and functional condition, and consequently on the quality of daily communication, whereby the information about the degree of a voice handicap experienced by individuals with hypokinetic dysarthria were obtained.

The relation between the values obtained by a spectral analysis and the score achieved on the VHI instrument is being increasingly studied today, and it has been determined that these values can be strong predictors of the type and severity of dysphonia (9). The main goal of this research was to determine the acoustic characteristics of voice and speech by spectral analysis in adults with Parkinson's disease being diagnosed with hypokinetic dysarthria. The ultimate goal was to determine the relation between the characteristics of speech and voice and quality of communication of individuals with hypokinetic dysarthria. More precisely, testing was performed in order to determine whether the altered position of vowel formants can be a predictor of the quality of communication in these individuals.

MATERIALS AND METHODS

Sample

The testing was performed on the sample of 30 elderly respondents of both sexes with Parkinson's disease and hypokinetic dysarthria. The respondents were aged between 59 and 94 years, with an average age of Me=82, with 11 (36.7%) being men and 19 (63.3%) being women. Demographic characteristics, educational level and smoking status of the respondents, as well as the presence of vocal professionals in the sample are shown in the Table 1.

The respondents did not have any associated disabilities that can affect the characteristics of speech and voice. The sample consisted inclusively of the respondents whose native language is Serbian so that voice characteristics of the respondents with hypokinetic dysarthria could be compared to the existing standards for adult typical speakers of the Serbian language.

Table 1. Structure of the sample

	N=30
Gender, n (%)	
Male	11 (36.7%)
Female	19 (63.3%)
Educational level, n (%)	
without school and primary school	10 (33.3%)
secondary school	10 (33.3%)
high and higher school	10 (33.3%)
Smoking status, n (%)	
Smoker	7 (23.3%)
Non-smoker	23 (76.7%)
Vocal professional, n (%)	
Yes	3 (10.0%)
No	27 (90.0%)
Average age, Median (min-max)	82.00 (59-94)

n – number of respondents, % - percentage

Instruments and procedure

The respondents which made the sample in the research are beneficiaries of several care homes for elderly and sick persons in Belgrade. Data about the type of pathology and dysarthria of respondents were obtained by an insight into their medical and logopedic documentation, and data about age, education, smoking status and profession were received from the respondents themselves. The consent to participate in the research was given by respondents and members of their families. The adults with Parkinson's disease and hypokinetic dysarthria answered the questions from the VHI instrument, thus evaluating the quality of their own communication. Recording of the voice and speech was performed individually, in a quiet room isolated from noise. Processing and analysis of data obtained by recording of the voice and speech of respondents were performed at the Department of Otorhinolaryngology at Medical Center „Zvezdara".

The computerized laboratory of *"Kay Elemetrics"* corporation, model 4300, was used for an acoustic analysis of voice and speech, to determine parameters of the spectral analysis of speech – formant structure of vowels. While the voice and speech were recorded, the respondents were reading "Balanced text" (10), which represents an instrument designed specifically for the analysis of voice and speech. The text represents a coherent semantic whole and contains complex utterances that are useful for speech analysis. The presence of all speech sounds is balanced in the text as in an everyday speech. The balance of the text refers to natural distribution of the frequency of syllables in semantic units of the Serbian language, as well as to the inclusion of all speech sounds in the Serbian language in the initial and medial articulation position and 14 most frequent speech sounds in the final position. The natural distribution of one-syllable, two-syllable and three-syllable words was also respected, as well as the distribution by types of words (10).

Voice Handicap Index (VHI) (8) is an instrument used in the research, and thus adults with hypokinetic dysarthria could conduct a subjective assessment of the degree of handicap they experienced because of the voice disorder and evaluate the quality of communication they achieved. The VHI instrument includes 3 subscales with 10 items each, physical which represents a patients' perception of their own voice, emotional which represents the emotional experience of a patient about the problem they have with their own voice, and functional which indicates patient's problems that occur during the communication. The results obtained in all three subscales indicate the possible problems which individuals with voice pathology have during communication. The VHI instrument indicates the level of self-assessed problem which a patient has with voice, which does not have to be in correlation with the objective measures of the voice. Each subscale contains 10 questions with 5 answer options of Likert scale type (0-4).

Statistical data analysis

As for descriptive statistics measures, a median with minimum and maximum for numerical variables was used, while the overview of categorical variables was given through frequencies and percentages. Differences between groups on numerical variables were tested by Mann-Whitney and Kruskal Wallis Tests. The relation of two categorical variables was tested by Chi square test. Spearman's correlation coefficient was used to test two numerical variables, while predictor features of the variables were tested by univariate linear regression analysis. Deviation of the values obtained by the analysis of the samples from normal values was tested by t test for one sample. Nonparametric tests were used given that deviation of distribution from the normal one was statistically significant.

Statistical significance was defined at the probability level of zero hypothesis of $p \leq 0,05$. Statistical analysis was done in the computer programme SPSS ver. 24 (Statistical Package for the Social Sciences).

RESULTS AND DISCUSSION

The Table 2 shows average values of formants of analysed vowels in the respondents with hypokinetic dysarthria, as well as results of testing of the difference between the achieved values on the sample and normal values of formants.

Table 2. Average values of formants and differences compared to normal values

	N	Min	Max	Median	T	df	p
F1 of vowel A	30	269	896	642,00	-1,933	29	0,221
F2 of vowel A	30	747	1554	1300,00	1,018	29	0,317
F1 of vowel E	29	269	687	508,00	-0,513	28	0,612
F2 of vowel E	29	867	2242	1674,00	-3,855	28	***0,001***
F1 of vowel I	30	179	717	328,00	4,619	29	***0,000***
F2 of vowel I	30	926	2682	1974,00	-4,638	29	***0,000***
F1 of vowel O	29	298	687	448,00	-1,523	28	0,139
F2 of vowel O	29	538	1225	1016,00	3,357	28	***0,002***
F1 of vowel U	30	209	538	358,00	-0,964	29	0,343
F2 of vowel U	29	508	1195	837,00	2,643	28	***0,013***

N-number of respondents, Mim – minimum value on the sample, Max – maximum value on the sample, t – t test, df – degrees of freedom, p – statistical significance

Differences were tested by t test for one sample. There is a statistically significant difference between normal values on the formant F2 of vowel /E/ (t=-3,855, p=0,001). The value of this formant on the sample (Me=1674) is lower than normal values (1720-2000 Hz). There is also a statistically significant difference compared to normal value on the formant F1 of vowel /I/ (t=4,169, p=0,000). Compared to normal values (170 – 300 Hz), higher average values (Me=328) were received on the sample. Lower average values on the sample (Me=1974) were calculated on the sample for the formant F2 of vowel /I/ compared to normal values (2100 - 2500 Hz). This difference is statistically significant (t=-4,638, p=0,000). The formant F2 of vowel /O/ is statistically significantly higher (Me=1016) than normal values (780 – 1000 Hz). The same applies to the formant F2 of vowel /U/ (t=2,643, p=0,013). The average values of this parameter are also higher (Me=837) than normal values (650-800 Hz).

Voice Handicap Index consists of three subscales: physical, emotional and functional. The scores on subscales are obtained by summing up the values achieved on items they consist of. Also, it is possible to have the total score for the whole scale measuring the impact of speech handicap on psychosocial functioning.

By processing the results, it was determined that theoretical range between the minimum and maximum is from Min=0 to Max=40. All three subscales have an average value which is close to the maximum: physical subscale (Me=6.50), emotional subscale (Me=8.50) and functional subscale (Me=2.00). Such low average values show the presence of minimum difficulties in all aspects of functioning that are assessed by the scale. Theoretical range on the score for the whole scale is from Min=0 to Max=120. The values achieved on the samples range from Min=0 to Max=94. The average is also very low (M=16.50), which indicates good psychosocial functioning and relatively good quality of communication.

The values achieved on the VHI instrument can be divided into three categories: mild, moderate and severe disorder in psychosocial functioning. When achieved values were divided into three categories in accordance with the instructions given by the scale constructor, the following results were received: 60% of the respondents belonged to the group of mild disorders, 13.3% to the group of moderate and 26.7% to the group of severe disorders on the functional scale. Mild disorder in emotional functioning was detected in 73.3% of respondents, moderate in 6.7% and severe in 20% of respondents. On the overall scale of psychosocial functioning, mild disorder was detected in 73.3% of respondents, moderate in 6.7% and severe in 20% of respondents.

Spearman's correlation coefficient was used to examine whether the score on the VHI instrument correlated with the value of formants (F1 and F2) of vowels (Table 3). The only statistically significant correlation was achieved between the formant F1 of vowel /A/ and functional subscale (rho=-0,393, p=0,032), as well as emotional subscale (rho=-0,363, p=0,049). Both correlations were statistically significant on the level p<0,05 and had negative values. So, the lower the values of formants, the higher the scores on functional and emotional subscales.

Table 3. Correlation between the values of formants with the results on VHI

		Functional subscale	Physical subscale	Emotional subscale	Total score
F1 of vowel A	Rho	***-,393****	-,254	***-,363****	-,346
	P	***,032***	,175	***,049***	,061

		Functional subscale	Physical subscale	Emotional subscale	Total score
F2 of vowel A	Rho	-,155	-,042	-,169	-,122
	P	,412	,826	,371	,519
F1 of vowel E	Rho	-,197	-,239	-,215	-,227
	P	,306	,211	,262	,236
F2 of vowel E	Rho	-,105	-,089	-,075	-,094
	P	,586	,646	,698	,627
F1 of vowel I	Rho	-,215	-,178	-,150	-,189
	P	,254	,347	,430	,318
F2 of vowel I	Rho	-,006	-,012	,122	,032
	P	,973	,951	,520	,868
F1 of vowel O	Rho	-,132	-,119	-,051	-,107
	P	,495	,539	,793	,581
F2 of vowel O	Rho	-,040	,134	,048	,052
	P	,839	,488	,805	,789
F1 of vowel U	Rho	-,034	,190	,077	,085
	P	,859	,316	,687	,654
F2 of vowel U	Rho	,261	,385*	,390*	,359
	P	,172	,039	,037	,055

rho-Spearman's correlation coefficient, p-statistical significance

Since the ultimate goal of the research was to determine the impact of the voice and speech characteristics on the quality of communication of adult respondents with hypokinetic dysarthria, it was also examined whether sociodemographic variables as control variables of the research were in a statistically significant correlation with the VHI instrument.

The correlation of the VHI instrument with the following variables was also tested: gender, educational level, smoking status and vocal professional, and it was determined that none of the variables was in a statistically significant correlation with subscales and total score of the VHI instrument (Table 4).

