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CONTENTS / SADRŽAJ

Vaccines are one of the most impressive scientific inventions of all time, helping to protect generations of people against infectious diseases. The result of more than 200 years of research, worldwide collaboration, and rigorous testing is the development of safe and effective vaccines for more than 25 diseases. The theme “Long Life for All” of World Immunization Week 2022 aims to unify people around the idea that vaccines make it possible for us to follow our dreams and live a long and healthy life, and that's something we all should be fighting for.

Vakcine su jedno od najimpresivnijih naučnih dostignuća svih vremena, koje omogućavaju zaštitu generacija ljudi od zaraznih bolesti. Rezultat preko 200 godina istraživanja, međunarodne saradnje i rigoroznog testiranja jeste razvoj bezbednih i efikasnih vakcina za više od 25 bolesti. Slogan Svetske nedelje imunizacije 2022. godine „Dug život za sve“ ima za cilj da ujedini ljude oko ideje da nam vakcine omogućavaju da sledimo svoje snove i živimo dug i zdrav život, a to je nešto za šta bi svi trebalo da se borimo.

ORIGINAL ARTICLES

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Diagnostic applications of the “pattern” electroretinography and visual evoked potentials in the evaluation of disorders of visual pathway function in Parkinson’s disease

Dijagnostička primena *pattern* elektroretinografije i vizuelnih evociranih potencijala u evaluaciji poremećaja funkcije vizuelnih puteva kod Parkinsonove bolesti

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Abstract

Background/Aim. In spite of continuous research efforts, specific laboratory, neuropsychological or neurophysiological tests for diagnosing Parkinson’s disease (PD) have not been established. The aim of the study was to determine the nature and extent of visual pathway disorders on “pattern” electroretinography (PERG) and visual evoked potentials (VEPs) in certain stages of PD. **Methods.** The study was carried out in a group of 60 persons of both sexes who were suffering from idiopathic PD at the I–IV stage of the disease according to the Hoehn and Yahr scale, and 30 healthy persons in the control group. The battery of noninvasive neurophysiological tests was used to estimate the functional status of the visual pathway − PERG and VEPs. **Results.** In the early phase of PD, there was a linear increase in the latency of the wave N50 of the PERG and the wave P100 of the VEPs, with a significant extension of the latency of the N50 and P100 waves in subsequent stages of PD. Diagnostic application of the PERG and VEPs enabled the confirmation of a disorder in the visual pathway function in PD. **Conclusion.** Applied neurophysiological techniques may record early changes in the function of retinal structures and the optic nerve in PD, which might be significant from both the diagnostic and therapeutic aspects.

Key words:
diagnosis; electroretinography; evoked potentials, visual; parkinson’s disease.

Apstrakt

Uvod/Cilj. Uprkos neprekidnim istraživačkim naporima, specifični laboratorijski, neuropsihološki ili neurofiziološki testovi za dijagnozu Parkinsonove bolesti (PB) nisu utvrđeni. Cilj rada bio je da se utvrdi priroda i obim poremećaja vidnog puta na *pattern* elektroretinogramu (PERG) i vizuelni evocirani potencijali (VEP) u određenim fazama PB. **Metode.** Ispitivanje je sprovedeno u grupi od 60 osoba oba pola, obolelih od idiopatske PB, u I–IV fazi bolesti prema skali Henove i Jara i 30 zdravih osoba u kontrolnoj grupi. Baterija neinvazivnih neurofizioloških testova upotrebljena je za procenu funkcionalnog statusa vizuelnog puta − PERG i VEP. **Rezultati.** U ranoj fazi PB došlo je do lineranog povećanja latence talasa N50 PERG i talasa P100 VEP, sa značajnim produženjem latencije talasa N50 i P100 u odmaklim fazama PB. Dijagnostička primena PERG i VEP omogućila je potvrđivanje poremećaja funkcije vizuelnog puta kod PB. **Zaključak.** Primenjene neurofiziološke metode mogu registrovati rane promene u funkciji retinalnih struktura i optičkog nerva u PB, koje mogu biti značajne i sa dijagnostičkog i sa terapijskog aspekta.

Ključne reči:
dijagnoza; elektroretinografija; evocirani potencijali, vizuelni; parkinsonova bolest.

Introduction

The diagnosis of Parkinson's disease (PD) is based on the clinical recognition of relevant symptoms and signs, as well as on a relatively good therapeutic response after the administration of levodopa. In spite of continuous research efforts, specific laboratory, neuropsychological or neurophysiological tests for the diagnosis of this disease have not been established [1].

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Neurophysiological methods have been used to identify subclinical bradykinesia and rigidity, as well as the differential diagnosis of an isolated static tremor. Certain results were obtained by examining the motion time in the paradigm of the reaction time, the quantification of the tremors, and the electromyographic response of the long latency. Early diagnosis of PD would allow not only differentiation of this disease from other parkinsonian syndromes, but also an adequate treatment and rehabilitation procedures.

The aims of the paper are to determine the nature and extent of visual pathway disorders on "pattern" electroretinogram (PERG) and visual evoked potentials (VEPs) in certain stages of PD, as well as to determine the diagnostic sensitivity of applied neurophysiological methods in PD.

Methods

Study design and ethical standards

The cross-sectional study was carried out at the Clinic of Neurology, University Clinical Center in Niš, during the period from 2017 to 2018. The study was carried out strictly in accordance with the principles of the Declaration of Helsinki as revised in 2000, ensuring full patient anonymity. Prior to the start of the study, the consent of the Ethics Committee of the institution was obtained.

Study subjects

The study group consisted of 60 patients of both sexes who came for regular neurological examinations for two years. They were suffering from idiopathic PD at the I–IV stage of the disease according to the Hoehn and Yahr scale. Computerized brain tomography excluded other possible etiological factors for the development of Parkinsonism (vascular lesions, expansive intracranial processes). The control group included 30 healthy subjects of both sexes and the appropriate lifespan. This group was formed by patients who underwent a neurological examination due to headache or dizziness but did not confirm the diagnosis of any neurological disease. All patients and subjects in the control group had previously undergone an ophthalmic examination to rule out any ocular disease.

Electrophysiological procedures

In this study, a battery of noninvasive neurophysiological tests was used to estimate the functional status of the visual pathway as a whole. These are PERG and VEP. Using these methods, detection of neuroelectric signals at different levels of the optical pathway, from the retina to the primary cortical optic center in the occipital lobe, was performed.

For the registration of PERG, a surface disk of electrodes placed below the lower eyelid is used. A "pattern" is triggered by a stimulus that is similar to the stimulus for the registration of VEP. The respondent is 1.25 m away from the monitor with a structured "pattern" stimulus, which looks at an angle of 13.91. This registered PERG has a three-phase format: positive, negative (N50), and positive wave. Values of latency and amplitude of PERG waves depend on the angle of stimulation, stimulus intensity, and type of electrodes for registration. The latency of the N50 wave is 40–50 ms in healthy persons.

VEP is a neurophysiological method for examining the function of the optical pathway from the ganglion layer of the retina to the visual cortex. VEP is registered using the "pattern" stimulus that represents a structured light stimulus according to the type of "chessboard". The form of evoked response depends, first of all, on the frequency of the stimulus. If the stimulus frequency is less than 5 stimuli per second, the V-shaped three-phase response, consisting of the first negative component N75, the positive component P100, and the negative component N145, is registered. Pattern VEP is stable and has a wide clinical application. The wave P100 comes from central neural elements of the field of vision, and its latency can be distinguished between the left and right eye. It has been accepted that the physiological interocular difference is up to 8 ms. The following equipment is required for VEP registration: a "pattern" monitor structured stimulation, in the form of a chess field with a series of white and black squares, size 32 min alternating signals, recording electrodes, an amplification system, and a computer for stimulating, with a response reading system. The square and the intensity of contrast are the most important variables when using the "pattern" stimulus. VEP recording is performed in a dark room. The patient is seated comfortably on the chair and looks at the center of the monitor screen, where at a certain frequency, a stimulus in the form of a chess field appears. The respondent is 1 m away from the monitor with a structured "pattern" stimulus, which looks at an angle of 17.34°. VEP registration is monocular, with a frequency of 1 to 2 stimuli per second. The frequency range is 30 and 300 Hz, the analysis time is 300 ms, and the 256 stimulus is copied, which is a sufficient number to obtain a reproducible response. During the recording, complete cooperation of a relaxed patient is required, as even the smallest movements cause artifacts. Patients with refractive anomalies wear glasses with appropriate diopter. Electrode position is determined by a 10–20 international electroencephalography (EEG) system. In the clinical practice for registration of VEP, three channels and the following assembly of electrodes are most commonly used: 1st channel: active Oz (5 cm above the inion) – reference Fz; 2nd channel: active electrode O1 (5 cm left of the inion) – reference Fz; 3rd channel: active electrode O2 (5 cm right of the inion) – reference Fz.

Statistics

Statistical analysis of the results obtained in this study was carried out using the standard Microsoft Excel program by analyzing the following statistical parameters: the arithmetic mean, the standard deviation, the variation of the results (minimum and maximum values), the coefficient of variation (CV), with the determination of the confidence interval, or the reliability limit of the estimated statistical parameters. The estimation of the statistical significance of the difference in the results was made using Student's *t*-test and

calculating the linear correlation coefficient. An analysis of the data necessary for assessing the reliability and accuracy of the tested method was performed using a linear logic regression model. The results obtained are presented in tabular and graphical form.

Results

This study included a group of 60 patients with PD (39 males and 21 females). There were more males than females, as men were more likely to suffer from PD (Table 1). In the control group, there were 30 healthy subjects (13 men and 17 women). The majority of patients belonged to the age group of 69 to 73 years (14 men) and from 59 to 63 years (9 women). The study included parkinsonian patients and healthy subjects of the control group approximately equivalent to the age structure. Based on the value of CV, it was found that the experimental group of parkinsonian patients and the control group of healthy subjects showed satisfactory homogeneity in relation to the age of the subjects. The average age of parkinsonian male patients was 66.1 years, and women with PD were 62.3 years old (Table 2). The average age of the males in the control group was 66.9 years, and the female age was 68.1 years.

In our study, all parkinsonian subjects were classified into groups according to the stage of the disease, using the Hoehn and Yahr scale (Table 2). The majority of parkinsonian males (13) were of the average age of 70.7 years and belonged to the third stage of PD. The highest number of parkinsonian women (7) was 65.1 years old and belonged to the IV stage of PD.

In Table 3, we compared the parameters of the N50 wave of PERG and the P100 wave of VEPs registered on the right and left eye in parkinsonian patients and the control group of healthy persons.

Table 1

The age and gender structure of parkinsonian patients

Age (years)	Parkinsonian patients, n (%)			Control group, n (%)		
	male	female	total	male	female	total
34–38	2 (5.1)	0 (0.0)	2 (3.3)	1 (7.7)	0 (0.0)	1 (3.3)
39–43	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
44–48	0 (0.0)	3 (14.3)	3 (5.0)	0 (0.0)	0 (0.0)	0 (0.0)
49–53	1 (2.6)	0 (0.0)	1 (1.7)	2 (15.4)	2 (11.8)	4 (13.3)
54–58	5 (12.8)	1 (4.8)	6 (10.0)	2 (15.4)	1 (5.9)	3 (10.0)
59–63	2 (5.1)	9 (42.9)	11 (18.3)	0 (0.0)	0 (0.0)	0 (0.0)
64–68	9 (23.1)	3 (14.3)	12 (20.0)	1 (7.7)	6 (35.3)	7 (23.3)
69–73	14 (35.9)	3 (14.3)	17 (28.3)	1 (7.7)	2 (11.8)	3 (10.0)
74–78	5 (12.8)	2 (9.5)	7 (11.7)	2 (15.4)	5 (29.4)	7 (23.3)
79–83	1 (2.6)	0 (0.0)	1 (1.7)	4 (30.8)	1 (5.9)	5 (16.7)
Total	39 (100.0)	21 (100.0)	60 (100.0)	13 (100.0)	17 (100.0)	30 (100.0)

Table 2

The age and gender structure of parkinsonian patients in relation to the stage of Parkinson's disease (PD)

Stage of PD (H&Y)	Male		Female	
	n (%)	age (years)	n (%)	age (years)
1	12 (20.0)	59.8	5 (23.8)	56.2
2	10 (16.7)	66.6	5 (23.8)	64.0
3	13 (21.7)	70.7	4 (19.0)	63.0
4	4 (6.7)	68.5	7 (33.3)	65.1
Total	39 (65.0)	66.1	21 (35.0)	62.3

H&Y – Stage of PD according to the modified Hoehn and Yahr scale.

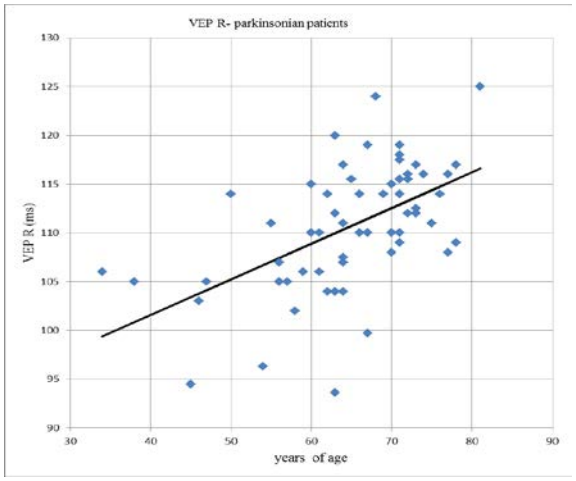
Table 3

Parameters of PERG (N50 wave) and VEP (P100 wave) for parkinsonian patients and the control group (healthy subjects)

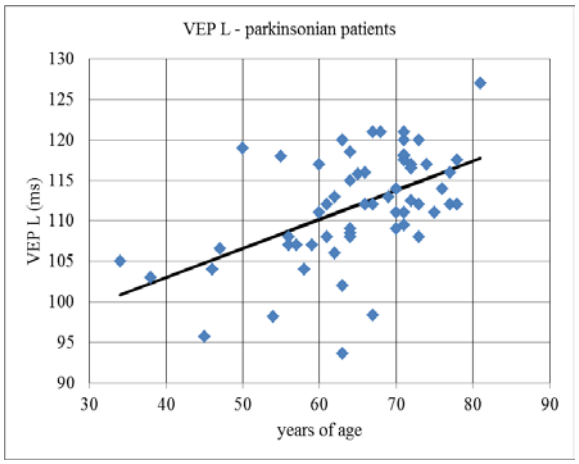
Study groups	PERG R	PERG L	VEP R	VEP L
PD patients				
mean	59.822	60.565	110.635	111.917
SD	4.963	5.263	6.507	6.801
Control group				
mean	52.440	52.480	104.083	104.150
SD	1.982	2.037	3.637	3.697

PERG – "pattern" electroretinograms; VEP – visual evoked potentials; PERG R – right-hand N50 wave of PERG; PERG L – left-hand N50 wave of PERG; VEP R – right-hand P100 wave of VEP; VEP L – left-hand P100 wave of VEP; PD – Parkinson's disease; SD – standard deviation.

Figures 1a, 1b, 2, 3a, 3b, and 3c show the regression lines for VEP parameters depending on the age and the stage of PD, as well as the regression lines for PERG parameters depending on the age and the stage of PD.



a)



b)

Fig. 1 – a) P100 wave latency of right-hand visual evoked potentials (VEP R) depending on the age of parkinsonian patients. Dots represent individual values [$p < 0.0001$ ($p = 1.13026435E-53$); $r = 0.545$]; b) P100 wave latency of left-hand visual evoked potentials (VEP L) depending on the age of parkinsonian patients. Dots represent individual values [$p < 0.0001$ ($p = 1.25723673E-58$); $r = 0.511$].

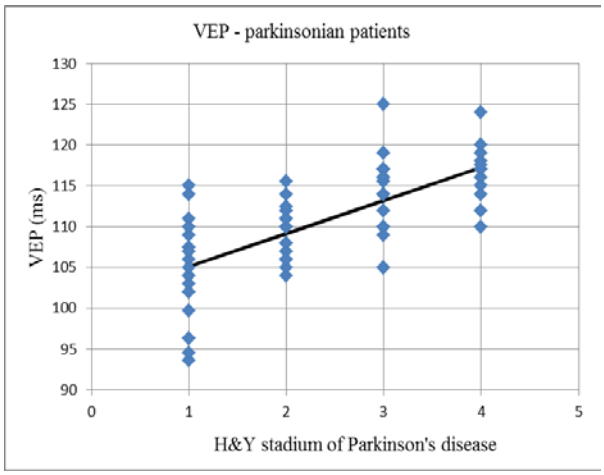


Fig. 2 – P100 wave latency of visual evoked potentials (VEPs) depending on the Hoehn and Yahr (H&Y) stadium of Parkinson's disease. Dots represent individual values [$p < 0.0001$ ($p = 3.5693995E-128$); $r = 0.678$].

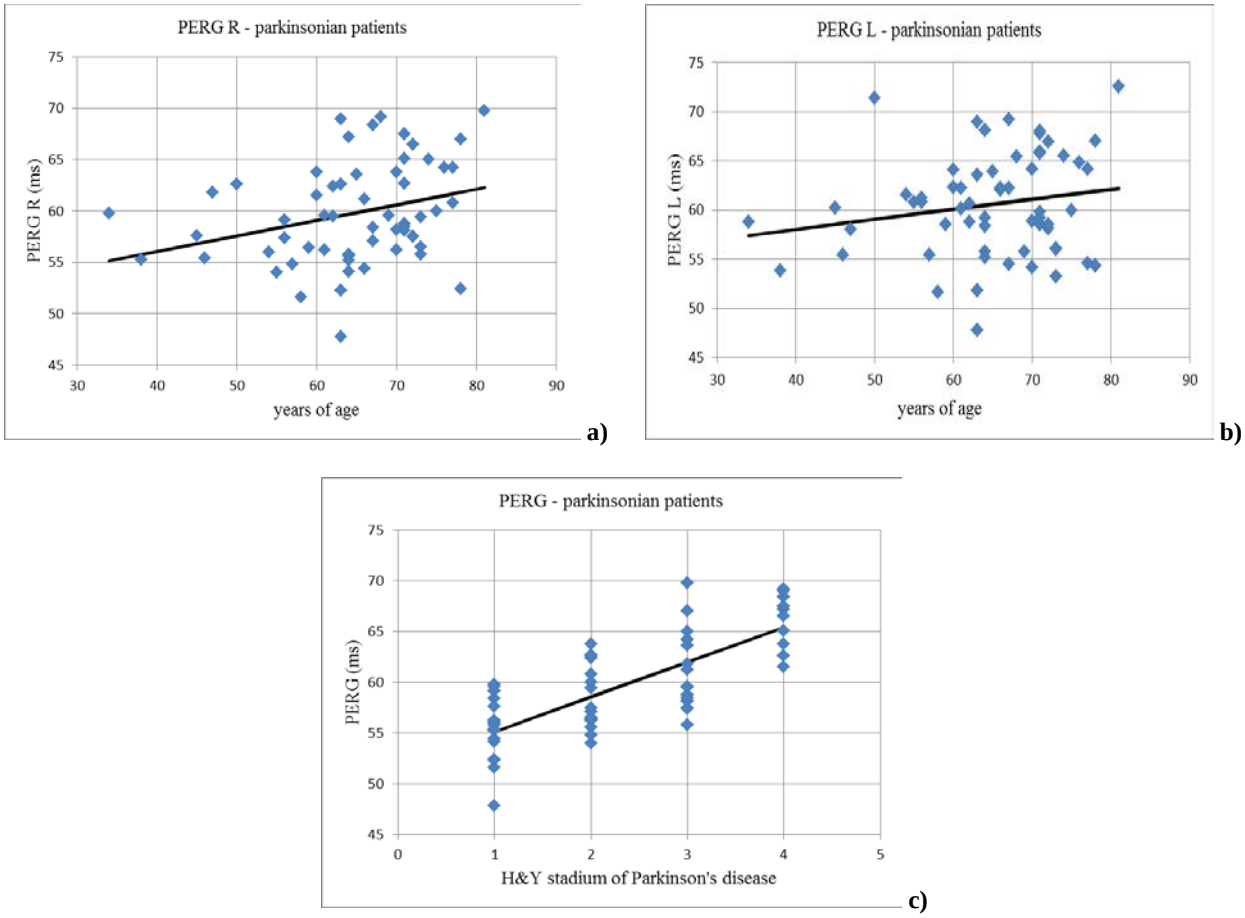


Fig. 3 – a) N50 wave latency of right-hand "pattern" electroretinograms (PERG R) depending on the age of parkinsonian patients. Dots represent individual values [$p < 0.0005$ ($p = 0.000309$); $r = 0.296$]; b) N50 wave latency of left-hand "pattern" electroretinograms (PERG L) depending on the age of parkinsonian patients. Dots represent individual values [$p < 0.005$ ($p = 0.001907$); $r = 0.188$]; c) N50 wave latency of "pattern" electroretinograms (PERG) depending on the Hoehn and Yahr (H&Y) stadium of Parkinson's disease. Dots represent individual values [$p < 0.0001$ ($p = 2.5692374E-110$); $r = 0.752$].

Discussion

In addition to significant movement problems, changes in visual acuity, contrast sensitivity, color discrimination, eye bulb movements, perception of motion, and speed of visual processing, have been described in patients with PD, especially for rapidly changing stimuli. Visual-spatial orientation disorders and visual hallucinations may also occur. Such a wide variety of visual problems can have a significant impact on the quality of life of parkinsonian patients [1]. The retina is the most distal site of visual dysfunction in PD, as demonstrated by electroretinography and optical coherence tomography studies to date. One of the most commonly studied neurotransmitters at the retinal level is dopamine, localized in amacrine cells and intersynaptic layers. Ganglion cells also include glycine and gamma-aminobutyric acid (GABA), responsible for transmission inhibitors.

Clinical electroretinography allows the detection of functional abnormalities of the retina before the onset of morphological changes. The PERG represents the focused and summarized electric potential of the macula. It is believed that the PERG generator in the retina is most likely in ganglion cells. Particularly sensitive to "pattern" stimulation are ganglion fibers that originate from the macula, and most of the PERG response is the electrical activity of that part.

VEP represents a neurophysiological method for examining the function of the optical pathway from the ganglion layer of the retina to the visual cortex. VEP represents the difference in electrical potential that is recorded on the head as a response to visual stimuli.

These methods have been used to detect impaired function of the retinal nerve fiber layer in parkinsonian patients [2].

Two years ago, the study performed by Živković et al. [3] analyzed changes in the thickness of the macular ganglion cell layer and the thickness of the inner plexiform layer in patients with PD. It was concluded that PD is accompanied by thinning of these macular complexes even in the earliest stages of the disease.

In recent years, the results of studies examining visual pathway disorders in parkinsonian patients have been published. The latencies of the VEP and PERG parameters were taken into account. However, the results of a study by Langheinrich et al. [4] also show a significant reduction in PERG amplitude.

Garcia-Martin et al. [5] examined the VEP and PERG parameters as well as the thickness of the foveal and macular region of the retina in parkinsonian patients and found that the symptoms of the disease were more severe in patients with more severe retinal damage.

The results of a study by Liu et al. [6] also show that visual pathways in the brainstem can be disturbed in parkinsonian patients.

A study by Hasanov et al. [7] examining pattern VEP and thickness of the retinal nerve layers revealed that electrophysiological and morphological changes are present at different levels of the visual pathway in the early stages of PD.

The results of our study indicate a median, positive linear relationship between the P100 wave latency values of VEP waves in Parkinson's patients and their lifespan (Figures 1a and 1b).

It can be assumed that the aging of parkinsonian patients further affects the already existing neurodegenerative process and further loss of dopaminergic receptors, both in brain structures and in the corresponding retinal layers.

This neurodegenerative process affects the significant prolongation of the latency of the N50 PERG wave and the P100 VEP wave in parkinsonian patients, which increases with age.

A weak positive linear relationship between the N50 PERG wave latency and the lifespan of parkinsonian patients was also recorded (Figures 3a and 3b).

By comparing the registered N50 PERG wave latencies and P100 VEPs in parkinsonian patients and healthy subjects of the control age-matched group, their significant prolongation was observed in the patients (Table 3).

This finding indicates the presence of a significant neurodegenerative process in the retinal structures of parkinsonian patients relative to healthy subjects. Degenerative changes of retinal structures with consequent disruption of dopaminergic neurotransmission significantly affect the function of visual pathways of parkinsonian patients. The greatest number of the patients was in the first three stages of PD, and that is the period when the dynamics and development of the neurodegenerative process can be monitored (Table 2).

By comparing the P100 wave latency values of VEP (Figures 1a and 1b) and N50 PERG (Figures 3a and 3b), it was observed that the P100 wave latency correlated significantly more positively with the patient's age than the N50 wave latency. A possible reason may be the location, and the length of the visual path, which is examined by the specified parameters. Specifically, the N50 wave of PERG is the response of retinal structures to the applied light stimulus, and the P100 wave of VEP is the response transmitted along the entire visual path to the primary cortical optic areas. The longer the impulse transmission path, the more pronounced are the consequences of the neurodegenerative process present.

In our study, it was observed that the latency values of P100 waves of VEP and N50 waves of PERG show a median or strong correlation with the stage of PD (Figures 2 and 3c). In this way, it was also found that the severity of the neurodegenerative process in the visual system more significantly depends on the stage of PD than on the age of the patient, as expected.

The results of our study are consistent with the results of the earlier conducted study [8], where bioelectric retinal dysfunction was observed in patients in the early stages of PD during the PERG test, possibly as a result of retinal dopamine deficiency. This finding indicates that PERG may be a useful test for understanding the causes of nonspecific visual disturbances that occur in parkinsonian patients. It can be concluded that PERG is useful for assessing retinal dopaminergic function as well as for monitoring the therapeutic action of dopaminergic drugs. Meta-analysis performed by He et al. [9] as well as the study made by Miri et al. [10] showed that P100 VEP wave latencies are longer in parkinsonian patients compared to healthy subjects.

These findings, as well as the results of our study, suggest that electrophysiological changes and functional deficits of visual pathways in parkinsonian patients may be important for understanding the pathophysiology of visual disorders in PD. Based on the previous presentation, it can be concluded that VEP and PERG may be sensitive parameters for the prognosis and assessment of the severity of PD. However, it is well known that VEP and PERG are not specific electrophysiological tests for a particular disease. This fact limits, to some extent, their diagnostic applicability. However, it is hypothesized that with careful selection of patients with an accurate exclusion of other diseases that may affect visual function, the results of future electrophysiological studies may significantly contribute to the differential diagnosis and treatment approach planning for parkinsonian patients. In this regard, future studies of VEP and PERG parameters in parkinsonian patients are needed.

Conclusion

Diagnostic application of the PERG and VEPs enabled the confirmation of a disorder in the visual pathway function in PD. Applied neurophysiological techniques may record early changes in the function of retinal structures and the optic nerve in PD, which might be significant both from the diagnostic and therapeutic aspects.

Conflict of interest

No conflict of interest exists for any of the authors listed in the article.

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Expression of collagen type IV in human kidney during prenatal development

Ekspresija kolagena tipa IV u ljudskom bubregu tokom prenatalnog razvoja

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Abstract

Background/Aim. Type IV collagen belongs to the group of nonfibrillar collagens and is an important component of the basement membranes, where it accounts for approximately 50% of its structural elements. The aim of the study was to describe the expression and distribution of collagen type IV in the embryonic and fetal metanephric kidney and to determine the volume density of collagen type IV in kidney tissue in each trimester of development. **Methods.** The material consisted of 19 human embryos/fetuses, in the gestational age from 8th to 37th week. Kidney tissue specimens were routinely processed to paraffin molds, stained immunohistochemically using polyclonal anti-collagen IV antibody and counterstained with Mayer hematoxylin and eosin. Stained slides were examined using a light microscope, and images of the selected areas under different lens magnification were captured with a digital camera. Volume density of collagen type IV was determined using ImageJ 1.48v and a plugin of the software, which inserted a grid system with 336 points. For the data comparison, the One-Way Analysis of Variance (ANOVA) was used. **Results.** Strong collagen IV immunopositivity was seen in all specimens, with a distribution in the basement membranes of urinary bud, parietal leaf of Bowman's capsule, glomerular basement membrane, basement membrane of interstitial blood vessels, and basement membranes of nephron tubules and collecting ducts. No statistically significant difference in the volume density of type IV collagen was found among the different trimesters of the embryonic and fetal development. **Conclusion.** The synthesis and secretion of collagen type IV simultaneously follow the development of nephron structures, collecting system and blood vessels. The volume density of collagen type IV remains constant throughout all the trimesters of metanephric kidney development, indicating that it plays a crucial role in the normal development of nephron and collecting system structures, as well as in maintaining the normal kidney function.

Key words:
growth and development; histological techniques; collagen type iv; fetus; immunohistochemistry; kidney.

Apstrakt

Uvod/Cilj. Kolagen tipa IV pripada grupi nefibrilarnih kolagena i predstavlja značajnu komponentu bazalnih membrana u kojima čini oko 50% u odnosu na sve strukturne elemente. Cilj rada bio je da se opiše ekspresija i distribucija kolagena tipa IV u embrionalnom i fetusnom bubregu i da se odredi volumenska gustina kolagena tipa IV u svim trimestrima razvoja. **Metode.** Materijal je činilo 19 humanih embriona/fetusa, gestacione starosti od 8. do 37. nedelje. Uzorci tkiva bubrega su rutinski obrađeni do parafinskih kalupa, bojeni imunohistohemijski upotrebom poliklonalnog anti-kolagen IV antitela i kontrastirani Mayer-ovim hematoksilinom i eozinom. Nakon bojenja, uzorci su analizirani na svetlosnom mikroskopu i upotrebom digitalne kamere, na različitim uveličanjima, napravljena je fotodokumentacija. Volumenska gustina kolagena tipa IV određivana je u programu ImageJ 1.48v uz upotrebu „plagina" kojim je na digitalne slike postavljana mrežica od 336 tačaka. Za statističku analizu dobijenih podataka korišćen je *One-Way Analysis of Variance* (ANOVA) test. **Rezultati.** Jaka imunopozitivnost kolagena tipa IV bila je prisutna na svim ispitivanim uzorcima, sa distribucijom u bazalnim membranama ureterskog pupoljka, parijetalnog lista Baumanove kapsule, glomerularnoj bazalnoj membrani i bazalnim membranama tubula nefrona i sabirnih kanalića. Nije pronađena statistički značajna razlika u volumenskoj gustini kolagena tipa IV između različitih trimestara embrionalnog i fetalnog razvoja. **Zaključak.**

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Sinteza i sekrecija kolagena tipa IV dešava se istovremeno sa razvojem struktura nefrona, sabirnog sistema i krvnih sudova. Volumenska gustina kolagena tipa IV ostaje konstantna u svim trimestrima razvoja metanefričnog bubrega, što ukazuje na to da kolagen tipa IV ima značajnu ulogu u normalnom razvoju struktura nefrona i sabirnog sistema, kao i u održavanju normalne funkcije bubrega.

Ključne reči:
rast i razvoj; histološke tehnike; kolagen, tip iv; fetus; imunohistohemija; bubreg.

Introduction

The human kidney is a complex organ consisting of functional units – nephrons, connected to a highly branched collecting duct system. Nephrogenesis, the process of kidney formation, ends around the 36th week of development and comprises a plethora of intertwined processes, such as epithelial-mesenchymal interactions, epithelial branching, cell migration, differentiation, and cell division, as well as cell-extracellular matrix interactions [1–3]. The unique feature of kidney development is the mesenchymal-epithelial transition that occurs during the formation of the nephron and the differentiation of highly specialized structures, such as the glomerulus [1, 3].

The definitive mammalian kidney development is a complex process that occurs through the formation of three excretory structures from intermediate mesoderm: pronephros, mesonephros, and metanephros, of which the first two are temporary and involute, while the metanephros will give rise to the definitive kidney [4]. The pronephros and mesonephros formations are necessary for the development of the metanephric kidney, and the interruption in the development of these two precursor excretory structures will lead to renal agenesis [5]. The metanephric kidney occurs during the 5th week of development and is a result of the interaction of the nephric duct and metanephric mesenchyme, both of which originate from the intermediate mesoderm [6]. The intermediate mesoderm is a narrow strip of mesoderm located between the somite and lateral plate mesoderm [7]. Its ventral part will give rise to the nephric duct, while the posterior part of the intermediary mesoderm, referred to as the nephrogenic cord, will become condensed near the hindlimb buds, thus giving rise to metanephric mesenchyme [6]. The nephric duct is a tubular structure covered with simple cuboidal epithelium, directed toward the cloaca of the embryo with whom it connects. It is shown that glial cell-line-derived neurotrophic factor (GDNF), a protein secreted by the metanephric mesenchyme cells, binds to the Ret receptors on the epithelial cells of the distal part of the nephric duct and initiates the formation of the ureteric bud [8]. The ureteric bud plays a crucial role in the formation of metanephros, and its branching in the metanephric mesenchyme will give rise to the collecting system of the kidney [9]. The metanephric mesenchyme contains multipotent self-renewing Six2+ progenitors that will give rise to the main body of the nephron, as well as self-renewing Foxd1+ progenitor cells that will give rise to the stroma of the interstitium, mesangium, and pericytes in kidney [10].

Type IV collagen belongs to the group of nonfibrillar collagens and is an important component of the basement membranes (BM), where it accounts for approximately 50% of its structural elements [11, 12]. In BM, collagen type IV forms a polygonal network that, along with other molecular components of the BM, has a supporting and barrier function [11]. Moreover, it has a role in supporting tissue integrity, cell survival, cell signaling, morphogenesis, and tissue regeneration [13]. The type IV collagen molecule is a heterotrimer (protomer) consisting of three alpha chains that have a similar primary structure [11, 14]. To date, six genes encoding alpha chains, denoted COL4A1-COL4A6, were identified [15]. Alpha chains are interconnected into a three-helix structure of type IV collagen molecules. Three molecular isoforms of type IV collagen are described: $\alpha1_2\alpha2$, $\alpha3\alpha4\alpha5$ and $\alpha5_2\alpha6$ [16]. The $\alpha1_2\alpha2$ collagen IV isoform is found in all basement membranes, the $\alpha3\alpha4\alpha5$ isoform is found in the kidney, lung, and at the neuromuscular junction, while the $\alpha5_2\alpha6$ isoform is present in smooth muscle and at the neuromuscular junction [16, 17].

The aim of the study was to describe the expression and distribution of collagen type IV in the embryonic and fetal metanephric kidney and to determine the volume density (Vv) of collagen type IV in kidney tissue in each trimester of development.

Methods

Material

The material consisted of 19 human embryos/fetuses, in the gestational age from 8th to 37th week, obtained following all legal and ethical guidelines. The material was obtained after spontaneous or artificial miscarriages and premature births due to prenatal deaths. There was no macroscopic damage or any pathological/autolytic changes in the specimens, and both sexes were represented in the sample. Gestation week was determined using medical history, as well as by measuring the crown-rump length. The study was performed at the Department of Histology and Embryology, Faculty of Medicine, University of Niš. All examined samples were allocated into three groups based on the trimester of development (Table 1).

Tissue preparation

Kidney tissue specimens were isolated and fixated in 10% buffered formalin and routinely processed to paraffin blocks. A 5 μm thick tissue section was cut on Leica RM2255 microtome (Leica Micro-Systems, Rueil-Malmaison, France) and stained with hematoxylin and eosin for histological examination.

Table 1

The number of samples, allocated to different groups based on the trimesters of the kidney development

Development period	Gestation week	Samples (n)	Total (n)
Embryo			
first trimester			
	8	2	6
	9	1	
	10	1	
	11	2	
second trimester			
	17	1	6
	19	2	
	20	1	
	21	2	
Fetus			
third trimester			
	29	1	7
	32	1	
	34	1	
	35	1	
	36	2	
	37	1	

Immunohistochemistry

Tissue sections were deparaffinized in xylene, rinsed in alcohols with descending concentrations (100%, 96%, 75%), and rehydrated with distilled water. Heat-induced antigen retrieval with citrate buffer (0.01M, pH 6) was performed for 30 minutes. The endogenous peroxidase was blocked with 3% H_2O_2 for 15 min at room temperature. The kidney tissue was then exposed to the anti-collagen IV antibody (Rabbit polyclonal, Abcam, USA, ab6586, 1:250) overnight at 4 ℃. The secondary antibody was applied for 45 min, and the tissue specimens were then stained with diaminobenzidine (DAB) and counterstained with Mayer hematoxylin. Secondary antibody and DAB were used from EnVisionFLEX, HighpH visualization system (Agilent, Denmark GV80011-2). The rinses between the steps were performed with phosphate buffer (0.1 M, pH 7.4). Stained slides were examined using a light microscope Olympus BX50 (Olympus, Japan), and images of the selected areas, under different lens magnification, were captured in TIFF format with a digital camera Leica DFC295 (Leica Microsystems, Germany).

Morphometric and statistical analysis

The Vv is a relative variable, which shows how much overall space is occupied by the observed space in volume units [18]. The Vv of collagen type IV was determined by using ImageJ v. 1.48v (Wayne Rasband, National Institute of Health, USA) and a plugin of the software which inserted a grid system with 336 points (Vt). The number of points overlapping the collagen IV positive structures (Vf) within the kidney tissue was counted. The Vv was determined using the following formula: $Vv = Vf/ Vt$ [18]. The obtained results were multiplied by 100 and presented in percentages. For each trimester, the Vv of collagen type IV was determined for the entire kidney tissue of the examined sample. In the kidneys obtained from fetuses belonging to the third trimester, the Vv of collagen type IV was determined in specific regions of the tissue: (1) cortex (renal corpuscles, proximal and distal tubules, Ferrein's pyramides (medullary rays) and blood vessels); (2) medulla (collecting ducts, loops of Henle, and blood vessels). The distribution of data was tested using the Kolmogorov-Smirnov test. For data comparison, the One-Way Analysis of Variance (ANOVA) was used.

Results

Collagen type IV was expressed in kidney tissue specimens in all the trimesters. In the histological sections of the kidney tissue during the 8th week of development, there were visible renal corpuscles and proximal and distal tubule (Henle's loops were not present in our tissue sections), along with the components of the ductal system and interstitial blood vessels. During the late first trimester (11th and 12th week), the number of nephrons was increasing, and all parts of the nephrons and ductal system were clearly visible. The collagen type IV was clearly and strongly expressed in the basement membranes of urinary bud, parietal leaf of Bowman's capsule, glomerular basement membrane (GBM), basement membrane of interstitial blood vessels, tubules, and collecting ducts (Figure 1a).

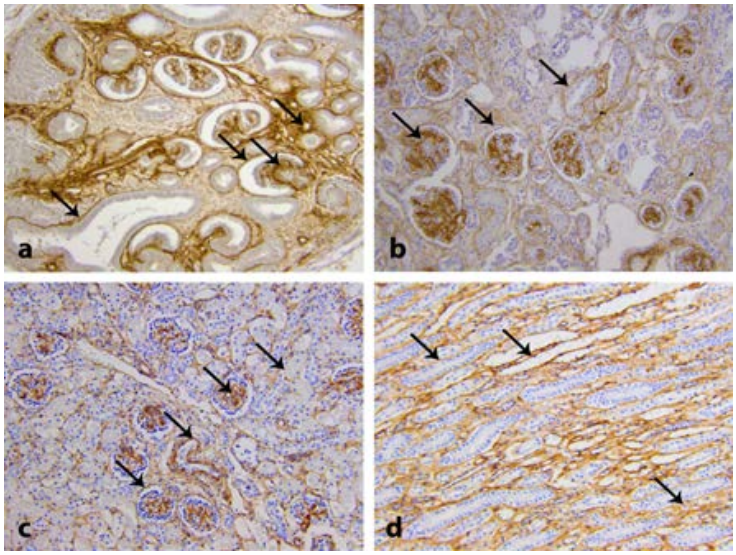

Fig. 1 – Expression of collagen type IV in the kidney in the 8th (a), 21st (b), 32nd (c) and 36th (d) week of development: a) The strong positivity is seen in basement membranes of the ureteric bud, parietal leaf of Bowman's capsule, glomerular basement membrane, and basement membranes of interstitial capillaries, ×200; b) Immunopositivity in the renal cortex is seen in the glomerular basement membrane, parietal leaf of Bowman's capsule, basement membrane of the distal tubule, ×200; c) Immunopositivity in the renal cortex is seen in the parietal leaf of the Bowman's capsule, glomerular basement membrane, basement membranes of interstitial blood vessels, and proximal renal tubule, ×200; d) Immunopositivity in the renal medulla is present in the basement membranes of collecting ducts, blood vessels, and Henle's loops, ×200.

Table 2

Volume density (Vv) of collagen type IV in the kidney, presented by the trimesters of the kidney development

Period of development	Vv (%), mean ± SD
1st trimester	15.97 ± 9.58
2nd trimester	14.34 ± 5.89
3rd trimester	16.25 ± 4.02

SD – standard deviation.

A clear difference between renal cortex and medulla was present in the kidney specimens of the second trimester. The growth of the fetal kidney was followed by an increase in the number of nephrons and extensive branching of the ductal system. During the second and third trimester of development, the collagen IV expression and distribution had the same pattern as in the late first trimester, i.e., in basement membranes belonging to the renal corpuscle and tubules of the nephron, collecting ducts, and blood vessels (Figures 1b, 1c, and 1d).

The volume densities of type IV collagen in the kidney, presented by trimesters of development, are shown in Table 2. No statistically significant difference in the volume density of type IV collagen was found between the different trimesters of the kidney development. Volume densities of type IV collagen in the elements of the renal cortex and medulla in the third trimester of development are shown in Figures 2 and 3.

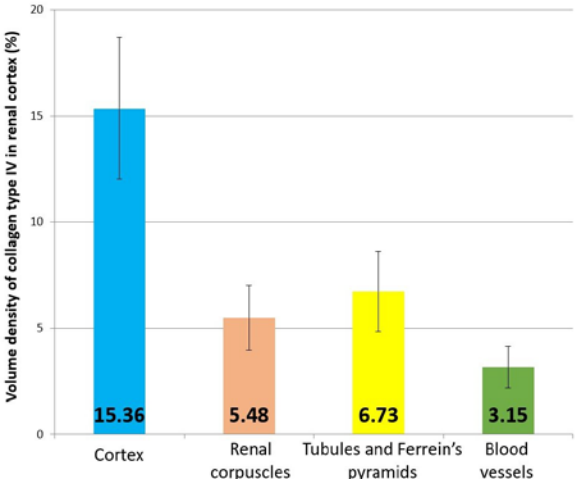

Fig. 2 – Volume density of collagen type IV in the renal cortex during the third trimester of the kidney development.

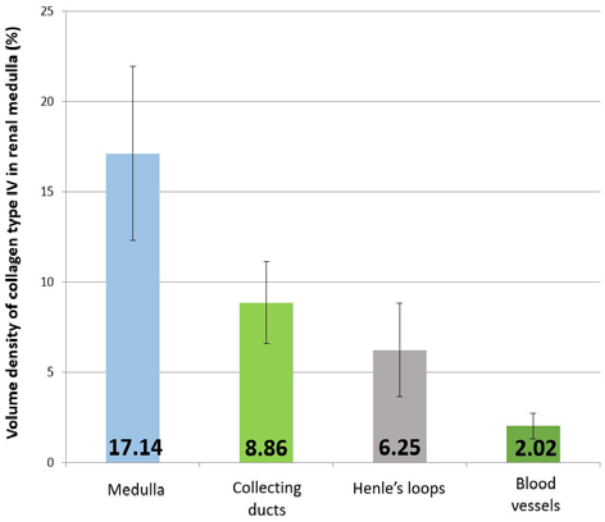

Fig. 3 – Volume density of collagen type IV in the renal medulla during the third trimester of the kidney development.

Discussion

Our results show that the expression of collagen type IV occurs early in kidney development and is limited to basement membranes of the ureteric bud, different parts of nephrons, collecting ducts, and blood vessels. These findings are in accordance with the previous reports that the formation of ureteric bud in the early stages of metanephros development is simultaneously followed by the assembly of basement membrane containing collagen type IV on the basal part of its cells [19–21]. During the further development, the formation of nephrons and collecting ducts, as well as blood vessels in the kidney, are followed by the deposition of basement membranes containing, among the other molecules, collagen type IV [22, 23]. Basement membranes have an important role in the modeling of parts of the nephron and collecting tubules of the kidney during development, and maintaining the normal tissue structure [23–25]. The results of type IV collagen testing on mouse kidney tissue show that developmental changes in different segments of the nephron and the collecting system are closely related to the expression of this type of collagen. From the aspect of nephrogenesis, type IV collagen is the most important protein of the basement membrane since its expression occurs immediately at the beginning of the formation of renal structures [26]. The collagen type IV is not only important for structural support of developing nephrons and collecting system, but it also has an important role in maintaining their function, i.e., the glomerular filtration, as well as the tubular reabsorption [27, 28].

