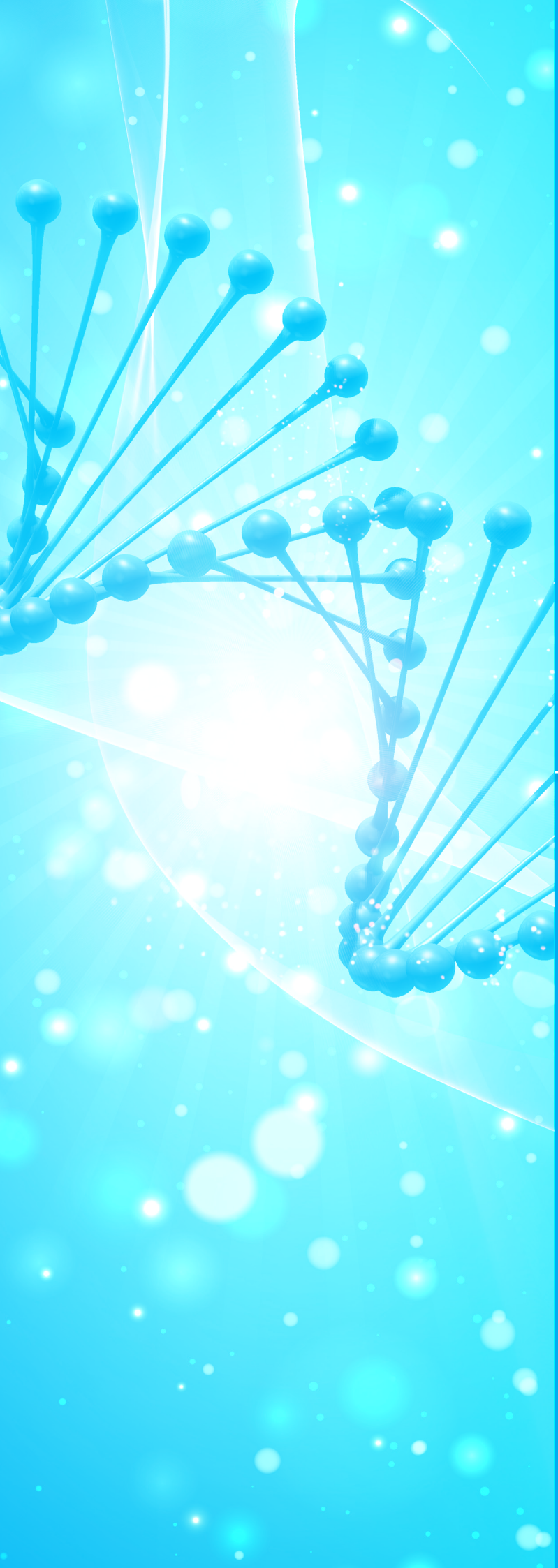

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PREGLEDNI RAD

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PREGLED LITERATURE O KOVID-19 I AUDIO-VESTIBULOLOŠKIH SIMPTOMIMA: ŠTA ZNAMO DO SADA?

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SAŽETAK

Uvod/Cilj: Iako smo već ušli u treću godinu pandemije, patofiziološki mehanizmi infekcije korona virusom teškog akutnog respiratornog sindroma (SARS-CoV-2) i dalje nisu u potpunosti razjašnjeni. Simptomi čula mirisa i ukusa su povezivani sa ovom infekcijom od početka, ali do sada se nije puno govorilo o drugim senzornim sistemima, na prvom mestu čulu sluha i ravnoteže. Cilj ovoj revijalnog rada je da sumira podatke i najnovija saznanja iz literature i da pokuša da odgovori na pitanje da li postoji uzročna povezanost između COVID-19 i oštećenja unutrašnjeg uva.
Metode: U okviru ovog preglednog rada su prikazani najnoviji podaci i dokazi dobijeni na osnovu pretraživanja baze podataka *PubMed* korišćenjem odgovarajućih ključnih reči: *COVID-19*, *hearingloss*, *tinnitus* i *vertigo*. Prilikom pretrage identifikovano je preko 460 publikacija. Nakon pregleda naslova i apstrakata, većina radova su isključeni iz dalje analize jer nisu ispunili kriterijume sistematskog pregleda.
Rezultati: Ukupno 16 radova koji su ispunili zadate kriterijume pregledani su u celini. Rezultati meta-analize su ukazali na to da je stopa pojave oštećenja sluha 3,1% (Interval poverenja - IP: 0,01-0,09), zujanja u uvu 4,5% (IP: 0,012-0,153) i nesvestice 12,2% (IP: 0,070-0,204).
Zaključak: Većina publikovanih radova ima nedostataka i veliki rizik pristrasnosti (bez kontrolne grupe, sa dosta nedostajućih podataka i bez osvrta na pridružene faktore). Za sada još uvek nije moguće sa sigurnošću tvrditi da postoji uzročna povezanost između ove infekcije i oštećenja čula sluha i čula za ravnotežu.