Table 4. Differences between patients of different characteristics on dimensions of the VHI instrument

	Functional subscale	p	Physical subscale	p	Emotional subscale	p	Total score	p
Gender								
Male	7,00 (0 – 33)	0,525[a]	15,00 (0 – 32)	0,232[a]	2,00 (0 – 24)	0,611[a]	26,00 (0 – 89)	0,395[a]
Female	6,00 (0 – 31)		15,00 (0 – 33)		2,00 (0 – 30)		15,00 (0 – 94)	
Educational level								
without school and primary school	5,00 (0 – 12)	0,154[b]	2,00 (0 – 14)	0,091[b]	0,00 (0 – 6)	0,125[b]	13,00 (0 – 27)	0,111[b]
secondary school	5,00 (0 – 22)		10,50 (0 – 29)		2,00 (0 – 24)		16,00 (0 – 74)	
high and higher school	13,00 (0 – 33)		16,50 (0 – 33)		7,00 (0 – 30)		33,00 (0 – 94)	
Smoking status								
Smoker	5,00 (0 – 33)	0,774[a]	12,00 (2 – 32)	0,061[a]	3,00 (0 – 24)	0,360[a]	18,00 (2 – 89)	0,245[a]
Non-smoker	7,00 (0 – 31)		5,00 (0 – 33)		2,00 (0 – 30)		15,00 (0 – 94)	
Vocal professional								
Yes	31,00 (0 – 33)	0,315[a]	32,00 (0 – 33)	0,283[a]	24,00 (0 – 30)	0,226[a]	89,00 (0 – 94)	0,315[a]
No	6,00 (0 – 29)		8,00 (0 – 29)		2,00 (0 – 24)		16,00 (0 – 74)	

[a]Mann-Whitney test; [b] Kruskal Wallis Test; p- statistical significance;
Medians (min – max) are shown in the table.

The initial goal of the research was to determine the predictors of the quality of communication. First, the univariate analyses were conducted. These analyses were conducted in order to determine which variables make a regression model. Specifically, only the variables which are in a statistically significant correlation with the dependant variable make a regression model. The only statistically significant correlation was identified between the formant F1 of vowel /A/ (independent variables) and functional and emotional scales (dependent variables). Regression analysis confirmed the previous findings. The first formant of the vowel /A/ has a statistically significant contribution to the explanation of the score achieved on the functional subscale, by explaining 15% of the functional subscale (Adjusted R Square=0,155), (-0,393 (11.30 – 47.37)). The same formant explains 10% of the emotional scale (Adjusted R Square=0,100), (-0,363 (-0,052 – 0,000)) (Table 5).

Table 5. Predictors of the quality of communication

Dependent variables	Independent Variables	Univariate linear regression analysis		
		Beta (95%CI)	p	Adjusted R Square
Functional subscale	F1 of vowel A	-0,393 (11,30 – 47,37)	***0,032***	0,155
Emotional subscale	F1 of vowel A	-0,363 (-0,052 – 0,000)	***0,049***	0,100

DISCUSSION

An acoustic analysis of the frequency of formants provided the values of formants of vowels (F1 and F2) in respondents with hypokinetic dysarthria. The first three formants carry the main features of vowels. However, the first two formants, which are the strongest in terms of energy, are enough to recognize the vowels, while the third formant F3 provides clarity and improves the quality of voice. In addition, the positions of the third formant for all vowels in the Serbian language are very close to each other, and they can be disregarded when it comes to discrimination against vowels (11). The results obtained in this research indicate the presence of statistically significant differences between the positions of formants (F1 and F2) for most of the vowels in the Serbian language in adult respondents with hypokinetic dysarthria compared to the position of the formants that are characteristic for typical speakers. Such differences indicate the presence of a pathological voice which occurs as a consequence of a speech disorder. Given that changes in the concentration of acoustic energy were determined for four vowels (/E/,/I/,/O/,/U/) out of five, the intelligibility of these respondents was significantly impaired. The change in the position of the first format (F1) was determined just for the vowel /I/, while changes were particularly identified in the position of the second formant (F2) for four stated vowels, and it is known that the second formant has the greatest dynamics at vowels.

Other studies also show that there is a reduction in the degree of articulation movement in individuals with Parkinson's disease, what disturbs the production of vowels which results in the change of the position of formants or it being noticed with a difficulty (12). The position of articulators defines three-dimensional characteristics of the vocal tract and affects the frequencies of formants, particularly the first (F1) and the second (F2) formant. As a result of limited articulator movements, an inadequate formation of vowels and restriction of normal formant production, which leads to lowering of typically high frequency of formants or to increasing of typically low frequency of formant, occur in these individuals (13). The results of our research show lower average values for the second formant of vowels /O/ and /I/ compared to the existing standards for typical speakers, and higher average values of the first formant of the vowel /I/, and the second formant of vowels /O/ and /U/. The research which examined the production of vowels in individuals with Parkinson's disease (14) showed the reduced transition of formants and limited acoustic space of vowels caused by hyperkinesia of articulators, which together lead to significant disruption of the intelligibility of speech. However, these authors also proved that speech intelligibility can be relatively preserved at mild forms of dysarthria which are characterized by monotonous speech, without the change in pitch.

Based on received results and a large amount of previous research, it has been noticed that communication changes in individuals with Parkinson's disease are almost inevitable (15). More precisely, about 80-90% of these individuals experience changes in voice, and 45-50% changes during the articulation (16). This paper shows changes in the position of formants of vowels determined by spectral analysis based on the speech of individuals with hypokinetic dysarthria caused by Parkinson's disease. However, in addition to these changes, hypokinetic dysarthria implies significant perceptual, acoustic and kinematic changes resulting from the deterioration of voice and speech (16, 17). The voice usually has monotonous pitch and volume, it is imprecise and dysfluent, with many acoustic changes that are exactly the result of the reduction of strength, amplitude, durability and intensity of the movement of speech apparatus. The intelligibility of an individual's speech becomes worse with time, speech production slows down, clarity is lost and murmur occurs, with long breaks and efforts at speech. Changes and variations in

speech are great even in the course of a day, which is very demoralizing for a speaker (15). However, some authors state that degradation during the production of vowels in individuals with dysarthria is more a consequence of the severity of a motor speech disorder than the fact that in overall it contributes to intelligibility deficits (12).

In the earlier studies, the individuals with a speech disorder were mainly neglected. More precisely, the focus of research was just a speech pathology and not a person. The research focused on the identification of dynamic or statistical acoustic values correlating with perceptual characteristics, i.e. the level of intelligibility. For instance, determination of the correlation between the speech intelligibility and dynamic measures, which indicate the instability of formants of vowels and reduced F2 slope (18). Since it was determined that there is a correlation between the position of the second formant of vowels and perceptual characteristics, it was started with examining the impacts of inadequate values of formants on the speech intelligibility in individuals with dysarthria. Thus, it was determined that there is a significant correlation between the positions of F2 of two vowels and intelligibility of sentences in individuals with dysarthria caused by amyotrophic lateral sclerosis and Parkinson's disease (12) as well as the correlation between the second formant and speech intelligibility in individuals with dysarthria caused by Parkinson's disease and stroke (18).

Today, more attention is directed towards what impact has dysarthria on individuals, their feelings related to problems during the conversation and their interaction with other people and the quality of their communication. The most important goal of communication, i.e. socialisation and transmission of information, is achieved though intrapersonal and interpersonal communication (19). A modern individual is believed to spend about 70% of the time in communication, out of which 50% is communicated by voice and speech which enable the realization of language as a symbolic system (20). In latest studies (3, 21) it is pointed out that in addition to impairing the speech intelligibility and successfulness of communication, acquired dysarthria also affects the psychosocial functioning of an individual. It is even pointed out that depression, anxiety, social exclusion and changes in the estimation and experience of oneself can occur as consequences of dysarthria (21). It has been determined that in patients tested after they experienced stroke, dysarthria has a significantly stronger impact than other diseases on social participation of an individual and their perception of their own identity (22). Other individuals with dysarthria of different etiology confirmed that due to speech problems they had come across barriers in communications because of attitudes of the environment (4).

In this research, the assessment of subjective experience of individuals with hypokinetic dysarthria of the effects of voice disorder was performed by the VHI instrument (8). The respondents expressed their opinions about the impact of the characteristics of their voice and speech on physical, emotional and functional condition, and consequently on the quality of everyday communication, whereby the information on the degree of speech handicap experienced by some individuals with hypokinetic dysarthria was also received. Unlike the previously stated studies, and given the impaired intelligibility of respondents' speech, the results of this research showed low scores on the overall VHI instrument. That indicates the presence of minimum difficulties in all aspects of functioning that are assessed by the scale, which indicates good psychosocial functioning and quality communication achieved by individuals from the sample despite the presence of speech pathology.

More precisely, the values achieved on the VHI scale can be divided into three categories: mild, moderate and severe disorder in psychosocial functioning, and received results showed that on the overall scale of psychosocial functioning, a mild disorder was identified in 73.3% of respondents, moderate in 6.7% and severe in 20% of respondents. However, there is research in which individuals with Parkinson's disease have low scores on the VHI instrument, which indicate nonexistence of communication difficulties or mild difficulties experienced by a respondent (23). Nonetheless, it is important to point out that the authors of the stated research see the lack of awareness of a speech disorder in patients with Parkinson's disease, which is an important aspect of communication deficit in these individuals, as the main reason for such score on the Voice Handicap Index.

A smaller number of studies dealt with establishing a correlation between objective data obtained by multidimensional assessment of voice and subjective assessment conducted by the VHI instrument. The researchers mainly studied the relation between acoustic parameters of voice, without special reference to the values received by spectral analysis, and the results received on subscales and total score on the VHI scale. In addition, the sample of those studies was not made of individuals with dysarthria but of individuals with different types of dysphonia (24-26). In this study, we wanted to determine whether the values of formants of vowels obtained by spectral analysis of the voice of individuals with hypokinetic dysarthria have changed compared to those of typical speakers, as well as whether the obtained values of formants as characteristics of an individual's voice and speech represent the predictors of the quality of communication in individuals with this type of speech disorder. The only statistically significant correlation was achieved between the formant F1 of the vowel /A/ and functional and emotional subscale. It was determined that the lower the values of formant F1, the higher the scores on the functional and emotional subscales. Even though only the first and the second formant of the vowel /A/ do not significantly deviate from the position of formants of the same vowel in typical speakers, the results show that the lower the values of the first formant, the higher the scores, or more precisely worse results on the above stated subscales. Thus, the first formant of the vowel /A/ has a statistically significant contribution to the explanation of the score obtained on the functional subscale, by explaining 15% of the functional subscale and the same formant explains 10% of the emotional subscale. These results

indicate that in this research only the position of the first formant of vowel /A/ in the sample of respondents with hypokinetic dysarthria can be a predictor of the quality of communication.

CONCLUSIONS

The obtained results showed, as expected, the changes in articulation and intelligibility of vowels in respondents with hypokinetic dysarthria. Speech disorder present in these individuals includes disorders in pronunciations of a large number of speech sounds, and changes occurring during the pronunciation of vowels have been presented through changed positions of the first and the second formants, and were received by a spectral analysis. However, regardless of the statistically significant difference between normal values of vowels and values of formants of vowels of the respondents from the sample, the individuals with Parkinson's disease and hypokinetic dysarthria believe that they do not have great problems in communication and that handicap caused by voice and speech disorder is not significant for them and does not largely affect their daily communication. However, this research needs to be conducted in respondents with other types of dysarthria and to determine whether there is a difference in the subjective experience of communication difficulties resulting from the voice and speech disorder of different etiology.