Studies with developing human kidneys showed that the $\alpha1_2\alpha2$ isoform of type IV collagen is first synthesized and secreted in the GBM, but at the late capillary stage, there occurs a substitution of this collagen IV isoform with $\alpha3\alpha4\alpha5$ [29, 30]. The experiments with murine developing kidneys show that collagen $\alpha3\alpha4\alpha5$ appears in discontinuous, nonlinear patterns in parts of laminin α5-positive GBM that does not contain either isoform of collagen IV [31]. The $\alpha1_2\alpha2$ isoform of type IV collagen in the renal corpuscle is secreted by endothelial cells, podocytes, and mesangial cells, while the $\alpha3\alpha4\alpha5$ isoform is secreted exclusively by the podocytes of the visceral leaf of the Bauman capsule [32]. It is believed that these isoform transitions play key roles in the establishment of the glomerular filtration barrier, as well as in the maintenance of endothelial cells and podocytes inside the glomerulus [33]. Concerning the other parts of the nephron, the α3 through α6 chains of type IV collagen are abundant in the distal tubular basement membrane (TBM) in humans, while α1 and α2 chains are found ubiquitously in TBM. In the basement membrane of the parietal leaf of Bowman's capsule, the major collagen IV chains are α1, α2, α5, and α6 [34].

Although there are several studies dealing with the temporal and spatial expression of different isoforms of collagen type IV in developing kidneys, there are virtually no data concerning the quantification of collagen type IV in prenatal human kidneys. Jalali et al. [35] used a semiquantitative approach to determine the amount of collagen type IV in a murine model of kidney development. Their results indicate that the first traces of collagen type IV were observed during the E13 and that its amount gradually increased until the E18, to finally reach its maximum around day 5 postnatally. The earliest specimen used in our research was at the 8th week of gestation, and strong collagen IV positivity was already seen around all the tubular structures in the developing kidney. The quantified Vv of collagen type IV showed no significant statistical difference compared to the later stages of development. Moreover, the Vv is a relative variable and does not reflect the absolute amount of collagen type IV in developing kidneys, but rather its volume presence expressed in percentages within the organ and compared to all the other structural kidney components.

The genetic disorders of collagen IV synthesis especially affect the kidney due to the dependence of its functions on the stability and normal morphology of the BM. Glomerular filtration and tubular filtration are highly specialized kidney features, and the failure of the kidney to perform these functions may lead to end-stage renal disease with life-threatening consequences. The two major syndromes occurring as a result of mutation of genes for collagen type IV are Alport's and Goodpasture's syndromes [36]. Alport's syndrome occurs as a result of mutations in any of the three genes encoding components of the $\alpha3\alpha4\alpha5$ collagen type IV network (COL4A3, COL4A4, and COL4A5). Most mutations prevent assembly and/or secretion of $\alpha3\alpha4\alpha5$ heterotrimers of collagen type IV such that all 3 proteins are absent from the GBM [37]. Clinically, it manifests with persistent hematuria, sensorineural hearing loss, and ocular abnormalities [38]. Goodpasture syndrome is an autoimmune disease caused by the development of autoantibodies against the GBM that leads to kidney failure [39].

Conclusion

The collagen type IV is an important part of basement membranes in the kidney, whose synthesis and secretion simultaneously follows the development of nephron structures, collecting system and blood vessels. The volume density of collagen type IV remains constant throughout all the trimesters of metanephric kidney development, indicating that it plays a crucial role in the normal development of nephron and collecting system structures, as well as in maintaining the normal kidney function.

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Conflict of interest

The authors declare no conflict of interest.

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Root canal treatment from patients' perspective: knowledge, awareness, and expectations

Lečenje kanala korena zuba iz perspektive pacijenata: znanje, svest i očekivanja

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Abstract

Background/Aim. Root canal treatment (RCT) is one of the most common endodontic procedures for which patients visit the dentist. Patient's knowledge, awareness, and attitude regarding root canal treatment are an interesting problem in everyday dentistry. Therefore, the study aimed to evaluate the knowledge, awareness, and attitude of patients coming for endodontic treatment. **Methods.** Questionnaire surveys were carried out in a group of 209 patients, including queries characterizing their knowledge, awareness, and attitude towards the RCT – their experiences, expectations, potential problems, and management expenses. **Results.** Exploring the knowledge of individuals concerning RCT, it was noticed that 51% of the surveyed patients in the past had experienced endodontics treatment. Fifty-two percent of participants reported that they knew the charges of the RCT, and 50% of them said that the price was sufficient for the difficulty of the process. The study confirmed that 47% of the participants still prefer specialists to perform the RCT. **Conclusion.** An enhancement of knowledge and awareness of people about the RCT has been observed, as well as a need of providing more information to patients about endodontics and the benefits of saving teeth.

Key words:
attitude to health; awareness; endodontics; knowledge; pain; root canal therapy; surveys and questionnaires.

Apstrakt

Uvod/Cilj. Lečenje kanala korena zuba (LKZ) je jedna od najčešćih endodontskih procedura zbog kojih pacijent posećuje stomatologa. Znanje, svest i stav pacijenta prema LKZ su zanimljiv problem u svakodnevnoj praksi stomatologa. Zbog toga je cilj rada bio da se procene znanje, svest i stav pacijenata koji dolaze na endodontsko lečenje. **Metode.** U grupi od 209 ispitanika sprovedeno je ispitivanje putem upitnika kojim se karakterišu znanje, svest i stav pacijenata prema LKZ – njihova iskustva, očekivanja, potencijalni problemi i troškovi procedure. **Rezultati.** Istraživanjem znanja pojedinaca koje se tiče LKZ, pokazano je da je 51% ispitivanih pacijenata u prošlosti lečeno endodontski. Od ukupnog broja ispitanika, 52% je izvestilo da je bilo obavešteno o troškovima procedure LKZ, a 59% da je cena bila odgovarajuća za kompleksnost procesa. Ispitivanjem je potvrđeno da bi 47% ispitanika za procedure LKZ izabralo lekara specijalistu. **Zaključak.** Zapaženo je poboljšanje znanja i svesti ljudi o LKZ, kao i potreba za pružanjem više informacija pacijentima o endodonciji i informacija o koristima očuvanja zdravlja zuba.

Ključne reči:
stav prema zdravlju; svest; endodoncija; znanje; bol; lečenje korenskog kanala; ankete i upitnici.

Introduction

Tooth disease may often lead the patient to seek dental care. Dental pain is the commonest reason observed among patients for seeking necessary management, which mainly comprises root canal treatment (RCT) or extraction of the diseased tooth [1]. Extraction of the tooth may lead to a chain of events such as causing the adjacent or opposing teeth to shift, affecting the masticating ability, and harming the natural smile and esthetics of the patient. The main outcome of securing oral health is the conservation of the inherent dentition. In modern dentistry, RCT is an achievable and effective method to preserve teeth [1,2].

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RCT is a process in which the damaged and contaminated dental pulp is eliminated and replaced with sterile and antibacterial material. It eliminates the focus on infection, allowing a person to keep tooth performance and aesthetics [2]. Tooth replacement, if indicated for esthetic and functional rehabilitation, is accomplished with prosthetic appliances, including implants making it a costly enterprise. Therefore, RCT should always be considered whenever indicated as it not only favors the preservation of natural teeth but also has excellent clinical outcomes [3, 4].

Current endodontics is constantly evolving. This development offers not only new gadgets but also new management modalities. The most basic rule of contemporary endodontics is a painless and effective treatment [2]. However, fear of dental treatment is somehow frequent in the population.

Although RCT is highly prevalent, it is still considered by several patients as a process to be feared. Studies have shown that fear and anxiety are the main deterrents in looking for RCT [5, 6]. These fears can be attributed to the ignorance of patients about root canal procedures. Patients often do not understand the nature of endodontic treatment and what it involves [7, 8]. Studies in the past have highlighted the need to provide more information about it [4, 9].

Awareness is defined as information that somewhat exists or understanding of a condition or matter at the current time based on knowledge or practice [2]. Awareness of endodontic management is significant in educating persons on saving their natural teeth. Many studies on the awareness of RCT are still limited. Some studies recommend that the level of knowledge is highest in people of developed countries. Research carried out by the American Association of Endodontics (AAE) observed that 76% of participants have a preference for RCT over tooth extraction [3]. Just to compare, only 20% of Indian people are interested in endodontic treatment, while 38.5% choose extraction [1]. In contrast, the level of knowledge on roots canal may be impacted by different factors such as the attitude about dental health and sociodemographic [2].

Janczarek et al. [6] reported that there is an enhancement of awareness and knowledge of individuals about the RCT.

This study aimed to evaluate patients' knowledge, awareness, and attitude toward endodontics treatment among the Lahore population. The criterion that directed the persons in deciding on root canal treatment has been investigated.

Methods

Questionnaire surveys were carried out on a group of 209 patients at the Department of Operative Dentistry, Lahore Medical and Dental College, Lahore, Pakistan. A multiple-choice questionnaire was designed comprising sociodemographic questions of participants, as well as their awareness about RCT – their experiences, expectations, treatment costs, and possible complications. The obtained data are presented in tabular form.

The inclusion criteria were male or female patients from 14–75 years old that attended the Outpatient Department of Lahore Medical and Dental College, Lahore, Pakistan, while the exclusion criteria for the participants were the mentally handicapped patients, pediatric patients, and those older than 75.

Statistical analysis

The analysis of data was carried out by descriptive statistics and expressed as a percentage. The answers of the participants were analyzed by the χ^2 test with a 5% level of significance using IBM Statistical Program for Social Sciences Version 23.0 (SPSS Inc., Chicago Illinois, USA).

Results

Out of the 209 participants in the study, 43.5% were males and 56.5% were females. Table 1 shows the sociodemographic characteristics of the participants. The mean age of participants was 34.72 ± 13.61 years, between 14 and 75 years of age.

Table 1

Socio-demographic profile of participants

Characteristics	n (%)
Gender	
male	91 (43.5)
female	118 (56.5)
Age range (years)	
14–29	94 (45.0)
30–39	49 (23.4)
40–49	33 (15.8)
50–59	19 (9.1)
60–69	11 (5.3)
≥ 70	3 (1.4)

Table 2 shows the patient's experience with endodontic treatment, in which 51% of subjects had experienced RCT in the past while 49% had no experience of the RCT. Out of the

Table 2

The patient's own experience regarding root canal treatment

Questions	Response	Respondents, %
Have you had any endodontic treatment done before?	Yes	51
	No	49
How do you recall your endodontic treatment?	Well	76
	Bad	24
Was it a painful treatment?	Yes	49
	No	32
	Don't remember	19
Who performed the treatment?	Student	6
	Intern	18
	Experienced dentist	33
	Don't Know	43

total, 76% of subjects who had undergone endodontic treatment recalled it as a good experience, but 24% of participants had a bad experience with it. Interestingly, almost half of the participants did not remember who performed the treatment.

Concerning the reasons behind the endodontic treatment of the respondents, the majority of them reported toothache while eating or biting (15%) or strong spontaneous pain (29%) (altogether 67%), but many of them reported some other symptoms as strong spontaneous toothache and color discoloration (1%).

In the case of toothache, the majority of participants (60%) used some home remedies to relieve toothache.

Among participants, 22% of them had average knowledge concerning the endodontic treatment, and they were willing to learn more information about it, but 42% reported that they did not know anything (Table 3).

The majority of the participants (47%) worried about possible pain associated with the endodontic treatment, and 23% indicated a high cost of endodontic treatment value such as breaking of the instrument in the canal or multiple X-rays/Orthopantomagraphs (OPGs).

The high price of endodontic treatment plays a vital role in choosing between the endodontic treatment or tooth extraction. Of the total number of participants, 86% answered that high price changes their decision on endodontic treatment, while only 14% answered that high prices did not affect the decision of choosing endodontic treatment. Only 37% of participants were willing to pay a high cost for proper endodontic treatment, and 63% were reluctant to pay a high cost for dental treatment. However, 70% of respondents would not go for extraction in any case.

Considering the criteria for selecting the person who would perform the endodontic treatment, most participants (47%) reported that they would choose a specialist to perform the endodontic treatment, 33% answered that they would choose a dentist recommended by a friend or relative, a dentist without specialization 6%, and a dentistry student 1%. However, 13% of participants reported that the person performing the endodontic treatment was irrelevant.

In the final part of the survey, the participants were inquired regarding the criteria for choosing the dental office. The majority of them answered that highly professional staff was the most significant criterion for selecting the dental clinic. Of the total, 21% participants reported painless treatment as the criteria for selecting a dental office, and 14% reported friendly service (Table 4).

Discussion

Nowadays, the RCT has been one of the fastest-growing fields of dental sciences. The use of the latest gadgets, such as new restoration materials, microscopes in daily practice, and rubber dams, considerably influences the durability and quality of the treatment. Conversely, the level of awareness concerning the RCT in individuals in the world varies from one area to another due to various technologies accessible, human population and resources, and many more reasons [2, 9, 10].

Pain experience causes patients to hesitate the treatment and become more frightened of it [8]. The main reason for patients to avoid going to the dentist was the fear of pain [10, 11]. This may be the reason why patients delay treatments until they experience a spontaneous toothache [12]. Nevertheless, further studies revealed that dental fear may create oral health problems, as well as psychosocial problems for the individual [13–15]. Participants stated that painless treatment is much more important than the expense of the treatment. Physicians should always try to reduce the stress level of the patient, and the dentist should always make decisions in favor of the patients [7, 16]. A few studies [11, 12, 17] showed that people avoid check-ups at the dentist due to fear. The participants most often take pain killers, anti-inflammatory drugs, or even antibiotics. Patients also said that they prefer custom-made preparations or locally applied cotton swabs soaked in alcohol, placing ash from a burnt newspaper on the tooth lesion, keeping the cigarette smoke in the mouth, rinsing the mouth with water, baking soda, and vinegar. One of the patients applied the acupuncture technique in reducing pain. Acupuncture was also applied in the practice of decreasing the stress of dental visits and

Table 3

Self-evaluation of patient's knowledge on the endodontic treatment

Knowledge parameters	Respondents, %
I know a lot	12
Average knowledge, I will ask from the dentist	22
Average knowledge, I am not interested in the course	6
I don't know anything	42
Will know from media/internet	18

Table 4

The criteria for selecting a dental office

Selection criteria	Respondents, %
Free of charge treatment	7
Reasonable price	7
Friendly service	14
Professional staff	44
Painless treatment	21
Easy access for you	7

brought shockingly good outcomes. It has been recommended that the most suitable puncture points are the hand's feet and ears [11, 18]. Nevertheless, due to the decreased number of studies, such information needs further study.

Prices related to the RCT are still debatable. Patients often complain that they are too high and the treatment should be financed by insurance companies. However, half of the respondents were ready to pay even higher amounts to prevent tooth loss [6].

According to the survey conducted by Daud et al. [2], 52% of the patients gained knowledge about the treatment through their relatives and friends. Subjects who were unaware of the specialized treatment, reported the use of home remedies for relieving pain, which comprised the use of clove oil, balm application, self-medications, and heat pack, but they were mainly among the geriatric group (12.73%) [8, 19].

Nearly half of the patients said they would prefer an endodontist to perform the treatment. This rate was much higher in other studies (68%). The proportion of patients who had not yet been acquainted with the concept of endodontists was much more than we expected. We believe that this was because patients were unaware of endodontics [2, 20]. In previous studies, the most important criterion for patients was the skill and experience of the staff [6, 8, 21], which we noticed, too. More than half of the participants reported their endodontics treatment done before. Previous studies revealed that the patient−dentist relationship strongly affects a patients' feeling to be safe and secure [19, 20]. Therefore, practitioners should explain the course of the treatment to patients, including alternative treatment plans.

Participants stated that almost half of them would prefer a specialist for their treatments (47%). However, 33% stated that the RCT should be performed by an experienced dentist. That reveals patients' lack of knowledge about the endodontic concept.

The awareness of people regarding the RCT has improved considerably over the past years. This is not only due to school education but also mostly due to mass media – television, newspapers, and the internet. People are aware of the chances accessible by the latest dental practices – not just to achieve instant relief of pain, but also to perform the management at the utmost level – ensuring long-term radiological surveillance, enabling observation of outcomes achieved, allowing immediate intervention in case of exacerbation of symptoms, and monitoring the effects of treatment.

Conclusion

Awareness levels of the patients concerning the RCT are different among races and populations. Knowledge of patients about endodontic treatment has been increased, and specialist dentist is the first choice of the majority of patients.

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How and when do we use continuous renal replacement therapy for acute kidney injury in Serbia? – The multicentric survey

Kako i kada koristimo kontinuiranu zamenu funkcije bubrega za lečenje akutnog oštećenja bubrega u Srbiji

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Abstract

Background/Aim. The absence of clear guidance in the definition, diagnostics, and indications for renal replacement treatment (RRT) is present. The aim of this study was to help outlining future clinical work in improving the treatment outcome and reducing complications of acute kidney injury (AKI) based on the current clinical practice. **Methods.** The questionnaires were distributed among physicians of different specialties who participated voluntarily and anonymously. The questionnaire was drawn up in accordance with the standard clinical practice. **Results.** We conducted a multicentric web survey among nephrologists (46.8%) and other physicians in Serbia. The sample consisted of 119 participants, out of which 78.9% filled out the survey forms correctly and were, therefore, included in the analysis. Most of them responded that the nephrologist indicates (76.8%) and prescribes (74.5%) continuous renal replacement therapy (CRRT). The application of the Kidney Disease Improving Global Outcomes (KDIGO) 2 criterion for "early" start of CRRT used 74.5% of the respondents, and 91.5% of them started "late" initiation of CRRT in the presence of complications associated with AKI or poor response to conservative treatment. Regarding the clinical experience of the respondents, 74.5% of them marked the "early" start of CRRT within 12 hours, whereas 56.4% of them considered the start of CRRT after 48 h as "late". The most commonly used modality was continuous venous hemodiafiltration (37.6%). Most participants used heparin as an anticoagulant (95.7%) with an average life span of filters less than 24 h (71.3%) and 25 mL/kg/h efficiency target dialysis effluent dose (45.2%) during CRRT. The most common complications of CRRT were hypotension (55.3%) and catheter-related infections (29.8%). **Conclusion.** The "early" start of CRRT is considered favorite by the majority of the participants. According to the obtained data, standardization of the strategy in the diagnostics and treatment of AKI is necessary.

Key words:
acute kidney injury; nephrologists; renal replacement therapy; continuous renal replacement therapy; surveys and questionnaires.

Apstrakt

Uvod/Cilj. Nedostaju jasne smernice u definiciji, dijagnostici i indikacijama za lečenje metodama zamene funkcije bubrega (ZFB). Cilj ove studije bio je da pomogne u kreiranju budućeg kliničkog rada u pogledu poboljšanja ishoda lečenja i smanjenja komplikacija akutnog oštećenja bubrega (AOB) na osnovu aktuelne kliničke prakse. **Metode.** Sprovedene su anonimne ankete među lekarima različitih specijalnosti. Upitnik je sastavljen u skladu sa

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standardnom kliničkom praksom. **Rezultati.** Istraživanje je sprovedeno kao multicentrična *web* anketa među nefrolozima (46,8%) i lekarima drugih specijalnosti u Srbiji. Uzorak je činilo 119 učesnika, od kojih su 78,9% korektno popunili anketu i bili uključeni u analizu. Većina učesnika je odgovorila da nefrolog ukazuje na potrebu korišćenja (76,8%) i određuje (74,5%) korišćenje kontinurane ZFB (KZFB). Na osnovu kriterijuma *Kidney Disease Improving Global Outcomes* (KDIGO) 2 „rani" početak KZFB koristilo je 74,5% ispitanika, a 91,5% među njima „kasno" počinju KZFB kod komplikacija povezanih sa AOB ili slabijeg odgovora na konzervativnu terapiju. U odnosu na kliničko iskustvo ispitanika, 74,5% je smatralo da bi „rani" početak KZFB trebalo da bude unutar 12 h, a 56,4% je odgovorilo da je preko 48 h „kasni" početak. Najčešće je korišćena kontinuirana veno-venska hemodijafiltracija (37,6%). Većina ispitanika (95,7%) je tokom KZFB koristila heparin kao antikoagulans, sa prosečnim trajanjem filtera kraćim od 24 h (71,3%), dok je najzastupljenija doza dijaliznog efluenta bila 25 mL/kg/h (45,2%). Najčešće komplikacije KZFB bile su hipotenzija (55,3%) i infekcije povezane sa prisustvom katetera (29,8%). **Zaključak.** Većina ispitanika daje prednost „ranom" početku KZFB. Prema dobijenim podacima u dijagnostici i lečenju AOB neophodna je standardizacija strategije.

Ključne reči:
bubreg, akutna insuficijencija; nefrolozi; bubreg, zamena funkcije; bubreg, zamena funkcije, kontinuirana; ankete i upitnici.

Introduction

Acute kidney injury (AKI) has a particularly high incidence in admissions to the intensive care unit (ICU), with reports of an incidence range of 16–36% and a three- to five-fold increase of in-hospital mortality (compared to those without AKI). Despite the evolution of the guidelines for diagnosing AKI, we are still largely indebted to serum creatinine, as well as urinary output, to determine the stage of AKI. Thus, while the unification of criteria for AKI is useful for furthering clinical research, it still permits only a relatively late diagnosis. In any case, these biomarkers (as well as others undergoing clinical research) are still too innovative for clinical practice and too expensive for widespread utilization in many low and middle-income countries and lower resource areas of high-income countries. In general, outcomes for AKI are poor, with one systemic review of over 300 cohort studies revealing overall mortality of 23.9% among adult AKI patients [1]. With AKI not requiring dialysis and AKI requiring dialysis both rising, it remains unclear whether this is due to changes in International Classification of Diseases coding, changes in AKI definition, awareness of AKI, or clinical practice [2]. As no specific pharmacological therapy is effective in AKI patients, their care is limited to supportive management in which continuous renal replacement therapy (CRRT) plays a central role [3]. Although there are many aspects of CRRT that are still under debate, its life-saving potential in severe cases of AKI can not be questioned [4].

The aim of this study was to help outlining future clinical work in improving the treatment outcome and reducing complications of AKI.

Methods

The study was conducted using a questionnaire that was distributed among physicians of different specialties. Nephrologists, anesthetists, intensivists, cardiologists, and internists, mostly from tertiary and secondary health care institutions, were invited to participate voluntarily and anonymously. The questionnaire was drawn up in accordance with the standard clinical practice [1]. The 40-question survey form included 4 parts: 1) information on the type of specialty, type of institution and length of service of the physician; 2) information on AKI definition and classification, indications and contraindications of CRRT, optimal start (regarding time, biomarkers, biochemical parameters) and termination of CRRT treatment; 3) information on the choice of vascular approach, type of modality, the dose administered, and anticoagulation; and 4) information on complications of CRRT, renal function recovery, dialysis dependence, and mortality. All the data regarding statistical analysis were compiled from hardcopy sources and processed using a Microsoft Excel database/ datasheet. All the data are presented either as a percentages or as absolute numbers.

Results

Out of the 119 survey participants, 78.9% fully completed the survey form.

According to the reported answers, 46.8% of the participants were nephrologists, 39.4% were specialists in anesthesiology, reanimatology and intensive care, and 13.8% were internists of different branches of internal medicine (9%) and other physicians. Out of them, 72.3% were employed in tertiary care institutions, 23.4% were secondary health care workers, and 4.3% worked in other institutions. Out of all participants, 40.4% had more than 20 years of service, 16.0% had 5–10 years of service, 13.9% had more than 10 years of service, and 1.5% had less than 5 years of service.

The most common AKI classification responses were: Risk, Injury, Failure, Loss of Kidney Function, End-Stage Kidney Disease (RIFLE) criteria – 59.6% in total, followed by Kidney Disease Improving Global Outcomes (KDIGO) criteria – 30.9%. Regarding the use of diuretics in oliguric patients, 43.6% of the participants confirmed administering high doses of furosemide (≥ 250 mg/day), 48.9% used low doses of furosemide (≤ 250 mg/day), whereas 7.0% of the participants did not use diuretics. The respondents had an almost equal distribution of responses in the prevalence of

patients requiring CRRT: < 5.0% (28.7% of the respondents); 5.0–10.0% (22.3% of the respondents); 11.0–20% (16.0% of the respondents); 21.0–30% (18.1% of the respondents) and > 30.0% (14.9% of the respondents). The majority of participants (74.5%) used the KDIGO 2 criterion when deciding on the "early" CRRT initiation, and 91.5% started "late" CRRT for AKI complications (oliguria/anuria, elevated creatinine, hyperkalaemia, metabolic acidosis, and/or refractory hypervolaemia) or a lower response to conservative therapy. In addition to the above criteria, regarding the clinical experience of the subjects, 46.8% thought that the "early" onset of CRRT should be within 6 h (Figure 1), whereas 22.3% said that the "late" onset was > 24 h, a similar percentage of respondents (21.3%) thought it was from 24–48 h, and the majority (56.4%) thought the "late" onset of CRRT was > 48 h. The prediction for the onset of renal replacement therapy (RRT) could be increased by functional tests (furosemide stress test), considered relevant by most of the participants (63.8%). Regarding the use of biomarkers as predictors of the onset of RRT, 43.6% of them indicated cystatin C in urine, 36.2% referred to neutrophil gelatinase-associated lipocalin in urine, and 20.3% indicated a combination of urine tissue inhibitor of metalloproteinase 2 (TIMP-2) and insulin-like growth factor (IGF) binding protein 7, whereas 3.2% were related to other biomarkers. As high as 77.7% stated that the severity and course of the disease were the determining factors for initiation of RRT, but that the decision was also influenced by the availability (apparatus, equipment, staff), the day of the week as well as the time of the day, the response to diuretic therapy, and comorbidities (Figure 2). Relative contraindications for the onset of CRRT in 56.4% of the responses were advanced malignant disease (except for multiple myeloma), followed by hypotension without vasopressor response (35.1%), older age, as well as other reasons in 4.3% of the cases. CRRT was indicated in 76.6% of the patients by a nephrologist, in 12.8% of the patients by a nephrologist in consultation with a specialist of anesthesiology, reanimatology, and intensive care, and in 7.4% specialists employed in the ICU/ semi-intensive care. A similar percentage was reported for the CRRT prescription (a nephrologist 74.5%; a nephrologist in consultation with anesthesia, reanimatology, and intensive care specialist 16%; a specialist employed at the intensive care unit 8.5% and an internist of another branch of internal medicine 1.1%). Moreover, 37.2% of the participants reported less than 6 h from the time the indication was given until the CRRT treatment began (Figure 3). Most of the respondents (51.1%) discontinued CRRT if diuresis was > 450 mL/day, 35.1% if creatinine clearance was > 20 mL/min while the others remain neutral.

In most institutions (87.2%), a specialist employed in Intensive Care Unit (ICU)/semi-ICU placed a dialysis catheter, whereas, in others, it was done by a nephrologist in consultation with a specialist of anesthesia, reanimatology, and intensive care. The catheter was mostly inserted using the Seldinger blind technique (in 69.1% of patients). The most often choice was the left/right internal jugular vein (77.7%), followed by ultrasonography (US) assessment in 12.8%, femoral in 5.3%, subclavian in 3.2%, and the jugular internal vein in obese and the femoral vein in non-obese in

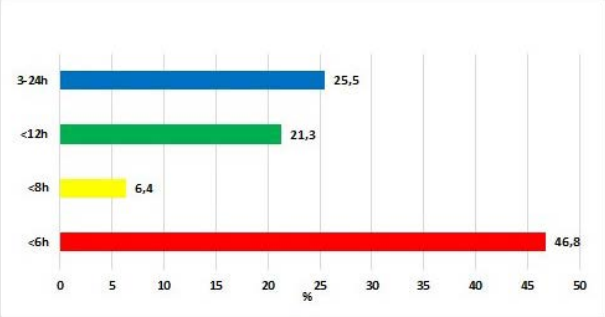

Fig. 1 – Opinion of respondents about timing of "early" continuous renal replacement therapy initiation in acute kidney injury.

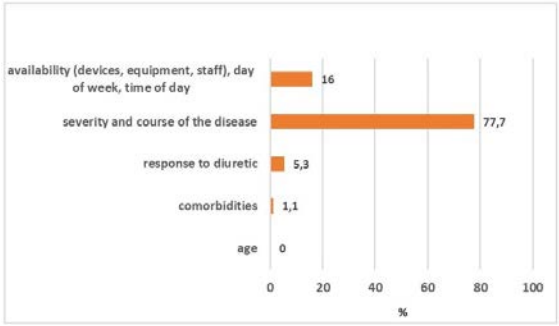

Fig. 2 – Opinion of respondents about factors influencing the decision to start continuous renal replacement therapy.

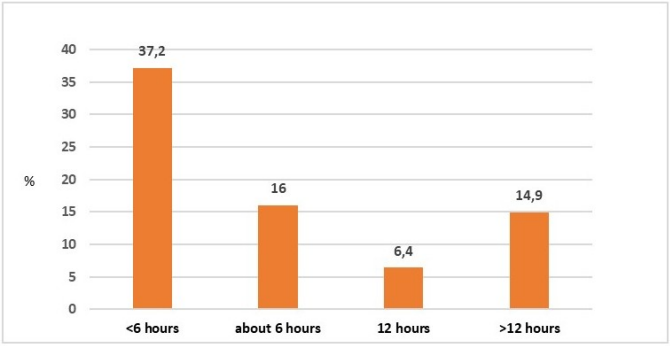

Fig. 3 – Opinion of respondents about "timing" from setting the indication to initiation of continuous renal replacement therapy.

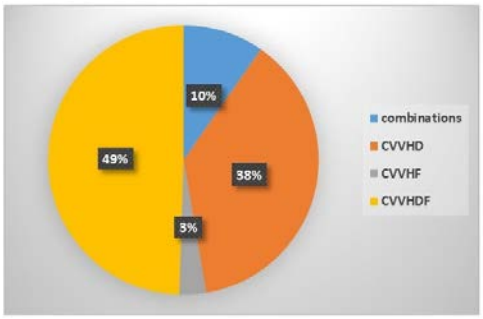

Fig. 4 – Most commonly used modalities of continuous renal replacement therapy.
CVVHD – continuous veno-venous hemodialysis;
CVVHF – continuous veno-venous hemofiltration;
CVVHDF– continuous veno-venous hemodiafiltration.

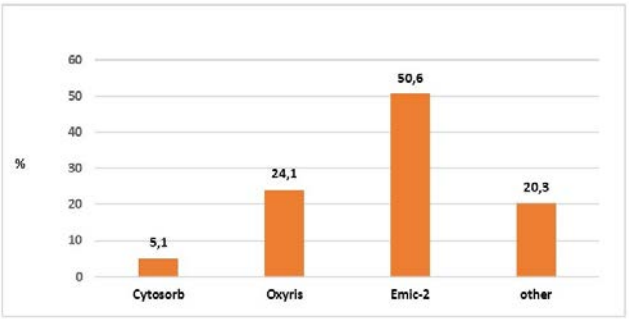

Fig. 5 – Adsorptive membranes use in the treatment of septic patients with acute kidney injury.

1.1% of the patients. The continuous veno-venous hemodiafiltration (CVVHDF) being most commonly used (49%) (Figure 4). The choice of modality depended on the clinical indication (38.7%), the decision of the nephrologist (34.4%), availability of modalities, logistics, personnel (20.4%), and other factors (6.5%). The most common target dose of CRRT was 25 mL/kg/h (45.2%), followed by 35 mL/kg/h (40.9%), 45 mL/kg/h (5.4%), and other values (8.6%). Systemic heparin-anticoagulation was prevalent – 95.7%, followed by regional anticoagulation (citrate) 3.2%, and another anticoagulation in 1.1% of the patients. The average filter life was in most respondents less than 24 h (71.3%). Adsorptive membranes in the treatment of sepsis in AKI were used by 60.6%, the most prevalent being Emic-2 in 50.6% of the cases (Figure 5).

The most common complications of CRRT were hypotension – 55.3% and catheter-associated infection – 29.8%, followed by electrolyte imbalance (8.5%), bleeding (2.1%), and other complications (4.3%). The incidence of catheter-related infections (1–3/1,000 catheter days) was reported by 43.6% of the participants, (4–6/1,000 catheter days) by 37.2% and (< 6/1,000 catheter days) by 19.1%. In patients who required CRRT, 41.9% of the respondents stated that 5–20% of the patients recovered their renal function by the period of 3 months, 35.1% reported that 5–20% of the patients had died, 47.9% said that the same percentage of patients lacked some of the stages of chronic renal failure, and 44.7% reported that 6–10% of the patients remained dialysis-dependent. Over the past year, 33% reported that 21–40% of the patients had "early" onset CRRT recovery in renal function, and 46.8% said that less than 5.0% of the patients had died, as opposed to "late" CRRT onset which reported an increase in renal function < 5.0% out of 44.7% of the participants, whereas 40.4% reported death in 5.0–20% of the patients (Table 1).

Discussion

The lack of clear guidelines in the definition, diagnosis, and treatment of AKI, but also the fact that standard biochemical and clinical parameters, as well as new biomarkers, did not optimize treatment outcomes, indicates the need for further research. The main aim of this multicentric research was to summarize CRRT clinical practice information for one year in order to gain insight into the most important issues, especially treatment timing, and to guide clinicians in their daily work.

Mostly nephrologists, followed by specialists in anesthesiology, reanimatology, and intensive care and physicians with other specialties, of whom 40.4% had work experience of over 20 years and most of them were employed in tertiary care institutions, participated in our questionnaire.

Table 1

Outcomes in patients with acute kidney injury who required renal replacement therapy (RRT) regarding "early" vs. "late" start of continuous RRT (CRRT) according to responses of participants

Patients (%)	Recovery (one year)	Mortality (one year)
Early start CRRT		
< 5	7.4	46.8
5–20	29.8	31.9
21–40	33.0	10.6
41–60	23.4	9.6
> 60	6.4	1.1
Late start CRRT		
< 5	44.7	19.1
5–20	26.6	40.4
21–40	19.1	21.3
41–60	8.5	10.6
> 60	1.1	8.5

According to the results, slightly more than half of the participants still use the RIFLE criteria to define the AKI, while in terms of representation, the following use the KDIGO criteria, which is different from the previous results, in which the KDIGO criteria are dominant [5]. The higher prevalence of the RIFLE classification has been reported in previous studies, and in our sample, it can be explained by the participation of non-nephrological specialty respondents and possibly by a high percentage of physicians with many years of experience who are somewhat skeptical about accepting novelties [6, 7].

Our subjects use almost equally high (≥ 250 mg/day) and low doses of furosemide (≤ 250 mg/day) in the conservative treatment of AKI, and a relatively equal representation in the range of up to 30.0% of AKI patients requiring CRRT is reported. These results are different from earlier study in which it was stated that most use high doses in oliguria patients and that 13.0% of patients require CRRT [5]. In our institutions, most respondents stated that they use the KDIGO 2 criterion for the "early" start of CRRT, and for already present complications associated with AKI or poor response to conservative therapy, participants start CRRT "late". Compared to comprehensive clinical work so far, almost 50.0% believe that the timeline for "early" start of CRRT should be within 12 h, which corresponds to the KDIGO 2 criterion, and slightly more than 50.0% believe that over 48 h is "late" start. These "early" start CRRT results rule out urgent indications and leave time for patient monitoring and clinical evaluation for the late start. However, Thakar et al. [8] reported in their survey that 53.0% of respondents felt that there was no benefit from "early"-start CRRT. Moreover, 35.0% of respondents believed that the risk of "early" CRRT outweighed the benefit. However, 46.0% of respondents indicated that they often initiate "early" CRRT in patients with AKI in ICU. The most influential parameters in determining dialysis initiation were complications of AKI, such as hyperkalemia and hypoxemia due to volume overload, whereas the degree of severity of kidney injury or markers of azotemia played a less important role in the "early" dialysis decision. In their work, Clark et al. [9] have shown that potassium levels and pulmonary edema are the most common indicators of "early" CRRT. The aforementioned surveys were conducted in 2012 when different biochemical and clinical parameters were used in deciding to initiate CRRT. By defining the KDIGO guidelines in the same year in the AKI classification, the use of the same was made possible in the following years, and the above-mentioned surveys are not comparable with ours. Most believe that the Furosemide Stress Test, cystatin C, and neutrophil gelatinase-associated lipocalin (NGAL) in the urine could increase the prediction for the start of CRRT. Our centers have no experience in using biomarkers other than cystatin C, and this may be the reason why only 20.3% of respondents said that (TIMP-2)•(IGFBP7) and other biomarkers would be good predictors. In a previous survey, 60.0% of participants indicated that they were implementing new biomarkers in their practice and research [9]. It is interesting to note that 77.7% still consider the severity and course of the disease to be the deciding factors for the start of CRRT, which indicates the importance of the "clinical scenario" as the most important part of the strategy in the treatment of AKI and also meets the current "watchful waiting" recommendations. However, the responses of the rest of the participants stating comorbidities, response to diuretic therapy, availability (appliances, equipment, staff), day of the week, and time of day are not negligible. It should be noted that none of the subjects indicated a decisive factor for the start of CRRT, but 4.3% indicated that older age was a relative contraindication for the start of CRRT. Just over 50.0% of participants cited advanced malignancy (except for multiple myeloma), and about a third of respondents reported hypotension with no response to vasopressors as relative contraindications for the start of CRRT. So far, many studies have been conducted towards the decision to initiate treatment, and there are fewer data about the patients with a very low probability of surviving where the used CRRT would be a source of inadequate information, as it would probably suggest that CRRT itself increases the risk of poor outcomes. The complexity of the clinician's decision-making in comprehensive consideration of the indications, prognosis, and outcome of the disease is sometimes hampered by subjectivity relative to the preferences of the patient or family, so CRRT is applied, although it is unlikely to modify the outcome. Therefore, the future consensus of the decision to start CRRT should include irrelative contraindications. Most participants stated that the nephrologist indicates the start and writes the CRRT prescription. Please note that in our region, due to the distance of CRRT institutions and some without employed nephrologists, training was conducted by nephrologists in previous years and consultative cooperation continued. About 50.0% of the respondents believe that from the diagnosis to the indication for RRT it takes up to 6 h and from the indication to the beginning of RRT around 6 h, although a quarter of them stated that the stated time depends on the availability of the apparatus, logistics, and staff.

In most of our centers, the specialist employed in the ICU places a dialysis catheter, predominantly by the blind Seldinger technique, most commonly in the left/right internal jugular vein. About half of the respondents use CRRT in their institutions and all modalities (CRRT/hybrid), and the most commonly used modality is CVVHDF. Although there is an upward trend in extracorporeal methods, the results indicate an under-representation of CRRT [5–7]. Most participants indicated that the choice of CRRT modality depends on the clinical indication and the decision of the nephrologist. The most commonly used target dose of CRRT is 25 mL/kg/h, systemic anticoagulation with heparin (95.7%) with an average filter life of less than 24 h (71.3%) is prevalent. Digvijay et al. [5] reported similar results, except in the use of anticoagulation (mostly unfractionated heparin followed by citrate, low molecular weight heparin, and regional anticoagulant therapy). Overberger et al. [10] stated that in their study CRRT was also the most commonly used modality of therapy as well as the applied dialysis effluent dose of 25 mL/kg/h. In another earlier study, over 90.0% of

subjects used CRRT, however, the most commonly prescribed dose was 35 mL/kg/h [8]. In our study, adsorptive membranes were used by 60.6% of subjects to treat sepsis in AKI (Emic-2–50.6% were the most prevalent).

The most common complications of CRRT are hypotension (55.3%) followed by catheter-related infection with an incidence of 1–6/1,000 catheter days reported by most subjects, similar to the results of certain previous studies [11–13]. In presented patients who required CRRT, the majority of respondents stated that up to 20.0% of patients had renal function recovery by 3 months and that, in the same percentage, some patients had some stage of chronic renal failure/dialysis dependency/death in the first year. Those that survive the initially high mortality rate associated with dialysis-requiring AKI mostly become independent of RRT within a year, but some of them do go on to develop chronic kidney disease and even progress to end-stage renal disease [14].

It is unclear whether a preventive/"early" strategy of the initiation of RRT in order to avoid complications associated with AKI leads to better patient outcomes and the use of health services, or a more conservative strategy in which RRT is started as a response to the development of complications provides better results [15]. About 50.0% of the respondents stated that the least patients died with the "early" start of RRT, as opposed to the "late" start of RRT, which was confirmed in our single-center retrospective study of 385 patients with AKI who were admitted between 2014 and 2017 [16].

About half of the physicians reported that patients with "late" dialysis start had the recovery of renal function in the lowest number, while one in three respondents said that 20–40% of patients who started dialysis "early" recovered the renal function. Recent meta-analyses are also remarkably clear, noting that increased mortality and recovery of renal function by "early" dialysis stems from lower quality data (i.e., high heterogeneity and/or higher risk of bias). Meanwhile, an analysis of high-quality pooled data shows no significant difference in mortality [17–22].

Conclusion

Most subjects consider the severity and course of the disease to be the determining factors for initiation of CRRT and favored the "early" start of CRRT – KDIGO 2 criterion within 12 h of diagnosis with an increasing prediction of the Furosemide Stress Test. Although there is an increasing trend in the use of extracorporeal methods, our data indicate underutilization of CRRT and a lack of citrate dialysis. Further research is needed to form a clinical model that, in addition to a functional test, would include one of the biomarkers or a combination of biomarkers in order to increase the prediction of initiation of CRRT treatment.

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Natural polymers for vaginal mucoadhesive delivery of vinegar, using design of experiment methods

Prirodni polimeri za vaginalnu mukoadhezivnu primenu sirćeta, korišćenjem dizajna eksperimentalnih metoda

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Abstract

Background/Aim. Vinegar is one of the main international traditional nutraceuticals, and it has been used as a vaginal health protectant due to vagina pH balance maintenance and antimicrobial properties. Since the main form of vinegar is liquid, it is difficult to apply vaginally due to its short retention. The aim of this study was to design a vaginal mucoadhesive gel made of vinegar. **Methods.** Xanthan gum and tragacanth were utilized as natural gel-forming polymers. The effects of xanthan gum and tragacanth on mucoadhesion strength and drug release of the gel formulations were optimized using a 3 level (3^2) factorial design. Several physicochemical properties of the gel formulations, including gel viscosity, lubricity, scanning electron microscopy (SEM) images of hydrogel chains, and chain release kinetic, were also investigated. **Results.** It was found that tragacanth possessed a statistically significant effect on release rate control (p-value = 0.0027), while both tragacanth and xanthan gum had a significant effect (p-value = 0.0001 and 0.0017, respectively) on mucoadhesion property. Formulation F7 with 5% xanthan gum and 1% tragacanth (mucoadhesion = 0.4632 N and release rate = 88.8% in 6 hours) considered as the optimum formulation with some modifications. **Conclusion.** Xanthan gum and tragacanth can be considered as appropriate natural polymers for vaginal drug delivery.

Key words:
acetic acid; gels; polymers; tragacanth; vaginal creams, foams, jellies; xanthan gum.