The research also showed that acoustic characteristics of speech and voice in adults with hypokinetic dysarthria can be predictors of the quality of communication. Thus, in this research, the position of the first formants of the vowel /A/ was the predictor which explains scores on the emotional and functional subscales of the VHI instrument. Since there is not much research examining the relation between the results obtained by a spectral analysis and scores achieved on scales, which enable self-assessment of one's own handicap, the future research should be focused on this problem and conducted on a larger sample in order to obtain more reliable results. Also, as already stated, such research should be also conducted in individuals with other types of dysarthria, in order to establish the predictors of the quality of communication for each type individually and to compare the obtained results with each other.

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OLANZAPINE - FOCUS ON THE CARDIOMETABOLIC SIDE EFFECTS

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OLANZAPIN - FOKUS NA KARDIOMETABOLIČKE EFEKTE

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ABSTRACT

In this article, we review the recent findings concerning weight gain, diabetes mellitus (DM), hyperlipidemia, cardiovascular side effects in patients receiving olanzapine. It will consider the OLZ is associated with an increase in metabolic syndrome or cardiovascular events, and knowledge of these risks is crucial for further monitoring of patients with OLZ-treatment. Although it is one of the most commonly prescribed and effective AATPs, olanzapine causes the most weight gain and metabolic impairments in humans. As noted with glucose abnormalities and antipsychotics, olanzapine has the greatest propensity for causing proatherogenic hyperlipidemia. The mechanism of dyslipidemia with OLZ is poorly understood, but OLZ has been shown to increase lipogenesis, reduce lipolysis, and enhance the antilipolytic effects of insulin in adipocytes. Olanzapine can induce cardiomyopathy in selected patients.

Taken together, all mentioned data indicate that interventions aimed at the amelioration of obesity and cardiovascular illness need to be as multipronged and complex as the contributing psychosocial, behavioural, and biological factors that make obesity and cardiovascular illness more likely in patients with severe mental illness, including schizophrenia.

Keywords: *olanzapine, weight gain, dyslipidemia, cardiovascular disease*

SAŽETAK

U ovom članku, razmatramo nedavna saznanja u vezi dobijanja u težini, šećerne bolesti (DM), hiperlipidemije i kardiovaskularnih neželjenih efekata kod pacijenata koji su na terapiji olanzapinom. Uz pretpostavku da je olanzapin u vezi sa povećanim rizikom za nastanak metaboličkog sindroma i kardiovaskularnih događaja, od presudnog je značaja poznavanje potencijalnih rizika kako bi se sproveo monitoring ovih pacijenata. Iako olanzapin (OLZ) predstavlja jedan od najčešće propisivanijih i najefektnijih atipičnih antipsihotika, ipak nosi i najvišu stopu rizika za nastanak metaboličkih smetnji kod ljudi. Olanzapin uzrokuje poremećaj metabolizma glukoze, povećava lipogenezu, smanjuje lipolizu, povećava antilipolitičke efekte insulina u adipocitima što uzrokuje dislipidemiju i doprinosi visokom proaterogenom potencijalu olanazapina. Opisani su i slučajevi kardiomiopatije usled primene olanzapina.

Sumarno posmatrano, literaturni podaci ukazuju na neophodnost složenih preventivnih i terapijskih protokola kod pacijenata sa mentalnim poremećajima, uključujući i shizofreniju, a koji su na terapiji olanzapinom, usmerenih na smanjenje psiholoških i bioloških faktora rizika za kardiovaskularne bolesti.

Ključne reči: *olanzapin, povećanje telesne težine, dislipidemija, kardiovaskularna oboljenja*

INTRODUCTION

Antipsychotics were first introduced into clinical practice in the 1950s and approved in 1996 by the FDA.

Antipsychotics are now frequently used beyond their core indications of schizophrenia and bipolar disorder. Off-label use of antipsychotics is frequent in major depressive disorder and other mood disorders, anxiety disorders and dementia (1-4). In recent years, the atypical antipsychotics or second-generation antipsychotics have become the drugs of choice for acute psychoses. They are "atypical" as they are differentiated from "conventional" or first-gen-

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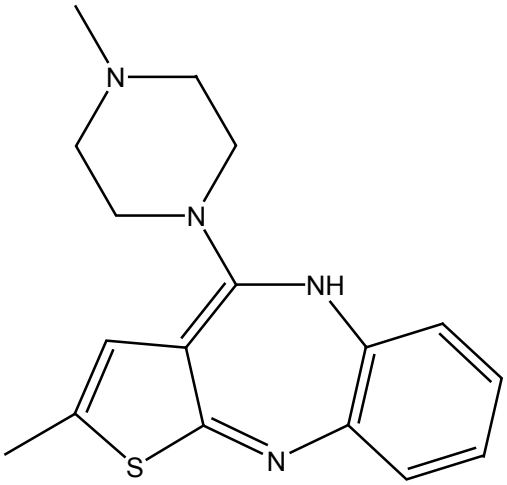

Figure 1. Olanzapine ($C_{17}H_{20}N_4S$) structure

eration antipsychotics based on their clinical profile. They have fewer side effects regarding extrapyramidal symptoms when compared to typical antipsychotics. Schizophrenia is a devastating illness that affects up to 1% of the population; it is characterized by a combination of positive symptoms, negative symptoms, and cognitive impairment. The atypical antipsychotic (APs) drugs have become the most widely used agents to treat a variety of psychoses because of their superiority with regard to safety and tolerability profile compared to conventional/'typical' APs (1-4).

Olanzapine (Figure 1) (OLZ; $C_{17}H_{20}N_4S$; 2-methyl-4-(4-methyl-1-piperazinyl)-10H-thieno[2,3-b][1,5]benzodiazepine) is an antipsychotic drug of the thienobenzodiazepine class that is effective in treating schizophrenia and acute manic episodes, and in preventing the recurrence of bipolar disorders (5). It is as has been shown to have some therapeutic advantages over other classic antipsychotics in terms of symptom reduction and its adverse event profile. It has a low propensity to cause extrapyramidal effects or sustained increases in prolactin levels (6, 7). Nevertheless, treatment with OLZ is associated with a higher risk of weight gain and, more extensively, metabolic syndrome than other typical and atypical antipsychotics (8).

Olanzapine is known as MARTA (multi-acting receptor targeted antipsychotics). Proposed mechanisms of action of atypical antipsychotics as well as olanazapine, is dopaminergic and serotonergic modulation and induction

Table 1. Potential clinical efficacy, benefits and possible effects related to the mechanisms of action of olanzapine. EPS, extrapyramidal symptoms

Mechanism of action	Clinical efficacy	Possible effects
D_2 antagonism	↓ positive symptoms	EPS ↓negative symptoms ↑ cognitive symptoms hyperprolactinaemia
D_2 partial agonism	↓ positive symptoms ↓ negative symptoms ↓ cognitive symptoms	little or no EPS behavioral activation
5-HT_2A antagonism	↓ negative symptoms	↓ EPS ↑ weight gain hyperphagia and obesity ↑metabolic syndrome
5-HT_1A partial agonism	↓ negative symptoms ↓ cognitive symptoms ↓ anxiety symptoms ↓ depressive symptoms	No adverse effects
Muscarinic antagonism	↓ EPS	↓ anticholinergic symptoms e.g. dry mouth, constipation, tachycardia
Muscarinic agonism	↓ psychotic symptoms ↓ cognitive symptoms	No adverse effects
Adrenergic α_1 and α_2 antagonism	No effects on negative and positive buhevior symptoms	↓adrenergic symptoms e.g. orthostatic hypotension and consequently induced tachycardia hyperphagia and obesity ↑metabolic syndrome
Histamine H_1 antagonism	↓ positive symptoms	↑ sedation ↑ weight gain hyperphagia and obesity ↑metabolic syndrome
Glutamate modulation	↓ positive symptoms ↓ negative symptoms ↓ cognitive symptoms ↓ illness progression	No adverse effects

of neuroplasticity. OLZ shares higher affinity to 5-HT_2A receptors than D_2 receptors (high 5-HT_2A/D_2 ratio). In comparison to the other atypicals, olanzapine presents high affinity for serotoninergic 5-HT_2A, 5-HT_2C, 5-HT_3, and 5-HT_6 receptors, medium affinity for dopaminergic D_1, D_2, D_3, D_4, D_5, and muscarinic M_1–M_5 receptors, low affinity for adrenergic α_1 and α_2 receptors, and the highest affinity for histamine H_1 receptors (olanzapine is the most potent histamine H_1 antagonist known) (*Table 1*) (9, 10).

Although the usual dose range for olanzapine is 5–15 mg/d, there are no standard reference values with respect to the expected concentrations of olanzapine after therapeutic administration. In clinical studies, steady state blood (plasma) concentrations of olanzapine are rarely over 150 ng/mL, but the potential for toxicity has been suggested at concentrations as low as 100 ng/mL (11).

Approximately 85 % of an oral OLZ dose is absorbed but, as about 40 % is inactivated by first-pass hepatic metabolism, its oral bioavailability is about 60 %. OLZ has a mean half-life in healthy individuals of 33 hours (range 21-54 hours). Peak plasma concentrations are reached within six hours. The drug is approximately 93 % bound to plasma proteins, mainly albumin (90 %) and alpha 1-acid glycoprotein (77 %). Its distribution volume is 16.4 ± 5.1 L (X±SD). Mean apparent plasma clearance is 26 L/h (range 12-47 L/h). After the administration of [14C]-OLZ in a single load pharmacokinetic study, approximately 87 % of the radioactivity was excreted, with 30 % appearing in the faeces and 57 % in the urine (10-12).

Psychiatrists have gradually prescribed antipsychotic drugs in come to reduce psychiatric symptoms, and extrapyramidal symptoms and tardive dyskinesia occur less frequently with atypical agents. Beside that, these medications may present a different set of adverse effects (10-12).

In this article, we review the recent findings concerning weight gain, diabetes mellitus (DM), hyperlipidaemia, cardiovascular side effects in patients receiving olanzapine. Evident increase in cardio-metabolic side effects during olanzapine administration inevitably leads to a question of its effect on the cardiovascular system of diseased psychotic patients. Bearing in mind that the treatment of these diseases is most often lifelong, it is clear that the degree of exposure of treated patients with olanzapine is long lasting, and for these reasons, not only the positive therapeutic effects of the drug must be seriously analyzed, but also the degree of impact of its adverse effects on the general health of the diseased.

Atypical antipsychotics are responsible for increasing cardiovascular risk by more than 30% in schizoid patients (10). Similar results were also seen in the increased risk for the development of metabolic syndrome and the risk of diabetes. Given that these drugs have a significant place in consumption, their impact on the health budgets in the countries where they are used is high (10). The treatment of cardiovascular and metabolic complications caused by the use of atypical antipsychotics is inevitable and this cost represents additional pressure on health funds (11, 12).

It will consider the OLZ is associated with an increase in metabolic syndrome or cardiovascular events, and knowledge of these risks is crucial for further monitoring of patients with OLZ-treatment. Recognizing these complications in addition to the necessary monitoring, opens space for the development of new drugs or procedures that need to eliminate or at least significantly reduce the consequences of cardiometabolic complications caused by the application of atypical antipsychotics, in this case olanzapine.

WEIGHT GAIN

Weight gain and obesity are critical issues in patients with schizophrenia. The abnormal nutritional status and 'developmental' obesity in schizophrenia have been described more than half-century ago. To date, there are over 2600 papers indexed by Medline on the topic of weight gain and obesity in schizophrenia. Patients with schizophrenia consume unhealthy food (13-15). A recent meta-analysis of 31 studies about dietary patterns identified a high consumption of saturated fat and low intake of fruit and dietary fiber. Also, controlled investigation indicated that patients with schizophrenia had higher daily intake of calories and protein per kilogram of body weight, which was independent of BMI. Social isolation, low interest in social achievement, and unmarried and unemployed status are common in patients with schizophrenia and lead to decreased levels of participation in sports and other mainstream physical activities (14).

The importance of neurotransmitter and hormonal effects in the weight accrual of patients with schizophrenia has been studied for olanzapine. Leptin levels were similar in schizophrenia patients and healthy control subjects with comparable BMIs (15). An inverse association was observed for baseline weight and leptin levels with the extent of weight gained during 3–6 months of antipsychotic monotherapy (16). This study suggesting a drug--mediated disruption of the hypothalamic appetite control, as well as prevous animal study, also indicated that olanzapine increased the orexigenic NPY mRNA and decreased the anorexigenic POMC in the arcuate nucleus (17) and upregulated ghrelin and ghrelin signaling, leading to hyperphagia.

Histaminergic transmission is involved in energy homeostasis and also seems to be relevant to antipsychotic-related weight gain, as the extent of histamine H1 receptor (H1R) antagonism of antipsychotics was the best predictor of the degree of weight gain in clinical studies. 5-HT2c antagonism has been implicated in antipsychotic drug-related weight gain too, and most second-generation antipsychotics, especially for olanzapine, which is a potent 5-HT_2c antagonist. Synergistic effects between the blockade of D_2 receptors and 5-HT_2a or 5-HT_2c receptors might play a key role in triggering a cascade of events that lead to increased energy intake and weight gain (18-22).

In a retrospective analysis of 1191 patients diagnosed with schizophrenia or schizoaffective disorder treated with olanzapine (23), approximately 15% of subjects had a rapid change of ≥7% body weight during the first 6 weeks of treatment, with a mean weight gain of 1.8–3.2 kg (about 4% of the baseline body weight) during the first 2 weeks. Increasing evidence indicates that antipsychotics have greater orexigenic weight gain potential in children and adolescents than in adults (24) and that young patients receiving antipsychotics are at increased risk of being or becoming overweight or obese. A recent comparison of pooled long-term studies (median followup = 201 days) of patients treated with olanzapine indicated a mean weight gain of 4.8 kg in adults, but 11.2 kg for adolescents (22-24). A debate is continuing with regard to the inverse relationship between baseline BMI and antipsychotic- induced weight gain. Pooled longitudinal data in patients treated with olanzapine (mean modal dose = 13.3 mg/day) indicated that the slowing in the rate of weight gain observed after 2–4 months of treatment was greatest for patients who were obese at baseline (25, 26).

METABOLIC SYNDROME

Also, atypical antipsychotics such as olanzapine often induce excessive weight gain and type 2 diabetes. In the past decade there have been numerous case reports, retrospective studies, and epidemiological investigations suggesting that certain OLZ may be associated with a great risk of DM. Although it is one of the most commonly prescribed and effective AATPs, olanzapine causes the most weight gain and metabolic impairments in humans. By World Health Organization criteria, 10.1% of patients developed diabetes mellitus (DM) after only 6 weeks of antipsychotic therapy (P =0.016) (27-28).

However, the mechanisms underlying these drug-induced metabolic perturbations remain poorly understood. Clinical studies have suggested the involvement of multiple genes, including those that encode the histamine, α-adrenergic, and serotonin (5-HT) receptors. Among them, *Htr2c* encodes the 5-HT 2C receptor, which acts in the brain to regulate food intake, body weight, and glucose metabolism (29, 30). Blockade of HTR2C signaling in mice leads to hyperphagia and obesity (31) that resemble AATP-induced metabolic symptoms in humans. Rates of metabolic syndrome are significantly higher in schizophrenia than in the general population. OLZ, as an atypical antipsychotic, has been associated with detrimental effects on metabolic risk factors. The pathomechanisms that underlie metabolic syndrome as a complication of antipsychotic treatment are not fully understood. Probably, the effects of OLZ on histamine H1, serotonin 5-HT2c and muscarinic M3 receptors are thought to play a central role. In addition, antipsychotics may have direct effects that cause leptin insensitivity as well as on appetite regulation (30-33).

Olanzapine, after clozapine, shows the strongest association with the risk for diabetes. Other studies have demonstrated significant changes in blood glucose levels with antipsychotic therapy despite not measuring other markers of glucose-insulin homeostasis. Lindenmayer and colleagues randomized 157 patients with schizophrenia to 14 weeks of therapy with clozapine, haloperidol, olanzapine, or risperidone. Fasting blood glucose was measured at baseline, at 8 weeks, and at end point (34, 35).

Olanzapine was associated with a significant increase in fasting glucose at end point (mean change from baseline 14 mg/dL, P < .02). Glycosylated hemoglobin (HbA1c) has been used as a surrogate marker for insulin resistance and glycemic control in the assessment of some antipsychotic medications. Olanzapine is associated with elevations in HbA1c levels. In some patients, a direct effect of olanzapine on pancreatic β-cell function may be present (36) but more commonly the accumulation of body weight with central adiposity, and the resultant increase in insulin resistance, would explain the development of diabetes mellitus over time.

Using the Food and Drug Administration (FDA) adverse events database, the risk of diabetes mellitus was increased for olanzapine, risperidone, clozapine and quetiapine, whereas a decreased risk was found for haloperidol, aripiprazole and ziprasidone (34, 36).

Both typical and atypical antipsychotics can cause significant increases in cholesterol, triglycerides and low-density lipoprotein cholesterol. The risk of hyperlipidaemia differs for individual antipsychotics. The risk of hyperlipidaemia appears higher for patients under treatment with clozapine and olanzapine (37) particularly for younger patients. *Simpson* and colleagues found that olanzapine, but not ziprasidone, significantly increased total cholesterol (median change from baseline to end point at 6 months, 13 mg/dL, P = .03) and low-density lipoprotein (LDL) cholesterol (median change from baseline to end point at 6 months, 17 mg/dL, P = .04) (37, 38).

In a further study, lipids were measured at multiple time points over 28 weeks, and olanzapine was associated with significant increases in total cholesterol. Olanzapine has been shown to be associated with unfavourable lipid derangements compared with aripiprazole. As noted with glucose abnormalities and antipsychotics, olanzapine has the greatest propensity for causing proatherogenic hyperlipidaemia. The mechanism of dyslipidaemia with OLZ is poorly understood, but OLZ has been shown to increase lipogenesis, reduce lipolysis, and enhance the antilipolytic effects of insulin in adipocytes (37-40).

CARDIAC DYSFUNCTION

Schizophrenia is associated with increased mortality and reduced life expectancy, with cardiovascular disease being the most frequent cause of death. Antipsychotics have detrimental effects on different risk factors for cardiovascular disease (41).

Patients with schizophrenia are at high risk of metabolic syndrome, a cluster of risk factors for cardiovascular disease. Prevous cohort study confirmed that 40% percent of 3470 French patients with schizophrenia (mean age at inclusion 39.3 years) died during an 11-year follow-up period. In the Olmstead County study, patients with schizophrenia had a significantly increased mortality, in particular from cardiovascular disease (42-44). Patients with schizophrenia frequently have multiple risk factors for cardiovascular disease. Firsty, excess prevalnce od obesity and increased BMI in patients with mental disorder in one of the major factor for develpment of cardiovascular disease (44). Monitoring glucose is crucial, and patients with risk factors for diabetes mellitus (e.g., obesity, family history of diabetes) who are starting treatment with olanzapine should undergo fasting blood glucose testing at the beginning of treatment and periodically during treatment. Beside abdominal obesity, dyslipidemia, hypertension and diabetes mellitus and have additive effects on an individual's risk of developing diabetes mellitus and cardiovascular disease. Rates of smoking are higher in schizophrenia patients than in the general population. Schizophrenic patients who smoke are at higher risk of death as well as death from cardiovascular disease than schizophrenic patients who do not smoke (45, 46).

Furthermore, typical and atypical antipsychotics are associated with a significant dose-related increase in the risk of sudden cardiac death. Some of the cases of sudden cardiac death have been associated with cardiac arrhythmia, in particular torsade de pointes, possibly secondary to a prolongation of the QT interval (45-47). But, OLZ was initially linked to potential QTc prolongation. Extensive studies have shown modest Qtc interval prolongations for these patients that are most likely not clinically relevant and with no evidence for increased mortality by disturbed Ot-changes by OLZ (48). But, study conducted by Morissette indicate that olanzapine possesses direct cardiac electrophysiological effects. They demonstrated that olanzapine can prolong cardiac repolarization in a reverse frequency- dependent manner by blocking time-dependent outward potassium current involved in cardiac repolarization. In fact, they shown that olanzapine 5.7 μM caused a significant prolongation of cardiac repolarization (13%) (49).

Adverse hemodynamic effects are possible with olanzapine, particularly orthostatic hypotension, bradycardia and tachycardia (50), which are most likely owing to adrenergic α1 blockade. Because of the antagonism of α1-receptors, OLZ is associated with orthostatic hypotension and consequently induced tachycardia, but in lowpotency. This risk of a OLZ-hypotension is not pronounced for olanzapine. Especially in eldery, OLZ is associated with increased risk of cardiovascular disease and also, this drug has also been associated with venous thromboembolism and pulmonary embolism. Again, OLZ seems to be associated with a low risk (51, 52).

Interestingly, some of previous clinical study, reported about the effects of olanzapine on inducing of specific cardiac disorders, such as myocarditis and cardiomyopathy. These rare but potentially fatal complications of antipsychotic treatment, myocarditis and cardiomyopathy are associated with antipsychotics are most frequently seen under treatment with clozapine, but can also occur with olanzapine treatment (53). Malays reported about 28-year-old male patient with bipolar disorder who taking olanzapine and lorazepam for almost 10 years and presented with weight gain, diabetes, and anasarca. Evaluation of the patient revealed he was in heart failure. The reason for his heart failure was ambiguous and an investigation into it revealed negative results. Literature search conducted showed a few reported cases of putative olanzapine induced cardiomyopathy and this is one of them. Well, cardiomyopathy is a less known side effect of OLZ (54). The main proposed mechanism for cardiomyopathy is myocarditis and myopericarditis by direct toxicity or allergic reaction. In animal studies, three months of olanzapine treatment was shown to induce ventricular hypertrophy of the heart. Cardiac lesions induced by neuroleptic drugs in the rabbit (55).