Apstrakt

Uvod/Cilj. Sirće je jedno od glavnih internacionalnih tradicionalnih nutraceutika koje se koristi kao sredstvo za zaštitu vagine, zahvaljujući osobini da održava pH vagine i poseduje antibakterijska svojstva. S obzirom na to da je sirće tečnost, zbog kratkog zadržavanja je teško primenljiv vaginalno. Cilj rada bio je da se od sirćeta dizajnira vaginalni mukoadhezivni gel. **Metode.** U studiji su kao polimeri koji formiraju prirodni gel korišćeni ksantan guma i tragakant. Efekti ksantan gume i tragakanta na jačinu mukoadhezije i oslobađanje lekova u formulacijama gela optimizovani su korišćenjem faktorijalnog dizajna на 3 nivoa (3^2). Takođe, istraženo je nekoliko fizičko-hemijskih svojstava formulacije gela, uključujući viskoznost gela, mazivost, slike *scanning* elektronske mikroskopije lanaca hidrogela i kinetiku oslobađanja lanaca. **Rezultati.** Utvrđeno je da je tragakant imao statistički značajan uticaj na kontrolu brzine oslobađanja ispuštanja (p = 0,0027), dok su i tragakant i ksantan guma imali značajan uticaj (p = 0,0001 i 0,0017, redom) na svojstva mukoadhezije. Formulacija F7, sa 5% ksantan gume i 1% tragakanta (mukoadhezija = 0,4632 N i stopa oslobađanja = 88,8% tokom 6 h) sa nekim modifikacijama, bila je optimalna. **Zaključak.** Ksantan guma i tragakant mogu se smatrati odgovarajućim prirodnim polimerima za vaginalnu „isporuku" lekova.

Ključne reči:
sirćetna kiselina; gelovi; polimeri; tragakant; vaginalne kreme, pene i želei; ksantan guma.

Introduction

The vagina is considered as a drug delivery site to obtain a local as well as systemic pharmacological effect [1, 2]. Thanks to the presence of *Lactobacilli*, the normal pH of the vaginal fluid in healthy women is maintained between 4 and 5.5 [3]. Low pH is one of the natural factors in resistance to the colonization of pyogenic organisms [4]. Lactic acid has been

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shown to deactivate a wide range of reproductive tract pathogens, including HSV-2 [5], *Neisseria gonorrhoeae* [6], and uropathogenic *Escherichia coli* [7]. In addition to direct inactivation of pathogens, vaginal acidity potentiates the slowing and trapping of HIV-1 virions by cervicovaginal mucus [8, 9]. Moreover, studies have confirmed the coincidence between the increase in vaginal pH along with bacterial vaginitis and trichomoniasis [10, 11].

Conventional vaginal drug delivery systems such as creams, tablets, capsules, pessaries, liquid dosage forms, etc., are associated with some disadvantages including leakage, messiness, and poor retention time (due to vagina self-cleaning action), which requires multiple daily doses and decreases patient compliance [12, 13]. In this way, mucoadhesive dosage forms seem to be a promising choice [14]. Among them, gels have received great attention due to their high water content [15] and rheological behavior causing increased vaginal retention time, lubrication, and patient compliance [16, 17]. Gels are also easy to manufacture and scale up and are the preferred vaginal drug delivery system among ladies [18, 19]. Natural polymers are valuable candidates for mucoadhesive gel formulations due to their proven safety, high biocompatibility [20, 21], ability to conjugate with other polymers [22], and eco-friendliness [23].

Xanthan gum and tragacanth are two examples of natural anionic polysaccharide gums that have been widely used in various industries including food, oil-recovery, cosmetics, water-based paints, petroleum, tissue engineering, biomedical, and drug delivery [24, 25]. Among several drug delivery systems incorporating these two polymers, mucoadhesive dosage forms are of interest due to their proper mucoadhesion property [26–28].

Vinegar called "*serkeh*" in Persian medicine [29] is produced from fermentable glucose in carbohydrate-rich foods such as grape, apple, fig, rice, etc., through fermentation processes (alcoholic and subsequently acetous fermentation) [30]. Vinegar has a long history and was widely prescribed by physicians of Persian medicine for several therapeutic purposes including oral consumption as an appetizer, digestive, thirst relief, treatment of warm headache, decreasing the bile flow [29, 31], and also in hypertension [32]. External dosage forms of vinegar separately or in combination with other medicines have been utilized for treating several illnesses including hemorrhoid, tinnitus, hearing loss, halitosis, gingivitis, mouth ulcers, toothache, intestinal worm infections, fire burning, removal of an attached leech from the pharynx [29, 31], and female genital infections [33]. Historically, the use of vinegar against infections dates back to Hippocrates (460-377 BC), who prescribed vinegar for treating sores and ulcers and its oral products in combination with honey named "oxymel" for improvement of persistent cough [34]. Furthermore, there are a few studies about the formulation of dosage forms using vinegar against vaginal infections [35, 36]. This liquid nutraceutical has also been studied in current medicine. Different studies show several health benefits of the vinegar such as improving lipid profile and suppressing fat accumulation, reduction of hyperglycemia and improvement of insulin secretion, inhibition of proliferation and induction of apoptosis in human cancer cells, antioxidant properties, exhaustion recovery effects, regulation of blood pressure, and natural disinfectant [30, 37].

Nowadays, in many analytical methods, experimental design is used for optimization instead of the traditional one variable-at-a-time (OVAT) approach [38, 39]. There are several reasons for the superiority of the design of experiment (DOE) approach over the OVAT approach. DOE yields the best possible formulation, while OVAT may find only suboptimal formulation. In addition, DOE is armed to estimate any synergistic or antagonistic interaction among constituents, whereas OVAT is inept to reveal any possible interaction. In the DOE approach, all response variables are quantitatively governed by a set of input factors (variables); therefore, any change in the optimized formulation for scale-up can be easily incorporated. On the other hand, the OVAT approach is restricted to the suboptimal point, and it is very difficult to modify the optimal points for any possible scale-up. Besides, the DOE approach is highly economistic in terms of resources and time. Factorial design is one of the most popular techniques of DOE. In situations when there are several factors (e.g., 2 or 3 factors), a three-level factorial design is certainly a possible choice by an experimenter who is concerned about curvature in the response function (Montgomery: design and analysis of experiments). The addition of a third-factor level allows the relationship between the response and the design factor to be modeled as a quadratic.

To the best of our knowledge, although there are vinegar douche products in the market, vinegar has not been formulated as a mucoadhesive gel yet. Therefore, in the present study, considering antimicrobial potential and pH modification of vinegar as a natural remedy, a 3-level factorial design was incorporated for the formulation of vinegar vaginal mucoadhesive gel, using tragacanth and xanthan gum natural polymers.

Methods

Materials

Tragacanth and xanthan gum were purchased from Gol Darou Co, Tehran, Iran, and vinegar was obtained from Bidestan co, Qazvin, Iran. Sodium hydroxide of analytical grade was obtained from Merck (Darmstadt, Germany). Dialysis bags with a molecular weight cut-off of 14 kDa were purchased from Sigma (Steinheim, Germany). All gel formulations were prepared with deionized water.

Experimental design

A 2 factor, 3-level (3^2) full factorial design was utilized for the optimization of vinegar gel using Design Expert 10 software (Stat-Ease Inc., Minneapolis, USA). Amounts (w/w %) of tragacanth and xanthan gum as independent variables were categorized in three (low, intermediate, and high) levels. Mucoadhesion strength and 6 hrs drug release were considered as dependent variables. Each design point was

tested three times, while the center point was assessed four times to consider the pure experimental error of the model. Furthermore, two formulations with zero percent of tragacanth or xanthan gum were also investigated. The design matrix and corresponding response values are presented in S1 (Supplementary Information) [40]. The statistical validation was confirmed by ANOVA at a significance level of $p < 0.05$ [41].

Gel preparation

According to the design matrix, a proper amount of each polymer was mixed with deionized water in a 250 mL beaker. The solution was stirred with a magnetic stirrer at 500 rpm, and vinegar was slowly added to reach the pH of 4 (around the vagina's natural pH). The final volume of each formulation was adjusted to 150 mL.

Viscosity studies

The viscosity of samples was measured using a DV-3 con viscometer (Brookfield, USA). A total of 50 mL of samples were applied to the viscometer container at 37 °C (n = 3).

pH measurement

To confirm the compatibility of the prepared formulations for vaginal mucosa, their pH values were measured by a pH meter (Mettler-Toledo GmbH, Switzerland) at room temperature (n = 3).

Gel spreadability

Of each gel formulation, 0.5 g was placed on a circular glass plate with 1 cm diameter, and another glass plate was placed on the gel. A 50 g weight was put on the upper glass for 5 min. The spreading area was calculated using the measurement of the increase in gel diameter (n = 3) [42, 43].

Mucoadhesion studies

The mucoadhesion study was performed according to Tasdighi's method [44] with some modifications. As is presented in Figure 1, 0.5 g of each gel formulation (A) was placed between two circle glasses (B) covered with sheep intestinal mucus (C). The bottom glass was fixed in a crystallizer, and the top glass was linked to a balance measuring the required force for detachment of the gel from the mucosal membrane. The test was performed in phosphate buffer medium pH = 4.5 and 37 °C.

In vitro release profile

The *in vitro* release was performed using a 14 kDa Dialysis tubing Cellulose Membrane. The membrane was soaked in distilled water for 24 hrs before the experiment. Five grams of each formulation was packed in a dialysis tube and placed in 200 mL distilled water (receptor medium) at 37 °C. Medium was stirred at 100 rpm during the release test, and samples were withdrawn at certain time intervals of 0.5, 1, 2, 4, and 6 hrs. Acetic acid was considered as the

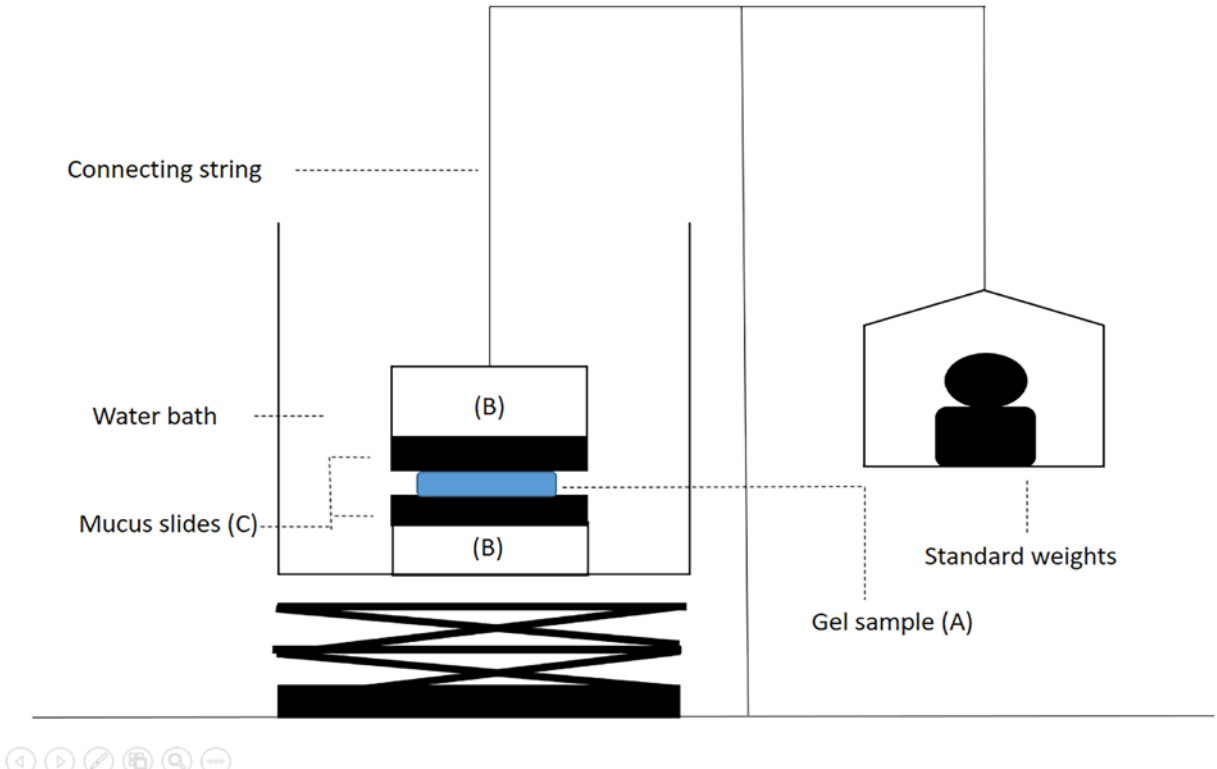

Fig. 1 – Schematic figure of apparatus used for assessment of gel's mucoadhesion.

main index in vinegar for quantitative analysis. The content of acetic acid in each sample was analyzed with pH metric titration with NaOH 0.005 M to equivalence point pH = 8.2 (Proline B210 pH meter Netherland). The amount of released acetic acid from each sample was calculated.

Release mechanism determination

The release kinetic of formulations F2, F3, F7, F9, and F10 were investigated by fitting the *in vitro* release data to various mathematical kinetic models. Models included zero order, first order, Higuchi, and Korsmeyer-Peppas [45].

Drug content

One gram of gel was vigorously stirred with 10 mL distilled water, using a sonicator and vortex, resulting in a transparent solution. Then the volume was adjusted to 100 mL, and the acetic acid content was measured using pH metric titration with NaOH as described in the release study (n = 3).

Scanning electron microscopy analysis

Scanning electron microscopy (SEM) evaluations were performed for the characterization of hydrogel polymeric chain micro-morphology.

Polymer chain morphology was investigated by FESEM Sigma VP (Zeiss, Germany) with an accelerating voltage of 10 kV under vacuum conditions. For sample preparation, gels were freeze-dried at -15 °C, and dried gels were gold sputter-coated before FESEM.

The study protocol was approved by the research committee of Guilan University of Medical Sciences by the ethics code of IR.GUMS.REC.1396.283.

Results

According to Table 1, gelling agents (polymers) were slowly dispersed in water with a range of 0–5% for both tragacanth and xanthan gum, and the pH was adjusted to 4, using vinegar.

The pH of formulations is one of the important parameters for vaginal compatibility. The vaginal pH of healthy women of reproductive age is around 4–5.5, changing during the menstrual cycle. Lactic acid produced by *Lactobacilli* present in the healthy vagina plays an important role in the control of infection by common pathogens. In our formulations, acetic acid as the index component of vinegar plays such a role.

The results showed that all the formulations were suitable for vaginal application as they could maintain the acidic pH value of the vagina (Table 1).

Viscosity influences other mechanical properties of formulations, such as spreadability and mucoadhesion, which are in direct correlation with patient compliance. The viscosity of each formulation was measured and stated in Table 1. The spreading area of each gel formulation was evaluated by measuring the diameter of the circle, which is formed under the pressure of glass. Spreading values are mentioned in Table 1.

Acceptable content of active pharmaceutical ingredient (API) or its indexes ensures the producer and consumer about receiving the adjusted or necessary dose. Since there is no defined monograph for vaginal vinegar gel in pharmacopeias, the prevalent range of 90–105% was considered acceptable in this study. The drug content of each formulation is presented in Table 1.

Four samples were subjected to SEM, including F9 and F10, which were simply made of xanthan gum and tragacanth, respectively, and F2 with the lowest amount of xanthan gum (2%) and the highest amount of tragacanth (5%), and F7 with the lowest amount of tragacanth (1%) and the highest amount of xanthan gum (5%). Figure 2 shows a homogenous wavy morphology for F9 (Figure 2a) and a relatively smoother morphology for F10 (Figure 2b), while in F2 and F7, a kind of polymeric chain integration is observed (Figures 2c, 2d). It seems that formulations containing both polymers show special polymeric interactions due to probable hydrogen or van der Waals bonds.

The acetic acid release profile was evaluated for all formulations in distilled water at 37 °C. Release profiles of F2, F7, F9, F10, and F3 (which is the center point of

Table1

Polymer composition and physical properties of formulations F1-F11

Formulation	XG (%)	TG (%)	pH	Viscosity (cp) mean ± SD	Diameter (cm) mean ± SD	Mucoadhesion (N x10^{-2})	Drug content (%) mean ± SD	Release rate (%)
F_1	2	1	4.01	21,100 ± 1,462.2	3.8 ± 1.3	41.9	95.5 ± 6.2	86.4
F_2	2	5	4.09	54,000 ± 2,581.5	3.0 ± 0.5	51.5	92.4 ± 4.4	73.3
F_3	3.5	3	4.21	34,000 ± 1,652.4	3.1 ± 0.9	45.2	95.1 ± 8.2	78.3
F_4	5	5	4.16	*	2.8 ± 0.4	56.5	89.3 ± 5.7	78.0
F_5	5	3	4.19	95,900 ± 4,235.9	3.7 ± 2.0	49.5	94.3 ± 7.0	84.7
F_6	3.5	5	4.08	*	2.9 ± 1.3	52.2	88.7 ± 9.3	76.4
F_7	5	1	4.14	29,000 ± 1,874.2	3.7 ± 2.8	46.3	91.3 ± 4.6	88.8
F_8	3.5	1	4.11	24,700 ± 1,423.5	3.6 ± 1.7	43.3	92.2 ± 11.6	88.2
F_9	5	0	4.23	28,600 ± 1,685.6	3.5 ± 1.4	46.0	97.6 ± 9.4	87.3
F_{10}	0	5	4.17	25,100 ± 1,584.5	3.0 ± 2.8	53.8	93.8 ± 6.3	86.4
F_{11}	2	3	4.16	26,400 ± 1,376.1	3.3 ± 1.7	44.3	84.0 ± 8.3	81.4

XG – xanthan; TG – tragacanth; SD – standard deviation.

***Due to very high viscosity, it was not measurable by the apparatus.**

experiment design) are presented in Figure 3. Release data for other formulations is accessible in a supplementary file.

According to the release results, F7 showed the highest (88.8%) while F2 showed the lowest (73.3%) release rate among other formulations in 6 hours. Considering F7 and F2 gums composition, they are a combination of both xanthan gum and tragacanth, in which F7 had the highest amount of xanthan gum (5%) with the lowest amount of tragacanth (1%) and F2 had the lowest amount of xanthan gum (2%) with the highest amount of tragacanth (5%).

Data obtained from *in vitro* release F2, F3, F7, F9, and F10 formulations were fitted to zero order, first order, Higuchi and Korsmeyer-Peppas models [44]. The drug release kinetic data are listed in Table 2, the highest regression coefficient (R^2) was considered the best fitted kinetic model for each vinegar gel formulation.

All chosen formulations showed the highest regression coefficient in the first-order model, which confirms that the drug release rate depends on its concentration.

Analysis of variances was used to assess the suitable response surface model and its significance. Tables 3 and 4

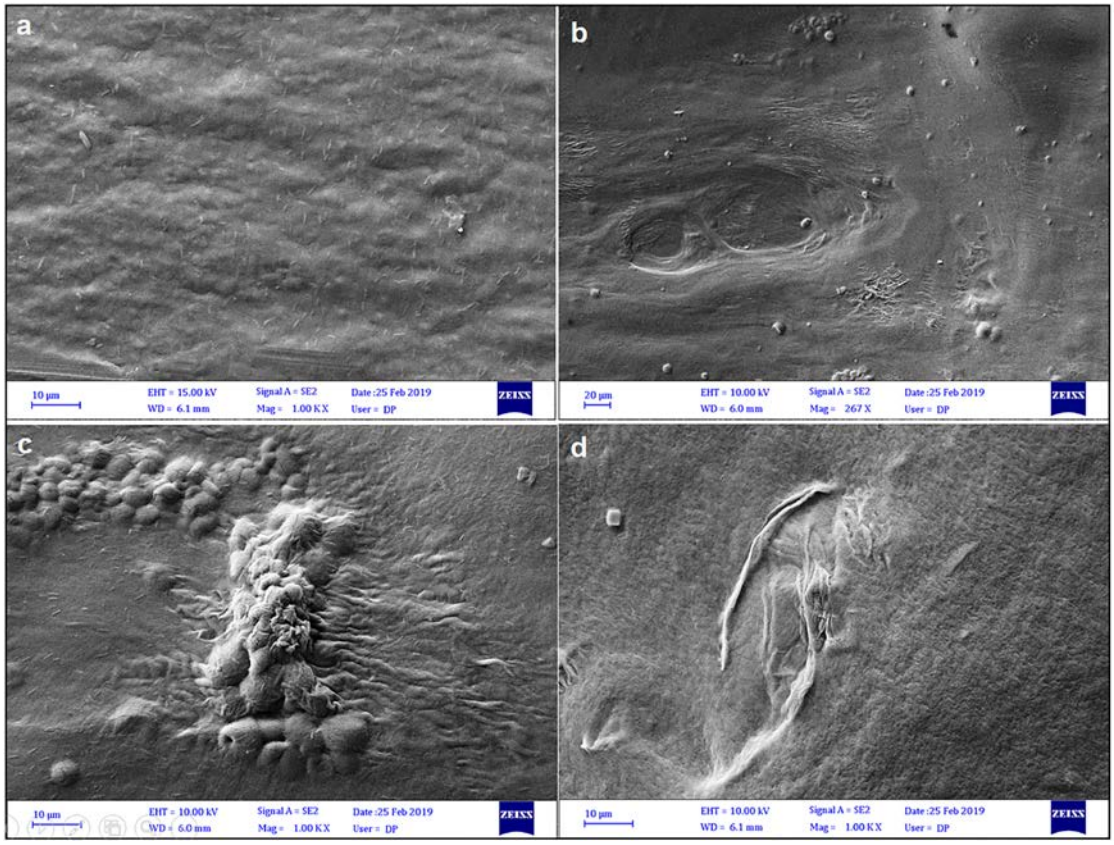

Fig. 2 – Scanning electron microscopy (SEM) images of hydrogel polymeric chain arrangement in: a) xanthan gum (5%), b) trgacanth (5%), c) xanthan gum (2%) and tragacanth (5%), d) tragacanth (1%) and xanthan gum (5%).

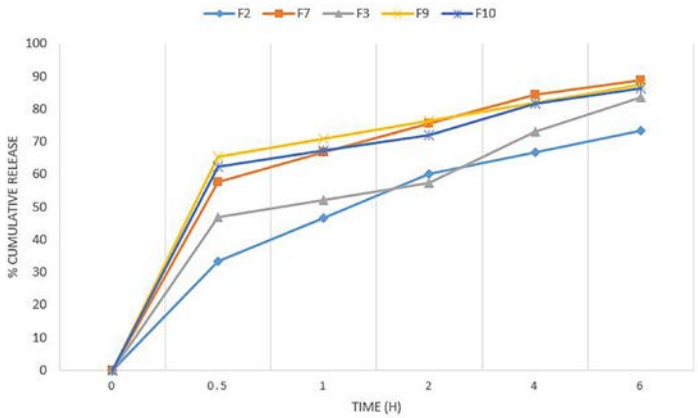

Fig. 3 – Release profile of formulations F2, F3, F7, F9, and F10 indicates the cumulative drug release vs. time.
Data on formulations are given in Table 1.

show ANOVA tables for mucoadhesion strength and release rate, respectively. A *p*-value lower than 0.05 was used to select significant parameters. As seems in Tables 3 and 4, the models are significant, and the *p*-value of the lack of fit implies that it is not significant relative to the pure experimental error and confirms the validity of the models.

Besides, the R-squares value and other statistical parameters of the models are all acceptable. Table S2 (supplementary materials) shows the statistical parameters of the models.

A dimensional graph of the effect of tragacanth and xanthan gum on A) vinegar *in vitro* release rate and B) mucoadhesion of gels is shown in Figure 4.

Table 2

Release kinetic parameters for F2, F3, F7, F9, and F10 formulations based on different mathematical models

Formulation	Zero order		First order		Higuchi		Korsmeyer-Peppas	
	K_0	R^2	K_1	R^2	K_H	R^2	K_K	R^2
F_2	13.709	0.9033	-0.1113	0.9843	13.709	0.9033	0.2954	0.5931
F_3	14.309	0.8549	-0.1383	0.9399	14.309	0.8549	0.2921	0.5526
F_7	15.229	0.7678	-0.1768	0.9627	15.229	0.7678	0.2941	0.5165
F_9	14.027	0.6676	-0.1544	0.8844	14.027	0.6676	0.2865	0.4858
F_{10}	14.126	0.7055	-0.1523	0.9074	14.126	0.7055	0.2875	0.4963

Data on formulations are given in Table 1.

Table 3

Analysis of variance (ANOVA) table for mucoadhesion strength of the formulations

Source	Sum of squares	df	Mean square	F-value	*p*-value	
Model	191.9	3	64.0	38.6	4.2 E-05	significant
A: Xanthan gum	35.5	1	35.5	21.4	1.7 E-03	
B: Tragacanth	137.0	1	137.0	82.6	1.72 E-05	
B^2	19.4	1	19.4	11.7	9.1 E-03	
Residual	13.3	8	1.6			
Lack of fit	4.8	5	0.9	0.4	0.9	not significant
Pure error	8.5	3	2.8			
Cor total	205.2	11				

Table 4

Analysis of variance (ANOVA) table for release rate of the formulations

Source	Sum of squares	df	Mean square	F-value	*p*-value	
Model	230.9	2	115.46	9.1	7.0 E-03	significant
A: Xanthan gum	18.0	1	18.0	1.4	0.3	
B: Tragacanth	212.9	1	212.9	16.7	2.7 E-03	
Residual	114.7	9	12.8			
Lack of fit	8.6	6	1.4	0.04	1.0	not significant
Pure error	106.1	3	35.4			
Cor total	345.6	11				

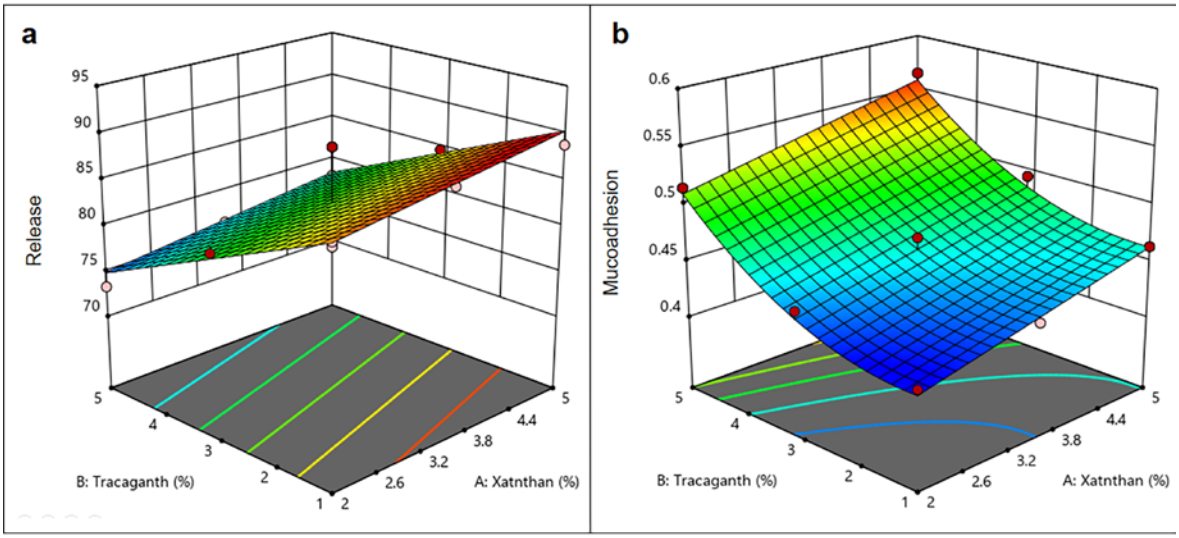

Fig. 4 – 3-Dimensional graph of effect of tragacanth and xanthan gum on a) vinegar *in vitro* release rate, b) mucoadhesion of gels.

Discussion

Paying attention to formulations F9 (5% xanthan gum) and F10 (5% tragacanth), it seems that an increase in tragacanth percent leads to decreases in vinegar release rate whilst xanthan gum increases the release rate of vinegar. Although tragacanth's behavior on release control has been observed in other studies [46], xanthan gum's influence on increasing the vinegar release rate is in contrast with former studies [47]. However, most studies have investigated the release of lipophilic API from tragacanth and xanthan gum matrices, while vinegar is a completely hydrophilic API. On the other hand, it's hypothesized that tragacanth can be adsorbed to the oil droplets and therefore possesses a more hydrophobic nature, while xanthan gum just acts through the viscosity increase [47, 48]. Therefore, it seems that in this study, tragacanth plays the main role in lowering the release rate, and xanthan gum modulates the rate by decreasing the tragacanth percent in the formulation because in this formulation maximum release of vinegar in 6 hours is desirable.

The release kinetic data is somehow in agreement with results of Salamanca et al. [47] that used xanthan and tragacanth gum in the formulation of a sustained-release tablet and drug release kinetic best fitted with first order and Higuchi models.

According to the models, tragacanth has a significant effect (p-value = 0.0027) on release rate, while xanthan gum has no statistically significant effect (p-value = 0.3). Figure 4a demonstrates the response surface graphically to help visualize the shape of the response surface of release rate. An increase in tragacanth weight (%) leads to a decrease in the release rate. As can be seen, there is no curvature effect of any parameter on the release rate. On the other hand, both tragacanth and xanthan gum, together with the curvature of tragacanth, have a significant effect on the mucoadhesive properties of the formulation. Figure 4b shows the response surface of mucoadhesive strength. Both tragacanth and xanthan gum have a positive effect on mucoadhesive strength.

Conclusion

The main objective of the optimization is to determine the optimum values of the parameters. After rigorous analysis of the selected models and their corresponding parameters, F7 has been selected as the best formulation of the study.

This study demonstrates that xanthan gum and tragacanth can be considered appropriate natural polymers for vaginal drug delivery. A certain combination of these two polymers with specific ratios (F7: 5% xanthan gum and 1% tragacanth) presented high mucoadhesion properties (0.4632 N) and good release characteristics (88.8%) in 6 hrs as a vinegar vaginal natural mucoadhesive gel.

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Conflict of interest

The authors declare that they have no conflict of interest.

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Correlation between cytological and histopathological diagnosis of non-small cell lung cancer and accuracy of cytology in the diagnosis of lung cancer

Korelacija citološke i histopatološke dijagnoze nemikrocelularnog karcinoma pluća i tačnost citologije u dijagnostici karcinoma pluća

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Abstract

Background/Aim. Lung cancer is one of the most common cancer types worldwide. More than 70% of patients are diagnosed with lung cancer in the advanced stages of the disease, with limited therapeutic options based on cytological and histopathological material. The value of cytology in diagnosing and subtyping non-small cell lung cancer (NSCLC) is very important for modern personalized therapies. The aim of this study was to find out the concordance between cytological and histopathological diagnosis of NSCLC and the accuracy, sensitivity, specificity, and the positive and negative predictive value of cytology in diagnosing lung cancer. **Methods.** A two-year retrospective study included 169 patients with cytological diagnosis of NSCLC, who, at the same time, had small biopsy and surgical specimens for histopathological diagnoses confirmation that were compared with cytological one. Histopathological diagnosis on surgical specimens was the golden standard for evaluation concordance to the cytological diagnosis of NSCLC and evaluation accuracy, specificity, sensitivity, and the positive and negative prognostic value of cytology as a diagnostic method for detecting lung cancer. **Results.** This study included 129 (76.3%) male and 40 (23.7%) female patients, aged between 39 and 83, with the average of 62.53 ± 7.6. There was no statistically significant difference between the ages of different genders ($p = 0.207$). The most frequent diagnosis among cytological diagnoses was NSCLC in 99 (58.58%) patients. Concordance between cytological and histopathological diagnoses of surgical specimens was 61.48%. There was no statistically significant difference between cytological diagnoses and histopathological diagnoses of small biopsies specimens ($p = 0.856$). The sensitivity, specificity, positive and negative prognostic value, and accuracy of cytology as a diagnostic method of lung cancer were 94.98%, 98.60%, 95.72%, 98.35%, and 97.71%, respectively. **Conclusion.** Cytological diagnosis of NSCLC is accurate, with high sensitivity, specificity, and benefits for patients. Most patients are diagnosed with advanced cancer when there is no surgical therapy option, and the only available diagnostic material is a small biopsy sampled during bronchoscopy.

Key words:
biopsy; bronchoscopy; carcinoma, non-small-cell lung; cytological techniques; histological techniques; prognosis; sensitivity and specificity.

Apstrakt

Uvod/Cilj. Karcinom pluća je jedan od najučestalijih karcinoma u svetu. Kod više od 70% bolesnika dijagnostikuje se u odmaklim stadijumima bolesti kada su terapijske mogućnosti ograničene i zasnovane na dijagnozi citološkog ili patohistološkog materijala. Kod novih personalizovanih vidova terapije veliki je značaj citologije u dijagnostici i subtipizaciji nemikrocelularnih karcinoma pluća (*non-small cell lung cancer* – NSCLC). Cilj rada bio je da se utvrdi podudarnost između citološke dijagnoze NSCLC i patohistološke dijagnoze, kao i tačnost, senzitivnost, specifičnost, i pozitivni i negativni prognostički značaj citologije u dijagnostici karcinoma pluća. **Metode.** Istraživanje je sprovedeno kao retrospektivno i obuhvatilo je 169 bolesnika kojima je tokom dve godine dijagnostikovan NSCLC na citološkom uzorku, pri čemu su bolesnici istovremeno imali i uzorak male biopsije, kao i hirurški uzorak za patohistološku dijagnostiku, čije dijagnoze su upoređivane sa citološkom dijagnozom.

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Patohistološka dijagnoza na hirurškim uzorcima bila je zlatni standard za utvrđivanje podudarnosti između citološke dijagnoze NSCLC i patohistološke dijagnoze, kao i tačnosti, senzitivnosti, specifičnosti, pozitivnog i negativnog prognostičkog značaja citologije kao metode u dijagnostici karcinoma pluća. **Rezultati.** U istraživanje je bilo uključeno 129 (76,3%) muškaraca i 40 (23,7%) žena, starosti između 39 i 83 godina, prosečno 62,53 ± 7,6. Nije bilo statistički značajne razlike u starosti bolesnika različitog pola ($p = 0,207$). Među citološkim dijagnozama najčešći je bio NSCLC kod 99 (58,58%) bolesnika. Podudarnost između citoloških i patohistoloških dijagnoza bila je prisutna kod 61,48% bolesnika. Nije bilo statistički značajne razlike između citoloških i patohistoloških dijagnoza materijala male biopsije ($p = 0,856$). Citologija, kao dijagnostička metoda za karcinom pluća pokazala je senzitivnost 94,98%, specifičnost 98,60%, pozitivni prognostički značaj 95,72%, negativni prognostički značaj 98,35% i tačnost 97,71%. **Zaključak.** Citološka dijagnostika NSCLC je tačna, visoko senzitivna i specifična i korisna za bolesnike. Kod većine bolesnika dijagnoza se postavlja u odmaklom stadijumu bolesti, kada je karcinom inoperabilan, a jedini dostupni materijal za postavljanje dijagnoze je mala količina materijala dobijeng tokom bronhoskopije.

Ključne reči:
biopsija; bronhoskopija; pluća, nesitnoćelijski karcinom; citološke tehnike; histološke tehnike; prognoza; senzitivnost i specifičnost.

Introduction

In the last seven decades, lung cancer has been the most common cancer worldwide, with 1.8 million new cases per year. It is the most common cause of cancer death worldwide [1]. More than 70% of patients were diagnosed in advanced stages of diseases; therefore, diagnostic possibilities are often limited to cytological diagnosis and/or histopathological diagnosis on small biopsies. For a few years, it was sufficient to distinguish between small cell lung cancer and non-small cell lung cancer (NSCLC) without further subtyping [2]. According to the last recommendations of the International Association for the Study of Lung Cancer, the American Thoracic Society, the European Respiratory Society, and the World Health Organization (WHO), novel therapeutic methods need subtyping of NSCLC even on cytological samples and small biopsies, whenever possible [3–5].

Based on WHO recommendations for diagnosing by examining cytological samples and small biopsies, lung adenocarcinoma is an epithelial malignant tumor morphologically with glandular differentiation, vacuolated cytoplasm, mucin production, enlarged nuclei, or specific immunohistochemical marker expression – napsin-A or thyroid transcription factor-1 (TTF-1) positivity after immunostaining. Squamous cell lung cancer is an epithelial malignant tumor morphologically with keratinization, dense cytoplasm, intracellular bridges, or specific immunohistochemical marker expression – p40 or p63 positivity after immunostaining. NSCLC, not otherwise specified (NOS), includes cancers without either morphological characteristics specific for adenocarcinoma or squamous cell carcinoma or immunostaining positivity [4, 6].

Accuracy, sensitivity, specificity, and positive and negative prognostic value of cytology in diagnosis and staging of NSCLC have been monitored since 1980. Plenty of cytological methods of sampling are in use, including exfoliative methods (sputum, bronchoalveolar lavage – BAL, bronchial aspiration, and brush cytology) and aspiration methods (transbronchial needle aspiration – TBNA) [7]. Nowadays specificity of cytology is up to 100% and sensitivity between 60% and 90%, depending on the sampling method. Ultrasound-guided TBNA has increased the sensitivity of cytology and decreased the number of false negative cytological diagnoses. The value of cytology in diagnosing NSCLC and subtyping it to adenocarcinoma and squamous cell carcinoma is very important for modern personalized molecular therapies and immunotherapy, while rapid diagnostic on small samples is preferred for patients' benefit, fewer complications while sampling, and appropriate therapy time [8].

The aim of this study was to find out the concordance between cytological diagnosis of NSCLC and histopathological diagnosis and the accuracy, sensitivity, specificity, and positive and negative predictive value of cytology in diagnosing lung cancer.

Methods

A two-year retrospective study was conducted at the Department of Cytology of the Institute of Pathology and Forensic Medicine of the Military Medical Academy (MMA) in Belgrade, Serbia. All the patients in this study first went on bronchoscopy because of clinical or radiological suspicion of lung cancer. Material for cytological and histopathological evaluation was taken during bronchoscopy. In those two years, the total number of patients with the suspicion of lung malignancy that was first diagnosed on cytological and small biopsy material, following histopathological confirmation on surgical material, was 1,047. Among those patients, 251 (23.97%) were cytologically malignant, and 169 (67.33%) had an NSCLC diagnosis. Those 169 patients with NSCLC cytological diagnosis were included in this study.

Criteria for inclusion of patients in the study were bronchoscopically or radiologically visible tumorous formation in the lungs and cytological, histopathological diagnosis on small biopsy and surgical material for each patient. Patients with previous chemotherapies, radiotherapies and malignancies were excluded from this study.

Demographical data (gender and age) and diagnostic procedure details were collected from patients' information databases of the MMA, Institute of Pathology, and Department of Cytology.

The material was sampled for cytological and histopathological analysis during video-assisted bronchoscopy (Olympus BF260 and Karl Storz, GmbH&Co.KG. Tuttlingen, Germany) of an analgosedated patient. Cytological methods of sampling included: TBNA using needle 19G (for tumors not visualized in bronchial lumen), brush cytology, bronchial content aspiration, sputum or BAL (for centrally located tumors in bronchial lumen), and "tru-cut" needle biopsy (for tumors localized on the periphery of the lung).

Among patients, 78.60% had material sampled using only one method, 20.20% of patients had material sampled using two, and 1.20% using three methods. Cytological methods of sampling are presented in Figure 1.

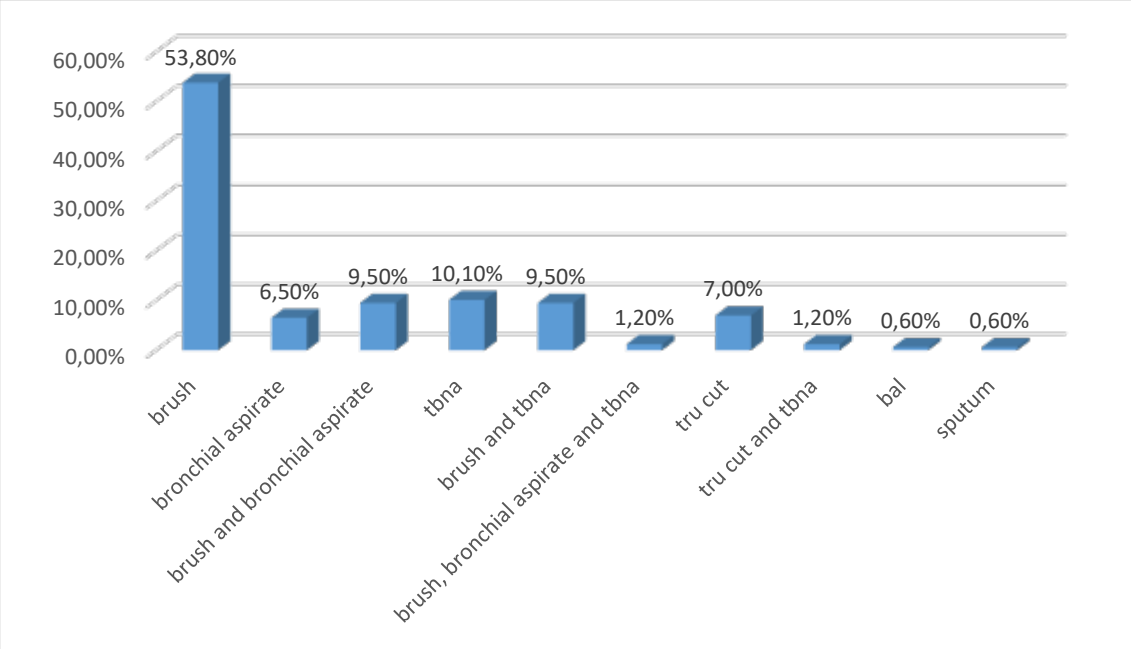

Fig. 1 – Cytological methods of sampling.
tbna – transbronchial needle aspiration; bal – bronchoalveolar lavage.

Cytological smears were made on microscopic slides from the material of each patient; moreover, cytospins were made in cases of large amounts of material. Microscopic slides were air-dyed and stained by May-Gruenwald-Giemsa (MGG) method. After microscopic evaluation, cytological diagnoses that were made were malignant – NSCLC, adenocarcinoma, or squamous cell carcinoma.

Material for small biopsy histopathological evaluation was sampled simultaneously with cytological material during bronchoscopy using bronchial biopsy or TBNA with a 19 G needle. It was fixed in buffered 4% formalin for 12 h, dehydrated by increased alcohol concentration, cleared by chloroform, embedded in paraffin, and cut by microtome (Leica) to slices measured 4 μm. After that, it was deparaffinized and stained by hematoxylin and eosin. Immunostaining methods were used in small biopsies samples in poorly differentiated tumors (CK7 and TTF-1 for confirmation of lung adenocarcinoma and p63 for confirmation of squamous cell lung cancer). Both cytological and histological slides were analyzed using a microscope (Olympus BX50) with a digital camera Olympus SC50 and computer software CellSense. Criteria for diagnoses on cytological and small biopsies material were according to the newest WHO 2015 recommendations [4].

Histopathological diagnosis on surgical specimens was the golden standard for the evaluation of specificity and sensitivity of cytology as a diagnostic method for detecting lung cancer. True positive is a malignant cytological sample confirmed after histopathological analysis as malignant. True negative is a benign cytological sample confirmed after histopathological analysis as benign. False positive is a malignant cytological sample and benign histopathological diagnosis. False negative is a benign cytological sample and malignant histopathological diagnosis.

Sensitivity measured a proportion of true positive cytological samples and the sum of true positive and false negative cytological samples. Specificity measured a proportion of true negative cytological samples and the sum of true negative and false positive cytological samples. The positive prognostic value measured a proportion of true positive cytological samples and the sum of true positive cytological and false positive samples. The negative prognostic value measured true negative cytological samples and the sum of true negative cytological and false negative samples. Accuracy measured a proportion of the sum of true positive and true negative cytological samples and the number of all samples [9].

All the data were statistically analyzed using the software package IBM SPSS 24. Statistical analysis included methods of descriptive statistics (mean value ± standard deviation and relative numbers), parametrical Student's *t*-test and ANOVA for numerical variables, non-parametrical Mc Nemmar and Kruskal-Wallis test for nominal variables, and

non-parametric Kendal-Tau correlation coefficient. The level of statistical significance was considered to be $p < 0.05$.

Results

A two-year retrospective study was done on 169 patients, including 129 (76.3%) male and 40 (23.7%) female patients. There was a statistically significant difference between the number of male and female patients, $p < 0.0001$.

Patients were aged 39 to 83 years, with an average of 62.53 ± 7.6. The age of the male patients was between 46 and 83 years, with an average of 63.29 ± 7.29. The age of the female patients was between 39 and 79 years, with an average of 60.1 ± 8.13. There was no statistically significant difference between the ages of different genders, $p = 0.207$.

The most frequent cytological diagnosis was NSCLC in 99 (58.58%) patients. Squamous cell carcinoma was diagnosed in 45 (26.63%) patients and adenocarcinoma in 22 (13.02%) patients. Only 3 (1.77%) patients had atypical cells suspicious of NSCLC in cytological samples.

The most common histopathological diagnosis, according to small biopsies samples, was squamous cell carcinoma in 79 (46.75%) patients. Adenocarcinoma and NSCLC, NOS were found in 77 (45.56%) and 10 (5.92%) patients, respectively. Non-Hodgkin lymphoma was diagnosed in 2 (1.18%) patients and plasmacytoma and metastasis of prostate adenocarcinoma each in 1 (0.59%) patient.

Squamous cell carcinoma, found in 78 (46.15%) patients, was the most frequent diagnosis in surgical histopathological specimens. Adenocarcinoma was found in 77 (45.56%) patients following NSCLC, NOS in 2 (45.56%), large cell lung carcinoma in 2 (1.18%), and Non-Hodgkin lymphoma 2 (1.18%) in patients (Figure 2). Large cell neuroendocrine carcinoma, plasmacytoma (Figure 3), epithelioid mesothelioma, carcinosarcoma, mucoepidermoid carcinoma, germ cell tumor, and prostate adenocarcinoma (Figure 4) were found each in 1 (0.59%) patient.

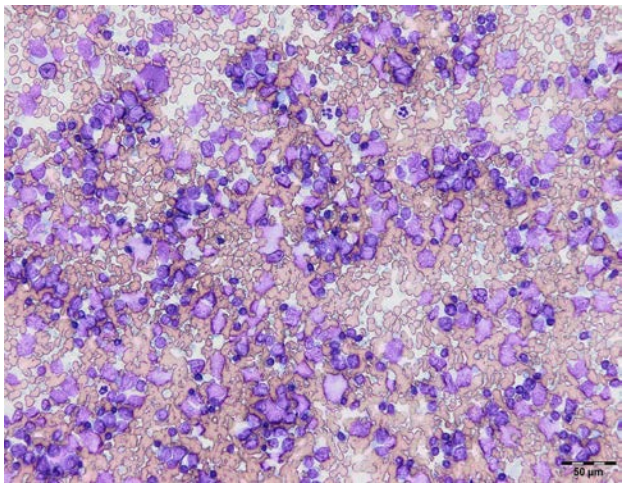

Fig. 2 – Cytologicially diagnosed non-small cell lung cancer with histopathological Non-Hodgkin lymphoma diagnosis (May Grunwald-Giemsa stain, ×200).

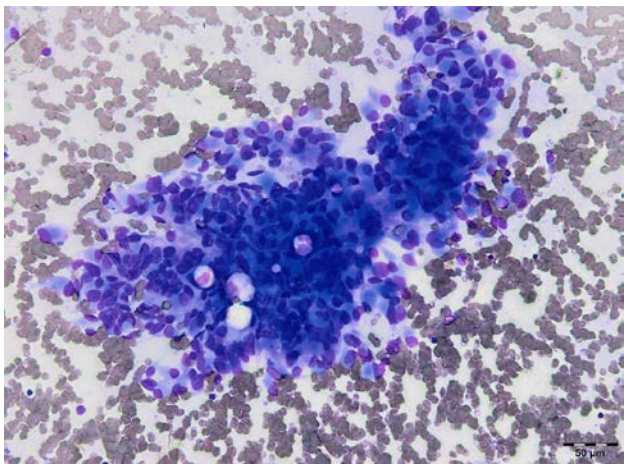

Fig. 3 – Cytologically diagnosed non-small cell lung cancer with histopathological plasmacytoma diagnosis (May Grunwald-Giemsa stain, ×200).

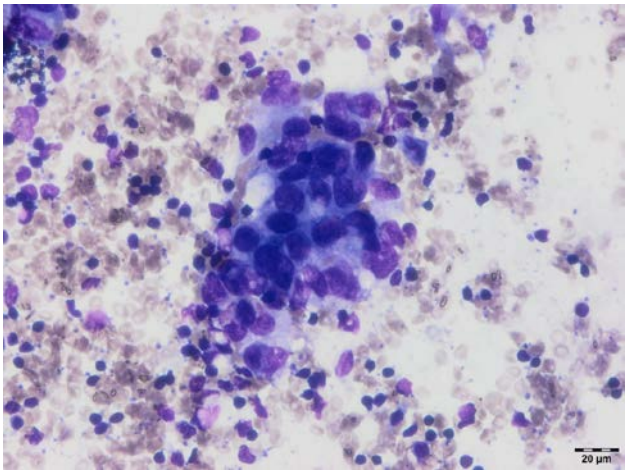

Fig. 4 – Cytologically diagnosed non-small cell lung cancer with histopathological prostate adenocarcinoma diagnosis (May Grunwald-Giemsa stain, ×400).

Concordance between cytological and histopathological diagnoses of surgical specimens was 61.48%. Unlike it, the concordance between histopathologic diagnoses of small biopsy specimens and surgical specimens was 95.2%.

There was neither statistically significant difference between cytological diagnosis and histopathological diagnoses of small biopsy specimens ($p = 0.856$) nor between cytological diagnosis and histopathological diagnoses of surgical specimens ($p = 0.196$). In addition, there was no statistically significant difference between histopathological diagnoses of small biopsies and surgical specimens ($p = 0.230$). Discordance in cytological, small biopsies, and histopathological diagnoses based on surgical specimens are presented in Table 1.

There was a statistically significant difference between diagnoses on cytological specimens, depending on the method of sampling ($p = 0.001$). There was statistically significantly less discordance in cytological diagnoses on material sampled by TBNA with histopathological diagnoses. Discordance in cytological and histopathological diagnoses on surgical specimens, depending on the cytological sampling method, is presented in Table 1.

Table 1

Discordance between cytological diagnoses and histopathological diagnoses on small biopsies and surgical specimens based on the method of sampling

Cytological diagnosis	Small biopsy histopathological diagnosis	Surgical specimen histopathological diagnosis	Sampling method for cytology
NSCLC	NSCLC	giant cell carcinoma	brush
NSCLC	undifferentiated carcinoma	carcinosarcoma	TBNA
NSCLC	NSCLC	large cell neuroendocrine tumor	TBNA
NSCLC	NSCLC	epithelial mesothelioma	brush
NSCLC	plasmacytoma	plasmacytoma	brush
NSCLC	NSCLC	sarcomatoid carcinoma	brush
NSCLC	NSCLC	anaplastic carcinoma	TBNA
NSCLC	prostate adenocarcinoma	prostate adenocarcinoma	TBNA
NSCLC	Non-Hodgkin lymphoma	Non-Hodgkin lymphoma	TBNA
NSCLC	Non-Hodgkin lymphoma	Non-Hodgkin lymphoma	TBNA
NSCLC	NSCLC	germ cell tumor	brush
squamous cell carcinoma	squamous cell carcinoma	mucoepidermoid carcinoma	brush

NSCLC – non-small cell lung cancer; TBNA – transbronchial needle aspiration.

In our study, sensitivity, specificity, positive and negative prognostic value, and accuracy of cytology as a diagnostic method were 94.98%, 98.60%, 95.72%, 98.35%, and 97.71%, respectively.

Discussion

Lung cancer is the most common cause of morbidity and mortality worldwide [10]. The highest incidence of lung cancer is in the ages between 65 and 74, on average 70 [11]. Patients in this study were slightly younger, with an average age of 60 for female and 63 for male patients. The youngest was a 39-year-old patient, similar to data in previous investigations [12]. Although the gender distribution of lung cancer patients is equal in developed countries, there were three times more male than female patients in this study, as was the case in other developing countries [10, 11, 13, 14].

An adequate sample for cytological and histopathological analysis has been obtained during bronchoscopy. The sample is fundamental for evaluation, confirmation, and in some cases, staging of tumor visualized during bronchoscopy [15]. The accuracy of diagnostic methods depends on the location of the tumor, its dimensions, type, and technical aspects, including the level of bronchoscopists' and pathologists' experience. Cytological diagnosis during bronchoscopy is preferable in centrally localized tumors, unlike tumors localized at the periphery of the lung when transbronchial biopsy, TBNA, or transthoracic biopsy should be done [16].

As it was in other studies worldwide, 26.63% of our patients had been diagnosed with squamous cell carcinoma based on cytomorphological criteria. Squamous cell carcinoma has been diagnosed in 46.75% of patients on small biopsy material, and all the diagnoses were confirmed on surgical specimens. The reason for fewer patients with cytologically diagnosed squamous cell carcinoma was poor differentiation of squamous cell carcinoma in approximately half of the patients. Those patients were diagnosed with NSCLC cytologically and needed further immunostaining for a more precise histopathological diagnosis [12, 17, 18].

In spite of 45.56% of lung adenocarcinoma histopathologically diagnosed on small biopsies material and confirmed on a surgical specimen, only 13.02% of lung adenocarcinoma were cytologically diagnosed. Similar results, with a small number of cytologically diagnosed lung adenocarcinoma in patients, were obtained in other studies [18, 19]. The majority of histopathologically diagnosed adenocarcinoma were cytologically diagnosed as NSCLC because of the lack of cytomorphological specific features significant for adenocarcinoma diagnosis according to the newest WHO 2015 criteria [4].

Besides the lack of cytomorphological characteristics for cytological adenocarcinoma diagnosis, a small number of viable cells, large amount of necrosis, tumor heterogeneity, and artifacts could also cause misdiagnosis [19, 20]. The precise diagnosis of adenocarcinoma on the cytological specimen is very important because of novel diagnostic methods. More cell blocks with paraffin-embedded cytological material and the possibility of further immunostaining are made from a part or from the rest of the cytological material. Furthermore, the necessity of cell viability for novel diagnostic methods is another advantage for cytological diagnosis [2, 8, 21]. In our study, the concordance of cytological and histopathological diagnosis was 61.48%, as was reported in the literature [12, 19, 21, 22]. There was a less statistically significant difference between cytological and histopathological diagnoses af-

ter sampling by the TBNA method in both our and other research. TBNA sampled material for cytological diagnosis is more abundant, better preserved, with more viable cells and less necrotic parts [12].

Avoiding diagnostic mistakes is very important because false positive diagnoses could lead to disease and even death. False negative diagnoses could, on the other hand, postpone earlier diagnosis and therapy [23]. Precise cytological diagnosis without immunostaining in poorly differentiated cancer is very difficult, as it was in our study. Cytologically misdiagnosed NSCLC in our study were later histopathologically diagnosed as large cell carcinoma, large cell neuroendocrine carcinoma, carcinosarcoma, epithelioid mesothelioma, Non-Hodgkin lymphoma, germ cell tumor, and plasmacytoma. According to the newest WHO recommendations, large cell carcinoma should not be diagnosed on cytological and small biopsies specimens [4]. Neuroendocrine characteristics are sometimes difficult to visualize during cytological diagnosing. Despite large single cells that can be seen in the majority of cytologically diagnosed Non-Hodgkin lymphoma, sometimes, because of material preservation, diagnosis can be a challenge [23].

Cytologically diagnosed lung adenocarcinoma, which was histopathologically diagnosed as metastatic prostate adenocarcinoma, was among cytological misdiagnoses in our study. Differencing primary and metastatic adenocarcinoma is necessary for cancer staging and adequate therapy. Without a patient's history and radiological findings, sometimes it is very difficult to do it only based on cytomorphological characteristics, as it was in our study [23, 24].

After cytologically diagnosed squamous cell carcinoma, histopathological diagnosis in one patient was mucoepidermoid carcinoma. Cytological smear contained only of necrotic background and squamous component – single cells and cluster of cells with basophilic cytoplasm, increased nucleocytoplasmic ratio, with an enlarged nucleus, without nuclei. Neither intermediate nor vacuolated cells, necessary for mucoepidermoid carcinoma diagnosis, did not exist on smear [25].

In our study, sensitivity, specificity, positive and negative prognostic value, and accuracy of cytology as a diagnostic method for lung cancer were 94.98%, 98.60%, 95.72%, 98.35%, and 97.71%, respectively. These results are similar to other results worldwide. In research by Tomar et al. [17], sensitivity, specificity, and positive and negative prognostic values of cytology were 88.88%, 100%, 100%, and 36.36%, respectively, in diagnostic material sampled by fine-needle aspiration and 65.07%, 75%, 97.61%, and 12%, respectively, in diagnostic material sampled by brush biopsy [17]. In the investigations of Ghildiyal et al. [13] and Pavani et al. [14], sensitivity, specificity, and positive and negative prognostic values of cytology as a diagnostic method in both neoplastic and non-neoplastic lesions were around 90%. Lower sensitivity was found in a few studies, including Gaur et al. [12], where it was 62%.

Conclusion

Cytological diagnosis of lung cancer is accurate, with high sensitivity and specificity. Even though there was some discordance between cytological and histopathologic diagnosis of NSCLC, it was not statistically significant. The value of cytology is high because less material and less time are needed for diagnosis, which is very important in advanced inoperable stages of diseases.

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Oral and perioral soft tissue lesions and oral functions in patients with dystrophic *epidermolysis bullosa*

Lezije oralnih i perioralnih mekih tkiva i oralne funkcije kod bolesnika sa distrofičnom buloznom epidermolizom

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Abstract

Background/Aim. Dystrophic epidermolysis bullosa (DEB) is characterized by distinct systemic and skin changes, as well as numerous oral manifestations. The aim of the study was to examine oral and perioral soft tissues and oral functions in DEB patients by monitoring changes over a period of one year. **Methods.** Twenty-four patients (1 month to 36 years old) were clinically examined initially (T0), after 6 months (T6), and after 12 months (T12). Appearance and localization of perioral and oral bullae and scars, maximum mouth opening, reduced vestibule depth, absence of lingual papillae and palatal rugae, and restricted tongue movement due to scarring were monitored. The values of maximum mouth opening at the initial examination were compared to those measured in the healthy control group of the same age. The age of patients and differences between the dominant and recessive subtypes of DEB were analyzed. **Results.** The average maximum mouth opening was significantly lower in DEB patients compared to healthy individuals. Oral and perioral bullae and scars, microstomia, and reduced vestibule depth were very common, with no statistically significant difference among T0, T6, and T12. The prevalence of restricted tongue movement due to scarring and the absence of lingual papillae and palatal rugae increased significantly over one year. Patients with microstomia, vestibule depth, and restricted tongue movement due to scarring were significantly older than patients without these characteristics. Lingual papillae and palatal rugae were more frequently absent in recessive than dominant DEB. **Conclusion.** DEB causes significant changes in oral and perioral soft tissues and oral functions impairment.

Key words:
blister; cicatrix; epidermolysis bullosa dystrophica; microstomia; mouth; preventive dentistry.

Apstrakt

Uvod/Cilj. Distrofijska bulozna epidermoliza (DBE) karakteriše se izrazitim kožnim i sistemskim promenama, kao i mnogobrojnim oralnim manifestacijama. Cilj rada bio je da se ispita stanje oralnih i perioralnih mekih tkiva i funkcionalnost usne duplje bolesnika sa DBE praćenjem promena u ovim tkivima u periodu od godinu dana. **Metode.** Klinički su pregledana 24 bolesnika sa DBE (uzrasta od 1 meseca do 36 godina), prvi put (T0), posle 6 meseci (T6) i posle 12 meseci (T12). Praćeni su pojava i lokalizacija perioralnih i oralnih bula i ožiljaka, maksimalno otvaranje usta, prisutnost smanjene dubine vestibuluma, odsustvo kvržica (papila) jezika i nabora (*rugae*) nepca i ograničena pokretljivost jezika usled formiranja ožiljka. Vrednosti maksimalnog otvaranja usta pri prvom pregledu upoređene su sa vrednostima izmerenim u zdravoj kontrolnoj grupi istog uzrasta. Analizirani su i uzrast bolesnika i razlike između dominantnog i recesivnog oblika DBE. **Rezultati.** Prosečna vrednost maksimalnog otvaranja usta je bila značajno niža u bolesnika sa DBE u poređenju sa zdravim osobama. Bule i ožiljci na oralnoj i perioralnoj regiji, mikrostomija i smanjen vestibulum bili su veoma često prisutni, bez statistički značajne razlike između T0, T6 i T12 pregleda. Učestalost ograničene pokretljivosti jezika i odsustvo papila jezika i nabora nepca su se u periodu od godinu dana statisitički značajno povećavali. Bolesnici sa mikrostomijom, smanjenim vestibulumom i ograničenom pokretljivošću jezika zbog formiranja ožiljka bili su značajno stariji u odnosu na bolesnike bez tih promena. Papile jezika i nabori nepca su bili značajno češće odsutni kod recesivnog u odnosu na dominantni oblik DBE. **Zaključak.** DBE dovodi do značajnih promena u oralnom i perioralnom mekom tkivu i poremećaja oralnih funkcija.

Ključne reči:
plik; ožiljci; epidermoliza, bulozna, distrofijska; mikrostomija; usta; stomatologija, preventivna.

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Introduction

Epidermolysis bullosa (EB) represents an inherited multisystemic, genetically and clinically heterogeneous group of diseases characterized by extreme fragility of the skin and mucous membranes leading to the development of bullae and erosions even after minor mechanical irritation [1, 2]. There are four basic types of this disease: EB simplex (EBS), junctional EB (JEB), dystrophic EB (DEB), and Kindler syndrome. DEB is among the most serious forms of the disease, and it is divided into two main subtypes. Depending on whether it is inherited in an autosomal dominant or recessive manner, dominant DEB (DDEB) and recessive DEB (RDEB) could be distinguished [2, 3]. DDEB is characterized by generalized recurrent bullae formation, which can become localized with age. Atrophic nail changes or complete loss of the nail plate may be present on fingers or toes. Other systemic changes, in addition to esophageal changes, are generally absent and these patients may have a relatively good quality of life [4]. RDEB is characterized by highly pronounced skin and systemic changes. Bullous changes cover large areas of the skin with their scarring during healing leading to joint contractures and pseudosyndactyly [1, 5]. Dexterity, ability to walk and maintain balance in these patients are impaired. Gastrointestinal and urogenital tract changes, eye changes, and cardiovascular system diseases are also present [6, 7]. Chronic loss of blood, iron, and nutrients combined with inflammation and damage to the small intestinal mucosa leads to malnutrition, anemia, and retardation in the physical development of these patients [8]. Squamous cell carcinoma may develop as the most serious complication of the disease, which is highly aggressive and produces metastases early [9].

Changes in the oral cavity are also very pronounced in patients with DEB. Bullae occur frequently all over the oral mucosa. When bullae rupture eroded areas are left behind that tend to undergo fibrotic healing [10, 11]. Continuous bullae formation and repeated processes of reepithelialization lead to the formation of large and numerous scars and the development of microstomia [10, 12, 13]. In addition, the absence of lingual papillae and palatal rugae, restricted tongue movement due to scarring, loss of vestibule depth, and leukoplakia of the mucous membranes could be observed on the buccal mucosa. These pronounced oral changes can be potential signs of malignant disease, and thus the risk of oral squamous cell carcinoma in these patients is very high [14, 15]. In addition to interfering with speech, chewing, and ingestion of food, these oral conditions make it difficult to maintain oral hygiene and carry out dental procedures [16]. In these patients, early occurrence and high prevalence of caries in both dentitions are reported, as well as orthodontic anomalies, due to underdeveloped alveolar arches caused by generalized growth and development retardation [17, 18].

As numerous oral manifestations may occur within the underlying disease, the aim of the present study was to examine the condition of the oral and perioral soft tissues and the oral functions in patients with DEB.

Methods

The protocol for the clinical study is in compliance with the Helsinki Declaration and was approved by the Ethics Committee. The research was conducted at the University Clinic during the period from June 2016 to December 2017. The study included 24 patients with DEB: 8 (33.0%) female and 16 (67.0%) male patients, with their ages ranging between 1 month and 36 years [(average age ± standard deviation (SD) was 9.55 ± 8.01 years)] that were monitored for one year. Four (16.7%) patients had a dominant and 20 (83.3%) patients had a recessive form of the disease. Patients were recruited directly from the DEBRA (Dystrophic Epidermolysis Bullosa Research Association). All patients, i.e., their parents, were DEBRA members, and almost all DEB population from the country where the research was carried out was included. A healthy control group consisted of the same number of healthy, sex and age-matched subjects (8 females and 16 males, average age 9.52 ± 8.04 years). After obtaining the written informed consent from adult patients and parents or guardians of minor patients to participate in the study, three dental clinical examinations were conducted: the first examination (T0), followed by the second examination at 6 months (T6), and the third examination at 12 months (T12). All examinations were performed by one investigator with experience in working with patients affected with EB. A dental examination was performed using standard diagnostic tools (dental mirrors), under artificial light, and according to the World Health Organization (WHO) criteria [19]. The findings obtained were recorded in the specially designed investigation charts.

Clinical examinations evidenced the presence of bullae in the perioral region on the skin of the lips, chin, and cheeks in DEB patients. Maximum mouth opening (MMO) was measured at the first examination in both groups (healthy control and DEB group) as the distance between the upper and lower border of the upper and lower lip vermilions at the maximal mouth opening [20]. Reduction of the vestibule depth was recorded upon intraoral examination in cases where it was almost nonexistent due to the attachment of soft tissues directly below the vestibular tooth surfaces [14]. Inspecting the oral mucosa, the presence and localization of the bullae and scars, absence of lingual papillae and palatal rugae, and restricted tongue movement due to scarring were noted. The frequency of occurrence of the inspected changes was also analyzed with respect to the DEB subtype (DDEB and RDEB) at T0.

At all examinations, patients and parents were advised on proper and regular maintenance of oral hygiene (advice on the choice of oral hygiene products, techniques, frequency, length of tooth brushing), tips on correcting diet, the use of chemoprophylaxis, advice for relief of problems caused by the formation of bullae in the oral cavity. Exercises that patients could perform independently in order to increase the possibility of mouth opening and tongue mobility were demonstrated.

Statistical analysis of the obtained data was performed using the software IBM SPSS version 21. Two independent

groups were compared using the *t*-test or Mann-Whitney *U* test depending on the type and distribution of numerical data. The arithmetic mean with SD was used for the description of continuous numerical data having a normal distribution, and for those that did not meet the criteria of the normality of the distribution, the median (med) and the range of values (min-max) were used. The frequencies of the studied changes are shown in absolute and relative numbers (n, %) and compared using the Fisher's exact probability test. Related samples of repeated measurements were analyzed using the Exact version of Cochran's Q and McNemar's tests. All statistical analytical methods were considered significant at the $p < 0.05$ level.

Results

The results of the average MMO at the first examination showed a statistically significant difference ($p = 0.005$, *t*-test) between DEB patients (35.92 ± 15.91 mm) and the healthy control group (52.90 ± 22.94 mm) at the first examination. The occurrence of the other investigated changes in DEB patients was compared among the examinations performed at six-month intervals. Upon all examinations, the majority of diseased children showed the presence of bullae on the perioral and oral soft tissues, as well as changes resulting in limited functions of the oral cavity, such as microstomia and restricted tongue movement due to scarring (Table 1). The appearance of a 5-year-old boy with DEB with noticeable microstomia and bullae on the lower lip and the tongue is presented in Figure 1. A statistically significant difference among the three examinations was found only for the presence of restricted tongue movement due to scarring ($p = 0.012$, Cochran Q test) and the absence of lingual papillae and palatal rugae ($p = 0.025$, Cochran Q test). The frequency of other changes observed in this study was not significantly different in the first, second, and third examinations (Table 2).

Table 1

The frequency of the studied characteristics at the first (T0), second (T6), and third (T12) clinical examination in patients with dystrophic epidermolysis bullosa (n = 24)

Characteristics	Patients exhibiting the disorders, n (%)		
	T0	T6	T12
Bullae in perioral region	16 (66.7)	17 (70.8)	15 (62.5)
Microstomia	17 (70.8)	17 (70.8)	17 (70.8)
Reduced vestibule depth	16 (66.7)	16 (66.7)	16 (66.7)
Bullae on oral mucosa	9 (37.5)	11 (45.8)	9 (37.5)
Scars on oral mucosa	6 (25.0)	6 (25.0)	6 (25.0)
Absence of lingual papillae and palatal rugae	14 (58.3)	18 (75)	19 (79.2)
Restricted tongue movement due to scarring	14 (58.3)	19 (79.2)	19 (79.2)

Table 2

The results of the statistical analyses of the frequency of the studied characteristics among the first (T0), second (T6), and third (T12) clinical examination and all in-between examination comparisons

Characteristics	*p*-value (McNemar's test)			*p*-value
	T0-T6	T6-T12	T0-T12	(Cochrane Q test)
Bullae in perioral region	0.500	0.250	0.500	0.667
Microstomia	1.000	1.000	1.000	1.000
Reduced vestibule depth	1.000	1.000	1.000	1.000
Bullae on oral mucosa	0.250	0.250	1.000	0.333
Scars on oral mucosa	1.000	1.000	1.000	1.000
Absence of lingual papillae and palatal rugae	0.063	0.500	0.031*	0.025*
Restricted tongue movement due to scarring	0.031*	1.000	0.031*	0.012*

*** – statistically significant values.**

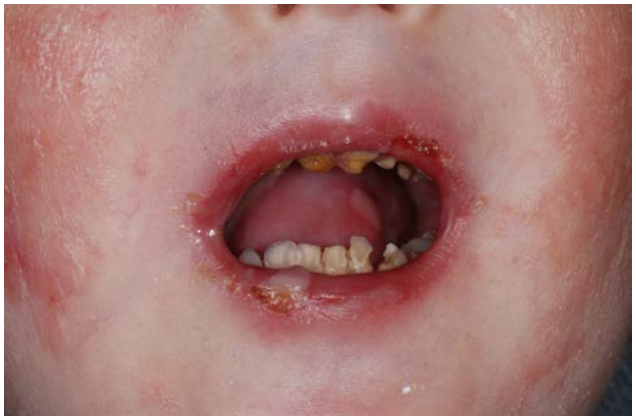

Fig. 1 – Microstomia in a 5-year-old boy with dystrophic epidermolysis bullosa (bullae present on the lower lip and on the tongue).

The frequency of the studied perioral and oral soft tissue changes in patients with DDEB and RDEB subtype of the disease at T0 is shown in Table 3. Lingual papillae and palatal rugae were not present in patients with DDEB, while the same changes were present in 70% of patients with RDEB (p = 0.020, the Fisher's exact test). For all other changes, there was no statistically significant difference in frequency between the two subtypes of EB.

The age of patients with oral, perioral, and functional changes was compared with the age of patients not exhibiting those changes at T0. Statistically significant age-related difference (the Mann-Whitney *U* test) was determined in patients with microstomia (p = 0.001), reduced vestibule depth (p = 0.012) and restricted tongue movement due to scarring (p = 0.006). Specifically, patients with these pathologic characteristics were significantly older than patients without them (Table 4).

The localization of the bullae on the oral mucosa is shown in Table 5.

Table 3

The frequency of the studied characteristics in patients with dominant dystrophic epidermolysis bullosa (DDEB) and recessive DEB (RDEB) subtype of the disease at T0 examination

Characteristics	DEB subtype, n (%)		*p*-value
	DDEB (n = 4)	RDEB (n = 20)	(Fisher's Exact test)
Bullae in perioral region	2 (50.0)	14 (70.0)	0.578
Microstomia	1 (25)	16 (80)	0.059
Reduced vestibule depth	2 (50.0)	14 (70.0)	0.578
Bullae on oral mucosa	0 (0.0)	9 (45)	0.259
Absence of lingual papillae and palatal rugae	0 (0.0)	14 (70.0)	0.020*
Restricted tongue movement due to scarring	1 (25.0)	13 (65.0)	0.272

DEB – dystrophic epidermolysis bullosa.
*** – statistically significant value.**

Table 4

The average age of the patients with dystrophic epidermolysis bullosa and without studied characteristics

Characteristics	Age (years)		*p*-value
	med (min–max)	mean ± SD	(Mann–Whitney *U* test)
Bullae in perioral region			
yes	8 (1–21)	8.44 ± 5.26	0.690
no	10 (0.1–36)	11.79 ± 11.94	
Microstomia			
yes	12 (3–36)	12.35 ± 1.89	0.001*
no	3 (0.1–7)	2.76 ± 0.95	
Reduced vestibule depth			
yes	11 (3–36)	12.12 ± 8.27	0.012*
no	4 (0.1–13)	4.41 ± 4.33	
Bullae on oral mucosa			
yes	17 (16–18)	17 ± 1.41	0.060
no	7.5 (0.1–36)	8.88 ± 8.02	
Absence of lingual papillae and palatal rugae			
yes	8 (3–36)	9.86 ± 8.37	0.883
no	9.5 (0.1–21)	9.13 ± 7.88	
Restricted tongue movement due to scarring			
yes	12 (3–36)	12.93 ± 8.45	0.006*
No	4 (0.1–13)	4.83 ± 4.25	

SD – standard deviation; median – median; min – minimum; max – maximum.
*** – statistically significant values.**

Table 5

The localization of the bullae on the oral mucosa in patients with dystrophic epidermolysis bullosa

Localization	Patients exhibiting the disorders, n (%)		
	1st clinical examination (T0)	2nd clinical examination (T6)	3rd clinical examination (T12)
On one site only			
tongue	6/9 (66.7)	4/11 (36.4)	2/9 (22.2)
buccal mucosa	2/9 (22.2)	3/11 (27.3)	3/9 (33.3)
On two or more sites			
tongue and buccal mucosa	–	3/11 (27.3)	1/9 (11.1)
tongue and lips	1/9 (11.1)	–	1/9 (11.1)
tongue and palate	–	1/11 (9.1)	–
tongue, buccal mucosa, and palate	–	–	2/9 (22.2)

Discussion

Patients with DEB have major oral and perioral changes resulting from the underlying disease. A change that is highly characteristic of the most severe forms of the disease is microstomia, and it was present in more than 2/3 of the studied patients upon all three clinical examinations. In addition to significant disturbance of the oral cavity function, substantially smaller maximum mouth opening than in healthy individuals makes it difficult to maintain oral hygiene, especially if it is associated with syndactyly, which can compromise patients' oral health. This also poses a major problem and a challenge for the dentist when treating these patients [12, 20, 21]. Dental care is most commonly recommended to be performed under general anesthesia because the relaxation of the musculature allows wider mouth opening, better visibility of the working field, and greater space for instruments manipulation. However, intubation is highly perilous, and the introduction of these patients into general anesthesia requires special apparatus and additional education of anesthesiologists [22–24]. Microstomia was also a major difficulty in performing dental examinations during this study because it was often very difficult even to insert mirrors to examine the distal parts of the oral cavity. To maximize the mouth opening ability, manual exercises that are easy to perform at home were demonstrated to patients and their parents. This study, however, found that there was no statistically significant difference in mouth opening at the follow-up examinations despite the exercises performed, as microstomia was always present in 17 patients. Similar results have been reported in previous studies. Kramer et al. [13] achieved in their patients a greater mouth opening ability (from 19–23 mm to 30 mm) with ten-minute exercises, but soon after the cessation of exercises, the original microstomia values returned. Moreover, it was observed that only 7 patients in this study did not have microstomia and were very young (age around 3 years). Patients who had microstomia were significantly older (age around 12 years), indicating that with age the possibility of maximal mouth opening was significantly reduced. All this points to the importance of exercising as early as possible, from infancy and before the onset of microstomia, to improve the effect of exercises on facilitating and maximizing the mouth opening ability [13].

Other intraoral lesions, as well as functional limitations, also became more pronounced with age. Patients who did not have restricted tongue movement due to scarring and reduced vestibule depth were about 4 years of age, whereas statistically significantly older patients were those with restricted tongue movement due to scarring and those who did not have reduced vestibule depth (about 12 years of age). In most patients, restricted tongue movement due to scarring was so pronounced that, in fact, the tip of the tongue was more mobile than the entire tongue. In the most severe forms of DEB, restricted tongue movement due to scarring is manifested at birth and prevents or impairs breastfeeding [25]. Later on, it compromises the child's speech and maintenance of oral hygiene [26, 27]. No significant differences in patients' age were observed for the other changes examined in this study.

When it comes to the two EB subtypes, although they differ widely in systemic characteristics [1, 21], the results of this study showed that they did not differ as much in changes in the perioral and oral soft tissue characteristics and the functionality of the oral cavity. The only statistically significant difference existed in the absence of lingual papillae and palatal rugae. Gradual atrophy of the lingual papillae and mucous membranes of the hard palate arised as a consequence of the continuous formation of bullae and frequent reepithelization of eroded surfaces [28]. This characteristic was significantly more frequently present in patients with RDEB in comparison to DDEB. The recessive form of the disease is otherwise considered to be a more severe form than the dominant one [1]. However, it should be emphasized that in this study, in the group of affected children, only 4 had DDEB type, while RDEB type was evidenced in 20 cases, which could affected the results of statistical analysis. Studies on a larger number of patients with both EB subtypes could provide different data. Moreover, in this study classification into two subgroups according to the subtype of the disease (DDEB and RDEB) was made based on standard diagnostic tests and skin biopsy, as well as according to their rather different clinical features based on which these two forms of EB can be distinguished with high certainty [1–7, 21]. It is important to note that patients with EB in the country where this research was performed are rarely subjected to genetic testing in order to determine what type of inheritance is involved, primarily because of the very high cost of such analyzes and because it is often necessary to conduct such tests abroad.

Furthermore, the number of patients who had restricted tongue movement due to scarring and the absence of the lingual papillae and the palatal rugae tended to change significantly during our performed clinical examination. That is, a significantly greater prevalence of restricted tongue movement due to scarring was evidenced at T6 and T12 examinations compared to T0 one. When lingual papillae and palatal rugae are in question, they were significantly more frequently absent at the T12 compared to the T0 examination. These results suggest that during the one-year follow-up period, significant mucous membranes of the tongue and palate changes occurred, as well as changes in the mobility of the tongue. Bullae and scars were present on the oral mucosa and in the perioral region at all examinations with no significant difference. This result indicates that bullae occur constantly, sporadically, and spontaneously, and are caused under the influence of minor local irritating factors. It was observed that intraoral bullae were most commonly localized on the tongue. In patients where they were localized on two or more sites, it was observed that the tongue was always involved in combination with bullae on the buccal mucosa, lips, or palate. In accordance with these findings, Serrano-Martinez et al. [11] and Wright [21] reported the mucosal membrane of the tongue as the most common site of onset of bullae. Furthermore, it was found that the occurrence of bullae was less frequent in DDEB and that repeated episodes of bullae did not lead to significant scar changes [20]. Conversely, the pronounced sensitivity of oral mucosa in RDEB was reported, where continuous bullae formation and repeated reepithelialization processes led to the formation of large and

numerous scars, which significantly impaired the function of the oral cavity, maintenance of oral hygiene, and dental treatment [11, 21, 29, 30]. Scar changes in the oral cavity, which additionally exacerbate microstomia and restricted tongue movement and compromise already shallow vestibule, are in the present study evidenced in 6 out of 24 examined patients (25%) upon all three examinations. However, their frequency was not increased over the one-year period.

Conclusion

Based on the results obtained in this study, it can be concluded that bullae and scars frequently appear on the mucous membranes of the oral cavity and the skin of the perioral region. Maximal mouth opening ability in persons with DEB is statistically significantly lower than in healthy patients. Specific changes, such as microstomia, restricted tongue movement due to scarring, and decreased vestibular depth, worsen significantly with patients' age. In the RDEB, the absence of lingual papillae and palatal rugae is very common. Therefore, the dental prevention program should be included as a mandatory part of the protocol for the complex treatment of these patients. Thus, the impact of oral diseases on the underlying disease and *vice versa* could be reduced, and the quality of life of these patients would be greatly improved.

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A comparative analysis of the efficacy of moxifloxacin and cefixime in the reduction of postoperative inflammatory sequelae after mandibular third molar surgery

Uporedna analiza efikasnosti moksifloksacina i cefiksima u smanjenju posledica zapaljenja posle hirurškog vađenja impaktiranih donjih trećih molara

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Abstract

Background/Aim. There is no scientific evidence that the prophylactic use of antibiotics as a part of the mandibular third molar surgery is effective in suppressing postoperative pain, edema, trismus, and dry socket. The aim of the study was to investigate the effects of antibiotics from the fluoroquinolone (moxifloxacin) and cephalosporin (cefixime) groups in reducing postoperative inflammatory sequelae (pain, edema, and trismus), as well as in possibly reducing the incidence of dry socket after mandibular third molar surgery. **Methods**. This double-blind study was completed by 157 subjects, comprising two study groups (who received the aforementioned antibiotics) and a control group, who received placebo tablets. Subjects were assessed on the first, second, and seventh day following surgery. In the postoperative course, patients were monitored for the occurrence, intensity, and duration of postoperative inflammatory sequelae and dry socket. **Results.** Both antibiotics, especially moxifloxacin, had a pronounced effect on reducing all inflammatory sequelae (pain, edema, and trismus) as the most common postoperative complaints following mandibular third molar surgery, and also contributed to reducing the incidence of dry socket. **Conclusion**. Antibiotic prophylaxis with cefixime and, especially moxifloxacin, reduced the occurrence of postoperative inflammatory sequelae and alleviated discomfort. It is interesting, that both antibiotics, especially moxifloxacin, also contributed to reducing the incidence of postoperative dry socket, which is not provoked by inflammation. Therefore, further research into the underlying mechanisms behind such an effect is warranted.

Key words:
anti-bacterial agents; dental prophylaxis; molar, third; postoperative complications.

Apstrakt

Uvod/Cilj. Ne postoje naučni dokazi da je profilaktička primena antibiotika posle hirurškog vađenja donjeg trećeg molara efikasna u suzbijanju postoperativnog bola, edema, trizmusa i alveolitisa. Cilj rada bio je da se istraže efekti antibiotika iz grupe fluorohinolona (moksifloksacin) i cefalosporina (cefiksim), na smanjenje zapaljenjskih postoperativnih sekvela (bol, edem i trizmus), kao i na eventualno smanjenje učestalosti nastanka alveolitisa posle hirurškog vađenja donjih trećih molara. **Metode**. Ovu dvostruko slepu studiju završilo je 157 ispitanika, od kojih su formirane dve studijske grupe (ispitanici koji su koristili navedene antibiotike) i kontrolna grupa, koja je koristila placebo tablete. Ispitanici su kontrolisani prvog, drugog i sedmog dana nakon operacije. U postoperativnom periodu praćeni su učestalost, intenzitet i trajanje postoperativnih zapaljenjskih sekvela, kao i pojava alveolitisa. **Rezultati.** Oba antibiotika, naročito moksifloksacin, imali su izražen efekat na smanjenje svih sekvela (bol, edem i trizmus), kao najčešćih tegoba nakon hirurškog vađenja donjeg trećeg molara i doprinela su smanjenju učestalosti pojave alveolitisa. **Zaključak.** Antibiotska profilaksa cefiksimom i, posebno, moksifloksacinom, smanjila je pojavu postoperativnih sekvela i ublažila tegobe. Interesantno je da su oba antibiotika, pogotovu moksifloksacin, doprineli smanjenju učestalosti alveolitisa, koji nije bio izazvan zapaljenjem. Neophodna su dalja istraživanja mehanizama ovakvog efekta.

Ključne reči:
antibiotici; profilaksa, stomatološka; molar, treći; postoperativne komplikacije.

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Introduction

The extraction of impacted mandibular third molars is among the most common oral surgeries [1] and is typically associated with postoperative clinical sequelae, such as pain, swelling, compromised mouth opening (trismus), and, occasionally, dry socket (in 1–12.6% of cases) [2] and wound infection. Consequently, many surgeons prescribe antibiotics following this intervention aiming to improve patient comfort during the postoperative period [3–6]. Antibiotic treatment is indeed indicated when the operative site is infected (in the presence of acute pericoronitis) [7], as well as when there is a need to protect the patient with endocardial lesions from transient bacteremia. However, the consensus is still lacking on the real benefits of antibiotic prophylaxis use in patients who are in good general health, in whom partially or completely impacted mandibular third molars were surgically removed in the absence of acute pericoronitis.

Antibiotic prophylaxis in healthy patients (without pericoronitis) is usually justified by the fact that surgical extraction of completely or partially impacted mandibular third molars induces surgical trauma to an already contaminated area, such as the oral cavity, thus providing conditions for subsequent infection. Given that postoperative problems and complications after this surgical intervention are common, antibiotics are often routinely prescribed for the immediate postoperative period [8, 9]. However, the unnecessary use of antibiotics is not without negative consequences as it promotes the development of resistant microorganisms and may lead to hypersensitivity to the applied antibiotic, which emphasizes the importance of correctly assessing indications for antibiotic prophylaxis. Moreover, there is no scientific evidence that the prophylactic use of antibiotics as a part of the mandibular third molar surgery is effective in suppressing postoperative inflammatory sequelae.

Authors of numerous articles published in professional and scientific literature advocate for the prophylactic use of antibiotics as a part of the surgical extraction of mandibular third molars and provide the reasons for this recommendation [10–13], while others offer equally compelling reasons for their disagreement with this approach [14–18]. However, neither of these opposing views is founded on scientific evidence. Moreover, even when evidence of antibiotic prophylaxis efficacy is statistically established using scientific methods before any recommendation is made, the clinical significance of such findings should be determined by assessing the relationship between the desired and adverse effects, as suggested by other authors [19, 20]. It is also noteworthy that the extant studies and the resulting recommendations are primarily based on evaluations of several antibiotics that have been in use for many years, most commonly amoxicillin with clavulanic acid, clindamycin, and metronidazole. There is an evident paucity of research involving other antibiotics, and the scant evidence indicates that there is no specific advantage of their prophylactic use following mandibular third molar surgery. A possible advantage of newer antibiotics stems from the fact that, in addition to their fundamental antimicrobial function, they can also exhibit immunomodulatory effects, which have a favorable contribution to the suppression of postoperative inflammatory sequelae in mandibular third molar surgery [21, 22].

Therefore, the aim of the study was to investigate the effects of antibiotics from the fluoroquinolone (moxifloxacin) and cephalosporin (cefixime) groups, which have potential immunomodulatory effects on inflammatory sequelae (postoperative pain, edema, and trismus), while possibly reducing the incidence of dry socket, too.

Methods

This clinical research was conducted for 8 months (from June 2019 to February 2020) at the Dentistry Clinic of Vojvodina, Serbia, adopting the double-blind prospective clinical study design. The study sample included 165 subjects with the same number of impacted mandibular third molars. All participants signed the informed consent. Only adult subjects over 18 years without a confirmed allergy to the drugs used in the study, in whom mandibular third molar surgery was indicated were included, while the exclusion criteria were pregnancy, breastfeeding, antibiotic allergy, and poor general health. Data pertaining to 8 subjects were subsequently excluded from the analyses, as these individuals either failed to adhere to the given instructions during the postoperative period or did not attend all the scheduled follow-up appointments.

The clinical research was approved by the Ethics Committee of the Dentistry Clinic of Vojvodina by decision number 01-33/8-2019.

Prior to data analyses, the participants were distributed into three groups. Those with prescribed antibiotics, moxifloxacin, from the group of fluoroquinolones (Elfonis®, Hemofarm, Serbia, 400 mg film-coated tablets) and cefixime, from the group of cephalosporins (Pancef®, Alkaloid, Northern Macedonia, 400 mg film-coated tablets), were assigned to the two study groups (groups M and C, respectively), while those that received placebo formed the control group (P). The medications, as well as the placebo, were in the form of film-coated tablets that were almost identical in shape and size, which is in line with the double-blind study design principles. Placebo tablets contained neutral substances that do not have any anti-inflammatory effect (99% microcrystalline cellulose, 0.5% silicon dioxide, and 0.5% magnesium stearate) and were made by Phytonet, Serbia. All treatments were administered once a day for the first five days postoperatively. Owing to this design, the three groups could only be formed upon the study completion after consulting the codebook used to provide the correct film-coated tablets for use in the postoperative period. At this point, it was revealed that, among the 157 individuals who completed the study period, 52 belonged to the Group M (receiving moxifloxacin), 53 formed the Group C (receiving cefixime), and the remaining 52 formed the Group P (the placebo control group).

All surgical interventions were performed under local anesthesia, using 2% lidocaine with adrenaline 1: 80,000 in a total 4 mL volume (2 mL solution for injection contained 40

mg lidocaine hydrochloride in the form of lidocaine hydrochloride monohydrate and 0.025 mg adrenaline in the form of adrenaline tartrate, Lidocaine 2% – adrenaline, 40 mg + 0.025 mg, Galenika, Serbia). In all participants, the surgery involved a triangular mucoperiosteal flap design, sutured using synthetic multifilament non-absorbable suture material (black silk 3–0).