In practice, olanzapine induced cardiac disorder should be considered in a patient who develops dyspnoea or other signs of the heart failure (56). Olanzapine should be withdrawn in those cases and treatment of heart failure should be done on a routine basis. Olanzapine can induce cardiomyopathy in selected patients. Early recognition and cessation of the drug is required to prevent irreversible myocardial damage. Cardiac functional assessment is periodically required for the patients taking antipsychotics. Cautious use is required in patients with known heart disease.

CARDIOMETABOLIC MONITORING OF PATIENTS WITH OLZ-TREATMENT

Patients with severe psychiatric disorders and with antipsyhotic therapy are at increased risk of cardiovascular disease, although some of this risk may be conferred by the psychiatric disease or lifestyle. Weight gain, obesity, metabolic and cardiovascular disorders in patients with schizophrenia and other mental disorders are associated with a host of adverse physical and psychiatric outcomes, as well as with OLZ treatment (*Table 1*). Therefore, body weight and related metabolic indices need to be monitored routinely and targeted as part of a comprehensive and integrated care programme in patients with OLZ-treatment (33, 35, 57, 58).

Ideally, a treatment algorithm should start with healthy lifestyle education/instruction and with lower cardiometabolic risk antipsychotic than OLZ. It is reccomendation that only consider higher risk agents, such as olanzapine, when it has become clear that the physically safer medication is not sufficiently effective or tolerated. Psychiatric care providers should aim for balancing acute

and long-term efficacy as well as tolerability, and engage other medical specialists as needed to improve the overall well-being of patients with schizophrenia. American Diabetes Association and American Psychiatric Association suggested that optimal management of patients with schizophrenia should include baseline assessment on their weight, waist circumference, blood pressure, blood glucose level and lipidogram and family history on obesity, diabetes, dyslipidemia, hypertension and cardiovascular illness (33, 35). During the first three months, weight gain should be monitored on monthly basis, while biochemical analysis should be performed after the first three months, and then once a year. In patients with significant weight gain, increase of blood glucose level or dyslipidemia, the first intervention should be switch to another antipsychotic. If necessary, a patient should be referred to an endocrinologist and advised on changing their life style (57).

Suggested algorithm for cardiometabolic monitoring of patients treated with OLZ is precisely described by *Manu* and coworkers. Suggested algorithm for managing antipsychotic-related weight gain is power tool for prevention of cardiovascular disease and for decreasing of mortality in patients with psychotic disorders. Nevertheless, it is also important to consider that antipsychotics are currently the only medication class with evidence for effective treatment of psychosis (58-60).

CONCLUSION

Taken together, all mentioned data indicate that interventions aimed at the amelioration of obesity and cardiovascular illness need to be as multipronged and complex as the contributing psychosocial, behavioural, and biological factors that make obesity and cardiovascular illness more likely in patients with severe mental illness, including schizophrenia. The use of olanzapine in the treatment of psychosis, especially schizophrenia, has revolutionized the treatment of these diseases, but has led to the opening of a question and price that we have to pay in terms of the development of cardio-metabolic complications and their impact on the quality of life of the diseased. By clearly recognizing the complications and mechanism of their emergence, we are given the opportunity to better implement new therapeutic procedures, by introducing drugs of similar therapeutic potential, but with a significantly lower impact on the development of cardiometabolic complications by applying adequate hygienic dietary regimes and changing lifestyle habits.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

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COMBINED SURGICAL APPROACH IN THE TREATMENT OF OCULO-ORBITAL COMPLICATIONS OF FRONTAL SINUS MUCOCELE: A CASE REPORT

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KOMBINOVANI HIRURŠKI PRISTUP U LEČENJU OKULO-ORBITALNIH KOMPLIKACIJA MUKOKELE ČEONOG SINUSA: PRIKAZ SLUČAJA

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ABSTRACT

Paranasal sinus mucoceles are benign cystic masses filled with mucous content. Mucoceles are locally destructive, causing pressure on sinus walls with their resorption, allowing them to spread on adjacent structures causing local, orbital or intracranial complications. They are most commonly found in frontal sinuses.

The aim of this report is to present case of oculo-orbital complications of frontal sinus mucocele, with focus on treatment using combined surgical approach.

A 75-year old female patient with frontal sinus mucocele which led to destruction of orbital roof and occurrence of complications in form of orbital cellulitis and palpebral abscess was successfully treated with a combination of external frontoethmoidectomy and endoscopic sinus surgery. After initial incision of the upper eyelid abscess with drainage of purulent content, modified external frontoethmoidectomy was performed using preformed defect of orbital roof. Finally, using endoscopic sinus surgery, natural drainage of anterior group of paranasal sinuses was achieved.

Various endoscopic and open approaches have been described in mucocele treatment. In this case we showed that the combined surgical approach in the treatment of frontal sinus mucoceles with destruction of sinus floor and appearance of oculo-orbital complications, provides an effective treatment and allows natural drainage of anterior group of sinuses.

Keywords: *Paranasal sinuses, mucoceles, oculo-orbital complications.*

SAŽETAK

Mukokele paranazalnih šupljina su benigne cistične tvorevine ispunjene mukoznim sadržajem. Mukokele su lokalno destruktivne, tako što pritiskom na zid sinusa uzrokuju njegovu resorpciju, što im omogućava da se šire na okolne strukture i izazovu lokalne, orbitalne i intrakranijalne komplikacije. Najčešće su lokalizovane u čeonim sinusima.

Cilj ovog rada je prikaz slučaja okulo-orbitalnih komplikacija mukokele čeonog sinusa, sa akcentom na lečenje kombinovanim hirurškim pristupom.

Pacijentkinja starosti 75 godina, sa mukokelom čeonog sinusa koja je dovela do destrukcije krova orbite i pojave komplikacija u formi orbitalnog celulitisa i palpebralnog abscesa, je uspešno lečena kombinacijom spoljne frontoetmoidektomije i endoskopske hirurgije sinusa. Nakon inicijalne incizije abscesa gornjeg očnog kapka sa drenažom purulentnog sadržaja, izvedena je modifikovana spoljna frontoetmoidektomija kroz preformirani koštani defekt krova orbite. Na kraju, endoskopskom hirurgijom sinusa omogućena je prirodna drenaža prednje grupe paranazalnih sinusa.

Različite endoskopske i otvorene hirurške tehnike su opisane u terapiji mukokela. U ovom slučaju smo pokazali da kombinovani hirurški pristup u lečenju mukokela čeonog sinusa sa destrukcijom poda čeonog sinusa i pojavom okulo-orbitalnih komplikacija, omogućava efikasan tretman i prirodnu drenažu prednje grupe sinusa.

Ključne reči: *Paranazalne šupljine, mukokele, okulo-orbitalne komplikacije.*

ABBREVIATIONS:

ESS - Endoscopic sinus surgery

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INTRODUCTION

Paranasal sinus mucoceles are slow-growing, cubic or pseudostratified epithelium-lined, mucous filled, cystic masses, usually resulting from obstruction of sinus ostia, that are locally destructive, causing bony resorption and displacement of adjacent structures (1, 2, 3). The occlusion of the ostia may be secondary to anatomic abnormalities, infection, allergy, trauma, tumours or sinus operations (3, 4, 5). It has also been suggested that a cystic degeneration of a goblet cell gland could result in a mucocele (4). The most commonly affected is the frontal sinus, followed by the ethmoid sinuses, with reports suggesting 70–90 % of mucoceles occur in these locations (1). Ten percent of mucoceles occur in the maxillary sinus while the sphenoid sinus is rarely affected (1). The clinical presentation of mucoceles varies with their anatomical site (5). The onset of symptoms is usually insidious, and intensification of symptoms usually indicate development of complications (3, 5). Patients may be referred with symptoms of varying severity according to the lesion location, size of the bone defect, and symptoms due to compression (3). Patients with frontoethmoidal mucoceles may develop frontal headache, facial asymmetry, or swelling, as well as ophthalmological manifestations, such as impaired visual acuity, reduced ocular mobility or proptosis (1, 5). Beside anamnesis, clinical examination and nasal endoscopy, the diagnosis is based on computed tomography (CT) and magnetic resonance imaging (MRI) (6). CT and MRI of the sinuses allow the assessment of mucocele extension in relation to adjacent structures (6).

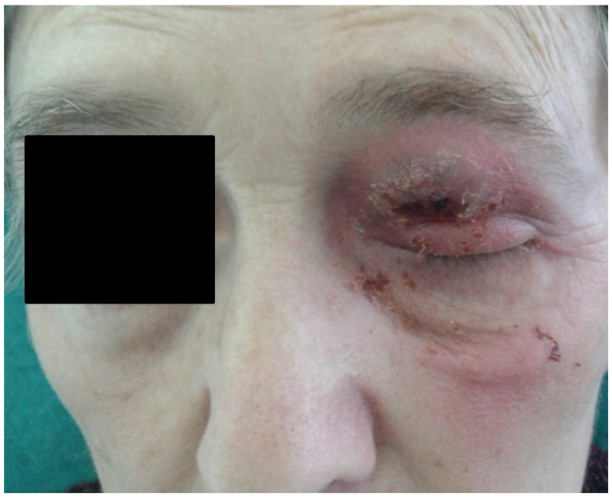
Surgery is the only method of treating paranasal sinus mucoceles (6). Considerations in the surgical approach for dysfunctional frontal sinuses include obliteration of affected sinus or restoring ventilation to the sinus through reestablishing the natural frontal sinus outflow tract (7). Initially, mucoceles were treated mainly by extirpation using external approaches and sinus obliteration to avoid recurrence (8). With introduction of endoscopic sinus surgery (ESS), mucocele marsupialization and enabling of sinus drainage became a valid choice for the treatment of selected types of mucoceles (8, 9).

In this paper we showed that the combined surgical approach provides an effective method in the management of a frontal sinus mucoceles involving the orbit. Since mucocele exteriorisation towards orbit led to sinus floor destruction, sinus obliteration was possible only through radical sinus surgery, such as Riedel procedure, that would lead to aesthetic impairment. Through already formed orbital roof defect, we were able to completely remove mucocele using modified frontoethmoidectomy, while ESS allowed us to enable drainage of the frontal sinus and ethmoid cells, thus sinus obliteration was not necessary.

CASE REPORT

A seventy five year old female patient with the swelling of left upper eyelid was admitted to Otorhinolaryngology Clinic, Clinical Center Kragujevac, Serbia, for additional radiographic diagnostics including multislice computed tomography (MSCT) of paranasal sinuses and left orbit and treatment, on account of suspected oculo-orbital complication of sinusitis. Anamnestic data showed that patient suffered sinusitis a month before, and that the left upper eyelid swelling started 3 weeks ago. Conservative therapy prescribed by otorhinolaryngologists and ophtalmologists did not give significant result. A clinical exam on admission showed intumescence, redness and ptosis of left upper eyelid, which resulted in completely closing the left orbit (Figure 1). In the central part of intumescence there was 10x10mm solid tumefaction, painful on palpation, indicating that palpebral abscess was formed. Eye bulb was compressed to the lower part of the orbit, with the patient suffering from diplopia. With nasal endoscopy we observed bilateral hiperemy and oedema of nasal mucosa with purulent secretion.