In the case of impacted teeth, the wound was sutured with individual sutures *per primam*, while in the case of partially erupted teeth, part of the wound healed *per secundam*. Sutures were removed on the seventh postoperative day. Subjects were advised to take an analgesic containing 200 mg ibuprofen and 325 mg paracetamol (Metafex® tablets, Pharmaceutical Works Polpharma SA, Poland) after surgery, as required.

Data related to intervention duration (from the first incision to the placement of the last suture) and its course (the need for tooth separation and mechanical bone manipulation) were entered into the research protocol. Participants were assessed on the first, second, and seventh day following surgery. In the postoperative period, patients were monitored for the occurrence and intensity of postoperative inflammatory sequelae (pain, edema, and trismus), as well as dry socket, and based on these indicators, potential favorable effects of the applied medication were evaluated. The degree of postoperative pain was established based on the number of analgesics having taken each postoperative day (till the suture removal). The extent of postoperative edema was determined by measuring the distance between selected reference points (chin tip-tragus) using a flexible ruler immediately before surgery (providing a baseline for subsequent comparisons), as well as 24 h, 48 h, and 7 days after surgery. The postoperative edema coefficient (Ec) for each of these periods was calculated according to the modified Carrillo et al. [23] formula (Ec = [postoperative distance – preoperative distance] ×100 / preoperative distance). Similarly, the degree of postoperative trismus was assessed 24 h, 48 h, and 7 days after surgery by measuring the distance (in cm) between the mesial incisal angles of the upper and lower central incisors at the maximum mouth opening ability. The dry socket was diagnosed based on reported severe pain in the wound area, accompanied by a specific local clinical appearance of the operative wound and absence of pus.

The SPSS 20.0 software package (IBM Corp. released 2011, IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY) was used for all data processing and statistical analyses. For descriptive data, absolute and relative values were used, along with the central tendency (arithmetic mean) and dispersion (standard deviation, percentiles) measures. Statistical analyses included parametric difference tests (ANOVA) and nonparametric tests (Kruskal-Wallis test, Fischer's test, and Pearson's χ^2 test), with the significance level set at 0.05.

Results

The study included 157 participants, of whom 52 comprised the Group M (moxifloxacin), 53 the Group C (cefixime), and 52 the Group P (placebo).

However, due to the circumstances beyond the researchers' control (due to the double-blind study design), the surgical intervention in the Group M was more than twice as long as in the other two groups (Figure 1). Although this discrepancy could have resulted in marked differences in the sequelae that occurred in the postoperative period, subsequent analyses revealed that this was not the case.

Postoperative pain was assessed by analyzing the number of tablets of the recommended analgesic taken daily during the postoperative period. The results showed that the subjects in the Group M used the fewest analgesics, even though the surgery in this group of patients, on average, lasted longer. The greatest number of analgesic tablets was taken by patients in the Group P, especially on the first and second postoperative day, but also later in the postoperative period, sug-

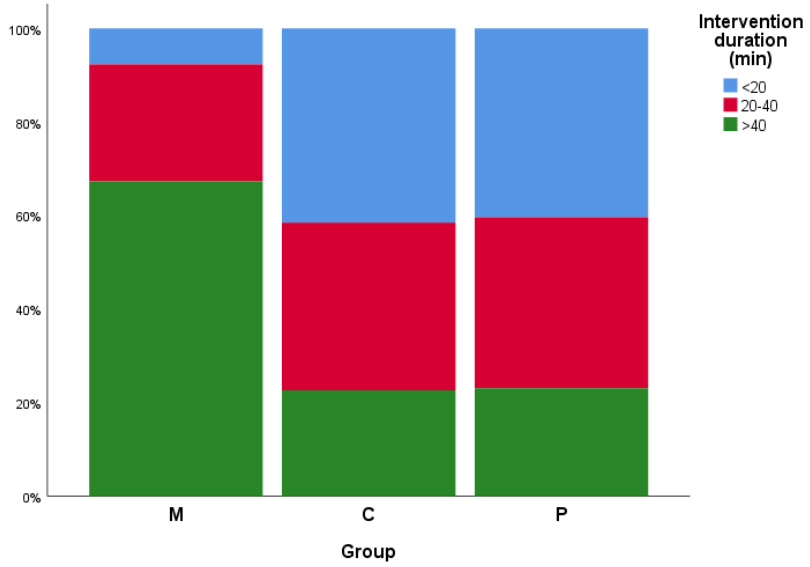

Fig. 1 – Comparison of the three research groups in terms of surgery duration. Group M – patients who received maxifloxacin; Group C – patients who received cefixime; Group P – patients who received placebo.

gesting that participants who were given a placebo experienced the strongest postoperative pain (Table 1). Analysis of changes in pain intensity as a function of time indicated a marked difference between the last and the first postoperative day in all three groups. However, the changes were least pronounced in the Group M, while pain intensity declined considerably in the Group C, and especially in the Group P, in which the pain was particularly intense on the first and second postoperative day.

Comparative analysis of postoperative pain intensity in the two study groups (M and C) and the control group (P), based on the same criterion (number of analgesic tablets taken daily), showed that the participants in the Group M experienced statistically significantly lower pain levels in all observation periods compared to those in the Group C (except on the last postoperative day) and the control group P (Table 2).

The mean value of the tragus-chin tip distance changed in all research groups during the postoperative period, most notably on the second postoperative day but also on the first, whereby this value was close to the baseline (preoperative) values on the seventh postoperative day. However, changes in the tragus-chin tip distance were less pronounced in the Group M than in the other groups, as shown in Figure 2.

When the postoperative edema values measured on the seventh postoperative day were compared to the baseline, a statistically significant difference was observed between the Group M and C, as well as between these study groups and the control group (Group P).

Table 1

Postoperative pain intensity at three follow-ups, based on the number of analgesic tablets taken daily

Group	Patients (n)	Tablets (n), mean ± SD
M		
1st postoperative day	52	0.9 ± 0.9
2nd postoperative day	52	0.2 ± 0.6
7th postoperative day	52	0.0 ± 0.0
C		
1st postoperative day	53	3.9 ± 0.9
2nd postoperative day	53	3.2 ± 0.8
7th postoperative day	53	0.5 ± 1.2
P		
1st postoperative day	52	4.5 ± 0.9
2nd postoperative day	52	4.3 ± 1.1
7th postoperative day	52	1.8 ± 1.8

Group M – patients who received maxifloxacin;
Group C – patients who received cefixime;
Group P – patients who received placebo;
SD – standard deviation.

Table 2

Statistical comparison of pain intensity at three follow-ups, based on the number of analgesic tablets taken daily

Follow-up appointment	Group M (*p*-value)	Group C (*p*-value)
1st postoperative day		
Group C	< 0.001	
Group P	< 0.001	0.025
2nd postoperative day		
Group C	< 0.001	
Group P	< 0.001	0.001
7th postoperative day		
Group C	0.051	
Group P	< 0.001	< 0.001

Group M – patients who received maxifloxacin;
Group C – patients who received cefixime;
Group P – patients who received placebo.

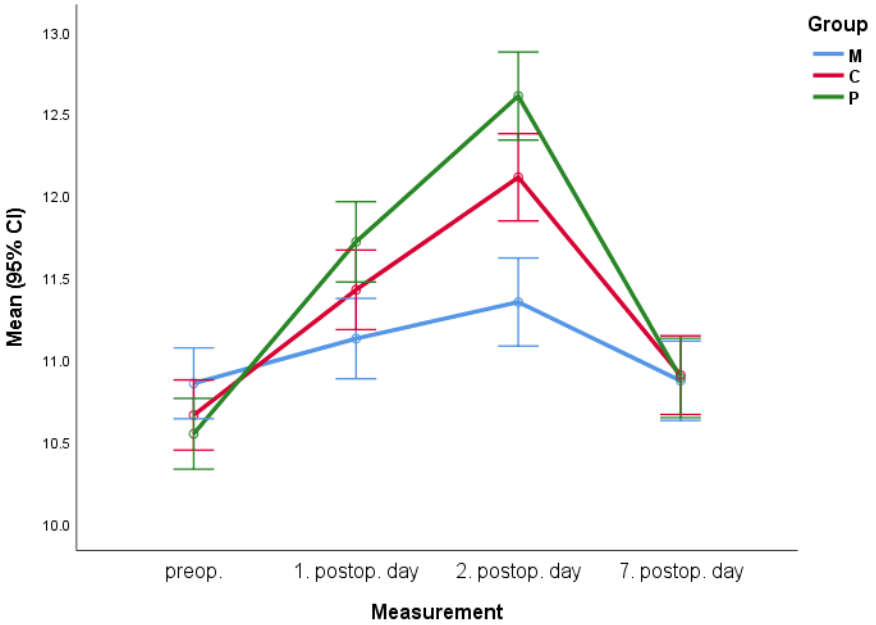

Fig. 2 – Postoperative edema progression, measured as the tragus-chin tip distance in the two study groups (M and C) and the control group (P).
Group M – patients who received maxifloxacin; Group C – patients who received cefixime; Group P – patients who received placebo; CI – confidence interval.

The postoperative Ec, calculated according to the modified Carrillo formula, further revealed that already on the first postoperative day, there was a statistically significant difference among the three groups, which persisted in the following days (Table 3). Moreover, the existence of statistically significant differences across the entire postoperative period was confirmed by comparison between individual groups (Table 4).

The degree of postoperative trismus, indicated by the interincisal distance between the upper and the lower central incisors expressed in centimeters, was measured preoperatively, as well as on the first, second, and seventh postoperative day (Table 5).

It is evident that, in all three groups, the degree of postoperative trismus was the greatest on the first postoperative day and gradually declined until the seventh postoperative day. Moreover, statistical analyses revealed that, relative to the baseline value, the trismus on both the first and second postoperative day was significantly lower in the Group M as well as compared to the Groups C and P. In addition, the degree of postoperative trismus in the Group C was statistically significantly lower compared to the Group P on the first as well as the second postoperative day. On the seventh postoperative day, the measured distance approached the baseline value in the Group M, and the differences in relation to the values measured in the other two groups were less pronounced.

The graph shown in Figure 3 indicates that postoperative trismus was least pronounced in the Group M and that the greatest differences in its degree were recorded on the first postoperative day, with a gradual tendency toward the baseline values by the seventh postoperative day.

The average incidence of dry socket in this study was 12.73%. The comparison of the three groups, however, revealed that none of the subjects in the Group M developed

Table 3

Postoperative edema assessment based on the edema coefficient (Ec) for the tragus-chin tip distance

Follow-up appointment	Patients (n)	Ec (mean ± SD)
1st postoperative day		
Group M	52	2.51 ± 3.18
Group C	53	6.99 ± 5.05
Group P	52	9.00 ± 5.02
2nd postoperative day		
Group M	52	4.13 ± 3.99
Group C	53	13.55 ± 6.83
Group P	52	17.37 ± 6.64
7th postoperative day		
Group M	52	0.13 ± 0.51
Group C	53	2.17 ± 2.26
Group P	52	3.28 ± 2.30

Group M – patients who received maxifloxacin;
Group C – patients who received cefixime;
Group P – patients who received placebo;
SD – standard deviation.

Table 4

Statistical comparison of the postoperative edema coefficient (Ec) measured for the tragus-chin tip distance among the three study groups

Follow-up appointment	Group M (*p*-value)	Group C (*p*-value)
1st postoperative day		
Group C	< 0.001	
Group P	< 0.001	0.036
2nd postoperative day		
Group C	< 0.001	
Group P	< 0.001	0.021
7th postoperative day		
Group C	< 0.001	
Group P		

Group M – patients who received maxifloxacin;
Group C – patients who received cefixime;
Group P – patients who received placebo.

Table 5

Mean postoperative trismus values relative to the baseline and the measurements obtained on each follow-up

Group	Patients (n)	mean ± SD
M		
baseline (preoperative) value	52	4.54 ± 0.61
1st postoperative day	52	3.55 ± 0.62
2nd postoperative day	52	3.92 ± 0.59
7th postoperative day	52	4.59 ± 0.60
C		
baseline (preoperative) value	53	4.67 ± 0.61
1st postoperative day	53	2.99 ± 0.88
2nd postoperative day	53	3.32 ± 0.87
7th postoperative day	53	4.18 ± 0.83
P		
baseline (preoperative) value	52	4.59 ± 0.59
1st postoperative day	52	2.44 ± 0.71
2nd postoperative day	52	2.65 ± 0.75
7th postoperative day	52	3.50 ± 0.93

Group M – patients who received maxifloxacin;
Group C – patients who received cefixime;
Group P – patients who received placebo;
SD – standard deviation.

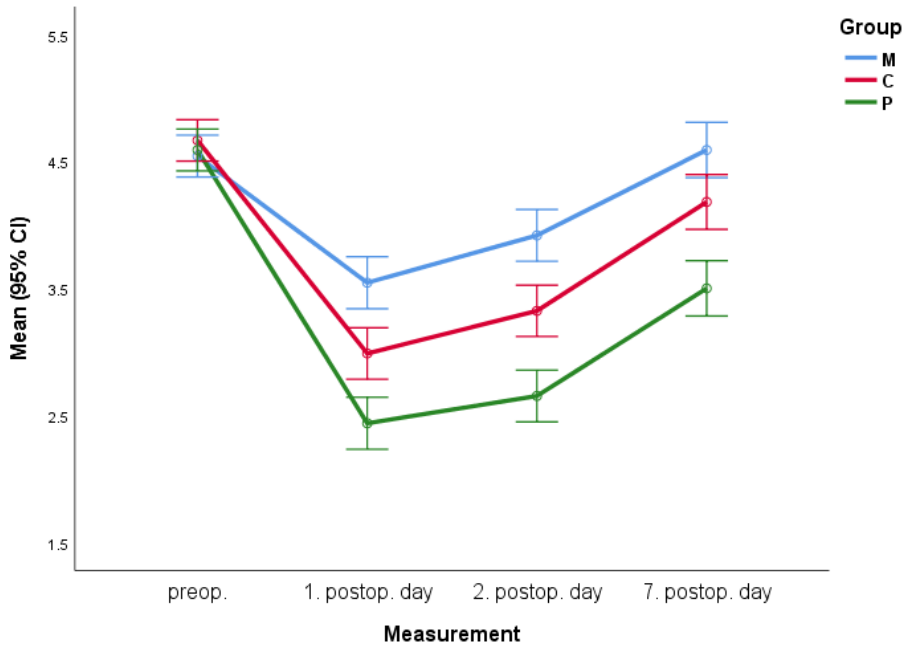

Fig. 3 – Postoperative trismus progression in the two study groups (M and C) and the control group (P). Group M – patients who received maxifloxacin; Group C – patients who received cefixime; Group P – patients who received placebo; CI – confidence interval.

Table 6

Dry socket incidence in the three researched groups

Group	Dry socket, n (%)	
	no	yes
M	52 (100.0)	0 (0)
C	46 (86.8)	7 (13.2)
P	39 (75.0)	13 (25.0)
Total, n (%)	137 (87.3)	20 (12.7)

Group M – patients who received maxifloxacin;
Group C – patients who received cefixime;
Group P – patients who received placebo.

Table 7

Statistical comparison of the significance of difference in dry socket incidence among the three researched groups

Group	M	P
C	0.013[b]	0.124[a]
P	< 0.001[b]	

[a]Pearson's χ^2 test; [b]Fisher's test.
Group M – patients who received maxifloxacin;
Group C – patients who received cefixime;
Group P – patients who received placebo.

dry socket, while 7 and 13 cases were recorded in the Groups C and P, respectively (Table 6). By subjecting findings pertaining to the three groups to the Fisher's nonparametric test, it was shown that the dry socket incidence was statistically significantly lower in the Group M in relation to the other two groups (Table 7).

Discussion

At present, as a part of the impacted mandibular third molar surgery, especially in private dental practice settings, antibiotics are often routinely prescribed due to the prevalent view (which is not based on scientific evidence) that this promotes a safer postoperative course, with fewer postoperative sequelae that commonly accompany this intervention. However, such potentially unnecessary use of antibiotics may lead to adverse consequences (development of resistant microorganism strains and increased risk of allergy to prescribed antibiotics). Thereby, many researchers do not support routine antibiotic use following mandibular third molar surgery unless preoperative infection (pericoronitis) is diagnosed [14–18]. Therefore, the aim of the present double-blind prospective study was to evaluate the efficacy of antibiotic prophylaxis in mitigating discomfort (inflammatory sequelae and dry socket) that most frequently occur after the impacted mandibular third molar surgery.

Due to the fact that many patients are allergic to antibiotics from the penicillin group [24, 25] (commonly prescribed in oral surgery), we investigated two non-penicillin antibiotics (moxifloxacin and cefixime) as potentially useful alternatives for all patients who are allergic to synthetic penicillin or are intolerant to other so-called "first line" antibiotics typically prescribed to treat oral infections (such as macrolides). Nevertheless, it is known that beta-lactam antibiotics, penicillin and its derivatives may have a cross-allergic reaction with cephalosporins (10–30%) and should, therefore, be administered with caution. Still, the latest research shows that the cross-allergic reaction is significantly lower and even negligible with cephalosporins of the second and especially the third generation [26, 27]. Although research on the use of moxifloxacin or cefixime in oral surgery is scant, it is interesting to note that in the few existing investigations, moxifloxacin was shown to be more effective in shortening the recovery period following the surgi-

cal extraction of mandibular third molars than amoxicillin with clavulanic acid [28]. Therefore, moxifloxacin (one of the newer fourth-generation fluoroquinolones used orally) and cefixime (one of the third-generation cephalosporins, also used orally) were examined in this study. Both antibiotics exhibit desirable activity against oral Gram-negative and multidrug-resistant Gram-positive bacteria [29, 30]. In addition, it was particularly advantageous that both antibiotics were manufactured in visually identical tablets and were used once a day, which facilitated the adoption of a double-blind design. Besides, the placebo tablets used by the patients in the control group also had the same appearance as the used antibiotics.

Nonetheless, owing to the double-blind study design, it was impossible to know in advance whether the research groups would be mutually comparable. After opening the codebook, we found that all three groups were comparable in terms of the number of subjects but differed in average intervention duration. This was a potential cause for concern, given that it could influence the incidence of postoperative inflammatory sequelae (pain, edema, trismus) [31, 32], which were the focus of the present investigation. Specifically, in the Group M, on average, the interventions lasted the longest, which indicates more difficult surgical procedures. However, the fact that the percentage of postoperative inflammatory sequelae was the lowest in this group suggested that the prophylactic use of moxifloxacin had a more influential effect on the postoperative course than the case complexity.

The greatest pain intensity, estimated by the number of analgesics used daily, was recorded on the first postoperative day, when pain was statistically significantly greatest in the control group (Group P), followed by the study group where cefixime was prescribed (Group C). Pain intensity gradually decreased during the postoperative period in all groups, as expected, due to the process of successful surgical wound healing. Subsequent comparison of the postoperative pain intensity across the groups revealed that the most favorable results were achieved in the Group M, as patients in this group took analgesics sporadically and only for the first two days, significantly less than in the other two groups.

Moxifloxacin is rarely used in the prophylaxis and therapy of odontogenic infections, which is surprising given that, for example, comparing moxifloxacin with the "gold standard" (amoxicillin with clavulanic acid), Limeres et al. [28] found that moxifloxacin significantly shortened the postoperative recovery time of subjects who underwent mandibular third molar surgery, with faster recovery of oral functions and decreased postoperative pain intensity. However, as the study design did not allow a placebo control group, further verification of the reported findings was needed.

When the three groups in our study were compared with respect to the extent of postoperative edema, a statistically significant difference between the study groups and the control group was noted. Moreover, postoperative edema was least pronounced in subjects who received moxifloxacin. The postoperative edema peaked between the first and the second day following surgery in all three groups, but the values measured at each follow-up were statistically significantly lower in the Group M relative to the other two groups.

In the only study identified during the literature search in which moxifloxacin was used prophylactically following mandibular third molar surgery, postoperative edema was not considered [28]. In other, much more numerous studies, some authors reported a significant reduction in postoperative edema after a course of amoxicillin or amoxicillin with clavulanic acid [12, 33], while others failed to observe such a beneficial effect [34–36].

Among the inflammatory sequelae that may arise following surgical extraction of mandibular third molar surgery, postoperative trismus is probably the most unpleasant for the patient. In our study, postoperative trismus was least pronounced in the Group M. Moreover, on the first and second postoperative day, the degree of postoperative trismus was also statistically significantly lower in the Group C compared to the Group P. These results can be attributed to the anti-inflammatory effect of the prescribed antibiotics.

Dry socket is probably the most challenging complication in wound healing after mandibular third molar surgery. In this study, the overall incidence of dry socket in the full sample was close to 13%, which is consistent with the results published elsewhere [37, 38]. However, it is interesting that the highest percentage of dry socket was, by far, recorded in the control group (in 25% of cases), while approximately half that percentage was recorded among the subjects who used cefixime (Group C), with no cases in the Group M (which received moxifloxacin).

Our findings have explicitly shown that both studied antibiotics (especially moxifloxacin) were effective in alleviating all inflammatory sequelae (pain, edema, and trismus) commonly occurring after surgical extraction of mandibular third molar surgery. This raises the question of the extent to which such a result can be attributed to the antimicrobial action of the applied antibiotics or their anti-inflammatory properties. It is well known that any trauma of soft and osseous tissue causes an acute inflammatory reaction and mobilization of several immune system cells. In this local defense mechanism, various mediators play a complex role, whereby pro-inflammatory cytokines [interleukin (IL)-1, IL-6, tumor necrosis factor (TNF)-α, TNF-β, and others] propagate inflammation through trauma-affected tissues and have been shown to have the capacity to activate prostaglandin secretion [39, 40]. It is noteworthy that fluoroquinolones, especially moxifloxacin, have an anti-inflammatory effect [41], thus influencing the production of IL-1, TNF-α, and IL-6. Hence, the stated anti-inflammatory properties of moxifloxacin can explain its favorable effect on the suppression of inflammatory sequelae after mandibular third molar surgery, as observed in our study.

Even though the results of this research point out favorable anti-inflammatory effects of antibiotics, as well as their positive impact on restoring oral functions in a shorter time and subsequently reduced morbidity, we should still

weigh these benefits against the risks of adverse effects of antibiotic use. To make relevant conclusions and solve the existing controversies, continuing research on this topic is necessary.

Conclusion

It is evident that the initial hypothesis guiding this investigation is largely confirmed. In fact, it is fully supported by the findings related to inflammatory sequelae, given that both antibiotics, especially moxifloxacin, had a pronounced effect on reducing pain, edema, and trismus as the most common postoperative complaints following mandibular third molar surgery. It is interesting, however, that both antibiotics, especially moxifloxacin, also contributed to reducing the incidence of dry socket in the postoperative period, which was unexpected because inflammation is not the cause of dry socket.

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Resistance index of the renal artery measured by doppler ultrasound as a predictor of graft function after kidney transplantation

Indeks rezistencije bubrežne arterije meren dopler ultrazvukom kao pokazatelj funkcije transplantiranog bubrega

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Abstract

Background/Aim. As an optimal treatment of choice for patients with the latest stage of chronic kidney failure (CKD), renal transplantation (Tx) is performed. The resistance index (RI) of the renal artery is measured by Doppler ultrasonography routinely at certain time intervals to show the condition of the renal graft. The value of RI > 0.75 is considered abnormal. The aim of the study was to determine the correlation between the values of the RI index and the function of the transplanted kidney. **Methods.** We analyzed retrospectively 63 patients in whom kidney transplant was done at the Clinic for Nephrology and Clinical Immunology, the University Clinical Center of Vojvodina, Novi Sad, Serbia, in the period from 2013 to 2017. Doppler of renal blood vessels was made to all examined patients in the first month after the renal transplantation. In addition to standard demographic data, all patients had the RI index and its relationship to the function of the transplanted kidney analyzed immediately after transplantation, as well as in the 6th, 12th, and 18th month, and in a certain number of patients in the 24th and 48th month after transplantation. **Results.** Out of 63 patients, 63.5% were men, and 26.5% were women, with an average age of 47.67 ± 13.62 years. The primary diseases in patients which led to the terminal CKD stage were hypertension in 33.3% and different forms of glomerulonephritis; while other diseases (diabetes mellitus, chronic pyelonephritis, eclampsia, polycystic kidneys, kidney agenesia, and unknown cause) were present in a lower percentage. RI < 0.75 was present in 73%, and RI > 0.75 in 27% of patients. There was no statistically significant association between RI and serum creatinine or creatinine clearance at a given time, and there was no connection between RI and gender, as well as length of previous treatment by HD. There was a statistically significant association between RI and age of kidney recipient, as well as Tx type. **Conclusion.** In the observed group of patients, RI of renal arteries did not prove to be a good predictor of the function of the transplanted kidney either in the early or later post-transplant periods. RI might have greater predictive significance if it were determined on or immediately after the transplantation procedure.

Key words:
graft survival; kidney transplantation; postoperative complication; prognosis; renal artery; ultrasonography; doppler.

Apstrakt

Uvod/Cilj. Transplantacija bubrega (Tx) je metoda izbora za lečenja bolesnika sa finalnim stadijumom hronične bubrežne bolesti (HBB). Indeks rezistencije (RI) bubrežne arterije meri se prilikom dopler ultrasonografskog pregleda bubrega i može se koristiti za procenu stanja renalnog grafta. Vrednosti RI > 0,75 smatraju se patološkim. Cilj rada bio je da se utvrdi korelacija između vrednosti RI indeksa i funkcije transplantiranog bubrega kod bolesnika lečenih Tx. **Metode.** Retrospektivno smo analizirali 63 bolesnika kod kojih je urađena Tx u Univerzitetskom kliničkom centru Vojvodine, Novi Sad, Srbija u periodu od 2013. do 2017. godine. U ispitivanje su bili uključeni svi bolesnici kod kojih je u prvih mesec dana nakon Tx urađen dopler renalnih krvnih sudova. Pored standardnih demografskih podataka, svim bolesnicima su analizirane vrednosti kreatinina u serumu, kreatinin klirensa i RI indeks i povezanost tog indeksa sa funkcijom transplantiranog bubrega neposredno nakon tranplantacije, kao i u 6, 12. i 18. mesecu, a kod određenog broja bolesnika i u 24. i 48. mesecu nakon Tx. **Rezultati.** U ispitivanoj grupi

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bolesnika, muškaraca je bilo 63,5%, a žena 26,5%, prosečne starosti 47,67 ± 13,62 godine. Osnovna oboljenja koja su dovela do terminalnog stadijuma HBB bila su hipertenzija i različiti oblici glomerolonefritisa sa zastupljenošću od po 33,3%, dok su ostale bolesti (dijabetes melitus, hronični pijelonefritis, eklampsija, policistična bolest bubrega, agenezija bubrega i nepoznat uzrok) bile zastupljene u nižem procentu. Indeks RI < 0,75 bio je prisutan kod 73%, a RI > 0,75 kod 27% bolesnika. Nije utvrđena značajna povezanost RI i kreatinina u serumu, klirensa kreatinina, kao ni povezanost RI sa polom i dužinom prethodnog lečenja hemodijalizom. Dokazana je značajna povezanost između RI i starosti primaoca bubrega, kao i vrste Tx. **Zaključak.** U posmatranoj grupi bolesnika indeks RI se nije pokazao kao dobar pokazatelj funkcije transplantiranog bubrega ni u ranom, niti u kasnijem postransplantacionom periodu. Taj indeks bi mogao imati veću prediktivnu vrednost ukoliko bi se merenje vršilo neposredno nakon završene transplantacione procedure.

Ključne reči:
graft, preživljavanje; transplantacija bubrega; postoperativne komplikacije; prognoza; *a. renalis*; ultrasonografija, dopler.

Introduction

The terminal stage of chronic kidney disease (CKD) requires active treatment by replacement of renal function. Methods available to do this are hemodialysis (HD), peritoneal dialysis (PD), and kidney transplantation (Tx).

Tx is the method of choice for treating patients in stage five CKD regardless of its etiology because, in addition to excretory, it replaces all other functions that a healthy kidney has. In addition to improving health, Tx reduces mortality, improves patient quality of life, and increases survival rates relative to HD and retroperitoneal dialysis methods [1–3]. Tx is a complex surgical procedure that replaces a nonfunctional organ with a new one in order to compensate for the tissue or organ function. During Tx, the organ is usually ileocecal. The donor is the person who gives the spool or transplant. Donors can be living-related donors, living-unrelated donors and cadaveric donors, that is, a person who has been diagnosed with brain death with the consent of the family [2].

There is a spectrum of complications that may occur after Tx. Vascular complications include hematoma, hemorrhage, renal vein, and artery thrombosis, lymphocele, pseudoaneurysm, renal artery stenosis. Urological complications are urine leakage and hydronephrosis [3].

The diagnostic method by which the occurrence of complications after Tx can be determined in the most rapid and noninvasive manner is Doppler ultrasonography. It is an imaging method for monitoring the condition after Tx [4, 5].

By calculating the resistance index (RI) at certain time intervals, the function of the renal graft can be monitored. The first examination is performed shortly after the transplant, and then after examination according to the appropriate protocols [6]. Arterial RI is a measure of pulse blood flow that shows resistance to blood flow caused by a microvascular bed distal to the site of measurement. It is usually measured in three places: the upper, middle, and lower poles of the kidney.

Doppler ultrasonography measures maximum systolic value (Vmax) and minimum diastolic value (Vmin), thus the RI is measured as 100x [1- (Vmin / Vmax)] [7]. The physiological value of RI, that is, the upper limit, is taken to be 0.7, while RI greater than 0.75 is interpreted as pathological peripheral resistance. Values > 0.7 and < 0.75 are considered borderline elevated. The physiological RI shows maintenance of high perfusion throughout the kidney [8].

An elevated RI, in comparison with a decreased RI, is a significant predictor of progressive renal dysfunction. RI can show different types of graft rejection, but it cannot distinguish between them [4].

The aim of this study was to investigate the correlation between RI values and transplanted kidney function in patients treated with Tx at the University Clinical Center (UCC) of Vojvodina, Novi Sad, Serbia.

Methods

We retrospectively analyzed the medical records of 63 patients undergoing kidney transplantation at the UCC of Vojvodina from 2013 to 2017. The study included all patients who underwent renal blood vessel Doppler in the first month after Tx. Color Doppler examination was performed with a 3.5 MHz convex-array transducer (Toshiba Ultrasound) in a supine position, at the angle of 30–60°. In interlobar and segmental renal arteries, RI was calculated from the Doppler spectra using the system software, according to the following formula: RI = (peak systolic frequency shift − minimum diastolic frequency shift)/peak systolic frequency shift.

This method was done sporadically in our Center from 2013 to 2015, after which it became a routine method. The study did not include patients whose surgical complications or cardiovascular comorbidities resulted in the termination of transplant operation and/or death in the immediate postoperative period. In addition to standard demographics, all patients were analyzed for RI and its association with renal transplant function (serum creatinine, creatinine clearance). We used the Modification of Diet in Renal Disease (MDRD) formula to determine creatinine clearance. The data obtained were analyzed statistically using the statistical software MedCalc and Microsoft Excel. Numerical data are presented using arithmetic means and standard deviations and median. Spearman's and Kendall's correlation coefficients were used in the analysis of one-way correlations. Comparisons were made by Student's *t*-test and the Mann-Whitney test. Statistical significance was defined by $p \leq 0.05$.

Results

General demographic data

The study included 63 patients who were followed for 18 to 48 months after Tx. The median time of follow-up was 24 months. The main demographic characteristics of our patients are shown in Table 1.

The main causes that lead to the end-stage of CKD, as well as the need for Tx, were hypertension in 21 (33.3%) patients and glomerulonephritis in 21 (33.3%) of patients. Other causes were shown in Figure 1.

The function of the transplanted kidney

Mean values of serum creatinine and creatinine clearance in the 1st, 12th, 18th, 24th, and 48th posttransplantation month are shown in Table 2. The last control of kidney function was within 3 months before

Table 1

Demographic characteristics

Characteristic	Values
Male gender, n (%)	40 (63.5)
Age at the time of transplantation (years), median (min–max)	48 (20–73)
Body mass index (kg/m^2), median (min–max)	24.49 (18.9–29.1)
Patients on hemodialysis before transplantation, n (%)	60 (95.2)
Preemptive kidney transplantation, n (%)	3 (4.8)
Time on HD (years), median (min–max)	5 (0.1–17)
Cadaveric kidney transplantation, n (%)	54 (85.7)
Living related transplantation, n (%)	9 (14.3)
Age of kidney donor (years), median, n (%)	52 (28–69)
Gender of kidney donor same as the recipient, n (%)	4 (44.4)
Immunosuppressive drugs (calcineurin inhibitor/mTOR inhibitor), n (%)	61 (96.8)/2 (3.2)
Immunosupressive drugs (corticosteroids), n (%)	100
Immunosupressive drugs (mycophenolic acid), n (%)	100

min – minimum; max – maximum; HD – hemodialysis.

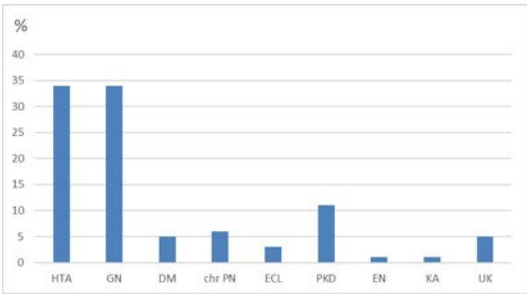

Fig. 1 – The main causes of chronic kidney disease.
HTA – hypertension arterials; GN – glomerulonephritis;
DM – diabetes mellitus; chr PN – chronic pyelonephritis;
ECL – eclampsia; PKD – polycystic kidney disease;
EN – endemic nephropathy; KA – kidney agenesis;
UK – unknown.

Table 2

Values of serum creatinine and creatinine clearance in the 1st, 12th, 18th, 24th, and 48th posttransplantation month

Parameter	Number of patients	Mean ± SD
Serum creatinine (μmol/L)		
1 month	63	192.89 ± 164.45
6 months	63	140.75 ± 49.45
12 months	63	132.61 ± 57.02
18 months	63	137.81 ± 59.90
24 months	38	135.39 ± 66.57
48 months	22	165.14 ± 101.27
Creatinine clearance (mL/min/1.73m^2)		
1 month	63	45.08 ± 25.96
6 months	63	46.14 ± 24.53
12 months	63	49.92 ± 24.64
18 months	63	47.13 ± 25.36
24 months	38	50.39 ± 24.06
48 months	22	49.90 ± 25.60

SD – standard deviaton.

analysis. As insufficient transplant function, we considered serum creatinine values greater than 200 μmol/L and they were found in the last control in 6 (9.5%) patients. Serum creatinine values in this group were from 206 μmol/L to 583 μmol/L; the median value was 216.5 μmol/L. The median value of creatinine clearance in this group of patients was 29.4 mL/min/1.73 m^2.

In the group of patients with serum creatinine values less than 200 μmol/L, they were in range from 68 μmol/L to 199 μmol/L; the median value was 116.0 μmol/L. The median value of creatinine clearance in this group of patients was 54.2 mL/min/1.73 m^2. In the observed group of 63 patients, one (1.6%) patient required active replacement of renal transplant function by HD 4 years after Tx.

Resistance index

Doppler ultrasound and RI measurement time range from 1 to 24 days after transplantation.

The physiological value of the RI < 0.75 was present in 46 (73%) patients, RI values were from 0.51 to 0.71. The median value was 0.63.

The pathological RI value > 0.75 was detected in 17 (27%) patients. RI values were from 0.75 to 0.97. The median value was 0.80.

Correlation of the pathological resistance index

We examined the correlation of the value of RI with the serum creatinine and creatinine clearance value in the 1st, 12th, 18th, 24th, and 48th posttransplantation month, as well as the correlation with gender and age of kidney recipients, type of transplantation, and previous dialysis duration time. The results are shown in Table 3.

Discussion

Tx is a method that replaces not only excretory but also all other kidney functions. Therefore, it is of great importance to maintain the adequate function of the transplanted organ for as long as possible. Numerous complications can occur after Tx. Over the years, research has focused on the detection of noninvasive diagnostic techniques that could allow early detection of complications and graft rejection [9].

RI is useful for showing different types of graft dysfunction, which can be: acute tubular necrosis (ATN), acute graft rejection, renal vein thrombosis, ureteral obstruction, and pyelonephritis but cannot differentiate between diseases [4].

Measuring RI over a longer period is a predictor for the early detection of chronic nephropathy [1, 4].

Our retrospective study included 63 patients treated with Tx at the UCC of Vojvodina from 2013 to 2017. Gender and age of patients were consistent with the literature [1, 6, 9].

The most common underlying disease leading to CKD was hypertension and some form of glomerulonephritis, which is similar to the findings in developed countries [10].

Cessation of graft function, i.e., the transfer of patients to another form of active treatment, in our study sample was determined in one patient four years after Tx. Similar results were obtained by Naesens et al. [6]. Therefore, according to the literature, we took a serum creatinine value greater than 200 μmol/L as a value indicating inadequate graft function. In our study, these serum creatinine levels at the last control of the nephrologist were observed in 9.5% of patients, which is slightly better than in the literature, where the incidence of inadequate renal transplant function was 23% [1]. A possible explanation for these results would be the rigorous selection of recipients due to the relatively small number of transplants

Table 3

Correlation of Resistance index (RI) with various factors

Correlation of RI with:	*p*	CI 95%
Serum creatinine after Tx		
1 month	0.9925	-0.191 to 0.215
6 months	0.5404	-0.160 to 0.246
12 months	0.6786	-0.228 to 0.160
18 months	0.6445	-0.259 to 0.143
24 months	0.572	-0.259 to 0.170
48 months	0.596	-0.385 to 0.206
Creatinine clearance after Tx		
1 month	0.0791	-0.201 to 0.251
6 months	0.1893	-0.224 to 0.157
12 months	0.1582	-0.251 to 0.188
18 months	0.1542	-0.261 to 0.194
24 months	0.1761	-0. 253 to 0.185
48 months	0.1598	-0.259 to 0.198
Gender of kidney recipient	0.486	-0.292 to -0.166
Age of kidney recipient in the time of Tx	0.0104	0.0333 to 0.382
Type of Tx	0.0499	0.000439 to 0.467
Period on HD	0.3853	-0.189 to 0.354

Tx – kidney transpantation; HD – hemodialysis; CI – confidence interval. Statistical significance was considered as values $p \leq 0.05$.

in our Center, as well as the fact that patients who had a permanent loss of graft function or death due to surgical complications in the immediate postoperative course were not included in the study.

RI in our patients is most commonly measured at the first outpatient check-up of the nephrologist during the first month after Tx, or in patients with delayed graft function, during hospitalization, also during the first month after transplantation. A pathological value of RI higher than 0.75 was present in 27% of our patients. According to the literature data, 20% of patients had pathological RI values, that is, RI > 0.75 [3–5].

In our study, we found no statistically significant association of RI with serum creatinine values at all time intervals tested. Such data may be due to the time of measurement of the RI, that is, the RI might have greater predictive significance if it were determined on or immediately after the transplantation procedure. In our center, we do not have a standard protocol that includes a Doppler ultrasound of a transplanted kidney on the day of the transplantation. This procedure is performed by a radiologist who is specialized in this field. Therefore, measurements were done when the radiologist was available. Data from the study of Cano et al. [4] show an association when measuring RI in the early period after transplantation as a valid marker for determining renal graft function, whereas in other literature data, this association has not been established [1, 3, 4].

According to other studies, we can conclude that a statistically significant association between the RI and serum creatinine values is shown over a period of 12 to 18 months [1, 4].

We did not demonstrate an association between RI and the gender of patients, which is consistent with the literature data [1, 3–6, 9, 10].

According to the literature data, a statistically significant correlation of the RI was found with recipient years, confirming that RI depends on the vascular characteristics of the recipient. We have reached the same conclusions in our research [1, 4].

A statistically significant correlation was shown between RI and type of transplantation, which coincides with results of studies already published [1, 11].

In the study, we proved that there is no statistically significant correlation between the RI and the length of previous dialysis treatment, as confirmed by the available literature data [11].

Conclusion

In the observed group of patients, the RI of renal arteries was not proven to be a good predictor of renal transplant function in the early or later posttransplant periods.

The RI might have greater predictive significance if it was determined on or immediately after the transplantation procedure.

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Potentially inappropriate medication prescribing among elderly patients with cardiovascular diseases

Moguće neodgovarajuće propisivanje lekova starijim bolesnicima sa kardiovaskularnim bolestima

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Abstract

Background/Aim. The growing number of older adults means higher medicine utilization. The aim of the study was to determine the frequency and identify risk factors of potentially inappropriate medication (PIM) in the elderly population with cardiovascular diseases. **Methods.** The retrospective, cross-sectional study was performed in 2018, and the relevant data were collected during the period from January 2016 to December 2017. The study sample included 1,500 patients over 65 years with cardiovascular disease who had medical records at the Institute for Gerontology and Palliative Care, Belgrade. Assessment of PIM was done by standard international criteria such as the American Geriatrics Society 2015 updated Beers Criteria for PIM use in older adults. **Results.** PIM frequency in the elderly population was 70.3%. In relation to gender, it was more frequent in female elders. The mean number of prescribed drugs was similar for 2016 and 2017, 7.2 and 7.3, respectively. The most common were: medium-acting benzodiazepines (70.9%), central α blockers (23.98%), and antipsychotics (typical and atypical) (20.94%). The most common comorbidity was noted in a group labeled with the International Disease Classification I00-I99, which includes heart and blood vessel diseases [n = 2,658 (36.9%)]. The most common diagnoses belonged to the subgroups I10-I15 [hypertensive diseases, n = 1,298 (18%)], I20-I25 [ischemic heart diseases n = 542 (7.5%)], I30-I52 [other forms of heart disease, n = 705 (9.8%)], I60-I69 [cerebrovascular diseases, n = 94 (1.3%)], and I80-I89 [diseases of veins, lymph vessels, and lymph nodes n = 12 (0.17%)]. The risk factors for PIM were: polypharmacy, gender, nicotine use, cognitive status, nutrition state, and the number of diseases registered in the study sample. **Conclusion.** Cardiovascular diseases in the elderly population are associated with a high prevalence of PIM. Creating health recommendations for prescribing drugs to the elderly that would emphasize these factors could reduce the prevalence of PIM in this population.

Key words:
aged; cardiovascular diseases; drug prescriptions; drug utilization; risk factors; serbia.

Apstrakt

Uvod/Cilj. Sve veći broj starijih osoba koristi lekove. Cilj rada bio je da se utvrdi učestalost i prepoznaju faktori rizika od mogućeg neodgovarajućeg propisivanja lekova (MNPL) starijoj populaciji sa kardiovaskularnim bolestima. **Metode.** Retrospektivnom studijom preseka, sprovedenom tokom 2018. godine, prikupljeni su bitni podaci za period od januara 2016. do decembra 2017. godine. Studijski uzorak obuhvatao je 1 500 bolesnika starijih od 65 godina sa kardiovaskularnim oboljenjima koji su bili korisnici zdravstvenih usluga i imali dostupnu medicinsku dokumentaciju na Institutu za gerontologiju i palijativnu zaštitu, Beograd. Procena MNPL obavljena je upotrebom standardnih međunarodnih kriterijuma poput *American Geriatrics Society 2015 updated Beers Criteria for potentially inappropriate medication use in older adults*. **Rezultati.** Učestalost MNPL u starijoj populaciji iznosila je 70,3%. U odnosu na pol, veća učestalost MNPL primećena je kod ispitanika ženskog pola. Prosečan broj propisanih lekova bio je sličan za 2016. i 2017. i iznosio je 7,2 i 7,3 lekova, redom. Najviše su propisivani: benzodiazepini sa srednjim vremenom delovanja (70,9%) centralni α blokatori (23,98%) i antipsihotici (tipični i atipični) (20,94%). Najviše komorbiditeta bilo je u grupi bolesti sa međunarodnom klasifikacijom I00-I99 koja obuhvata bolesti srca i krvnih sudova [n = 2 658 (36,9%)]. Najčešće dijagnoze bile su iz podgrupa: I10-I15 [hipertenzivne bolesti, n = 1 298 (18%)],

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I20-I25 [ishemijske bolesti srca, n = 542 (7,5%)], I30-I52 [ostale oblika bolesti srca, n = 705 (9,8%)], I60-I69 [cerebrovaskularne bolesti n = 94 (1,3%)], i I80-I89 [bolesti vena, limfnih sudova i limfnih čvorova n = 12 (0.17%)]. Faktori rizika od MNPL bili su: polifarmacija, pol, upotreba nikotina, kognitivni status, uhranjenost, kao i broj oboljenja zabeležen kod ispitanika. **Zaključak.** Kardiovaskularne bolesti u starijoj populaciji povezane su sa visokom prevalencijom MNPL. Kreiranje zdravstvenih preporuka za propisivanje lekova starijim osobama koje bi naglasile navedene faktore moglo bi uticati na smanjenje prevalencije MNPL u navedenoj popilaciji.

Ključne reči:
stare osobe; kardiovaskularne bolesti; lekovi, propisivanje; lekovi; korišćenje; faktori rizika; srbija.

Introduction

Elderly people are a vulnerable population in a pharmacological sense for two primary reasons: the physiological changes occurring in the elderly population affect and change the pharmacokinetics and pharmacodynamics of the administered drugs, as well as the presence of at least two or more chronic diseases (multimorbidity) [1–3]. The polypharmacy is most commonly present among people aged 65 and over, and it is one of the main causes of the drug-drug or the drug-disease interactions, which results in more frequent adverse drug reactions (ADRs), a poor medicine adherence, faster cognitive decline, unplanned hospitalizations, and higher health costs [4–6]. Now and in the future, increased consequences are expected with population aging and the prolongation of life expectancy.

Many studies have shown a higher risk of drug-drug or drug-disease interaction with the increased number of prescribed drugs. The prevalence of drug-drug interaction in nursing home residents who take two drugs was only 6%, but it significantly rises up to 100% for persons on co-medications with 8 drugs [7], while the probability for drug-disease interaction has the range of 15–40% in frail elderly people [8]. So far, it is generally known the medicines that have the higher potency for the occurrence of ADRs are also widely administered among the elderly. These include non-steroidal anti-inflammatory drugs (NSAIDs), anticoagulants, cardiovascular medicines (including diuretics and statins), antiepileptic drugs (AEDs), benzodiazepines, antibiotics, and oral hypoglycemic agents [8]. Numerous ADRs, as a consequence of drug-drug interaction, can be predicted and prevented by the use of scientific literature, databases, and software for their detection (Lexi-Interact, Micromedex, Drug Interactions, Medscape, and Epocrates) [9].

Moreover, polypharmacy is a significant risk factor leading to potentially inappropriate medication (PIM) prescribing associated with a high rate of disability and mortality (1.6 times higher risk in a more recent systematic review) in the elderly, reducing the quality of life, whether they are in nursing homes or hospitals [10, 11]. The hazard ratio of hospitalization was 1.73 due to the higher prevalence of PIM (ranging between 21.9% and 48%) among elderly nursing home residents in two European counties [12, 13].

PIM was observed in the primary care hospitalized patients but also in community-dwelling older people and nursing home residents [14]. The results of studies conducted both in the outpatient and hospital settings showed that approximately 60% of the elderly use at least one unnecessary medicine. Additionally, the use of the over-the-counter (OTC) preparations and dietary supplements is very common, up to almost 50% in community-dwelling elderly adults. The number of prescribed drugs (more than 9) is increasing in nursing home residents, and the most commonly used drugs were: diuretics, cardiovascular drugs [angiotensin-converting enzyme inhibitors (ACE) inhibitors, calcium channel, and beta-blockers], statins, antipsychotics, benzodiazepines, selective serotonin reuptake inhibitors, and proton pump inhibitors [8].

The prevalence rate of PIM has a wide range in various health settings worldwide, and studies recorded its much higher values in persons living in nursing homes (≈ 45%) compared to the community-dwelling older people (7.5%) [10]. The differences in the used screening tools for its detection and in the quality of prescribing drugs or the status of medication review practices between countries and geographical regions contribute significantly to this [15]. During the last decades, PIM has been a part of the global healthcare concern, and several guidelines worldwide [the Beers criteria, STOPP (Screening Tool of Older Person's Prescription) and START (Screening Tool to Alert doctors to Right Treatment) criteria, PRISCUS, and the Laroche list] provide explicit definitions and lists of PIMs in the geriatric population [16–19]. The Beers criteria was the first published list developed in the twentieth century (1991 by M. Beer) and adapted by the American Geriatrics Society for PIM detection in older people, and up to date, the original list has undergone 5 revisions (the latest in 2019). The original Beers criteria or revised versions with their own health standards are often used worldwide, both in the USA and the European countries [20].

Apart from polypharmacy and multimorbidity in the geriatric population, PIMs are linked with several physician errors summarized as follows: drug prescription without the obvious reason or diagnosis, lack of important information about a patient during prescribing medicines or lack of teamwork between physicians or pharmacists, presence of inaccurate medical records and insufficient knowledge or education about drugs whose prescription should be avoided in the geriatric population [21]. The results of the studies showed that the continuous and more frequent medical education of physicians, pharmacists, and all the medical staff taking care of nursing home residents could be effective and lead to the significant reduction and improvement of the PIM in the geriatric population [22].

According to all available information about the factors that really affect prescribing, it is clear that there is a long list of factors, but it should be emphasized which factor is vulnerable for a specific population such as the elderly, pregnant women, or pediatric patients, etc.

The aim of our study was to present frequencies of PIM in the elderly population with cardiovascular diseases and to identify the factors with a significant impact on PIM present in the study population.

Methods

Study design and *respondents*

The research was designed as an observational, retrospective cross-sectional study conducted at the Institute of Gerontology and Palliative Care (IGPC) in Belgrade and included elderly respondents living in ten Belgrade municipalities. The study was performed for three months in 2018, when data were collected for the period from January 2016 to December 2017. The number of patients who used certain types of health care in this institution for the observed period was 3,131, of which 1,500 patients met the criteria for inclusion in the study sample based on the precisely defined exclusion and inclusion criteria.

The inclusion criteria were: diagnosis of cardiovascular disease, patients' age of 65 or older, who took two or more medications prescribed daily by a medical doctor (MD) at the IGPC, and availability of complete medical documentation with demographic, socio-epidemiological and clinical data on patients.

The exclusion criteria were age below 65 years, patients in the terminal phase of the disease, and incomplete medical documentation.

The study was approved by the Ethics Committee of the relevant health institution, and each respondent was asked to sign an informed consent form to participate in the study before the start of the study. In the case of cognitive disorders, the consent was signed by the closest relatives.

Variables monitored in the study

Relevant data for the patients' analysis were taken from the Helliant electronic database and medical history, and the following data were taken into account: demographic characteristics (sex, age), epidemiological data (education, occupation, nicotine use, drug and food allergies), clinical data, cardiovascular system diseases, cognitive, emotional and nutritional status, as well as data on prescribed medications. The medical documentation was used as a source of information on the functional ability of patients, motivation for rehabilitation, subjective assessment of the health condition, existence of certain functional disabilities (visual and hearing impairment), speech disorders, and information about genetic predispositions.

Information about cognitive status was extracted from the patient's medical record where, according to psychological and psychiatric assessment, patient's cognitive status was noted as normal, with dementia or delirium presence. Besides cognitive status, there was information about emotional status, which was categorized as normal, depression, anxiety, fatigue, or other.

The assessment of nutritional status in the examined population was performed based on body mass index calculated as a quotient of body weight (expressed in kilograms) and body height in square meters.

The prevalence of PIMs in elderly patients was evaluated using explicit criteria, defined by the American Association for the Elderly, Beers Criteria, version 2015 [20].

Statistical analysis

Descriptive statistics, such as percentages and means or median, according to variable nature, were used to describe patient characteristics and to estimate the prevalence of PIM use among the studied population.

Variable normality was assessed by Kolmogorov-Smirnov test. Continuous variables with normal distribution were examined through the Student *t*-test, while Mann Whitney test was used for variables not showing normal distribution. Categorical variables were statistically processed by χ^2 with no comparison for cells, i.e., fields whose values are less than 5 pts, which are not taken into account considering the sample size. The influence of the observed factors on PIM prescribing was determined by a multivariable logistic regression model. The statistically significant value was smaller than 0.05.

The obtained data were analyzed by SPSS software package version 23 [Statistical Package for Social Sciences software (SPSS Inc., version 23.0, Chicago, IL)].

Results

Subject characteristics

The basic characteristics of the sample and the information about the prescribed drugs are shown in Table 1. Regarding the sample size, it included 1,500 respondents, whereby the mean age was 82.7 years, while even 35.6% were older than 85, a very old population. In terms of gender, there was a higher number of females in the study sample compared to the number of males (1,158 vs. 342, respectively).

Polypharmacy was present in a large number of examined participants, both groups (PIM and non-PIM) showed the use of more than 5 drugs. There was a statistically significant difference in polypharmacy between PIM and non-PIM groups.

The distribution of the number of drugs used is shown in Table 1, with the largest number of respondents, as many as 46.7%, using 5 to 8 drugs at the same time. Pronounced polypharmacy (concomitant use of 9 or more drugs) was observed in 31.7% of subjects with PIM. Regarding gender, there was a statically significant difference between PIM and non-PIM groups. The female gender was in a significant correlation with PIM presence.

The cognitive status in the PIM and non-PIM groups clearly indicates a statistically significant difference in

Table 1

Baseline characteristics of study participants

Variables	PIM	Non-PIM	Total	t (χ^2*)	p
Age (years), n (%)					
65–74	194 (18.4)	71 (16.0)	265 (17.7)		
75–84	495 (46.9)	206 (46.3)	701 (46.7)	1.892*	0.387
≥ 85	366 (34.7)	168 (37.8)	534 (35.6)		
Total	1,055 (70.3)	445 (29.7)	1,500 (100.0)		
Gender, n (%)					
male	211 (20.0)	131 (29.4)	342 (22.8)	15.663*	0.000
female	844 (80.0)	314 (70.6)	1,158 (77.2)		
Number of medicines 2016					
mean	7.2	5.2	6.7		
median	7.0	5.0	6.0	11.288	0.000
SD	3.4	3.1	3.4		
Number of medicines 2017					
mean	7.3	5.4	6.8		
median	7.0	6.0	6.0	11.600	0.000
SD	3.3	2.9	3.3		
Number of used drugs, n (%)					
2–4	228 (21.6)	216 (48.5)*	444 (29.6)		
5–8	493 (46.7)	159 (35.7)*	652 (43.5)	122.86*	0.000
> 9	334 (31.7)	70 (15.8)*	404 (26.9)		
Total	1,055 (70.3)	445 (29.7)	1,500 (100.0)	248.06	0.000
Nicotine use, n (%)					
yes	179 (11.9)	53 (3.5)	232 (15.5)	6.060*	0.014
no	876 (58.4)	392 (26.1)	1,268 (84.5)		
Education level, n (%)					
primary school	252 (16.8)	83 (5.5)	335 (22.33)		
intermediate degree	494 (32.9)	183 (12.2)	677 (45.13)		
fifth degree	22 (1.4)	10 (0.6)	32 (21.33)	11.637*	0.040
higher education	56 (3.7)	33 (2.2)	89 (5.93)		
university education	229 (15.2)	124 (8.2)	353 (23.53)		
PhD degree	10 (0.6)	4 (0.2)	14 (0.93)		
Marital status, n (%)					
married	299 (19.9)	136 (9)	435 (29.0)		
divorced	90 (6)	54 (3.6)	144 (9.6)	8.224*	0.042
widower	589 (39.2)	220 (14.6)	809 (53.9		
unmarried	62 (4.1)	50 (3.3)	112 (7.5)		
Nutritional level, n (%)					
normal	876 (58.4)	377 (25.1)	1253 (83.5)		
undernourished	140 (9.3)	37 (2.4)	177 (11.8)	13.416*	0.001
obese	39 (2.6)	31 (2)	70 (4.7)		
Cognitive status					
normal	753 (50.2)	330 (22)	1083(72.2)		
dementia	285 (19)	84 (5.6)	369 (24.6)	3.351*	0.000
delirium	25 (1.6)	23 (1.5)	48 (3.2)		
Emotional status, n (%)					
normal	362 (24.1)	201 (13.4)	563 (37.6)		
depression	227 (15.1)	60 (4)	287 (19.1)	15.685	0.003
anxiety	155 (10.3)	66 (4.4)	221 (14.7)		
fatigue	105 (7)	42 (2.8)	147 (9.8)		
other	189 (12.6)	93 (6.2)	282 (18.8)		
Motivation, n (%)					
high	79 (5.3)	63 (4.2)	142 (9.5)		
usual	563 (37.5)	271 (18)	834 (56.2)	13.395*	0.001
low	396 (26.4)	128 (8.5)	524 (34.9)		
Subjective health assessment, n (%)					
great	36 (2.4)	36(2.4)	72 (4.8)		
very good	24 (1.6)	8 (0.53)	32 (2.1)		
good	284 (18.9)	152 (10.1)	436 (29.0)		
poor	490 (32.6)	214 (14.2)	704 (46.9)	15.990*	0.007
bad	101 (6.7)	27 (1.8)	128 (8.6)		
does not know	102 (6.8)	26 (1.7)	128 (8.6)		

PIM – Potentially inappropriate medication; SD – standard deviation.
The statistically significant value was considered $p < 0.05$.

inappropriate drug prescribing in the group of patients with dementia.

A statistically significant difference was observed in emotional status between the PIM and non-PIM groups, where depression was a condition with a relatively high degree of presence in PIM subjects. A significant difference in the nutritional status was noted between the PIM and non-PIM groups. In these groups, there was a similar number of obese subjects, while the undernourished patients were more frequent in the PIM group.

Comparing the mean age values in the study sample according to PIM presence did not show statistically significant differences.

Analyzing the number of prescribed medicines, by the average number or by category, there is always a statistically significant increase in the number of medicines prescribed in the PIM group of respondents, and that in 2017 compared to 2016 there is a slight increase in the number of prescribed medicines in both groups observed (PIM and non-PIM), although this change was not statistically significant.

Comorbidity of respondents

In addition to the existing basic diagnosis for which they were admitted, the subjects also had accompanying comorbidities. On average, we obtained 4.8 comorbidities in the examined sample. Figure 1 shows the distribution of respondents according to the number of comorbidities. Most subjects (n = 266) had 4 comorbidities with a basic diagnosis.

Table 2 shows the distribution of the respondents' diagnoses. The group I00-I99 includes diseases of the heart and blood vessels, where the most common diagnoses were from subgroup I10-I15.

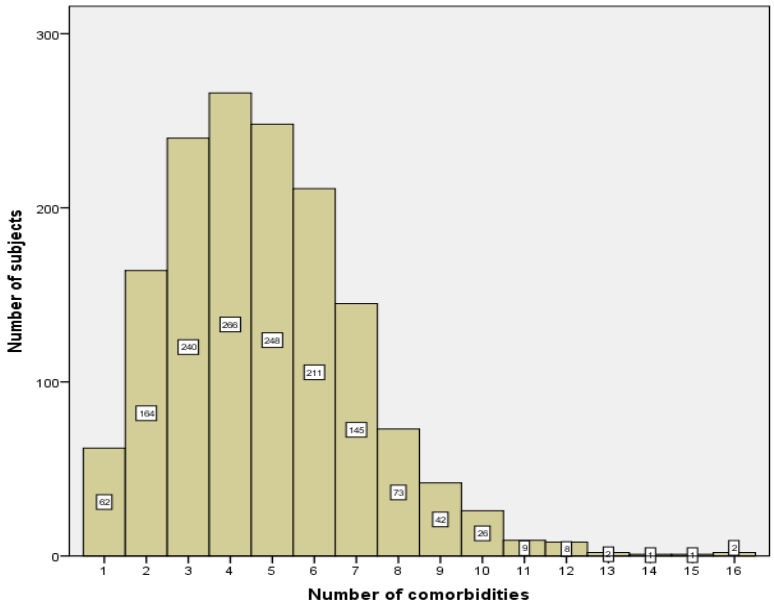

Fig. 1 – Distribution of respondents according to the number of diagnoses.

Table 2

Distribution of respondents according to the diseases diagnoses

Diagnosis	IDC code	n (%) (∑ = 7,199)
Mental disorders and behavioral disorders	F00-F99	1,735 (24.1)
Heart and blood vessel diseases	I00-I99	2,658(36.9)
arterial hypertension	I10	1,298 (18.02)
angina pectoris	I20	501 (6.95)
acute myocardial infarction	I21	16 (0.22)
chronic ischemic heart disease	I25	25 (0.34)
non-rheumatic trefoil disease	I36	4 (0.05)
heart muscle diseases	I42	150 (2.08)
cardiomyopathy	I429	108 (1.49)
atrial fibrillation and ventricular flutter	I48	164 (2.27)
other heart rhythm disorders	I49	169 (2.34)
heart failure	I50	120 (1.66)
brain infarction	I63	33 (0.45)
consequences of cerebrovascular disease	I69	61 (0.84)
phlebitis and thrombophlebitis	I80	12 (0.16)
Diseases of the urinary system	N00-N99	702 (9.8)
Diseases of the endocrine glands, nutrition, and metabolism	E00-E90	506 (7.02)
Diseases of the respiratory system	J00-J99	484 (6.7)

IDC – International Disease Classification.

Table 3

Adjusted analysis for factors associated with PIM

Variables	Wald coefficient	*p*	OR	95% CI for OR	
				lower	upper
Number of drugs	106,135	0.000	0.586	0.416	0.827
Gender	10,711	0.001	1.660	1.225	2.249
Smoking	7.477	0.004	1.511	1.108	2.261
Hypertension	0.251	0.616	1.068	0.826	1.382
Education (year)	2.833	0.726	1.115	0.308	2.037
Subjective health assessment	2.100	0.404	0.125	0.007	2.264
Motivation	1.482	0.477	0.687	0.375	1.260
Emotional status	2.050	0.133	1.149	0.783	1.685
Cognitive status	7.303	0.026	2.464	1.228	4.944
Marital status	4.122	0.249	1.341	0.654	2.749
Nutrition	15,358	0.000	4.108	2.025	8.333
Number of diseases	8.114	0.002	3.992	2.716	5.105
Constant	0.094	0.759	1.324		

Hosmer and Lemeshov χ^2 was 11.718; $p = 0.164$; Cox&Snall R^2 was 0.166 and Nagelkerke R^2 was 0.250.
OR – Odds ratio; CI – confidence interval.
The statistically significant value was considered $p < 0.05$.

Logistic regression analysis clearly indicates the influence of a number of diseases on PIM presence in the examined population (Table 3).

Potentially inappropriate drugs according to Beers criteria

The total number of PIMs determined by the Beers criteria was present in 1,055 (70.3%) subjects compared to prescribing adequate drugs in 445 (29.7%) subjects. Figure 2 shows the distribution of respondents according to the number of PIMs. The largest number of respondents, as many as 577 (38.4%), had 1 PIM, 305 (20.3%) respondents had 2, while 142 (9.5%) respondents had 3 PIMs. Subjects had an average of 1.2 PIMs in therapy during the year (range of 1–8).

Analyzing the pharmacological subgroups inducing PIM in our sample showed that the most common drug classes were short and medium-acting benzodiazepines (in 70.9%), antipsychotics (typical and atypical) (in 20.94%), and central α blockers (in 23.98%). Concerning gender, there was no statistical difference. The only significant differences by gender were reported for bromazepam (higher in female subjects; $\chi^2 = 11{,}931$; $p = 0.000$) and in doxazosin, where a higher number (3.3%) of male respondents received this drug (Fisher test, $p = 0{,}041$). In the benzodiazepines group, short and middle-acting benzodiazepines showed the highest rate of PIM prevalence, where bromazepam had the highest rate, even 49.8%, followed by lorazepam in 23.9% of PIM subjects. Long-acting nonbenzodiazepines were not present in so many cases; diazepam was found in 9.6% of PIM subjects.

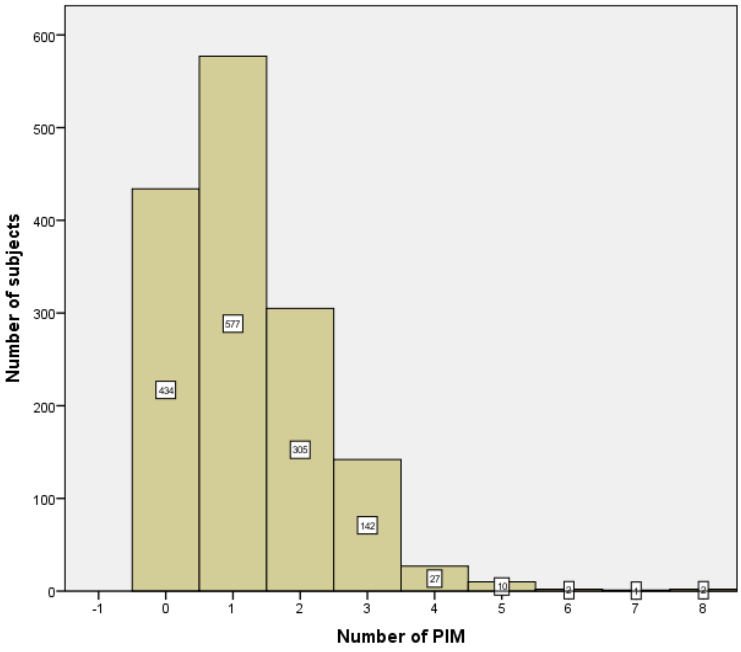

Fig. 2 – Distribution of respondents according to the number of potentially inadequate medication (PIM).

In order to identify factors associated with PIM, multiple logistic regression was conducted (Table 3) that examined the influence of 28 different variables, of which even 11 had statistical significance. After the adjustment, factors identified as significant for PIM in the elderly population by the logistic analysis were polypharmacy, gender, nicotine use, cognitive and nutritional status, and a number of diseases (Hosmer and Lemeshov χ^2 was 11.718; p = 0.164; Cox&Snall R^2 was 0.166 and Nagelkerke R^2 was 0.250).

Discussion

Reduction and timely prevention of PIM prescribing in elderly patients can significantly affect the health care approach and improve clinical and economical results for this specific vulnerable group. Specific pharmacological and pharmaceutical approach is necessary for these patients, and additional monitoring after drug administration is of great importance and creates a field of need for continuous monitoring of desired/adverse events in this area. The routine use of specific tools for PIM identification should become an unavoidable component of health care policy in most countries with a high prevalence of polypharmacy which is, according to literature-based evidence, one of the most important prediction factors [21–25]. Previous research of PIM in our country was not based on Beers criteria as specific tools but showed a relatively high prevalence of PIM; the study from 2014 [23] showed 27.3% of PIM, while data from 2016 [26] indicated a higher value, even 41.3%. To our knowledge, this is the first study in our country that used Beers criteria version 2015 for PIM research in elderly patients, which can bring significant results to the professional and general public, especially due to the specific modification of this version compared to the older versions from 2012 or 2011.

Our results clearly point to the alarming facts about PIM among the elderly population, even 70.3% with PIM prevalence. This high value was most frequent in female patients (Table 1). An interesting observation was that the average number of prescribed medications was similar during the two years, 7.2 and 7.3, respectively. The presence of polypharmacy was in accordance with the above data for both years. In 2016, even 827 patients out of 1,500 had concomitant use of 5 or more drugs, while significant polypharmacy (use of ≥ 9 drugs) was present in 40.4% of the mentioned number of patients. Similar observations were for 2017; polypharmacy was present in 834 patients, i.e., 39.92%. The significance of mentioned variables has been proven by multivariate logistic regression, where gender was characterized by adjusted odds ratio (OR) 1.660 and polypharmacy category with adjusted OR 0.586. The polypharmacy category of used drugs showed as a protective factor which can be explained by the more careful approach by doctors in a specific population such as elderly community-dwelling patients. The presence of polypharmacy as a predictor factor of PIM has been observed in numerous clinical studies. However, an interesting observation was made regarding data from Serbia. The data show that this factor was protective in our study in contrast with the earlier data, where it was the risk factor [adjusted OR 2.85, 95% confidence interval (CI) 1.97–4.14 from 2014 study and 3.05, 95% CI 1.59–5.85] [23, 24]. Our findings are not surprising, considering the research chronology of PIM in the elderly in our country and the consequent growing awareness of medical doctors after this research. On the other hand, there is a large pool of biomedical evidence in conflicting reports on polypharmacy effect on PIM, and further cross-sectional national study will further clarify this topic in detail in different geographic areas in Serbia [26–28].

The presence of polypharmacy as a PIM significant factor in our study is somewhat unexpected due to the presence of a large number of comorbidities at the level of the entire study population and due to the relatively high average number of comorbidities per subject, as much as 4.8. It should be emphasized that 2 cases were recorded where the subjects had 16 comorbidities, which can significantly affect the caution and reservations of physicians when defining a therapeutic approach for such patients. According to this data from our research, we should point out a very important observation – the influence of the number of diseases on PIM presence in the study sample. Multivariable logistic regression showed a strong influence of this factor and presented a significant risk factor through an adjusted OR value of 3.99. This can be explained by a large number of medical specialist examinations of patients with comorbidity and consequently the simultaneous prescribing of drugs that enter into potential interactions according to Beers criteria [22, 25–29].

The study result in our research point out women as a specific risk factor for PIM (adjusted OR 1.660, 95% CI 1.225–2.249). Females are probably more prone to PIM due to the longer life expectancy of the female population worldwide, which many national-wide socio-epidemiological studies noted, and consequently the higher number of medical conditions that can develop due to physiological and pathophysiological processes due to aging. In line with our results, there are many studies with the same conclusion in terms of gender as a risk factor for PIM [27–29].

According to the obtained results, benzodiazepines are the most common PIM in the examined elderly population. Inappropriate use of benzodiazepine in elderly people inevitably leads to the increase in adverse effects, such as increase of sedation effects, and in rare cases results in depression of the cardiovascular and respiratory centers. Numerous information based on literature clearly points out a positive correlation between benzodiazepine use and the high rate of morbidity and mortality among elderly patients [30, 31]. As our study group was created based on the presence of cardiovascular diseases, it is very important to emphasize the significance of the awareness of benzodiazepine prescribing in community-dwelling elderly people with this disease. Benzodiazepine adverse effects derived from PIM can affect not only individuals but also families and society, which can present a great economic cost for the whole mentioned subpopulation.

The majority of studies reported prescription of PPI (proton pump inhibitors) and NSAID besides prescription of benzodiazepine as the most common PIM factor [27–29], contrary to our reports. A possible explanation for this inconsistency can be the increased awareness of doctors about the liquid retention by NSAIDs which can induce exacerbations of cardiovascular disease. For other inconsistencies concerning the PPI drugs, there is no logical justification, thus further clinical analysis may bring us some reasonable explanation.

The use of antidepressants as a PIM category in the study population was expected due to the presence of depression in 19.13% of subjects, which was a higher frequency compared to other mood disorders. The results of research on the use of anticholinergics in elderly patients clearly indicate a positive correlation with outcomes such as reduced cognitive ability and the occurrence of dementia [32]. These data correlate with our results where the presence of dementia in the group of subjects with PIM was 19% compared to non-PIM subjects with dementia in only 5.6% of subjects. Due to the stated reasons, physicians must avoid prescribing psychotropic drugs in the population of elderly patients.

Paroxetine as the antidepressant drug turned out to be inappropriate in our research, according to Beers criteria, in as many as 51 patients. This antidepressant agent should be avoided among elderly people because of the higher risk of mortality outcome, which is the conclusion of different epidemiological studies [28, 29, 33]. Our results for typical and atypical antipsychotics indicate a relatively high incidence of their use which is not an adequate approach according to Beers criteria, where quetiapine has the highest rate in as many as 109 patients.

In the additional pharmacological group, which included an inappropriate medication for our subjects according to Beers criteria, were antipsychotics. The higher incidence of death followed the use of antipsychotics due to pneumonia has been reported, where atypical antipsychotics have a higher rate than the typical ones [34]. The most frequently prescribed antipsychotic was quetiapine (10.33% of the PIM population), which is in accordance with the national cross-section study conducted among the Norwegian population [35]. There was scientific debate about the increase of cardiovascular risk in a patient undergoing antipsychotic therapy, but up to now, there are no scientific data that strongly indicates this correlation and specific guidelines for unsafe use of antipsychotic drugs by patients with cardiovascular diseases [36]. However, medical experts should keep in mind the recommendation of the Beer criteria, which says: “Antipsychotics are associated with great risk of cerebrovascular accident (stroke) and mortality in persons with dementia” [16].

Cardiovascular diseases are one of the leading causes of death, according to the World Health Organization. As many as 31% of all deaths worldwide are caused by these diseases. Heart attack and stroke are on the pedestal of cardiovascular events related to lethal outcomes, while hypertension is the most common chronic non-communicable disease in this group. As many as 1.13 billion people in the world have this diagnosis [37]. As the use of certain medications can affect the consequent increase in the risk of cardiovascular events, it is especially important to monitor the administration of such drugs in persons diagnosed with cardiovascular disease. The beneficial clinical effects of multiple drug therapy are often overshadowed by the side effects they cause on both the underlying disease and other organ systems, so falls and consequent fractures are common with inappropriate cardiovascular therapy [38, 39].

Nicotine use is recognized as a risk factor for numerous health conditions in different populations, specifically in the elderly population, due to their physiological and pharmacological differences, which can reflect the administered therapy and the consequently achieved therapeutic goals. Most health professionals know that nicotine induces the activity of liver enzymes P450 isoform 1A2 and 2B6 [40], as these two forms are involved in the metabolic pathway of the drugs from antidepressants and antipsychotics groups. This should alert their prescription to persons using nicotine daily.

The two potential risk factors for the PIM in the elderly are cognitive status and nutrition. The cognitive status in our study was defined as a risk factor with an OR value of 2.464, which induces cognitive impairment when a healthcare professional selects one or more drugs affecting the central nervous system activity. The research has shown that specific nutritionals associated with the reduction of certain cardiovascular risk factors have a protective effect on cognitive status and prevent the development of dementia in the elderly [41, 42]. There is also evidence in references confirming an inversely related correlation between nutrition status and the number of used drugs that can be correlated with our result. This can be explained by bad habits of elderly people presented by the lower intake of specific vitamins (such as A, D, E, and B vitamins) and a higher intake of cholesterol, glucose, and sodium, which can lead to the development of numerous cardiovascular diseases and consequently produce the need for the drugs prescribing in large amounts [43, 44]. According to these scientific facts, our result, highlighting nutrition status with adjusted OR 4.10, presents valuable information for health care professionals.

It should be emphasized that there were specific limitations in our research. First, the study design had a retrospective character. Secondly, we used the 2015 Beers Criteria for PIM assessment, and that is not the last version of this PIM tool. The last revision was done in 2019 when some drugs were added while others were eliminated regarding the 2015 one, but when we started the data collection (2018) this was the most often used version. An additional limitation was the cross-sectional design of the study, which could not determine the real causality between factors and outcomes but could indicate the most significant points.

Conclusion

Our study suggests that elderly patients are more prone to PIM prescription due to several risk factors on which health professionals should be focused during the health assessment of this vulnerable patients group. Moreover, the results of this study point out how the Beer criteria can be a

useful tool and highlight the importance of their further integration into health policy. Healthcare education and widespread dissemination of the Beer criteria should be imperative as a potential method for a better health approach and the resulting quality of life for elderly people.

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Detection of diabetic polyneuropathy in a family medicine clinic by using monofilament

Detekcija dijabetesne polineuropatije u ambulanti porodične medicine korišćenjem monofilamenta

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Abstract

Background/Aim. Diabetic polyneuropathy (DPN) is the most common microvascular complication of diabetes mellitus (DM), which may be present at the time of disease detection. Screening for DPN is performed for the patients with type 2 diabetes at the time of diagnosis and for type 1 diabetes 5 years after diagnosis. The primary aim of this study was to determine the prevalence of DNP among family medicine patients with DM aged 18 to 70 years using nylon monofilament. **Methods.** The cross-sectional study estimated the prevalence of DPN among primary care patients with DM in Banja Luka, Republic of Srpska, Bosnia and Herzegovina. Semmes-Weinstein nylon 10 g monofilament was used to detect DPN. Age, gender, duration of DM, type of therapy, symptoms, glycosylated hemoglobin (HbA1c), and risk factors (hypertension, smoking, dyslipidemia, obesity, physical inactivity) were analyzed. Data collection took place from June 1st, 2017 to May 31st, 2018. **Results.** The study included 228 patients, 132 (57.9%) men and 96 (42.1%) women. There was a statistically significant difference in the presence of all symptoms of DPN (tingling, burning, light burning, and stinging) among patients with different duration of DM ($p < 0.01$). Multivariate logistic regression revealed that patients who had hypertension [odds ratio (OR) = 26.2; 95% confidence interval (CI): 4.070–168.488; $p = 0.001$], used oral antidiabetic therapy (OR = 12.3; 95% CI: 1.300–116.309; $p = 0.029$), had tingling (OR = 5.2; 95% CI: 1.431–18.571; $p = 0.012$) and a longer duration of diabetes (OR = 4.27; 95% CI: 1.983–9.175; $p = 0.000$) were more likely to have DPN. **Conclusion.** The prevalence of DPN in family medicine patients with DM using nylon monofilament was 24.2%. Determinants of DNP were the presence of symptoms of tingling, duration of diabetes, hypertension, and the use of oral antidiabetic therapy alone.

Key words:
bosnia and herzegovina; diabetes mellitus, type 2; diabetic neuropathies; prevalence; primary health care; risk factors.

Apstrakt

Uvod/Cilj. Dijabetesna polineuropatija (DPN) je najčešća mikrovaskularna komplikacija dijabetesa melitusa (DM) koja može da bude prisutna i u trenutku samog otkrivanja bolesti. *Screening* na DPN se radi svim obolelim od DM tipa 2 u trenutku postavljanja dijagnoze, a kod DM tipa 1, 5 godina od postavljene dijagnoze. Osnovni cilj ovog istraživanja bio je da se utvrdi prevalencija DPN kod bolesnika sa DM korišćenjem najlonskog monofilamenta u ambulanti porodične medicine. **Metode.** U istraživanju sprovedenom po tipu studije preseka praćena je učestalost DPN kod bolesnika sa DM na području Banja Luke. Za detekciju DPN korišćen je *Semmes-Weinstein* najlonski monofilament od 10 g. Analizirani su dob, pol, trajanje DM, simptomi, vrsta terapije, glikozilirani hemoglobin (HbA1c) i faktori rizika (hipertenzija, pušenje, dislipidemija, gojaznost, fizička neaktivnost). Podaci su prikupljani u periodu od 01. juna 2017. do 31. maja 2018. godine. **Rezultati**. Istraživanje je obuhvatilo 228 pacijenata, 132 (57,9%) muškarca i 96 (42,1%) žena. Utvrđena je statistički značajna razlika u prisustvu svih simptoma DPN (trnjenje, gorenje, peckanje i žarenje) kod bolesnika sa različitim trajanjem DM ($p < 0,01$). Multivarijantnom logističkom regresijom utvrđeno je da najveću verovatnoću pojave DPN ima bolesnik sa hipertenzijom [*odds ratio* (OR) = 26,2; 95% *confidence interval* (CI): 4,070–168,488; $p = 0,001$], koji koristi oralnu antidijabetesnu terapiju (OR = 12,3; 95% CI: 1,300–116,309; $p = 0,029$), ima simptom trnjenje (OR = 5,2; 95% CI: 1,431–18,571; $p = 0,012$) i duže trajanje DM (OR = 4,27; 95% CI: 1,983–9,175; $p = 0,000$). **Zaključak.** Prevalencija DPN kod bolesnika sa DM, korišćenjem najlonskog mono

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filamenta u ambulanti porodične medicine, iznosila je 24,2%. DPN je bila udružena sa prisustvom simptoma trnjenja, trajanjem dijabetesa, hipertenzijom i upotrebom samo oralne antidijabetesne terapije.

Ključne reči:
bosna i hercegovina; dijabetes melitus, tip 2; dijabetičke neuropatije; prevalenca; zdravstvena nega, primarna; faktori rizika.

Introduction

Diabetic polyneuropathy (DPN) is the most common microvascular complication of diabetes mellitus (DM), which may also be present at the time of disease detection. It is mainly distal sensorimotor polyneuropathy, which is responsible, in 75% of cases, for the early amputation of parts of the extremities and whole extremities in patients with diabetes [1–4].

A study conducted in England found the onset of symptoms of painful neuropathy in one-third of the total number of diabetic patients at the community level examined [5].

Small A-delta and C fibers become damaged first. Initially, the disease is asymptomatic in 50% of cases [6], but later on, there are symptoms such as tingling, burning, loss of sensation of touch, temperature or pain, trophic changes on the skin with the onset of ulcer development. The intensity of symptoms is greatest when resting, especially at night. DPN is the leading cause of foot ulceration, as well as a prerequisite for the development of Charcot's neuropathy or Charcot's foot, also increasing the risk of falls and fractures [7, 8]. It was found that 45–60% of diabetic foot ulcers are of neuropathic origin, and patients with DM are 3.5 times more likely to develop ulcers than non-diabetic patients.

The American Diabetes Association (ADA) [9] recommends DNP screening for all patients with type 2 DM at the time of diagnosis and with type 1 DM, 5 years after diagnosis. According to the International Diabetes Federation (IDF), every family physician should provide foot examination at least once a year to his/her patients [10]. There are several clinical diagnostic modalities for diagnosing DPN. Quantitative sensory testing (QST) has been available for more than 2 decades using cold and warmth thresholds to detect small fiber neuropathies. Semmes-Weinstein 10 g monofilament test is commonly used to detect DNP in family medicine setting. Further evaluation includes clinical imaging and nerve conduction studies (NCS). The study by Park and Kim [11] established the need for simple and non-invasive tests, including a Semmes-Weinstein 10 g monofilament test for DPN in patients with type 2 DM.

According to the previous studies, DPN can develop as early as in pre-diabetes (glucose tolerance impairment – IGT) [12, 13]. In addition to hyperglycemia, one of the important factors involved in the pathogenetic mechanism of DPN is hyperlipidemia [14, 15].

Many prospective studies have confirmed that loss of pressure sensitivity by 10 g monofilament is an important predictor of possible onset ulceration and diabetic foot leading to possible lower limb amputations [16, 17]. The monofilament test is the best choice for DPN detection because it is portable, fast, non-invasive, inexpensive, and patient-friendly.

The epidemiological research found that the prevalence of DPN in the world is greater than 50% when adjusted for diabetes duration and age [18–20]. However, data for diabetes patients in the Republic of Srpska are lacking. The primary aim of this study was to determine the prevalence of DNP among family medicine patients with DM aged 18 to 70 years by using nylon monofilament. The secondary aim was to determine the risk factors (hypertension, smoking, dyslipidemia, obesity, physical inactivity), duration of diabetes, type of therapy, and regulation of DM (glycosylated hemoglobin – HbA1c) associated with DNP.

Methods

The cross-sectional study explored the prevalence of DPN in patients with DM registered with the family practices and affiliated with Primary Health Center in Banja Luka, Republic of Srpska, Bosnia and Herzegovina. With a population size of 15,617 diabetic patients, an error of 5%, confidence level of 95% and confidence interval (CI) of 6.44, the estimated sample size was 228. Patients were selected randomly from the electronic registry of patients with DM. Data collection took place between June 2017 and May 2018.

Inclusion criteria were as follows: age 18 to 70, type 1 and type 2 DM diagnosis according to International Classification of Diseases (ICD), and written consent to participate in the study obtained from each respondent. Patients with DM who had ulcers or amputations, associated peripheral arterial disease, and those with multiple complications of diabetes were excluded from the study. Written and electronic records of DM patients were used in the data collection.

For this research, a checklist was created for each participant individually. The participants underwent inspection, palpation, and physical examination of the foot. Semmes-Weinstein nylon 10 g monofilament was used to detect DPN.

The examiner demonstrated first the strength of the monofilament touch on each participant's arm, then asked them to close their eyes and performed testing on both feet. The examined points included the first metatarsal-phalangeal joint of the thumb, the dorsum of the thumb, the plantar side of the thumb, and the plantar side of the heel. The participant should relay when he or she feels the touch. The total score is eight. According to a previous study, more than four wrong answers screened positive for DPN [4]. Moreover, data on subclinical manifestations (tingling, burning, light burning, and stinging) of DPN are collected in an interview with each participant (Yes, No).

Age, gender, duration of diabetes, type of therapy, glycemic regulation (HbA1c), and risk factors (hypertension, smoking, dyslipidemia, obesity, physical inactivity) were recorded for each participant.

Participants were divided into 4 age groups: 20–30, 31–40, 41–50, and 51–60 years. They were also divided into 4

groups according to the duration of diabetes: duration of diabetes up to 5, 10, 20 years, and over 20 years.

According to the type of therapy, they were divided into 3 groups: those using oral antidiabetic therapy, insulin therapy, and combination therapy. For glycemic control assessment, HbA1c was used. HbA1c levels were evaluated in the central laboratory of the Primary Health Center in Banja Luka (Bioanalyzer Arhitekt c 8000). HbA1c < 7% was considered good glycemic control and HbA1c ≥ 7% as poor glycemic control. Blood pressure values greater than 130/80 mmHg were considered unregulated hypertension. Dyslipidemia was diagnosed if total cholesterol value was > 4 mmol/L, and/or LDL cholesterol > 2.6 mmol/L and/or triglyceride > 1.7 mmol/L [21, 22]. Obesity was recorded if the participant's body mass index (BMI) was > 30 kg/m^2 and waist circumference (WC) > 94 cm for men and 80 cm for women. According to physical activity, participants were rated as inactive, moderately active, and extremely physically active [23].

The consent of the institutional Ethics Committee was obtained for this research.

Statistical analysis

All analyzes were performed using SPSS version 25 (SPSS Inc., Chicago, IL, USA). The results were analyzed and presented using descriptive statistics (absolute and relative numbers, measures of central tendency, standard deviation). Demographic data and risk factors in the respondents were analyzed using adequate statistical tests (χ^2-test and Student's *t*-test of independent samples). Univariate and multivariate logistic regression were used to determine the association between DNP and risk factors. A probability level or *p*-value of less than 0.05 ($p < 0.05$) was considered statistically significant.

Results

The study included 228 patients, 132 (57.9%) men and 96 (42.1%) women. The average age of the participants was 55.8 ± 9.2 years. The prevalence of DNP among our participants with DM, using nylon monofilament, was 24.2%.

Participants with a longer duration of diabetes and report-

Table 1

Frequency of DPN according to gender, age, HbA1c, duration of diabetes, presence of symptoms (tingling, burning, light burning, and stinging), and type of therapy

Parameter	Presence of DPN, n (%)		*p*
	yes	no	
Gender			
male	38 (69.1)	94 (54.7)	0.059
female	17 (30.9)	78 (45.3)	
Age (years)			
< 18	0 (0.0)	0 (0.0)	
18–30	1 (1.8)	6 (3.5)	
31–40	2 (3.6)	10 (5.8)	
41–50	54 (7.3)	21 (12.2)	0.712
51–60	25 (45.5)	73 (42.4)	
61–70	23 (41.8)	62 (36.0)	
> 70	0 (0.0)	0 (0.0)	
HbA1c (%)			
≤ 7.00	20 (36.4)	64 (37.4)	0.007
> 7.00	35 (63.6)	107 (62.6)	
Duration of diabetes (years)			
5–10	19 (34.5)	106 (61.6)	
10–15	11 (20.0)	41 (23.8)	< 0.001
15–20	13 (23.6)	18 (10.5)	
> 20	12 (21.8)	7 (4.1)	
Presence of symptoms			
tingling			
yes	44 (80.0)	55 (32.0)	< 0.001
no	11 (20.0)	117 (4.1)	
burning			
yes	27 (49.1)	26 (15.1)	< 0.001
no	28 (50.9)	146 (84.9)	
light burning/stinging			
yes	32 (58.2)	38 (22.1)	< 0.001
no	23 (41.8)	134 (77.9)	
Therapy			
oral antidiabetics			
yes	13 (23.6)	30 (17.4)	0.307
no	42 (76.4)	142 (82.6)	
insulin			
yes	33 (60.0)	70 (40.7)	0.012
no	22 (40.0)	102 (59.3)	

DPN – diabetic polyneuropathy; HbA1c – glycosylated hemoglobin.

ing all symptoms of DPN (tingling, burning, burning, and burning) were more likely to have DNP in comparison with those without symptoms and short disease duration ($p < 0.001$). The statistically significant difference in the DNP presence was found among patients on insulin therapy and those who used other types of therapy ($p = 0.012$). No statistically significant dofference was found between patients with lower and higher HbA1c than 7%, nor between both types of diabetes with respect to the occurrence of polyneuropathy (Table 1).