Figure 1. Preoperative local findings



MSCT pointed out intumescence with central hypodense zone in the region of left upper eyelid and subcutaneously in the region of frontal bone arc, which spreads into medial part of the orbit (Figure 2A, B). There was a bone defect of the orbital roof, together with the spreading of the frontal sinus content towards the orbit, which was liquid to viscous secretion attenuation, without clear radiography signs of continuity with described intumescence of left upper eyelid (Figure 2C, D). MSCT findings of other paranasal sinuses indicated that the patient suffered from pansinusitis. Microbiological analysis of the left upper eyelid material showed *S.aureus* infection. All examinations suggested that the patient suffered from pansinusitis with secondary infected mucocele of left frontal sinus (pyocele) with oculo-orbital complication in form of cellulitis and palpebral abscess.

After adequate preoperative preparation, which included preanesthetic assessment, internistic and ophtalmic examination as well as laboratory diagnostics, we performed a surgical treatment. Initially we made incision of the left upper eyelid abscess with drainage of purulent content. Then, we performed modified external frontoethmoidectomy using preformed defect of orbital roof, with extirpation of the left frontal sinus mucocele (Figure 3).

Finally, we carried out ESS of the left side of the nose including infundibulectomy, medial meatotomy and anterior ethmoidectomy enabling natural drainage of anterior group of sinuses. Histopathological analysis of inflammatory altered left frontal sinus mucosa confirmed clinical diagnosis. The patient was discharged from the hospital on 9th postoperative day, without complications and signs of recurrence on follow up examinations.

Figure 2. MSCT findings pointing out the presence of palpebral abscess on transfersal (A) and sagittal (B) section. Frontal sections showing bone defect of orbital roof with spreading of the frontal sinus content towards the orbit on the soft tissue (C) and bone (D) CT window

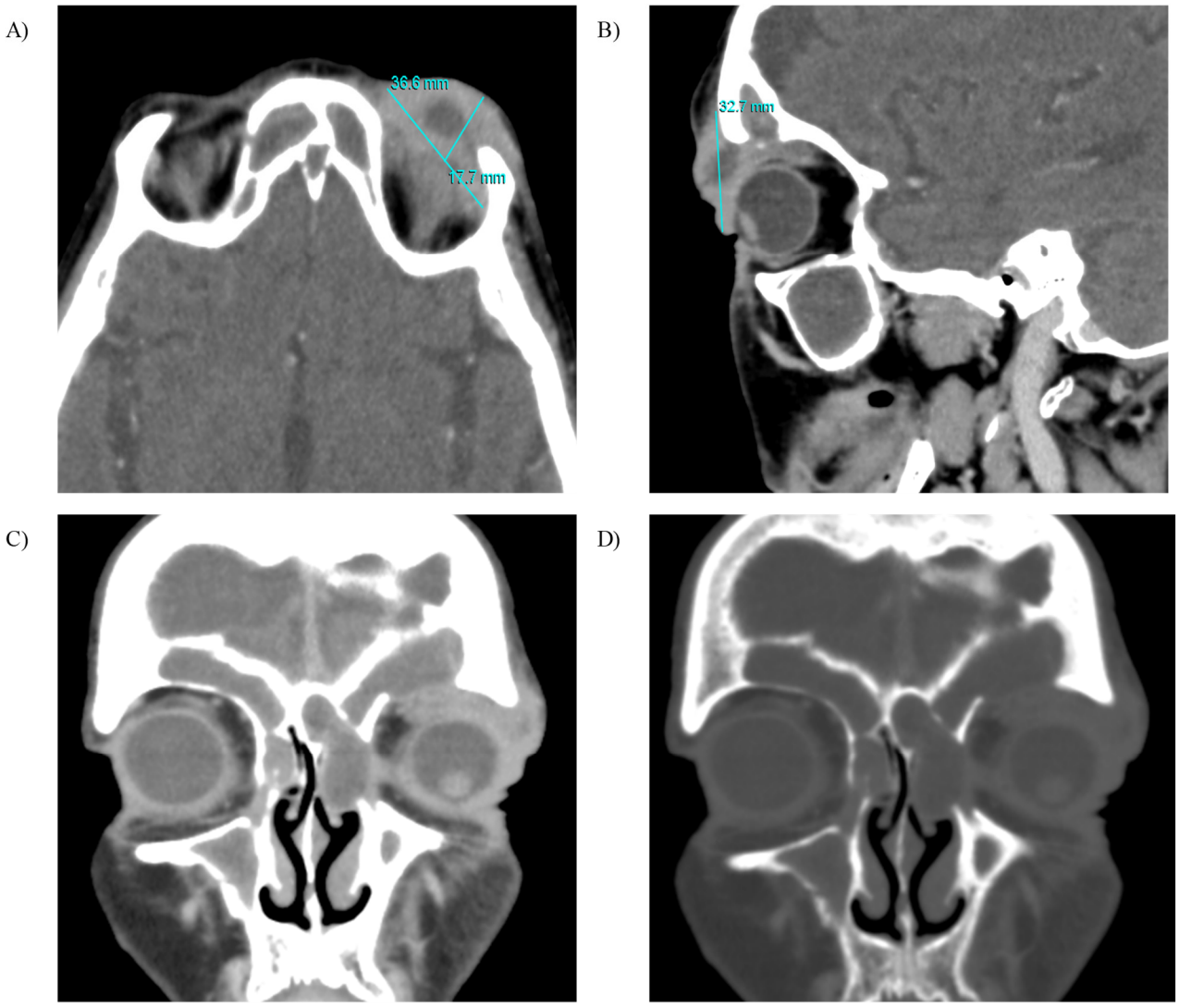
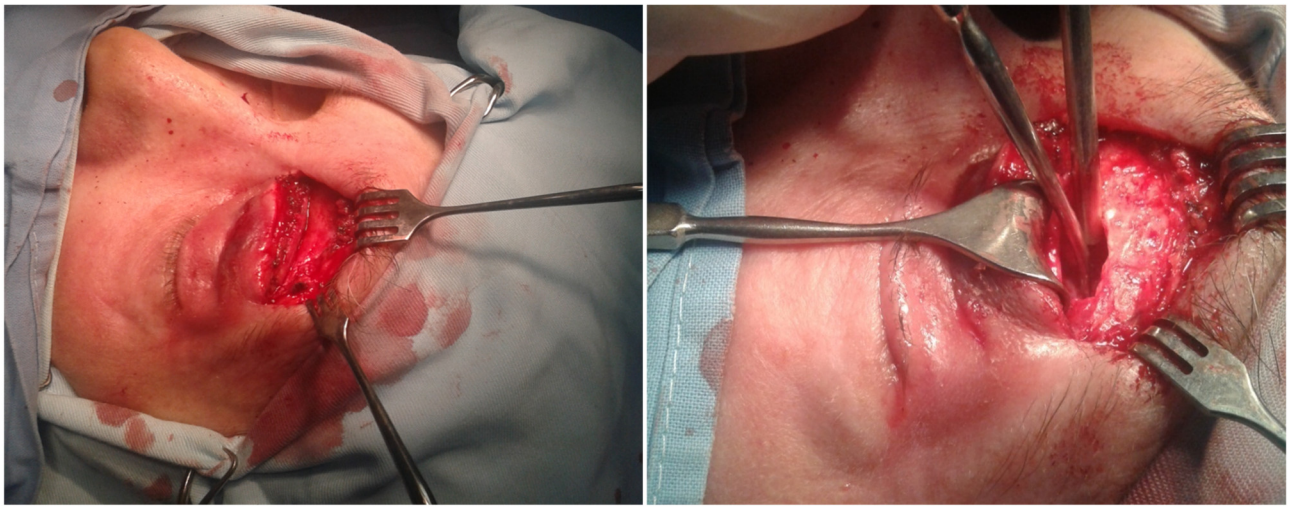


Figure 3. Intraoperative findings while performing modified external frontoethmoidectomy



DISCUSSION

Mucoceles are epithelium-lined mucous filled cystic formations which usually develop when the ostium of paranasal sinus became obstructed (10). They most commonly occur in the third or fourth decades of life with a slight male predilection (1). The mucoceles are benign, slow-growing lesions that commonly occur in the frontal or ethmoidal group of sinuses (5). In the period from 2006-2017 we had 16 cases of mucoceles in our Clinics, 9 (56.25%) of those primarily localised in frontal sinuses, 4 (25%) in ethmoids and 3 (18.75%) in maxillary sinuses, with no cases of sphenoid sinus mucocele. Symptoms usually develop slowly, and their intensification indicates occurrence of complications (5). Mucocele complications can be local, orbital and intracranial and they appear as a consequence of bone destruction and secondary mucocele infection (pyocele) (2, 11). Bone degradation is caused by increased size of mucocele, continuous augmentation of pressure on sinus walls, and the release of inflammatory mediators such as prostaglandin E2 and collagenase at the capsule of the mucocele, which degrades the bone (12). Patients may be referred with symptoms of varying severity according to the lesion location, size of the bone defect, and symptoms due to compression (3).

The most common symptoms of patients with frontoethmoid sinus mucoceles are headache, swelling or facial asymmetry and ophthalmic symptoms, such as visual disturbance, proptosis and diplopia (13). In the case of local complication, frontoethmoid mucoceles are often associated with a palpable mass in the superonasal and medial canthal region (14). Destruction of the posterior frontal sinus wall results in intracranial mucocele complication which can be presented as meningitis, meningoencephalitis, brain abscess, seizures, or cerebrospinal fluid fistula (11). Unilateral proptosis is the most common presenting sign of an oculo-orbital frontal mucocele complication, as in our case (15, 16). Other ophthalmic symptoms include outward and downward displacement of the globe, restriction of eye movements, diplopia, visual loss, retroorbital pain or headache (17). In our case, proptosis was associated with upper eyelid hyperemia and oedema, conjunctival chemosis, downward displacement of the globe and diplopia. Since the swelling of the upper eyelid was the main presenting feature, this condition could have been easily misdiagnosed and treated inappropriately. However, the red flags that should be pointed out in our cases were the unresponsiveness to the treatment, a palpable well-circumscribed mass in the upper palpebral region and clinical signs of sinusitis. Most common nasal symptoms such as nasal blockage and secretion, as well as loss of sense of smell can be signs of nasal expansion of mucocele, or sinusitis as in our case (18).

The diagnosis of the mucocele is based on anamnesis, clinical examination, nasal endoscopy with the aid of CT and MRI (14). CT provides information about regional anatomy, degree of bone destruction and lesion extension (1, 14). Mucoceles are typically presented on CT as homogenous, isodense masses, with clearly defined margins surrounded by osteolyitic sinus wall lesions (10). MR imaging is useful in differentiating mucoceles from neoplasms and surrounding soft tissue in cases of their expansions, using contrast enhancement (1, 14). Dermoid cysts, histiocytosis, fungal and tuberculosis infections, fronto-orbital cholesterol granuloma, and neoplasms must be considered in the differential diagnosis (5). In our case, we based our diagnosis on clinical examination, nasal endoscopy and MSCT. Examination indicated the presence of the left upper eyelid abscess, with left orbit cellulitis causing proptosis and diplopia. Nasal endoscopy examination showed signs of chronic rhinosinusitis exacerbation presented as bilateral nasal mucosa hyperemia and oedema with purulent secretion originating from the middle nasal meatus. MSCT imaging provided us with the information about superior orbital wall lesion with extension of clearly demarcated cystic formation towards left orbit and confirmed

clinical diagnosis of orbital cellulitis, palpebral abscess and pansinusitis.