Differences in DNP presence were not found regarding hypertension ($p = 0.276$) and smoking ($p = 0.607$). However, DNP was more frequently found among participants with dyslipidemia compared to those without it ($p = 0.046$) (Table 2).

The multivariate logistic regression model was adequate for the data available ($\chi^2 = 80.794$, $p < 0.001$), with 63.6% of the variability of the dependent variable explained by the selected model. Additionally, when predicting polyneuropathy using the characteristics of patients who entered the model, 85.9% of cases would be successful.

In univariate regression models, associations were found between DNP and the following variables: the presence of symptoms of tingling (8.509), burning (5.415), light burning and stinging (4.906), hypertension (3.380), and the use of insulin therapy (2.075) (Table 3).

Table 2

Frequency of risk factors in a patient with and without DPN

Risk factor	Presence of DPN		*p*
	yes	no	
Hypertension > 130/80 mmHg, n (%)			
yes	19 (34.5)	46 (26.9)	0.276
no	36 (65.5)	125 (73.1)	
Smoking, n (%)			
ex-smoker	9 (16.4)	35 (20.3)	0.607
smoker	14 (25.5)	34 (19.8)	
non-smoker	32 (58.2)	103 (59.9)	
Dyslipidemia, n (%)			
yes	30 (57.7)	111 (72.5)	0.046
no	22 (42.3)	42 (27.5)	
LDL (mmol/L), mean ± SD	2.939 ± 0.95	3.353 ± 1.019	0.012
HDL (mmol/L), mean ± SD	1.3075 ± 0.397	1.293 ± 0.435	0.825
CHOL (mmol/L), mean ± SD	5.1172 ± 1.152	5.468 ± 1.248	0.068
Tg (mmol/L), mean ± SD	2.007 ± 1.45	2.1605 ± 1.509	0.516
Obesity (BMI > 30 kg/m^2), n (%)			
yes	24 (44.4)	56 (33.7)	0.155
no	30 (55.6)	110 (66.3)	
Physical activity, n (%)			
inactive	13 (23.6)	35 (20.3)	0.857
moderately physically active	34 (61.8)	109 (63.4)	
extremely physically active	8 (14.5)	28 (16.3)	

DPN – diabetic polyneuropathy; LDL – low density lipoprotein; HDL – high density lipoprotein; CHOL – cholesterol; Tg – triglycerides; BMI – body mass index; SD – standard deviation.

Table 3

Univariate and multivariate logistic regression of DPN-related variables

Variable	Univariate logistic regression				Multivariate logistic regression			
	p	OR	95% CI for OR		*p*	OR	95% CI for OR	
			lower	upper			lower	upper
Gender	0.061	0.539	0.283	1.029	0.790	0.850	0.257	2.813
Duration of diabetes	0.000	2.085	1.534	2.833	0.000	4.266	1.983	9.175
Presence of symptoms								
tingling	0.000	8.509	4.083	17.733	0.012	5.155	1.431	18.571
burning	0.000	5.415	2.761	10.618	0.105	3.368	0.777	14.600
light burning, stinging	0.000	4.906	2.572	9.357	0.066	4.054	0.914	17.980
HbA1c	0.097	1.153	0.975	1.365	0.407	1.159	0.817	1.645
Cholesterol	0.070	0.787	0.607	1.020	0.150	0.556	0.250	1.238
LDL	0.013	0.641	0.450	0.912	0.077	0.440	0.177	1.093
CCr	0.107	0.637	0.368	1.103	0.133	0.516	0.218	1.222
AvgTA	0.008	1.033	1.008	1.058	0.028	0.934	0.879	0.993
Hypertension > 130/80 mmHg	0.001	3.380	1.667	6.852	0.001	26.186	4.070	168.488
Oral antidiabetic therapy	0.045	0.526	0.282	0.985	0.029	12.296	1.300	116.309
Insulin therapy	0.025	2.075	1.098	3.919	0.069	6.014	0.870	41.578

DPN – diabetic polyneuropathy; OR – odds ratio; CI – confidence interval; HbA1c – glycosylated hemoglobin; LDL – low density lipoprotein; CCr – creatinine clearance; AvgTA – average blood pressure.

Multivariate logistic regression revealed that patients who had hypertension [odds ratio (OR) = 26.2; 95% CI: 4.070–168.488; $p = 0.001$], used oral antidiabetic therapy (OR = 12.3; 95% CI: 1.300–116.309; $p = 0.029$), had tingling (OR = 5.2; 95% CI: 1.431–18.571 $p = 0.012$;) and a longer duration of diabetes (OR = 4.27; 95% CI: 1.983–9.175; $p = 0.000$) were more likely to have DPN (Table 3).

Discussion

The prevalence of DNP among family medicine patients with DM aged 18 to 70 years, using nylon monofilament, was 24.2%. The determinants of DNP were hypertension, using oral antidiabetic therapy, having tingling, and a longer duration of diabetes.

The prevalence found in the current study was lower than in previously conducted research. Salvotelli et al. [24], investigating DPN in patients with type 2 DM, based on a clinical examination of the foot, detected a prevalence of 30%. A study conducted in Tanzania found that more than half of patients included in that study had neuropathy with a severe form, and the main risk factors were: increasing age, increasing duration of diabetes, obesity, and hypertension [25].

Age and gender were not associated with the prevalence of DNP, which is in disagreement with a study by Gill et al. [26] in which the association between prevalence of DPN, and age, and duration of symptoms was found.

A study by Abbott et al. [5] from North West England showed that type 2 DM patients, women, and the South Asian population have a higher incidence of DNP. Studies in Jordan and England have found a prevalence of DPN of 30.3–39.5% in patients with type 2 DM over the age of 18. Furthermore, they detected higher prevalence in the secondary health care versus primary health care setting level, as well as a higher occurrence of DPN among patients with type 2 DM compared to the patients with type 1 DM [27, 28]. Our study showed no association between glycemic control via HbA1c and the presence of DPN, even though a greater number of patients with DPN had HbA1c greater than 7% (average HbA1c = 7.98 ± 2.07%).

Although statistical analyses showed that participants with dyslipidemia have a higher prevalence of DNP, no associations between DNP and lipid serum levels were found in multivariate regression analysis. A meta-analysis of several observational studies has demonstrated an association between LDL cholesterol fraction and systolic blood pressure with DPN [29]. Moreover, a study done in Jordan found a significant association of dyslipidemia with increased OR for DPN [27].

A Chinese study carried out on patients with type 2 DM found a higher prevalence of DNP among overweight and obese patients (33.1%) compared to patients who had optimal BMI [30]. On the other hand, a recent Indian study found no associations between obesity and DNP, which corroborates our findings [31].

Considering the type of therapy, this study found that the use of oral antidiabetic therapy alone was a predictor of DPN together with the duration of diabetes and hypertension as the present risk factor (OR = 12.296, 95% CI: 1.300–116.309; $p < 0.05$). A cross-sectional study conducted in Peru found that patients who were on both oral and insulin therapy were 40% more likely to have DPN than those with a diabetes duration longer than 10 years [32].

Several limitations need to be considered. Only Semmes-Weinstein nylon 10 g monofilament test was used to detect DNP. Although this test presents a good, inexpensive and accessible screening tool, more objective diagnostic procedures are required to confirm the diagnosis of DNP. The study measured HbA1c at a single point in time, which may not reflect the real level of glycemic control. The study was carried out in one region of the Republic of Srpska, Bosnia and Herzegovina, and the results may not be generalized to the whole country.

Conclusion

The prevalence of DPN in family medicine diabetic patients was 24.2%. DPN was associated with hypertension, the presence of symptoms (tingling), the duration of diabetes, and the use of oral antidiabetic therapy alone. Screening of diabetic polyneuropathy is justified in a family medicine setting. Early and rigorous management of diabetes and associated risk factors may have an essential role in the prevention of diabetic complication development and progression.

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Anxiety and depressive symptomatology among children and adolescents exposed to the COVID-19 pandemic – A systematic review

Anksioznost i simptomi depresije među decom i adolescentima u pandemiji COVID-19 – sistematski pregled

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Abstract

Background/Aim. Children and adolescents are sensitive groups for the development of mental disorders during the crisis. The aim of this systematic review was to assess the impact of the COVID-19 pandemic on anxiety and depressive symptomatology in the population of children and adolescents. **Methods.** The investigation was based on a systematic review followed by PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) protocol, including Ovid MEDLINE, Embase, Global Health, and APA Psyc Info databases, to identify relevant studies reporting anxiety and depressive symptoms among children and adolescents during the COVID-19 pandemic. A total of 27 articles were included. **Results.** Anxiety symptoms increased from 28.3% before the pandemic to 49.5% during confinement [General Anxiety Disorder (GAD)-7 ≥ 11)] (McNemar test, $p < 0.0001$). More depressive symptomatology was found, as well as weight and sleep disturbances which are the characteristics of children and adolescents' mental health. Additionally, female teenagers were experiencing grater declines in mood disorders than male teenagers during the COVID-19 crisis. On the one hand, different positive correlations between anxiety and other variables, were found, such as clinical depressive symptoms and anxiety (3/14), smartphone and internet addiction (2/14), lower levels of family income (2/14), perceived threats (2/14), higher grades at school (2/14), and loneliness (1/14). On the other hand, positive correlations were reported between depression and children and adolescents that were socially disconnected (3/17). Finally, mothers with higher level of education and income were associated with higher level of happiness (2/17). **Conclusion.** COVID-19 has a strong impact on the mental health of children and adolescents regarding depression and anxiety symptoms. Prevention programs focused on coping strategies should be conducted in elementary schools, middle schools, and high schools. Mental health should become a priority matter for governments, and the current pandemic could be an opportunity to highlight the importance of mental well-being and to invest in the betterment of clinical trainings, treatments and mental health research.

Key words:
adolescent; anxiety; child; covid-19; depression.

Apstrakt

Uvod/Cilj. Deca i adolescenti su osetljiva grupa za razvoj psihičkih poremećaja tokom krize. Cilj ovog sistematskog pregleda literature bio je da se proceni uticaj pandemije COVID-19 na razvoj simptoma anksioznosti i depresije u populaciji dece i omladine. **Metode.** Sistematski pregled literature je urađen pomoću PRISMA (*Preferred Reporting Items for Systematic Reviews and Meta-Analyses*) protokola, uključujući *Ovid MEDLINE, Embase, Global Health i APA Psyc Info* baze podataka, u cilju identifikovanja relevantnih istraživanja o simptomima anskioznosti i depresije kod dece i adolescenata u toku pandemije COVID-19. Pregledano je 27 radova. **Rezultati.** Simptomi anksioznosti su se povećali sa 28,3% pre pandemije na 49,5% tokom „zatvaranja" usled pandemije [*General Anxiety Disorder* (GAD)-7 ≥ 11)] (*McNemar* test $p < 0{,}0001$). Ustanovljen je i skok u simptomima depresije, kao i u telesnoj masi i poremećajima spavanju, koji karakterišu smetnje u mentalnom zdravlju dece i adolescenata. Poremećaj raspoloženja (pad) je bio veći kod tinejdžera ženskog, u odnosu na tinejdžere muškog pola. Pokazane su pozitivne korelacije između anksioznosti i drugih varijabli: kliničkih depresivnih simptoma i anksioznost (3/14), zavisnosti od pametnih telefona i interneta (2/14), nižeg porodičnog prihoda (2/14), percipirane opasnosti (2/14), viših razreda u školi (2/14), i

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usamljenosti (1/14). Pokazane su i pozitivne korelacije između depresije i socijalne isključenosti dece i adolescenata (3/17). Konačno, pokazana je povezanost između majki sa višim nivoima obrazovanja i prihoda i osećanja sreće (2/17). **Zaključak.** Pandemija COVID-19 ima snažan uticaj na mentalno zdravlje dece i adolescenata odnosno pojavu simptoma depresiije i anksioznosti. Zbog toga bi u osnovnim, srednjim i višim školama trebalo sprovoditi programe prevencije, sa fokusom na razvoj strategija prevladavanja. Mentalno zdravlje bi trebalo da postane prioritetna tema vlada zemalja, a trenutna pandemija mogućnost da se istakne važnost brige o mentalnom blagostanju i ulaganja u poboljšanje kliničkih treninga, tretmana i istraživanja u oblasti mentalnog zdravlja.

Ključne reči:
adolescenti; anksioznost; deca; covid-19; depresija.

Introduction

According to the World Health Organization (2020), childhood and adolescence are critical developmental stages, when social and emotional skills are shaped. Thus, the environment is crucial for children and adolescents' well-being, and early negative experiences could be a risk factor for developing emotional disorders like depression and anxiety [1].

The Coronavirus Disease 2019 (COVID-19) pandemic is the hardest global crisis we have faced in the past 75 years. Since its emergence in Asia, the virus has spread to every continent, causing death to over 1.6 million people worldwide [2]. Throughout the outbreak, people have lost relatives, social connections, jobs, and income [3]. In addition, some governments implemented strict socializing measures to control (or try to reduce) the spread of the virus, including isolation, 15-day quarantines, remote schooling, and national lockdowns. Fear about the virus's impact, uncertainty about the future, and social distancing are considered among the factors that explain sharpened anxiety and depressive symptoms among children and adolescents.

According to the Diagnostic and Statistical Manual of Mental Disorders (DSM-5), depressive symptomatology's most common features are sadness, emptiness, or irritable moods, followed by somatic or cognitive changes. More specific symptoms include irritable mood, diminished interest in normal activities, insomnia or hypersomnia, significant weight loss or weight gain, diminished ability to concentrate or think, and recurrent thoughts involving death. On the other hand, the most common feature of the anxiety symptomatology DSM-5 is excessive fear and anxiety related to behavioral disturbances. Excessive worrying, difficulty controlling the worry, restlessness or feeling keyed up or on edge, being easily fatigued, irritability, sleep disturbances, muscle tension, and difficulty concentrating or mind going blank are the main symptoms of anxiety.

Studies that have examined the direct link between viral diseases and affective disorders symptomatology have found correlations with diseases such as influenza (H1N1), varicella-zoster, and herpes simplex virus [4]. Moreover, previous systematic reviews and meta-analyses have evaluated anxiety and depression in health workers and adults during SARS/MERS/COVID-19 [5] . Nevertheless, none has examined the impact of the pandemic on children and adolescents. Therefore, the aim of this systematic review was to assess the impact of the COVID-19 pandemic on anxiety and depressive symptomatology in this population.

Methods

A literature systematic review was carried out with the aim of selecting relevant studies from January to October 2020, using four databases. Ovid MEDLINE, Embase, Global Health, and APA PsycInfo that were examined thoroughly up to November 25th, 2020 using the combination of the following search terms: (child* OR "kid" OR "student" OR "infant") OR (adolesc* OR "teen" OR "young people" OR "Middle school students" OR "High School students" OR "College Students" OR "University Students" OR "Undergraduates students") AND (covid* OR "covid19" OR "coronavirus" OR "covid-19 pandemic") AND ("depress*" OR "major depression" OR "mood disorders*" OR "affective disorders" OR "MDD" OR "anxi*" OR "Agoraphobia" OR "GAD" OR "General Anxiety Disorder", OR "Separation Anxiety Disorder", OR "Social Anxiety Disorder", OR "Social Phobia", OR "School Phobia", OR "Panic Disorder" OR "Panic Attack" OR "Obsessive Compulsive disorder" OR "OCD"). These key terms were chosen to find the most relevant studies regarding the COVID-19 impact in emotional disorder symptomatology among children and adolescents. Upon finalizing duplication at Ovid, the studies found were downloaded into Endnote (X9) and its abstracts were reviewed, as well as screened with the inclusion and exclusion criteria. Subsequently, the remaining studies (full text) were read and assessed for eligibility using Rayyan platform. Finally, a manual review was followed to obtain references and additional articles.

Inclusion criteria

Information was extracted and summarized from the remaining studies, and one author was contacted and asked for a full text paper. Our inclusion criteria consist of: a) individual studies; b) studies conducted in children and adolescents; c) studies conducted during the COVID-19 pandemic; d) studies evaluating depressive or anxiety symptomatology, comprised of statistical data and established instruments, tools, or scales; e) reviews focused only on studies published in English.

Data extraction

One researcher extracted data from every study that was included. A summary of selected variables included: first author, year of publication, countries, population, sample size, age range, sex (% female), instruments, and primary outcome (Figure 1).

Quality assessment

The overall results of the meta-analysis depend indeed also on a rigorous evaluation of the studies' quality. Among all the possible tools for this complex evaluation, the Newcastle Ottawa Scale (NOS) is one of the most used worldwide, above all, for observational studies [6]. The NOS is a validated instrument and has a long history of reliability commonly used in medicine in the field of psychiatry, in both observational and interventional studies' meta-analyses.

Each study was assessed for risk of bias with the Newcastle-Ottawa Quality Assessment Scale adapted from the NOS for cohort studies to cross-sectional studies developed by the collaboration between the University of Newcastle Australia and Canada [7].

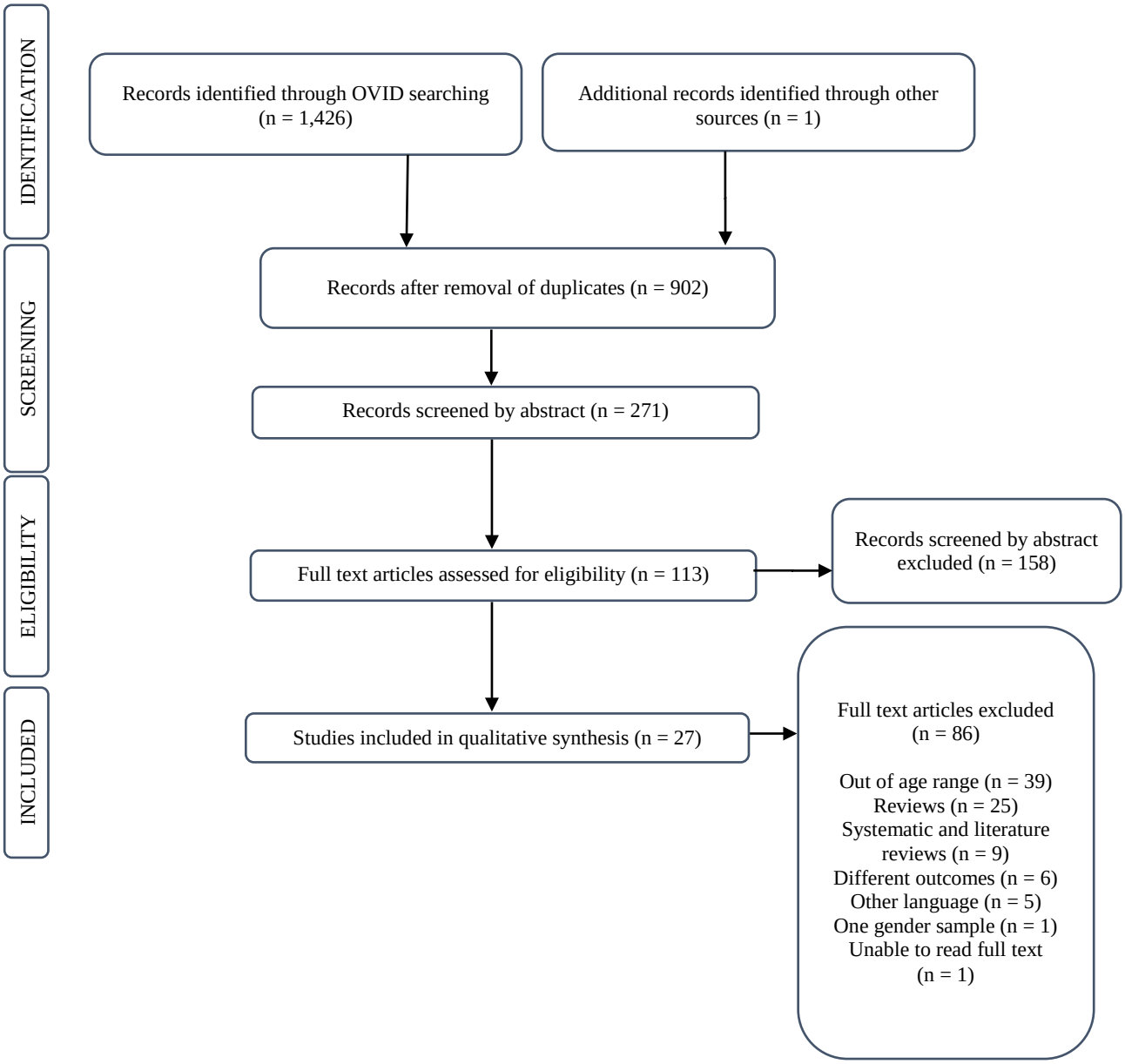

Fig. 1 – PRISMA flow diagram for systematic review.

The NOS assesses the quality of nonrandomized studies with a star system in order to have a proper understanding of each study. The maximum score is 10 points (10 stars) (Table 1) [8–34].

Table 1

Quality assessment with Newcastle-Ottawa Scale adapted for cross-sectional studies

Study	Score
Abawi et al. [8]	7 – Good study
Adıbelli et al. [9]	7 – Good study
Asanov et al. [10]	8 – Good study
Chen et al. [11]	7 – Good study
Garcia de Avila et al. [12]	7 – Good study
Duan et al. [13]	7 – Good study
Fazeli et al. [14]	8 – Good study
Giannopoulou et al. [15]	8 – Good study
Guo et al. [16]	8 – Good study
Hou et al. [17]	6 – Satisfactory study
Kılınçel et al. [18]	6 – Satisfactory study
Li et al. [19]	7 – Good study
McGuine et al. [20]	6 – Satisfactory study
Metwally et al. [21]	7 – Good study
Oosterhoff et al. [22]	8 – Good study
Qi et al. [23]	7 – Good study
Seçer et al. [24]	7 – Good study
Smirni et al. [25]	8 – Good study
Tang et al. [26]	8 – Good study
Xie et al. [27]	8 – Good study
Zhang et al. [28]	6 – Satisfactory study
Ellis et al. [29]	7 – Good study
Magson et al. [30]	7 – Good study
Ougrin et al. [31]	8 – Good study
Alves et al. [32]	7 – Good study
Rogers et al. [33]	7 – Good study
Dilek et al. [34]	6 – Satisfactory study

Results

The initial search identified 1,426 records; upon duplication, it was reduced to 902 and screened by date since the COVID-19 global pandemic in early 2020; studies published in 2019 were excluded. After the first screening, 271 articles were reviewed by their abstracts, and 158 records were omitted for failing to meet the inclusion criteria; for instance, studies that did not include the desired population age range (n = 39), reviews without a quantitative method (n = 25), systematic and literature reviews (n = 9), studies with different outcomes (n = 6), studies written in another language (n = 5), and one gender sample (n = 1).

Synthesis includes 27 observational studies with 21 cross-sectional studies, 4 longitudinal cohorts, 1 case-control, and 1 mixed-method study. Twenty different countries were represented: Asia (20%), Europe (50%), America (20%), Africa (5%), and Oceania (5%). The overall population is 52,797 children and adolescents from 6 to 19 years old (out of which 54.12% were females) (Table 2).

The desired outcome (anxiety and depressive symptomatology) was measured by each study with different instruments and a combination of clinical interviews and assessments. The main characteristics of the studies, including the most common instrument for anxiety – the 7-item Generalized Anxiety Disorder (GAD)-7 Scale [15, 17, 19] and for depression, The Pediatric Quality of Life Inventory 4.0 (PedsQL) [8, 14, 20], are shown in Table 2 [8–34].

Results for anxiety symptomatology

Ten out of 27 studies looked only for anxiety symptoms and 14 looked for anxiety and depressive symptomatology (Table 2). Fourteen found that anxiety levels were significantly higher among children and adolescents during the pandemic compared with previously published norms. For example, the proportion of the sample who screened positive for anxiety (GAD-7 $\geq$ 11) increased from 28.3%, before the pandemic, to 49.5% during confinement (McNemar test, $p < 0.0001$); those scoring within the severe anxiety range (GAD-7 $\geq$ 17) increased from 3.8% to 20.5% (McNemar test $p < 0.0001$). The comorbidity, defined as a positive screen for depression and anxiety, increased from 24% to 45% (McNemar-Bowker, test $p < 0.0001$) [15]. Some studies also looked at different positive correlations between anxiety and other variables, like clinical depressive symptoms and anxiety [15, 21, 25–28, 33], smartphone and internet addiction [13, 18], lower levels of family income [12, 30], perceived threats [13, 16, 17], higher grades at school [11, 17], loneliness [29], poor academic records [34], TV as the main source of communication [29], social stigma [19, 22], friends or family conflict [34], and emotion-focused coping style [24].

Table 2

Main characteristics of the studies included (data extraction)

Study	Country	Study design	Sample size (n)	Age range (years)	Sex (% female)	Instruments	Primary outcome
Abawi et al. [8]	Netherlands	Cross-sectional	75	7–15	52	PedsQL Questionnaire, Phone Interview (20–30 min)	anxiety symptomatology
Adıbelli et al. [9]	Turkey	Cross-sectional	597	7–13	55	Generic Health-related, Quality of Life, Questionnaire for Children	depressive symptomatology
Alves et al. [32]	USA	Observational	64	9–15	63	State-Trait Anxiety Inventory for Children (STAIC), Positive and Negative Affect Schedule for Children (PANAS-C)	anxiety symptomatology
Asanov et al. [10]	Ecuador	Cross-sectional	1320	14–18	53	5-item MHI-5 index of Veit and Ware (1983)	depressive symptomatology
Chen et al. [11]	China	Cross-sectional	1036	0–19	49	Depression Self-Rating Scale for Children, Screen for Child Anxiety Related Disorders	anxiety symptomatology
Garcia de Avila et al. [12]	Brazil	Cross-sectional	289	6–12	54	The Children's Anxiety Questionnaire (CAQ) and the Numerical Rating Scale (NRS)	anxiety symptomatology
Dilek et al. [34]	Turkey	Case control	30	8–18	64	The State-Trait Anxiety Inventory (STAI), Hospital Anxiety and Depression Scale (HAD)	anxiety and depressive symptomatology
Duan et al. [13]	China	Cross-sectional	3613	7–18	50	Spence Child Anxiety Scale, Child Depression Inventory and Coping style Scale	anxiety and depressive symptomatology
Ellis et al. [29]	Canada	Longitudinal cohort	1054	14–18	74	COVID-19 Stress Questions, Brief Symptom Inventory (BSI), UCLA Loneliness Scale, Godin Leisure-Time Exercise Questionnaire	depressive symptomatology
Fazeli et al. [14]	Iran	Cross-sectional	1512	13–18	44	Depression, Anxiety, and Stress Scale-21 (DASS-21), Insomnia Severity Index(ISI), Internet Gaming Disorder Scale-Short Form (IGDS9-SF), Pediatric Quality of Life Inventory 4.0 Short Form (PedsQLTM 4.0 SF15)	anxiety symptomatology
Giannopoulou et al. [15]	Greece	Cross-sectional	442	16–18	68	Anxiety was measured using the 7-item Generalized Anxiety Disorder Scale (GAD-7)	anxiety and depressive symptomatology
Guo et al. [16]	China	Cross-sectional	6196	11–18	52	Post-traumatic stress symptoms (PTSS) were assessed with the self-report PTSD Checklist for DSM-5 (PCL-5), Anxiety was assessed by the Zung self-rated anxiety scale (SAS)	anxiety symptomatology
Hou et al. [17]	China	Cross-sectional	335	16	39	The 9-item Patient Health Questionnaire (PHQ-9), the 7-item Generalized Anxiety Disorder Scale (GAD-7), and the Impact of Events Scale - Revised (IES-R)	anxiety and depressive symptomatology
Kılınçel et al. [18]	Turkey	Cross-sectional	745	12–18	70	State Anxiety Inventory (STAI-S), Trait Anxiety Scale (STAI-T): UCLA loneliness scale	anxiety symptomatology
Li et al. [19]	China	Cross-sectional	1172	8–18	58	Social Cognition and Behaviour Investigation of COVID-19, Children's Revised Impact of Event Scale (CRIES-8), Generalized Anxiety Disorder scale (GAD-2) adapted from the Perceived Threat of the Middle East Respiratory Syndrome (MERS) scale	anxiety symptomatology

Table 2 (continued)

Study	Country	Study design	Sample size (n)	Age range (years)	Sex (% female)	Instruments	Primary outcome
Magson et al. [30]	Australia	Longitudinal cohort	248	13-16	51	The Generalized Anxiety subscale (e.g., "I worry about things") of the Spence Children's Anxiety Scale (SCAS-C), The Short Mood and Feelings Questionnaire – Child Version (SMFQ-C; Angold et al. 1995),The Student's Life Satisfaction Scale (SLSS; Huebner 1994),COVID-19 related stress	anxiety and depressive symptomatology
McGuine et al. [20]	USA	Cross-sectional	13002	9–19	53	General Anxiety Disorder-7 Item (GAD-7),Patient Health 16 Questionnaire-9 Item (PHQ-9), The Pediatric Functional Activity Brief Scale (PFABS) and the Pediatric Quality of Life Inventory 4.0 (PedsQL)	anxiety and depressive symptomatology
Metwally et al. [21]	Egypt	Cross-sectional	2040	8-12	51	Designed Questionnaire, Children completed self-report rating scale, Child psychiatrists interview with the DSM-IV panic symptoms	anxiety symptomatology
Oosterhoff et al. [22]	USA	Cross-sectional	683	13–18	75	8-item Patient-Reported Outcomes Measurement Information System Anxiety Scale	anxiety and depressive symptomatology
Ougrin et al. [31]	England, Scotland, Ireland, Austria, Italy, Hungary, Serbia, Turkey, Oman and the United Arab Emirates	Cohort	1795	NA–18	88	Hospital interview for self-harm	anxiety and depressive symptomatology
Qi et al. [23]	China	Cross-sectional	7202	14–18	NA	Patient Health Questionnaire-9, Chinese version of the 7-item Generalized Anxiety Disorder scale, Social Support Rate Scale	anxiety and depressive symptomatology
Rogers et al. [33]	USA	Mixed methods	609	14–17	NA	Open-ended questions, six-question Children's Depression Inventory−Short (CDI-S), generalized anxiety	anxiety and depressive symptomatology
Seçer et al. [24]	Turkey	Cross-sectional	598	14–18	61	Questionnaire is a self-reported seven-point Likert-type measurement tool adapted to the Turkish culture	anxiety and depressive symptomatology
Smirni et al. [25]	Italy	Cross-sectional	148	17–19	57	Self-Rating Anxiety Scale (SAS) Italian Emotion Awareness Questionnaire (EAQ) for children and adolescents	anxiety symptomatology
Tang et al. [26]	China	Cross-sectional	4391	1–12 (grade)	49	Depression, anxiety, and stress scale (DASS-21)	anxiety and depressive symptomatology
Xie et al. [27]	China	Cross-sectional	2330	6–12 (grade)	44	Children's Depression Inventory–Short Form (CDI-S) and the Screen for Child Anxiety Related Emotional Disorders, respectively	anxiety and depressive symptomatology
Zhang et al. [28]	China	Cohort	1271	4–8 (grade)	NA	Study questionnaire (with ethical approval)	anxiety and depressive symptomatology

NA – not available.

Six studies reported that females have consistently higher (32.2%) prevalence rates of anxiety disorders symptomology, although we can also see an increase in males (23.9%) using GAD-7 anxiety scale [17]. The data showed that being a female was a risk factor for anxiety symptomatology during the pandemic outbreak. Post-traumatic stress disorder (PTSD) symptomatology was also common at this sample with three studies outlining high rates.

Social distancing was also mentioned and studied statistically as a potential source of anxiety and depressive symptoms in three studies. Anxiety levels of children who "stayed at home" were more than five standard deviations [32] higher than before the pandemic [10]. Vulnerable groups with obesity and multiple sclerosis had a high percentage of anxiety symptoms [34].

Finally, one study found modest differences in anxiety with 0.2 to 0.6 standard deviations [30].

An overview of studies, including the findings of anxiety or depression in young examinees, is shown in Table 3 [8, 11, 12, 14, 16, 18, 19, 21, 25, 32] and Table 4 [9, 10, 13, 15, 17, 20, 22–24, 26–31, 33, 34].

Table 3

Results and main findings

Study	Main findings anxiety outcome	Main findings depression outcome
Abawi et al. [8]	Anxiety symptoms were reported for 24 out of 75 (32%) children.	NA
Alves et al. [32]	Anxiety levels of children who "stayed at home" were higher than in pediatric populations prior to the pandemic.	NA
Chen et al. [11]	Female adolescents presented a higher risk of presenting mood disorders. Nevertheless, there was no association with different ages or anxiety.	Older adolescents in the sample were more depressed than the younger ones. The data indicated that adolescents without companionship during the week were more likely to be depressed.
Garcia de Avila et al. [12]	Higher levels of anxiety were found among children with a prevalence of 19.4% (using the CAQ) and 21.8% (using the NRS).	NA
Fazeli et al. [14]	Anxiety is correlated with depression.	Quality of life in the pandemic is also affected by internet gaming disorder. Findings showed that variables such as internet gaming, insomnia, anxiety, depression, and stress had positive correlations, with small to large effects.
Guo et al. [16]	Exposure to COVID-19 predicted higher levels of PTSD and anxiety (0.06–0.15) (standardized betas).	NA
Kılınçel et al. [18]	Positive correlations were found between loneliness and anxiety (r:0.175, P:.001), (r:0.194, P:.000).	NA
Li et al. [19]	Children and adolescents are considered a vulnerable group that might experience anxiety and PTSD symptoms. The stigma around socializing and the perception of a threat could represent risk factors related to PTSD and GAD in the coronavirus pandemic.	NA
Metwally et al. [21]	Phobia, expressed through stress, avoiding people and panic, was the most prevalent disorder among young children.	NA
Smirni et al. [25]	Over half of the individuals reached a high anxiety score. Items regarding sleep, anxiety, panic, and negative expectation of the future reached high average scores.	NA

NA – not available.

Table 4

Results and main findings

Study	Main findings anxiety outcome	Main findings depression outcome
Adıbelli et al. [9]	Not available.	41.5% of children gained weight, 34.2% of children increased their sleeping time, and 69.3% stated that their internet use increased as a result of the COVID-19 lockdown.
Asanov et al. [10]	Not available.	16% of this sample scored for major depression disorder, while 68% reported being happy.
Dilek et al. [34]	Adolescents with multiple sclerosis (MS) diagnosis presented more anxiety than the control group (healthy group). Results show that 100% of MS children present anxiety symptoms, whereas this ratio was 34% for the control group.	During COVID-19, 43.3% of patients gained weight, and 73% had less exposure to sunlight.
Duan et al. [13]	91.06% of children and adolescents reported higher levels of social phobia, generalized anxiety, panic disorder, physical injury, and separation anxiety. These levels also correlated with depression symptoms, smartphone and internet use.	Depressive symptomatology among children and adolescents (22.28%) was higher than in prior studies (13.2%)
Ellis et al. [29]	Not available.	Social media frequency use increased during the COVID-19 pandemic while 28% of the respondents showed depression symptoms.
Giannopoulou et al. [15]	The number of samples who screened positive for anxiety was higher (49.5%) (GAD-7 $\geq$ 11) in the home confinement than before the COVID-19 pandemic (28.3%) (GAD-7 $\geq$ 11). The proportion of those with severe anxiety rose from 3.8% to 20.5% (GAD-7 $\geq$ 17) (McNemar test $p < 0.0001$).	The proportion of all respondents indicating positive screen for depression increased from 48.5% before coronavirus to 63.8% for the time of social distancing (McNemar test $p < 0.0001$) and of those, scoring within the severe depressive symptomatology (PHQ-9 $\geq$ 20), increased from 10% to 27% ($p < 0.001$).
Hou et al. [17]	54.5% presented anxiety symptoms, and 85.5% presented PTSD symptomatology.	The results indicated that 71.5% presented some depression symptoms.
Magson et al. [30]	While significant decrements in mental health were demonstrated, the size of these effects is modest with 0.2 to 0.6 standard deviations.	Feeling lonely without social connections during the pandemic was correlated with higher levels of depression symptoms and low life satisfaction.
McGuine et al. [20]	Data reveals that athlete adolescents from lower-income families experienced high levels of depression and anxiety while physical activity and quality of life drops.	The prevalence of depression symptoms was higher in team sports (74.1%) and lowest in individual sports (64.9%).
Oosterhoff et al. [22]	Several reasons and motivations for social distance have different impacts on mental and social health. Young people who were persistent in social distancing in order to stay safe reported less burdensomeness and greater anxiety. On the contrary, adolescents who were doing social distancing to avoid social judgement felt more anxious than depressed.	Depression symptoms were found in the youth that was following social distancing due to peer recommendation, while adolescents who preferred to stay home presented lower depressive symptoms.
Ougrin et al. [31]	The proportion of youth with an emotional disorder increased from 58% in 2019 to 66% emotional disorder in 2020. The proportion of youth presenting with self-harm tendencies increased from 50% in 2019 to 57% in 2020, and out of the clinical diagnoses that present self-harm, emotional disorders (anxiety).	The proportion of children and adolescents self-harming with suicide intent was 49% in 2019 and 55% in 2020 across all areas. However, this apparent increase in 2020 did not reach the threshold required for significance ($p = 0.057$).
Qi et al. [23]	The prevalence of anxiety symptoms is 38%. The rate is high even though the pre-pandemic prevalence is unknown.	Prevalence of depressive symptoms is higher than anxiety symptoms by 44%.

Table 4 (continued)

Study	Main findings anxiety outcome	Main findings depression outcome
Rogers et al. [33]	Adolescents reported distinctly challenging poor social interaction during the outbreak, the ability to get out of the house was the second hardest thing to cope with, followed by the lack of privacy and family conflict.	Although the quantitative results revealed a low prevalence of mental health problems, paired samples t-test showed an increase in depression and feelings of loneliness.
Seçer et al. [24]	The fear of getting infected with the virus is a risk factor for anxiety symptoms in adolescents.	The negative emotional reactivity caused by the fright of virus infection predicted depression symptoms in adolescents. Depression and anxiety are predictors of OCD.
Tang et al. [26]	Anxiety symptoms were the most prevalent, especially in middle school pupils who had more pressure on academic performance. The prevalence of anxiety was 24.9%.	Depression symptoms were found in 19.7% of the sample, which was similar to other findings.
Xie et al. [27]	The anxiety prevalence of the sample was 18%. On the other hand, most children worried about being infected with the COVID-19 virus.	22% of the sample reported depressive symptoms and low optimism regarding the pandemic.
Zhang et al. [28]	An increase in anxiety symptomatology was not found.	Depression symptoms are present and higher in students in the second wave of the cohort.

Results for depressive symptomatology

Three out of 27 studies looked only for depressive symptoms and 14 for anxiety and depressive symptomatology. Weight and sleep changes were mentioned in 3 different studies with disturbances in children and adolescents' mental health. Sixteen percent of a sample had the criteria for a major depressive disorder using the 5-item MHI-5 index of Veit and Ware (1983) [10]. One study was consistent with higher reports of female depression. After a multivariate analysis, the main difference was found in gender; girls presented higher depression than males [mean (M) = 2.85, standard error (SE) = 0.06 *vs* M = 2.26, SE = 0.15, respectively], loneliness (M = 2.64, SE = 0.03 *vs* M = 2.50, SE = 0.09, respectivel), and COVID-19 stress (M = 2.92, SE = 0.03 *vs* M = 2.69, SE = 0.06, respectively) [11].

Some positive correlations between depression and other factors were found in 17 included articles. Children and adolescents that were socially disconnected and did not have social support experienced more depressive symptoms (3/17). Children from lower-income families presented more depressive symptoms (1/17), mothers with higher levels of education and income were associated with happiness (2/17) as well as internet access (1/17). Age was also an important variable since three studies reported higher depression in higher grades.

The most consistent statement reported in seven studies was that higher levels of depression were found. Prevalence of youth with an emotional disorder increased from 58% in 2019 to 66% in 2020. The increase of emotional disorders tested statistically significant with an estimated odds ratio of 1.58, 95% confidence interval 1.06 to 2.36; $p = 0.025$). In 2019, 49% of children and adolescents were engaged in self-harm behavior with suicide intents, and in 2020, it was 55% ($p = 0.057$) [31]. Finally, there was one sample that reported that 68% of their population was feeling happy [10].

Discussion

To the best of our knowledge, this is the first systematic review to have exhaustively searched for the impact of the SARS/MERS/COVID-19 pandemic on children and adolescents' depression and anxiety symptomatology. On the one hand, the current studies demonstrate that the overall proportion of youth with an emotional disorder has increased. Anxiety symptomatology is one of the main concerns since it was found in 24 of our included studies. On the other hand, clinical depression was the second symptomatology most frequently found with a higher prevalence than the generally estimated in previous years. It is important to highlight that some of our included studies report greater symptoms of depression than anxiety [23]. However, more data directly reveal greater anxiety; thus, we can argue that anxiety has been more studied than depression in the current outbreak, and, therefore, more data can be found.

Additionally, different variables played an important role in several studies; some of the most consistent ones affecting anxiety and depression are children living in isolation without their parents or with no social support [33]. The level of parents' education was also a risk factor; children whose guardians had a postgraduate education ($p = 0.019$) had lower anxiety scores [12] and felt happier [10]. Moreover, data suggest that as the level of poverty increases, symptoms of anxiety and depression grow as well [20].

Furthermore, the usage frequency of social media, the internet, smartphones, and television [18] has escalated, in contrast to the past, prior to the pandemic [11]; there are positive relationships between online gaming with insomnia, depression, anxiety, and stress, with small to large effects. These findings indicate that as one variable increases, so do others [14]. The major comorbidity of anxiety is depression and

vice versa; thus, it is estimated that one variable affects the other in terms of risk factors.

Regarding population differences, the data show impressive and consistent results: female teenagers are experiencing greater declines in mood disorders than male teenagers during the COVID-19 crisis [11]. Briefly examining those numbers, the report of the NHS Mental Health of Children and Young People in England (2017) states that young women (17–19 years old) have been identified as a high-risk group in relation to emotional mental health. Therefore, this information confirms that the impact of the COVID-19 outbreak was not an exception, and rates were also higher within this population. It is hypothesized that young women rely more on their social circles as a coping strategy, and in the outbreak, this was difficult to achieve. A difference was also found in the prevalence of anxiety and depression regarding age. The older you are, the more likely you are to present symptoms of an affective disorder during SARS/MERS/COVID-19 [11]. At-risk populations with medically diagnosed children and adolescents experienced higher rates of anxiety due to the threat of infection and depressive symptoms like gaining weight [34].

Limitation of the study

A considerable limitation is that results are mainly from cross-sectional studies, so we cannot attribute causality to the rise of psychopathology criteria and COVID-19. Furthermore, some of the instruments included are not standardized and were designed uniquely for the study. Either way, these highlight key data and information to be examined. It is now crucial to observe whether these high levels of anxiety and depression will persist over time, disappear, become a clinical disorder, or whether there will be risk factors for other outcomes. Another limitation is that data collection was completed by online questionaries, and results are based on the comprehension of the instrument by young children and adolescents. Moreover, we are providing a range of quantitative data; however, the analysis is qualitative one, thus, a meta-analysis study will give us more accurate numbers and a broader understanding of this mental health matter. It is important to highlight that even though there was anxiety and depressive symptomology, a mental health disorder cannot be confirmed. Finally, the only two outcomes studied were anxiety and depression, which are two difficult outcomes to define.

Conclusion

The COVID-19 pandemic is having a strong impact on the mental health of children and adolescents regarding anxiety and depressive symptomatology. Public health systems should provide efficient and gold-standard interventions to this vulnerable group that could be damaged on a long-term basis. Prevention programs focused on coping strategies should also be conducted in elementary schools, middle schools, and high schools. Finally, mental health should become a priority matter for governments, and the current pandemic could be an opportunity to highlight the importance of mental well-being and to invest in the betterment of clinical training, treatments, and mental health research.

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Challenges, standards, and prospects in the therapy of orthodontic traction of impacted maxillary canine – A surgical phase

Izazovi, standardi i pravci razvoja terapije izvlačenja impaktiranog maksilarnog očnjaka – hirurška faza

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Ključne reči:
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Introduction

The maxillary canine is the third tooth from the midline, positioned between the frontal and lateral zone. It is usually characterized by the longest root of all teeth and the longest eruption period. It is exposed to tremendous masticatory pressure and located in the smile zone. The tooth erupts between 11–12 years of age, while the root completion occurs at the age of 13–15 [1, 2]. The impacted tooth (*impactio dentis*) is a tooth whose eruption is considerably delayed and for which there is clinical or radiographic evidence that further eruption may not take place due to the existence of various barriers. In dentistry, the terms *impactio* and *retentio* are explained as a failure of tooth eruption caused by detectable and undetectable reasons for tooth emergence, respectively.

The etiology of canine impaction is multifactorial, encompassing both genetic and local factors [3, 4]. The delay or complete cessation of tooth eruption may be due to a long and tortuous eruption pathway, trauma, improper position of the tooth germ, fibrous callus in mucoperiosteum, sclerotic changes in bone, mucosal thickening, existing impacted teeth, hyperdontia, odontoma, cysts, tumors, etc.

Upper canines are very frequently reported impacted teeth, second only to third molars. The prevalence of impacted maxillary canines ranges between 0.92% and 2.56% [5–7]. Upper canine impaction is reported in 3% of the population, with gender prevalence rates being 70% and 30% in females and males, respectively. The incidence of the palatal location of impacted maxillary canines is three times more frequent than that of labial location. In fact, 85% of palatally impacted canines have sufficient space to erupt into the dental arch, contrary to only 17% of labially impacted ones.

Unerupted maxillary canine can be extracted or preserved. Extraction of the deciduous canine is an interceptive treatment in children with canines positioned palatal [8]. Therapy options include the following: interceptive extraction of primary canine and placement of space maintainer; surgical extraction of the canine and moving the first premolar into its site; surgical extraction of the canine and use of fixed denture (bridge or implant); exposure of the canine to enable spontaneous eruption and growth; exposure of the canine and active traction to move the tooth into the dental arch; exposure of the canine and active traction to move the tooth into the dental arch using the orthodontic implant as an anchorage; transplantation of the canine with follicle; no active treatment (monitoring) [5]. For several purposes, preservation and adequate positioning of the canine into the dental arch is desirable. Treatment decision is made exclusively by an orthodontist. Preservation of the tooth implicates the removal of physical barriers in its eruption path and enabling its spontaneous positioning to the desired site (provided that there is evidence of potential growth, incomplete growth, and sufficient space). Exposure of impacted tooth is managed using open and closed surgical techniques. The term “tooth denudation” is used for the open method. After the surgical procedure, the tooth is uncovered from tissues, becoming visible in the oral cavity. The term “tooth liberation” is associated with the closed surgical

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technique in which the tooth remains invisible after the surgery. The method was first described by Hunt [9] and Mc Bride [10]. In case the cycle of tooth root growth and development has not yet been completed, the tooth might be allowed to erupt spontaneously. If the growth cycle is completed or if the tooth is improperly positioned, orthodontic traction management is required. In the closed method, the elements of traction appliances are placed intraoperatively. Moisture and blood contamination of conditioned enamel results in weakening the adhesive bond with the tooth. Activation of the traction (after surgery) can lead to adhesive bond failure, thus, an additional surgical procedure might be required. A range of traction methods and appliances for managing the impacted tooth is available. The system should be safe, immobile, permanent, and stable. The adhesion bond itself is highly complex.

Radiographic examination – timely diagnosis of impacted canine

Radiographic examination is indicated in case the tooth has not erupted after the expected optimal eruption time, while the same tooth erupted normally on the other side. The absence of the tooth (anodontia) and the exact position of the impacted tooth should be determined. Scan analysis identifies the position of the impacted tooth, root morphology (curvature of the apex), relationship to adjacent teeth, distance from the alveolar bone limbus, relationship towards maxillary sinus and nasal cavity, possible adjacent tooth root resorption (mostly second incisor) [11], presence of a follicular cyst, odontoma and supernumerary teeth. Periapical radiography offers only basic information [12]. The position of the teeth was determined based on two images, the phenomenon of parallax, Clark's rule. Commonly, the term rule Buccal Always Moves Away (BAMA) is used. Sometimes, an occlusal radiographs image was used as a supplement.

Orthopantomogram (OPG) is standard radiography, which is widely applied in routine practice. Lateral cephalometry and posteroanterior cephalometry provide data that are critical for adequate therapy planning. Novel digital technologies and software analysis increased the processing speed and accuracy for a range of parameters, which undoubtedly led to the improvement of the therapeutic approach. Ericson and Kurol [13] defined the term "sector analysis" that involves three sectors in an OPG scan that enable differentiation between diverse impaction types. Four lines in the OPG scan determine three sectors defining impaction types. These include medial line (interincisal) and lines extending along the axes of the first premolar and first and second incisor. The position of the canine crown tip within a specific sector is determined. Moreover, the Ɵ-angle formed by the longitudinal axis of the canine tooth with interincisal line is defined. This analysis is vital for the prognosis of the success of the orthodontic traction procedure. The risk of root resorption of the lateral incisor is increased by some 50% if the canine is positioned in sectors 1 and 2 and with a Ɵ-angle above 25°.

Cone beam computed tomography (CBCT) enables an accurate and precise indication for tooth traction or extraction. The method prevents diagnostic errors that were common in the past and should evolve into the new standard of imaging [14–18].

Major reasons for therapy failure and surgical techniques

Therapy failure mainly considers the dropping out of active traction therapy. In an ideal situation, extraction should be indicated before starting the orthodontic therapy. The tooth is surgically extracted to prevent wasting of time for creating sufficient space to accommodate teeth in the jaws.

According to the relevant protocol, a presence of an orthodontist is required during the surgical procedure in order to get an insight into the position of the tooth and surrounding tissue structures and estimate the appropriate position of traction appliances [19, 20]. Extremely rarely, the orthodontist might change his/her decision about the therapeutic approach during the first surgical tooth exposure. In that case, upon removal of tissue structures covering the crown, the orthodontist may decide that tooth extraction is highly indicated and informs the oral surgeon and parents thereof.

The failure of adhesive bonds can occur during the period of tooth traction and consequent repeated surgical procedure. Quite often, the patients lack motivation to continue with the treatment due to possible failure [21]. The selection of relevant surgical procedures highly determines further treatment course. Open surgery has certain advantages, such as the presence of an orthodontist is not required, and detachment of the bracket does not indicate repeated surgery. The advantages of closed surgery include fast wound healing, less discomfort for the patient, good postoperative hemostasis, lesser functional disorder and bone removal, immediate application of orthodontic traction, more consistent tooth-to-adhesive bond, and feasibility of the procedure even in close proximity of adjacent tooth root resorption. In the case of closed surgery, the presence of an orthodontist is highly required [22, 23].

Intraoperative reasons for therapy failure

Poor (or broken) bond of the orthodontic appliance at the impacted tooth can compromise the success of orthodontic surgical treatment [24].

The advancements in the field of adhesives and composite materials enabled the placement of orthodontic brackets onto the impacted tooth. In the case of the closed technique of tooth exposure, the adhesion bond between unerupted tooth and traction anchorage must be strong because of the resistive force of surrounding soft tissues. Such force acts in the opposite direction of tooth traction and has a tendency to break the bond between the tooth and orthodontic appliance. In case of bond failure, a repeated surgical procedure will be inevitably indicated. This is the

major problem to be avoided and the issue of reinforcement of the bond is to be emphasized. The basic bond is between the enamel and adhesive material, according to Miletić and Santini [25]. Furthermore, important bonds include adhesive-to-composite, composite-to-bracket, bracket-to-connector, and connector-to-orthodontic appliance attachments.

A comprehensive, team-based approach to bracket positioning is imperative. The placement of traction elements is performed by the orthodontist. Hemostasis performed by a surgeon is a prerequisite for the successful outcome of this stage. Sometimes, persistent hemorrhage requires an application of pressure on the bleeding spot using a blunt instrument, bone wax, or cauterization. How to overcome the problem of conditioned enamel contamination and failure of the enamel-to-adhesive bond? Intraoperative placement of traction elements is complicated by humidity in the mouth cavity (saliva, blood), according to Varga and Šlaj [26]. After the removal of the acid and enamel drying, the adhesive is applied onto the tooth and light-polymerized. The majority of orthodontic adhesives used for bracket sealing are hydrophobic, i.e., their bond strength decreases significantly in a humid environment. Thus, the procedure should be performed quickly, and blood penetration must be prevented. If contamination still occurs, blood should be removed with alcohol and cotton pellet and dried using sterile gauze.

The emergence of the novel 7th generation monophase (self-etch) hydrophilic adhesives can substantially improve the tooth-to-adhesive bond strength in the presence of contamination [27]. A one-step procedure for conditioning and application of the adhesive decreases the risk for moisture contamination of the tooth. These adhesives show lower adhesion strength as compared to hydrophobic ones, yet only under ideal conditions. However, in humid conditions, hydrophilic adhesives demonstrate higher adhesion strength than hydrophobic ones.

It has been observed that the self-etching adhesives demonstrated higher bond strength to dentin than enamel. The fifth protocol, "selective etching", has been introduced to improve the enamel-to-self-etching adhesive bond. The protocol entails enamel treatment with orthophosphoric acid during 15 s. The self-etching monocomponent adhesive removes potential moisture remaining from etched enamel. The adhesive is applied and allowed to penetrate for 20 s. This step is followed by polymerization for 20 s [25, 28].

A wide variety of adhesive systems is available on the market. All of them consist of three components – acid, primer, and bond. The systems can be divided into two groups: total-etch and self-etch adhesives. Total-etch adhesives encompass three-step acid-etch systems (acid, primer, and bonding agent as three separate components) and two-step acid-etch systems (acid as a separate component and single bottle of primer+bonding agent). Self-etch systems can be either two-step self-etch adhesive (self-etching primer and bond) or monophase self-etching adhesive (all components in a single bottle).

The clinician should be focused on providing a dry operating environment and fixing the brackets or chain with composite, whereas adhesive application itself is not considered a crucial factor for the stability and durability of the bond. The knowledge of orthodontists and oral surgeons about adhesive systems and protocols is still limited. Modern adhesive application protocols were developed and described by Van Meerbeek et al. [29] in 2003. Two-step acid-etch adhesive is most commonly used. One-step self-etch adhesives are not widely used in everyday routines.

The answer to the question of whether to use monophase self-etching adhesive can be obtained only from an *in vitro* experiment [30, 31]. The use of extracted teeth enables simulation of specific conditions and measurement of shear bond strength. The majority of studies investigated the force applied at an angle of 90° (pull-out strength), commonly in ideal and very rarely under humid conditions. Experimental settings mimicking authentic surgical environment (blood, moisture) and shear de-bonding are very rare [32, 33]. Clearly defined protocols with guidelines for the selection of an adhesive to be used in conditions of contamination (moisture, blood) are still lacking. The authors of this paper carried out a study on these basics in order to introduce a safer work protocol. The improvement of micro-mechanical bonding in conditions of enamel contamination and recommendations for the type of adhesive to use is the goal of future research. Oonsombat et al. [34] and Sfondrini et al. [35] pointed out the drop of shear bond strength associated with blood contamination. The authors also emphasized that the application of self-etch primers produces stronger bonds, which is due to hydrolysis that facilitates partial cleaning. The qualitative properties of 7th generation monophase self-etch adhesive systems have not yet been adequately investigated in a surgical environment, where blood and physiological saline can cause enamel contamination. Some of these agents do not contain hydroxyethyl methacrylate and bisphenol A (either its derivatives), and acetone provides evaporation of residual water. Such adhesive systems show high shear bond strength along with fluoride-releasing behavior, while conditioning, priming, and bonding processes take part simultaneously [36].

After adhesive polymerization, the composite material is applied onto the orthodontic bracket, placed onto the adhesive zone on the tooth, and light-polymerized. Bracket conditioning entails the placement of ligature wire onto the bracket head. This technique is most commonly practiced [37].

In closed surgery technique, where the impacted tooth is covered with a flap and thus invisible, the only link with the outside environment is established *via* wire ligature, gold-chain, or elastic bands attached to the bracket before bonding [5, 19]. Such mediators are termed connectors. Surprisingly, gold chains have been widely accepted and approved thanks to their adequate strength and easy handling and placement, regardless of their high cost and poor availability on the market. Ligatures made of stainless steel are a simpler alternative. Stainless steel wire is safe in the hands of both the orthodontist and oral surgeon.

Standard brackets are considerable in size and with a high, wide, and sharp profile. The body of the bracket moves along with the tooth during its eruption and produces

irritation of the mucosa. The tension of wire ligature twisted around the bracket towards the arch increases friction against the soft tissue, thus leading to inflammation and potentially irreversible damage of periodontal tissues. Novel trends in orthodontics involve direct placement of 14 k gold chains [19] with small chain links that are gradually removed as the tooth erupts. In this way, the maximum enamel surface is conditioned (wider than bracket surface), the chain is vertically bonded and covered with the composite. Contrary to the brackets, there is no coalescence of the composite and soft tissue; thus, the resistance of soft tissue is minimal. Consequently, every bracket placed on the tooth (when using the closed technique) should be small enough and low-height profile in order to produce minimum adverse effect on surrounding soft tissues.

Mini implants are increasingly used as an anchoring center when pulling impacted canines [38].

There is a growing trend to use mini implants in certain circumstances.

Conclusion

The prerequisites for successful treatment outcome of orthodontic traction of retained upper canines include patient's motivation and compliance with a long-term therapy as well as systematic and comprehensive planning of treatment course. A team-based approach is indispensable. From the technical point of view, maximal concentration of both the orthodontist and oral surgeon is essential, especially after enamel conditioning. In case of blood contamination, the use of 7th generation adhesive is indicated. Application of gold chain attachment is recommended, along with maximal conditioning of the enamel area. The chain should be covered with composite to minimize the resistive force of the tissue.

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CASE REPORTS

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Incidental finding of an ovarian epithelial tumor, adequate approach and fertility preservation – A case report

Slučajni nalaz epitelnog tumora ovarijuma, odgovarajući pristup i očuvanje plodnosti

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Abstract

Introduction. Ovarian carcinoma is the fifth leading cause of death in women. In 3–14% of cases, it occurs in women under the age of 40 who intend to have children. Studies have shown a high survival rate if the tumor is diagnosed and treated at an early stage, with a 5-year survival rate of 91.2%, which makes a conservative treatment a valid option. Preserving fertility is safe for grade 1 and 2 of the International Federation of Gynecology and Obstetrics (FIGO) stage I epithelial ovarian carcinomas. A sparing operation involves salpingoophorectomy on the tumor side, multiple biopsies of suspected sites, blind biopsies and infracolic omentectomy, as well as cytological analysis of the wash. **Case report.** A 25-year-old patient, G0, P0, went to the gynecologist due to severe pain in the lesser pelvis. An ultrasound examination revealed a cystic hypoechoic alteration in the right ovary of about 5 × 6 cm, suspected for torsion, and the patient was urgently operated. A right cystectomy was performed. The histopathological finding of the surgically removed cyst was: endometrioid adenocarcinoma of the ovary, histological grade 2 (HG2) and nuclear grade 2 (NG2), without lymphovascular invasion and no penetration of the capsule, submitted in parts. As the surgery performed did not reveal the degree of ovarian malignant tumor spreading, the FIGO stage could not be determined, and a second operation was necessary to stage the disease according to the FIGO protocol for ovarian cancer. **Conclusion.** Fertility preservation in patients with malignant ovarian epithelial tumors is a major challenge. The intense desire of the patient to have children has to be satisfied without reducing the success of treatment for this type of disease. The staging of the disease spreading is of paramount importance in order to make an adequate decision regarding the treatment.

Key words:
diagnosis; fertility preservation; gynecologic surgical procedures; histological techniques; incidental findings; neoplasm staging; ovarian neoplasms; ultrasonography.

Apstrakt

Uvod. Karcinom jajnika je peti vodeći uzrok smrti kod žena. Od ukupnog broja obolelih žena, 3–14% su mlađe od 40 godina i imaju želju za rađanjem. Studije su pokazale visoku stopu preživljavanja ukoliko se tumor dijagnostikuje i leči u ranom stadijumu, a petogodišnje preživljavanje iznosi 91,2%, pa se konzervativni način lečenja može smatrati validnom opcijom. Očuvanje fertiliteta je bezbedno za gradus 1 i 2 FIGO (*International Federation of Gynecology and Obstetrics*) stadijuma I epitelnih ovarijalnih karcinoma. Poštedna operacija podrazumeva salpingooforektomiju na strani tumora, multiple biopsije sumnjivih mesta, biopsije na slepo i infrakoličnu omentektomiju, kao i citološku analizu lavata. **Prikaz bolesnika.** Bolesnica stara 25 godina, G0, P0, javila se ginekologu zbog jakih bolova u maloj karlici. Ultrazvučnim pregledom dijagnostikovana je cistična hipoehogena promena na desnom jajniku promera 5 × 6 cm, suspektna na torziju zbog čega je bolesnica hitno operisana. Učinjena je desna cistektomija. Histopatološki nalaz operativno odstranjene ciste bio je: endometrijalni adenokarcinom ovarijuma, histološkog gradusa 2 (HG2) i nuklearnog gradusa 2 (NG2), bez limfovaskularne invazije i bez proboja kapsule, dostavljen u delovima. Kako urađenom operacijom nije utvrđen stepen rasprostranjenosti malignog tumora jajnika FIGO stadijum nije određen zbog čega je bilo neophodno uraditi reoperaciju u cilju određivanja stadijuma oboljenja prema FIGO protokolu za karcinom ovarijuma. **Zaključak.** Očuvanje fertiliteta kod pacijntkinja sa malignim tumorima jajnika predstavlja veliki izazov. Potrebno je zadovoljiti snažnu želju za ostvarivanjem materinstva, a da se ne smanji uspešnost u lečenju tog tipa oboljenja. Određivanje stadijuma raširenosti bolesti je od izuzetne važnosti za donošenje adekvatne odluke o načinu lečenja.

Ključne reči:
dijagnoza; plodnost, očuvanje; hirurgija, ginekološka, procedure; histološke tehnike; slučajni nalazi; neoplazme, određivanje stadijuma; jajnik, neoplazme; ultrasonografija.

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Introduction

Ovarian carcinoma is the fifth leading cause of death in women. In the US, 22,140 new cases were registered in 2017, among which 14,080 patients died of this disease. It is most often discovered in an advanced stage, and it is more common among older women aged between 55 and 64 [1]. In 3–14% of cases, it occurs in women under the age of 40 who intend to have children. Fertility preservation can safely be achieved in germinative type ovarian tumors, in stromal cell tumors, and in the case of borderline tumors. In the case of ovarian tumors of epithelial origin, enabling women to give birth represents a major challenge. About 30% of patients with epithelial ovarian carcinoma (EOC) are diagnosed with the International Federation of Gynecology and Obstetrics (FIGO) stage I, and 13% want to have a child, making the radical approach unacceptable for such patients [2]. Studies have shown a high survival rate if the tumor is diagnosed and treated at an early stage, with a 5-year survival rate of 91.2%, which makes a conservative treatment a valid option [3]. Preserving fertility is safe for grade 1 and 2 of the FIGO stage I EOC. In cases of grade 3 tumors, aggressive clear cell type tumors, anaplastic, and small cell tumors, a conservative approach is not advised [4]. A sparing operation involves salpingoophorectomy on the tumor side, multiple biopsies of suspected sites, blind biopsies and infracolic omentectomy, as well as cytological analysis of the wash.

We presented a case of a young woman who had not given birth and had been surgically treated due to lesser pelvis pains when an early stage EOC was discovered.

Case report

A 25-year-old patient, G0, P0, went to the gynecologist due to severe pain in the lesser pelvis. An ultrasound examination revealed a cystic hypoechoic alteration in the right ovary of about 5 × 6 cm, suspected for torsion. The left ovary and the uterus presented usual ultrasound characteristics for the age; there was no free fluid in the pouch of Douglas. Laboratory and biochemical analyses were regular, with cancer antigen 125 (Ca 125) tumor marker being 18.56 U/mL (reference value 0–35 U/mL). Due to severe pain, the patient was urgently operated on after being admitted to the hospital. Intraoperatively, on the right ovary, a torsioned cyst was found presenting a smooth capsule with clear content, whilst there was no free fluid in the abdomen. A right cystectomy was performed. The histopathological finding of the surgically removed cyst was: endometrioid adenocarcinoma of the ovary, histological grade 2 (HG2) and nuclear grade 2 (NG2), without lymphovascular invasion and no penetration of the capsule, submitted in parts.

As the surgery performed did not reveal the degree of ovarian malignant tumor spreading, the FIGO stage could not be determined, and a second operation was necessary to stage the disease according to the FIGO protocol for ovarian cancer. An additional computerized tomography (CT) of the abdomen and lesser pelvis, as well as the X-ray of the lungs, performed preoperatively, did not reveal pathological changes.

One month after the first surgery, the patient underwent a second operation. On this occasion, the lesser pelvis and abdomen were explored. No abnormality of the uterus and the adnexa was found, as well as no pathological changes or the serous intestine injury, parietal peritoneum was smooth, the liver was smooth with sharp margins, and nothing abnormal detected, lymph glands in the lesser pelvis and paraaortally were not palpable. A lavage fluid sample was sent for cytological analysis, and right salpingoophorectomy and lymphadenectomy of the right iliac and obturator region were performed as well as the infracolic omentectomy. The material was sent for histopathological analysis.

The histopathological results after the second operation showed that there were no significant pathological changes on the tissue samples taken, and the cytological test detected no abnormalities so that based on this and the previous operation, a FIGO stage IC1 of the disease was determined. Considering the fact that the patient had a strong desire to have children, having discussed this with her, the multidisciplinary team decided to have her undergo regular controls every 3 months and that after her pregnancy she has to undergo a radicalization of the previous surgery. A repeated verification of the Ca 125 tumor marker, as well as the CT scan of the abdomen and lesser pelvis at the check-ups, were satisfactory.

Discussion

EOC is the most common type of ovarian tumor and occurs in 90% of the patients. It exhibits various genetic mutations, and a subdivision in two types has been introduced based on genetic and pathohistological characteristics: type 1, which comprises serous low grade, endometrioid, mucinous and clear cell epithelial tumors of the ovary, and type 2, which comprises high grade serous, high grade endometrioid, malignant combined mesodermic and undifferentiated ovarian tumor. The serous pathohistological type is characterized by a mutation on the TP53 gene, in clear cell mutation it occurs on ARID1, PK3Ca, which is also characteristic of the endometrioid carcinoma, although they also present a mutation of the CTTNB1 gene. On the other hand, in mucinous ovarian carcinoma, the KRAS gene mutation is dominant. It is believed that tumors belonging to type 1 originate from the same forms of benign or borderline lesions or appear in the field of endometriosis, such as the endometrioid and clear cell histological type of the tumor. Those belonging to type 2 are more aggressive, and it is believed that they most likely originate from the oviduct fimbriae and that their manifestation on the ovaries is primary tumor metastases. If diagnosed at an early stage, they usually belong to type 1 as opposed to type 2, which is detected at a late advanced stage [5].

The incidence of endometrioid ovarian carcinoma is 10% concerning all epithelial carcinoma. It occurs in the

field of endometriosis or borderline form of adenofibromas, it's mainly diagnosed at an early stage as a unilateral cyst and is highly sensitive to platinum. All this gives encouraging indications that, in case of early detection, fertility preservation can be taken into consideration in patients who have yet to bear children [6, 7]. The European Society of Gynaecological Oncology (ESGO) recommendations indicate that fertility can be preserved in all epithelial low-grade tumors, G1-G2 endometrioid carcinoma, or expansive mucinous tumors.

The first reports on the preservation of fertility for EOC appeared in the 1960s and 1970s. Studies have shown that in case of stage IA, grade 1 and 2, the percentage of patients without relapse is 93.4% and 87.5%, respectively, and the survival rate is 98% and 95%, respectively. At this stage, no additional chemotherapy is needed because the prognosis is very good. In stage IC cases, chemotherapy is recommended for a better treatment outcome [8, 9].

Fertility preservation is not recommended in case of any stage of high-grade tumors, IC tumors, clear cell histological subtype of tumor irrespective of grade because the prognosis is very poor [10].

The decision regarding the type of surgery and the degree of radicality is affected by the histological type of the tumor, the degree of differentiation as well as the FIGO stage in which the tumor has been diagnosed. Adequate disease staging is extremely important because malignant tumors in young patients are mainly detected incidentally – like in the case of our patient. If all the procedures needed to stage the disease are not carried out during the first operation, a second surgical intervention is required. One study has shown that in a patient initially diagnosed as the FIGO stage IA, after a pelvic and paraaortic lymphadenectomy, the disease was staged as the FIGO IIIA [11]. In one study, Cass et al. [12] have found that 14 out of 96 (15%) women in the early stage of ovarian cancer had occult metastases in the lymph nodes at the moment of surgery. It is believed that patients with a relapse of the disease, which was discovered at an early stage, have not been adequately staged and that they already had occult metastases in retroperitoneal lymph nodes [13]. Pelvic and paraaortal metastases are found in 10–15% of such patients [14]. It is, therefore, essential that a complete procedure of disease diagnosis is carried out when making a decision to preserve the uterus and the remaining ovary. Studies comparing outcomes in patients with conservative treatment and those that underwent radical surgery for the FIGO I stages of epithelial ovarian tumors have shown that there was no significant difference in the outcome for these two types of treatment nor that the patients who had undergone radical surgery had a better prognosis [15, 16].

The main factors important for making a decision to preserve fertility are the FIGO stage of the disease, the histopathological type, and the tumor grade.

Many studies have confirmed that the histological grade of the tumor is the most significant prognostic factor. Namely, high-grade tumors are more aggressive and more often lead to a relapse. Vergote et al. [17] covered 1,545 patients with the FIGO stage IA in a retrospective study and came to the conclusion that a high grade correlates with the worst possible prognosis. The grade 3 is a significant predictor of tumor aggressiveness and survival level. The recommendation is that conservative treatment is not advisable for this histological grade nor for the aggressive forms of tumors such as clear cell, anaplastic, and small cell tumors [8, 9].

No significant difference in the disease outcome has been found by comparing FIGO stages IA and IC. Many researchers disagree with the view that the stage IC implies a worse prognosis. By comparing and analyzing the stage I EOC of the patients that have undergone sparing surgery, it has been shown that there was no difference in the survival and disease relapse between IA and IC stages [16, 18]. Additional application of chemotherapy in the stage IC cases significantly improves survival and delays disease recurrence [19].

Adjuvant chemotherapy is used in patients who have an increased risk of disease relapse, the FIGO stage IC1 and higher, and grade 2–3 tumors. In the first stage patients with the FIGO IA-IB, grades I and II there is no need to be applied [20]. The number of cycles ranges from three to nine, and they are administered after surgical treatment.

Many authors point out the good oncological and reproductive response in patients that met the conditions for sparing surgery in order to preserve fertility. The survival rate in these patients is not affected by the type of surgery but rather by the aggressiveness of the tumor, and the lethal outcome occurs more frequently due to other reasons than the relapse of the disease itself [10].

Conclusion

Fertility preservation in patients with malignant ovarian epithelial tumors is an important issue because the treatment has to meet multiple requirements. The intense desire of the patient to have children has to be satisfied without reducing the success of treatment for this type of disease. The staging of the disease spreading is of paramount importance in order to make an adequate decision regarding the treatment. Staging implies salpingoophorectomy on the tumor affected side, a biopsy of suspected sites and adhesions, blind biopsy, infracolic omentectomy, pelvic lymphadenectomy, and cytological analysis of abdominal cavity wash. If, regardless of the expected benign pathology, the histopathological examination confirms a malignant process, a reoperation is necessary to adequately stage the disease, as in the case of our patient. Subsequently, a decision is made on the extent of the surgical therapy. The preservation of fertility is safe with stage IA and IC, grade I and II, but adjuvant chemotherapy is recommended in the stage IC patients.

The patient declined the suggested chemotherapy. Regular Ca 125 tests, abdomen and lesser pelvis ultrasound and CT were normal, and the patient was feeling well.

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CASE REPORT

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Upper limb replantation: surgical strategy and the prophylaxis of acute renal failure due to ischemia reperfusion injury – A report of two cases

Replantacija ruke: hirurška strategija i profilaksa akutne bubrežne slabosti usled ishemijskog reperfuzionog oštećenja

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Abstract

Introduction. The arm replantation is an extremely rare and challenging procedure. The recognized risk is myoglobinuria and, consenquently, ischemia reperfusion-induced renal failure. **Case report.** We presented two patients aged 24 and 46 years who were admitted after traumatic arm amputation. Ischemia time was six and two hours, respectively. Postoperative intensive care treatment with assisted ventilation, sedation, and obtaining sufficient urine output prevented myoglobin-induced renal injury. In the case where ischemia time was shorter, there was only one delayed reconstruction of skin defects after fasciotomy, but in the case where ischemia lasted longer, the patient had two secondary look procedures with acceptable definitive results. **Conclusion.** Arm replantation is a safe procedure even in cases with longer ischemia time. Postoperative control of urine output, correction of acidosis, and preventing myoglobin-induced tubular injury are crucial for stable postoperative recovery and.

Key words:
hand; intensive care units; reconstructive surgical procedures; replantation; respiration, artificial.

Apstrakt

Uvod. Replantacija ruke je izuzetno retka i izazovna procedura. Jedna od komplikacija ove intervencije je mioglobinurija i posledična bubrežna insuficijencija izazvana ishemijsko reperfuzionim oštećenjem. **Prikaz bolesnika.** Prikazana su dva bolesnika starosti 24 i 46 godina, primljena nakon amputacije ruke izazvane traumom. Vreme ishemije iznosilo je šest, odnosno dva sata. Postoperativni tretman i intenzivna nega primenom asistirane ventilacije, sedacije i postizanja optimalne diureze sprečili su bubrežnu slabost izazvanu mioglobinom. U slučaju kada je vreme ishemije bilo kraće, urađena je samo jedna odložena rekonstrukcija defekata kože nakon fasciotomije, a u slučaju kada je ishemija trajala duže, bolesnik je imao dva sekundarna postupka sa prihvatljivim konačnim rezultatima. **Zaključak.** Replantacija ruke je siguran postupak čak i u slučajevima u kojima je vreme ishemije trajalo duže. Postoperativna kontrola diureze, korekcija acidoze i sprečavanje mioglobinom izazvane tubularne nekroze bubrega su presudne za stabilan postoperativni oporavak.

Ključne reči:
ruka; intenzivna nega, odeljenja; hirurgija, rekonstruktivna, procedure; replantacija; disanje, mehaničko.

Introduction

Reconstruction of upper extremity injuries is challenging, especially after traumatic amputation. There is a lack of knowledge about the impact of ischemia duration on the development of possible complications, such as myoglobinuria and acute renal failure.

Rhabdomyolysis is a clinical syndrome characterized by acute damage to the sarcolemma of the skeletal muscle leading to the release in the circulation of myoglobin,

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creatine kinase (CK), alanine aminotransferase (ALT), aspartate aminotransferase (AST), etc. [1].

During rhabdomyolysis, necroptosis and ferroptosis occur in the form of non-apoptotic cell death resulting in the accumulation of reactive oxygen species (ROS) [2], which could lead to skeletal muscle cell death [3]. Due to the toxic effects of free radicals, tubular necrosis occurs, resulting in acute renal failure [4]. We presented two patients with upper limb replantation pointing out the significance of the intensive therapy after replantation in order to prevent ischemia-reperfusion injury.

Case report

Case 1

A 46-year-old male patient was admitted with traumatic amputation of the right upper extremity that happened one hour prior to admission. The injury was caused by a wood cutting machine.

The patient underwent emergency surgery, and revascularization was achieved after two hours from injury time. External skeletal fixation was applied, followed by debridement of the wound edges, arterial and venous anastomosis by the technique of running suture. Immediately after the creation of vascular anastomoses, a heparin single dose of 10,000 I.U. was administered. Neurorrhaphy of median, radial, and ulnar nerves and musculography were performed. The skin was closed with interrupted stitches. Longitudinal fasciotomies were performed on the forearm. During surgery, the blood transfusion started with an overall amount of 1,400 mL of red blood cells (RBC) and 1,100 mL of fresh frozen plasma.

During the first two days in the Intensive Care Unit (ICU), the patient was intubated on bilevel positive airway pressure (BiPAP) ventilation mode, using midazolam sedation. The values of fraction of inspired oxygen (FiO_2), pH, and base excess (BE) in the blood are shown in Table 1. The renal excretory function was controlled by monitoring hourly diuresis that was reaching 200 mL/h in the first two days. Muscle damage was controlled through myoglobin and CK concentrations (Figures 1 and 2). C-reactive protein (CRP) reached its peak on the fourth day, measuring 164 mg/L (the normal range is < 5 mg/L) (Table 2). The high doses of bicarbonate were used for the correction of negative blood BE. Daily urine output was sufficient during recovery.

Table 1

Values of FiO_2, pH, and BE in the Case 1

Post-admission day	Time	FiO_2	pH	BE
1	21 h	100	7.225	-7.2
2	2 h	60	7.222	-7.5
2	12 h	50	7.258	-7.4
2	19 h	50	7.381	-1.4
3	7 h	21	7.400	-0.7
3	12 h	21	7.339	0.3
3	18 h	21	7.378	2.7

FiO_2 – fraction of inspired oxygen; BE – base excess.

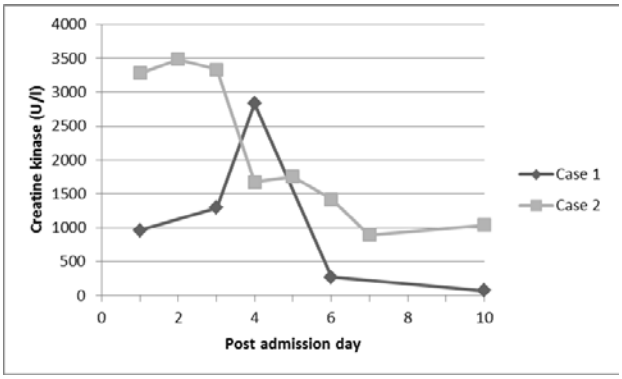

Fig. 1 – Serum creatine kinase (CK) values in the Case 1 and Case 2.

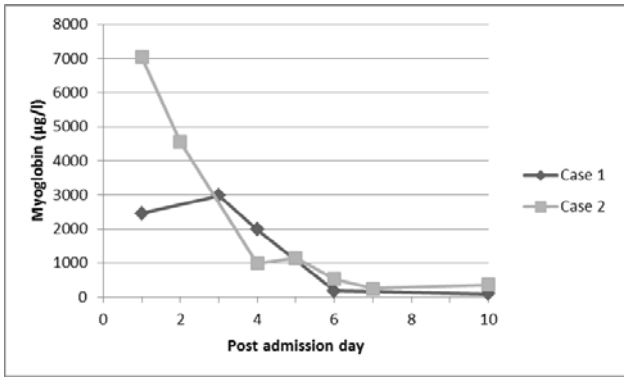

Fig. 2 – Serum myoglobin levels in the Case 1 and Case 2.

Table 2

Values of white blood cells (WBC) and C-reactive protein (CRP) in the Case 1

Post-admission day	WBC ($\times 10^9$/L) (ref ≤ 9.0)	CRP (mg/L) (ref ≤ 5)
1	11.5	6.6
3	13.7	117.4
4	9.0	164.0
6	8.9	30.4
10	7.7	15.3

The parameters of the renal function were in normal reference values at all times. On the fifth day, a second look procedure and additional debridement on the distal wound edge were performed. After that, the wound was closed. The second surgery was performed on the fifteenth day when skin defects at the site of fasciotomy were skin grafted. The patient was discharged on the twenty-seventh day.

Case 2

A 24-year-old male patient was admitted after traumatic amputation of a right upper extremity, accompanied by fractures of the lower jaw, left clavicle, and left fibula that happened four hours before admission. The injury was caused when a huge rock from the ceiling in a mining shaft fell on the patient. Revascularization was achieved one and a half hours after admission, but the total ischemia time was six hours. Debridement of devitalized bone tissue was made proximally and distally cc 2.5 cm in length converting irregular fracture lines into a sharp cut and obtaining bone fragments covered by the periosteum. All steps of surgery were the same as in the Case 1 but after performing an arterial anastomosis, bleeding from the brachial vein was uncontrolled for 20 seconds in order to eliminate the initial products of anaerobic metabolism, as well as reactive oxygen species (ROS). A drain was placed into the amputation line, and fasciotomies were performed. Because of an unstable double lower jaw fracture, a temporary Risdon ligature was applied. Intraoperative blood transfusion was administered in the amount of 1,700 mL of resuspended RBC and 880 mL of fresh frozen plasma.

During the first two days in ICU, the patient was on BiPAP ventilation mode with proper sedation. The values of FiO_2, pH, and BE, as well as leukocytes, CRP, AST, and ALT are shown in Tables 3–5. Diuresis was stimulated with 10% mannitol 125 mL/h during the first two days. Renal function was controlled by monitoring hourly diuresis, which was 245 mL/h in the first two days. The high doses of bicarbonate were used to correct the acid-base status. Muscle damage was monitored by myoglobin and CK concentrations (Figures 1 and 2). Anticoagulant therapy was administered intraoperatively by administering 10,000 I.U. of heparin, and low-molecular-weight heparin (LMWH) was given for ten days postoperatively. Anti-aggregation therapy was started on the seventh day by administering acetylsalicylic acid at a dose of 500 mg daily. The parameters of the renal function were in reference values all time.

Table 3

Values of FiO_2, pH, and BE in the Case 2

Post admission day	Time	FiO_2	pH	BE
1	11 h	60	7.296	-8.8
1	18 h	50	7.299	-7.5
1	22 h	50	7.322	-6.4
2	5 h	50	7.331	-5.7
2	11 h	50	7.446	-6.9
2	21 h	21	7.391	-3.3
3	21 h	21	7.384	-1.7
4	7 h	21	7.358	0

FiO_2 – fraction of inspired oxygen; BE – base excess.

Table 4

Values of white blood cells (WBC) and C-reactive protein (CRP) in the Case 2

Post-admission day	WBC ($\times 10^9$/L) (ref ≤ 9.0)	CRP (mg/L) (ref ≤ 5)
1	22.2	16
2	12	114.7
3	7.2	79.2
4	5.7	39
5	6.2	24.4
6	7.2	30
7	9.0	28.7
10	9.6	108
22	7.9	49.2

Table 5

Activity of aspartat aminotransferase (AST) and alanine aminotransferase (ALT) in the Case 2

Post-admission day	AST (U/L) (ref ≤ 37)	ALT (U/L) (ref ≤ 40)
1	111	37
2	154	47
3	184	49
4	105	42
10	103	162
22	42	54

The patient was operated for the second time on the seventh day when the lower jaw, left clavicle, and left fibula were stabilized by plates. Skin defects at the site of forearm fasciotomy were covered with a skin graft on the seventeenth day. Due to the pseudoarthrosis of the humerus after nine months, a bone graft of lyophilized bone was inserted, and the humerus was healed properly. Two years after surgery, the patient had a full range of motion in the elbow and wrist, but hand muscles were atrophic and functionless (Figure 3). The patient was not motivated for further treatment.

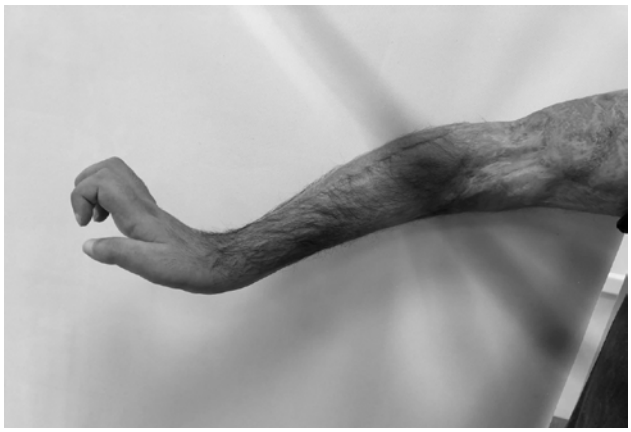

Fig. 3 – Upper limb replantation two years after surgery in the Case 2.

Discussion

The replanted and revascularized segments have numerous functional restrictions and need various corrective secondary procedures [5]. Therefore, prompt surgical care of the injured, with a short period of ischemia and subsequent adequate

intensive therapy and follow-up of patients, are the basic postulates of surgical strategies in preventing acute renal failure.

Rhabdomyolysis can be caused by a variety of factors, including muscular trauma after surgery [6], lower extremity exercise training [7], undifferentiated connective tissue disease [8], as well as non-traumatic muscle breakdown, including chemical [9] and biological agents [10, 11]. However, the highest incidence of rhabdomyolysis is associated with the onset of compartment syndrome in the extremity injuries. Diagnosis of rhabdomyolysis is confirmed by elevated plasma myoglobin levels and increased CK levels. The CK level usually rises within two to twelve hours of muscle injury, reaching maximum values at 24–72 hours [12].

In both presented cases, we found markable changes in serum CK and myoglobin levels after arm replantation (Figures 2 and 3).

CK activity was above 2,800 U/L (reference value up to 195 U/L) in the Case 1 and above 3,400 U/L in the Case 2. It is important to point out that the patient in the Case 2, who had multiple injuries and a longer ischemia time, had higher values of CK on the tenth day compared to those in the Case 1.

In the Case 1, the myoglobin level was up to 2,973 µg/L (reference value up to 92 µg/L) compared to the patient in the Case 2, whose myoglobin level was up to 7,035 µg/L. In our review of the literature, we found that the duration of surgery (about five and a half hours) had crucial risk factors for developing rhabdomyolysis and acute renal failure [13], but our two cases had not developed any sign of it.

The activity of AST and ALT, as markers of liver injury and a part of rhabdomyolitic laboratory disorders, were transitory elevated in the Case 2 (Table 5). Liver dysfunction molecular mechanisms have not yet been clarified. Literature data state that released proteases from injured muscles can be significant causes of liver damage [14].

Although the surgical requirement itself was more demanding, bearing in mind multiple injuries as well as a longer period of ischemia in the Case 2 compared to the Case 1, we can conclude that the rapid recovery of the patient in the Case 2 from compression syndrome was a result of prompt fasciotomy and adequate surgical care and proper therapy approach in ICU. Urgent fasciotomy in both patients improved venous and lymphatic drainage, in addition to the decompression of the subcutaneous and compartment pressure of the extremity in preventing compartment syndrome after revascularization and ischemic reperfusion tissue damage.

Leukocyte count in both patients were above the reference values for the first six days of surgery (max: 13.7×10^9/L, min: 7.7×10^9/L in the Case 1 and max: 22.2×10^9/L, min: 5.7×10^9/L in the Case 2), while moderately elevated CRP values were present for a longer period of time, whereby significantly more CRP values were present in the Case 2 (Tables 2 and 4).

Other laboratory parameters, including urea, creatinine, potassium, and calcium, as renal function assessment parameters, were within the reference values, which may be explained by rapid and good rehydration oxygenation and bicarbonate infusion in high doses. For the same reason, despite extensive destruction of muscle mass most severe complications of rhabdomyolysis-acute renal failure did not develop.

Conclusion

Arm replantation is a safe procedure even in cases with longer ischemia time. Postoperative control of urine output, correction of acidosis, and preventing myoglobin-induced tubular injury are crucial for stable postoperative recovery.

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Legende za ilustracije pisati na posebnom listu, koristeći arapske brojeve. Ukoliko se koriste simboli, strelice, brojevi ili slova za objašnjavanje pojedinog dela ilustracije, svaki pojedinačno treba objasniti u legendi. Za fotomikrografije navesti metod bojenja i podatak o uvećanju.

Skraćenice i akronimi

Skraćenice i akronimi u rukopisu treba da budu korišćeni na sledeći način: definisati skraćenice i akronime pri njihovom prvom pojavljivanju u tekstu i koristiti ih konzistentno kroz čitav tekst, tabele i slike; koristiti ih samo za termine koji se pominju više od tri puta u tekstu; da bi se olakšalo čitaocu, skraćenice i aktinome treba štedljivo koristiti.

Abecedni popis svih skraćenica i akronima sa objašnjenjima treba dostaviti pri predaji rukopisa.

Detaljno uputstvo može se dobiti u redakciji ili na sajtu: www.vma.mod.gov.rs/vsp