Until the late 80s, paranasal sinus mucoceles were treated with open surgical procedures such as frontal sinus osteoplasty, external frontoethmoidectomy and maxillary sinus trepanation (1, 8). Traditional teaching emphasized the need for a complete removal of the sinus mucocele lining and obliteration of the sinus to avoid recurrence (8). Management of mucocele has significantly changed since the introduction of ESS, so that mucocele marsupialization and enabling of sinus drainage became a valid choice for the treatment of selected types of mucoceles (8, 9).

Kennedy et al. marsupialized 9 of 11 frontal mucoceles and reported no cases of relapse, and Har-El marsupialized endoscopically 108 paranasal sinus mucoceles including 66 frontal and frontoethmoid, 17 ethmoid, 7 sphenoethmoid, 12 sphenoid, and 6 maxillary mucoceles with recurrence of a frontal mucocele seen in 1 patient (0.9%) (19, 20). Lund treated 48 mucoceles in the frontal, frontoethmoidal and sphenoidal sinuses during the period of five years, 20 by an entirely endonasal endoscopic approach and 28 by a combination of an external procedure and an endoscopic approach (21). Nevertheless, most of the authors agree that even though ESS is nowadays the surgical method of choice, the treatment of mucocele depends on many factors (5).

The location, magnitude, and expansion of the lesion are main determinants of the appropriate surgical procedure (3). Many authors consider lateral localization of frontal mucocele a contraindication to ESS (22). Martel-Martin et al. indicate an external approach for frontal mucoceles which only affect the most external and posterosuperior region of the sinus, when the appearance of the mucocele is the consequence of an endosinusal process which septates the sinus, such as an osteoma or major sclerosis in the region of the frontal recess (2). On the other hand, Sharouny et al. suggest that endoscopic sinus surgery is the treatment of choice in most cases of frontal sinus mucoceles including lateral frontal mucoceles (23). While smaller lesions can be treated endoscopically, larger lesions can usually be managed only via external procedures and sinus obliteration to prevent the ascending infections (14). Trimarchi et al. suggest that it is essential to determine mucocele extension beyond a virtual sagittal plane tangential to the medial side of the ocular globe and consider mucocele extension medially to the virtual sagittal plane as the main selection criterion for ESS, more important than the size of the mucocele (8). Endoscopic trans-sinusal approach is not recommended in cases of intracranial extension of mucocele because of the higher risk of intracavitary residual and postoperative recurrences due to a narrow access to the lesion (24). On the other hand, obliteration of the involved sinus is not recommended if there is an erosion of the sinus bony wall with extension of the mucocele into the orbit, because mucosa lining the mucocele becomes adherent to orbital periosteum and cannot be removed during the surgery without significant risk of injury to the adjacent structures (9). And, if mucosa is left behind and the sinus obliterated, a recurrence of the mucocele is highly likely (9). As most cases of symptomatic mucocele have erosion of the bony sinus wall, the obliteration of the sinus should not be considered (9). Kim et al. note that minimally invasive surgery to remove ethmoid mucoceles with orbital complications is relatively straightforward and avoids the complications associated with these lesions (13). In cases where intranasal treatment presents difficulties, it is possible to use an external route or a combined approach with external treatment under endoscopic control (5). Beigi et al. suggest that the combined internal/external approach provides a viable and effective method for managing frontal sinus mucocele involving the orbit (25).

In our Clinics, in the period from 2006-2017 we treated 3/9 (33.3%) frontal sinus mucocele endoscopically, while for the 6/9 (66.7%) we decided to use external approach. In the case of ethmoid sinus mucocele we used ESS in 4/4 (100%) cases, while 2/3 (66.7%) mucoceles were treated endoscopically and recurrent 1/3 (33.3%) with the open technique. We suggest that in any case of asymptomatic mucocele, as well in the cases without exteriorisation of mucocele, ESS with mucocele marsupialization and allowing sinus drainage should be performed. If mucocele is inaccessible, recurrent or complications occurred, we recommend using an adequate external or combined surgical approach. In this case, since oculo-orbital complication occurred, we decided for a combined surgery. External frontoethmoidectomy allowed us to completely remove mucocele through already formed orbital roof defect. Endoscopically we managed to enable drainage of the frontal sinus and ethmoid cells, thus sinus obliteration through radical sinus surgery that would lead to aesthetic impairment was not necessary.

CONCLUSION

Because of the tendency to destruct sinus wall, mucoceles can cause complications at some point. To prevent complications, early diagnosis and timely surgical treatment is necessary. Endoscopic sinus surgery and marsupialization should be the treatment of choice for asymptomatic and simple frontal mucoceles. More radical approaches are required if the size of mucoceles is large and if there appears to be extensive bone erosion causing orbital or intracranial complications. We believe that obliterative sinus surgery should be avoided whenever possible and reserved mostly for the cases of intracranial complications in order to avoid spreading the ascending infection, since obliteration of sinus ostia by itself can be cause of mucocele recurrence. Postoperative long-term follow-up with endoscopic surveillance is mandatory for every patient because recurrence of mucocele could occur many years after surgical management.

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TAKOTSUBO CARDIOMYOPATHY PRECIPITATED BY THYROIDECTOMY - A CASE REPORT

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TAKOCUBO KARDIOMIOPATIJA ISTALOŽENA TIROIDEKTOMIJOM - PRIKAZ SLUČAJA

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ABSTRACT

Takotsubo cardiomyopathy (TC) is an acute cardiac condition triggered by emotional or physical stress. General anesthesia and sympathetic activation are possible triggers for TC. However, little is known about the role of sympathovagal activity in TC. In our report, we present a female patient, aged 62, who underwent thyroidectomy and at the end of the surgery developed cardiac complications. The patient had no chest pain, but had ST depression and negative T waves on the electrocardiogram (ECG). Cardiospecific enzyme troponin was elevated. Cardiac catheterization revealed unobstructed coronary arteries. Echocardiography revealed the enlargement of the left ventricle and ejection fraction of 40%. The patient was diagnosed with TC and dual antiplatelet therapy was introduced, a beta blocker and ACE inhibitor.It is possible that TC in perioperative period after thyroidectomy in this patient occured due to both sympathetic and parasympathetic activation. Probably, extraction of large thyroid induced vagal stimulation which resulted in hypotension and bradicardia. The patient was subsequently treated with adrenaline and atropine. In this case, sympathetic and parasympathetic activation in different intervals could result in the development of this condition.

***Keywords**: takotsubo, cardiomyopathy, thyroid gland, sympathetic, parasympathetic.*

SAŽETAK

Takocubo kardiomiopatija (TC) je akutno kardiološko stanje čiji je okidač emocionalni ili fizički stres. Opšta anestezija i simpatička aktivnost su mogući okidači za nastanak TC. Do danas se vrlo malo zna o simpatovagalnoj aktivnosti u TC. U našem slučaju, predstavljana je pacijentkinja, 62 godine, kojoj je urađena tireidektomija. Na kraju operacije razvile su se srčane komplikacije. Pacijentkinja nije imala bolove u grudima, ali ST depresija i negativni T talasi su postojali na elektrokardiogramu (ECG). Kardiospecifični enzim troponin je bio povišen. Kateterizacijom je pokazano da koronarne arterije nisu bile zapušene. Ehokardiografijom je pokazano povećanje leve komore kao i smanjenje ejekcione frakcije na 40%. Pacijentkinji je dijagnostifikovan TC i data dvojna antiagregaciona terapija, beta blokatori, ACE inhibitori. Moguće je da je kod naše pacijentkinje uzrok nastanka TC nakon tiroidektomije zajedničko dejstvo simpatičke i parasimpatičke aktivnosti. Vađenje velike tiroidne žlezde je stimulisalo vagus što je rezultiralo hipotenzijom i bradikardijom. Pacijentkinji je dat adrenalin i atropin. U našem prikazu slučaja pokazano je da aktivacija simpatikusa i parasimpatikusa u različitim vremenskim intervalima može dovesti do TC.

***Ključne reči:** Takocubo, kardiomiopatija, tiroidna žlezda, simpatikus, parasimpatikus.*

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INTRODUCTION

Takotsubo cardiomyopathy (TC) is an acute cardiac condition triggered by emotional stress or acute illness. It is also known as “transient left ventricular (LV) apical ballooning syndrome (ABS)” or “broken heart syndrome“ or “stress-induced cardiomyopathy” (1, 2). This cardiomyopathy is characterized by symptoms of acute myocardial infarction and electrocardiographic results show an acute coronary syndrome. Usually, these patients do not have obstructive coronary artery disease. Patients, who have been reported to have TC, had greater vulnerability to stress (3).

Although multiple cases of TC developed during perioperative period have been reported, it is not a common complication of general anesthesia or any particular surgical procedure (4-7).

The pathophysiology of TC is not well established (5). However, some possible etiological factors have been proposed, such as catecholamine cardiotoxicity, metabolic disturbances, impairment of coronary microvascular circulation and multivessel epicardial coronary artery spasm (8). It is also possible that a range of other pathogenetic factors, including neurogenic injury, genetic polymorphisms of adrenergic receptors and the disbalance of sympathovagal activity may play a role in the development of this rare condition (9).

The aim of this case study is to show the importance of a proper diagnosis of TC during perioperative period, with an emphasis on differential diagnosis which includes acute coronary syndrome, so that adequate treatment can be provided. We also expect that this case could shed more light on the role of sympathovagal misbalance in the development of TC.

BACKGROUND

Female patient, 62 years old, with substernal multinodular thyroid goiter underwent thyroidectomy. At the end of the perioperative period, the patient developed bradycardia with heart rate of 30/min and hypotension (80/50). Adrenaline was administered (0.5 mg i.v. 1:1000) as well as atropine (0.4 mg) and oxygen therapy. Cardiospecific enzyme troponin was elevated (5.2 μg/L). The patient was admitted at the coronary unit. During hospitalization, the patient had no chest pain, no signs of heart failure, or rhythm disturbances. On the electrocardiogram (ECG), ST depression and negative T waves at DIII, aVF, V4, V5 were observed (Figure 1). Fraxiparin (0.4 ml) was prescribed immediately, but the dual antiplatelet therapy was introduced 7 days post-surgery due to the risk of postsurgical bleeding. Patient’s previous diseases were hypertension, ventricular extrasystolic arrhythmia and hypercholesteremia. Her regular therapy included carvedilol (12.5 mg, 1x1), perindopril (2.5 mg, 1x1), propafenon (150 mg, 1x1) and aspirin (100 mg, 1x1).

Echocardiography was performed during hospitalization. The left ventricle was larger with normal wall thickness, only basal segments of the walls were contractile. Ejection fraction (EF) was about 40%. Mitral regurgitation was grade 1. Diastolic flow represented slower relaxation of the left ventricle. Right ventricle was normal. Tricuspid regurgitation was grade 2. Right ventricle systolic pressure was 41 mm/Hg. There was pericardial effusion behind the left ventricle posterior wall of up to 9 mm lateral and around the right ventricle about 4 mm (Figure 2). On the control echocardiografic examination, 10 days after the initial assessment, an improvement in myocardial contractility was registered. The segments that had been akinetic were hypocontracting. EF was about 55% (Figure 3). Control ECG showed sinus rhythm of 90 beats per minute with negative T waves in leads DI, DII, aVF, V2-V6. Cardiac catheterization revealed unobstructed coronary arteries. Coronary arteries were without angiographically significant stenosis (Figure 4). The X-Ray of the heart and lungs were normal. Laboratory analysis were in range except for increased cholesterol (6.7).

Based on the echocardiographic and angiographic findings, as well as the elevated troponin, the diagnosis of TC was confirmed.

The patient was treated with dual antiplatelet therapy (aspirin 100 mg, 1x1 and clopidogrel 75 mg, 1x1), beta blocker (labetalol 25 mg, 3x1), ACE inhibitor (zofenopril 3.75, mg 1x1), thyroxin (100 mg, 1x1), calcium supplementation ($CaCO_3$, 500 mg, 3x1), statin (atorvastatin 10 mg, 1x1) and sedative (bromazepam 3 mg, 1x1).

This study was carried out in accordance with the recommendations of the Ethics Committee of the Faculty of Medicine, University of Belgrade, Belgrade, Serbia with written informed consent of our patient. Subject gave written informed consent in accordance with the Declaration of Helsinki.

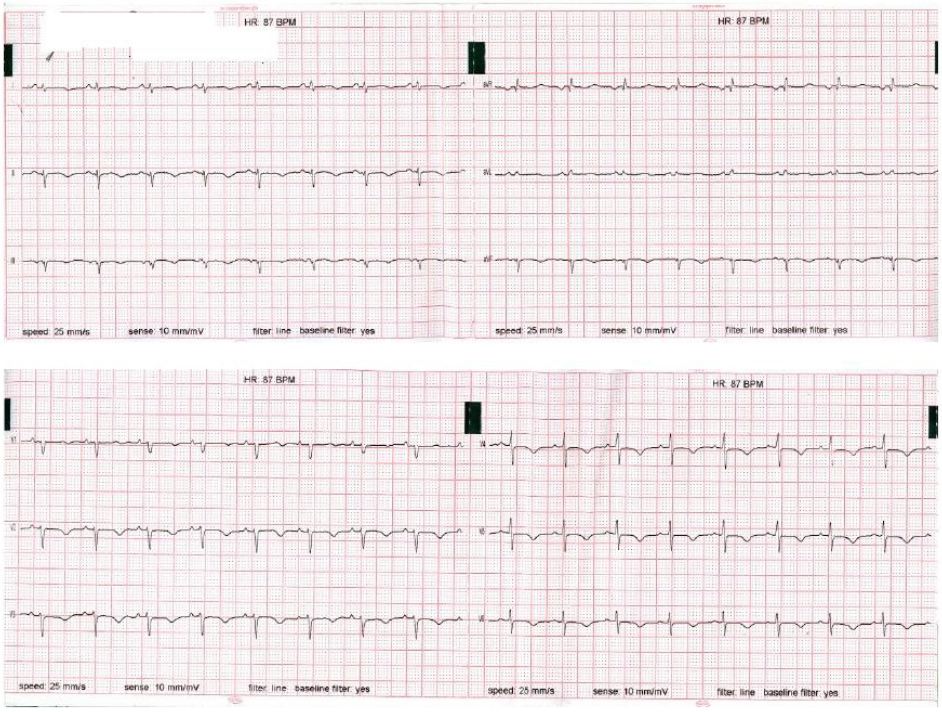


Figure 1. Electrocardiogram on admission shows negative T waves in leads DII, DIII, aVF and V2-V6

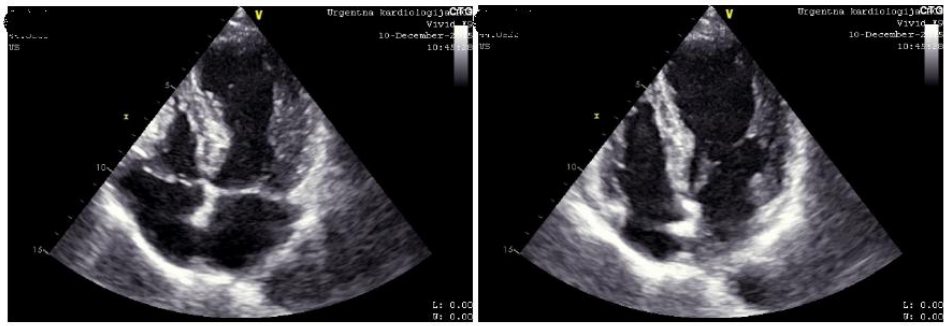


Figure 2. Apical 4-chamber view showing apical ballooning during acute phase.

A) Systole B) Diastole

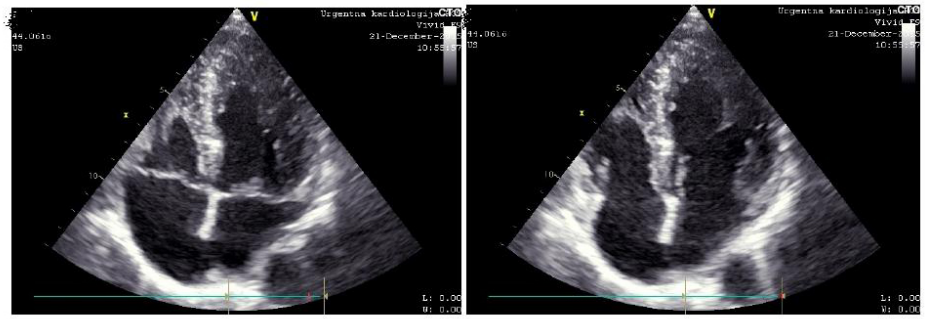
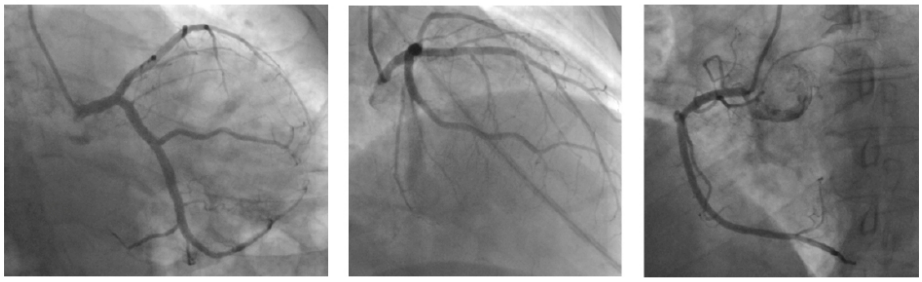


Figure 3. Apical 4-chamber view after 11 days of admission shows an improvement in left ventricular systolic function. A) Systole B) Diastole



Cx LAD RCA

Figure 4. Coronary angiography shows no significant stenosis on coronary arteries

Cx-Circumflex coronary artery, LAD-Left anterior descendent coronary artery, RCA-Right coronary artery

DISCUSSION

Takotsubo cardiomyopathy often occurs after emotional or physical stress. It is characterized by transient left ventricular apical ballooning with the absence of coronary occlusion (10).

Currently, there are no official guidelines for the diagnosis and treatment of TC. However, the European Society of Cardiology (ESC) suggests following criteria for diagnosing TC which include newly observed ECG abnormalities, transient apical dyskinesia or akinesia, detected by echocardiography, beyond a single coronary artery distribution, nonobstructive coronary artery disease (stenosis < 50%) at angiography in the absence of: myocarditis, pheochromocytome, head trauma, intracranial haemorrhage and hypertrophic cardiomiopathy (11).

Diagnosis in our case was performed on the recommendation of Mayo Clinic Criteria for TC (12). All four proposed criteria were present: transient hypokinesis, akinesis, or dyskinesis of the left ventricular mid segments with or without apical involvement; absence of obstructive coronary disease; new electrocardiographic abnormalities (either ST-segment elevation and/or T-wave inversion) or modest elevation in cardiac troponin; absence of pheochromocytoma or myocarditis (8).

There were cases where hypothyroidism, hyperthyroidism and hormone disturbance induced TC (13, 14). In our case, thyroid hormones were in reference range.

Noradrenaline levels are in general unusually high in the acute phase of TC, suggesting a hyperadrenergic mechanism. In our patient, levels of catecholamines in urine were within normal range, but the test was done the day after surgery when catecholamines levels are already expected to be normalized. However, the patient was treated with adrenaline at the end of the perioperative period due to bradicardia and hypotension. It is known that catecholamines are included in the pathogenesis of TC. Earlier, the importance of β-blockers in acute-phase management of TC was investigated. Isogai et al. (15) found no significant connection between early β-blocker use and in-hospital mortality in patients with TC. Our patient got β-blocker labetalol which improved heart function.

Little is known about the role of parasympathetic activity in patients with TC. In one study, ten women sympathetic and parasympathetic activity were assessed (16). Sympathovagal activity was assessed at resting state and during baroreflex stimulation (Valsalva maneuver and tilt testing). The level of catecholamines in plasma did not differ between TC women and controls. During baroreflex stimulation indexes of parasympathetic (vagal) modulation of heart rate were decreased in women with TC versus controls. Women with TC had greater sympathetic response and impaired parasympathetic modulation and baroreflex control of heart rate (16). It is possible that, due to surgical maneuvers, during thyroidectomy which could stimulate vagus, our patient had a strong parasympathetic response which resulted in hypotension and bradycardia. One of the possible mechanisms of the recovery of heart rate after strong parasympathetic stimulation is so called “vagal escape”. This mechanism occurs when bradycardia and hypotension are compensated by the increased sympathetic activity which affects mainly ventricles which are not supplied by vagus (17). Also, the general anesthesia is a known risk factor for TC. Anesthetic management of patients with TC requires special care throughout the perioperative period. Cases of patients with a confirmed diagnosis of TC during anesthesia in perioperative period were reported (5, 6, 7, 18). Proper monitoring during surgery is important as well as team of experts for treating this syndrome (2). Our patient also had hypertension and ventricular extrasystoles preoperatively, which indicates increased sympathetic activity and was administered adrenaline at the end of the surgery. All these factors could potentially contribute to the development of TC in our patient.

CONCLUDING REMARKS

This is the first case which presents activation of sympathetic and parasympathetic action in different intervals which could result in the development of TC.

CONFLICT OF INTEREST

There is no conflict of interest.

ACKNOWLEDGEMENTS

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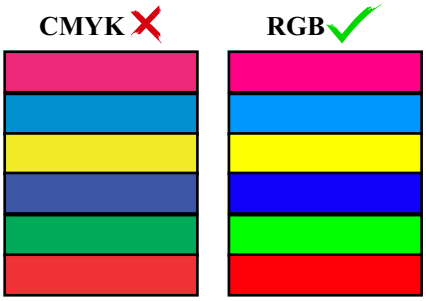

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