Ključne reči: Covid-19, oštećenje sluha, SARS-CoV-2 virus, tinitus, vrtoglavica

Uvod

Svetska zdravstvena organizacija (SZO) je objavila pandemiju bolesti izazvane korona virusom (engl. *Coronavirus Disease* – COVID-19) 11. marta 2020. godine i u ovom trenutku u toku smo treće godine pandemije sa skoro 500 miliona zaraženih osoba i više od 6 miliona umrlih (1). U početku su samo respiratorni i gastrointestinalni simptomi bili dobro opisani, ali tokom prethodne dve godine mnoštvo drugih simptoma povezanih sa ovom infekcijom je prijavljeno u literaturi. Poremećaji čula mirisa i ukusa (hiposmija/anosmija i hipogeuzija/ageuzija) navedeni su kao karakteristični simptomi na početku pandemije i ostaju najčešće prijavljivani simptomi povezani sa uhom, grlom i nosem do sada.

Primećene su neurotropne karakteristike virusa, a do 30% pacijenata iskusi neurološke manifestacije ove bolesti (2,3). Ranije je dokazano da virusi mogu da dovedu do naglog, akutnog senzorineuralnog oštećenja sluha (eng. *Sudden SensoriNeural Hearing Loss* – SSNHL) ili akutne unilateralne periferne vestibulopatije (4,5). Predloženi mehanizmi uključuju direktno virusno oštećenje lavirinta ili nerva, reaktivaciju latentnog virusa, ili posredovani imuni mehanizam kod sistemske virusne infekcije (6,7). Desetak studija slučaja i serija slučajeva je publikovano u literaturi od početka pandemije ukazujući da virus SARS-CoV-2 takođe može da dovede do oštećenja sluha ili poremećaja ravnoteže preko nekoliko prethodno opisanih

REVIEW ARTICLE

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REVIEW OF COVID-19 AND AUDIO-VESTIBULAR SYMPTOMS: WHAT WE KNOW SO FAR?

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SUMMARY

Introduction/Aim: Although we have already entered the third year of the pandemic, the pathophysiological mechanisms of Severe Acute Respiratory Syndrome Corona Virus 2 (SARS-CoV-2) infection are still not fully elucidated. While symptoms of smell and taste dysfunction have been associated with this infection from the beginning, not much has been reported on other sensory systems, in the first place the hearing and balance. The aim of this review paper is to summarize data from the literature and the latest publications and answer the question whether there is a causal link between COVID-19 and inner ear damage.
Methods: This review paper presents the latest data and evidence obtained from search of PubMed database by keywords: COVID-19, hearing loss, tinnitus and vertigo. The search identified more than 460 publications. After reviewing the title and abstract, most papers were excluded due to non-compliance with the eligibility criteria.
Results: A total of 16 papers fulfilled the eligibility criteria and were reviewed in their entirety. The results of the meta-analysis indicated that the incidence of hearing loss was 3.1% (Confidence Interval - CI: 0.01-0.09), of tinnitus 4.5% (CI: 0.012-0.153), and dizziness 12.2% (CI: 0.070-0.204).
Conclusion: Most published papers are characterized by limitations and high risk of bias (no control arm, missing data, and no reference to confounding factors). For now, no certain causal relationship between this infection and damage to the senses of hearing and the sense of balance can be established.

Key words: Covid-19. hearing loss, SARS-CoV-2, tinnitus, vertigo

Introduction

The pandemic of corona virus disease (COVID-19) was announced on 11 of March of 2020 by the World Health Organization (WHO) and at this point, we are into its third year, with almost 500 million infected individuals and over 6 million dead (1). In the beginning, only respiratory and gastrointestinal symptoms were well described, but during previous two years, a plethora of other symptoms connected with this infection were reported in the literature. A dysfunction of smell and taste (hyposmia/anosmia and hypogeusia/ageusia) were listed as hallmark symptoms early in the pandemic and remain most commonly reported ear, nose and throat symptoms up to date.

The neurotropic properties of the virus were observed and up to 30% of patients experience neurological manifestations of the disease (2,3). It has been shown previously that viruses can lead to sudden sensorineural hearing loss (SSNHL) or acute unilateral peripheral vestibulopathy (4,5). The proposed mechanisms include direct viral invasion of the labyrinth or nerve, reactivation of latent virus, or immune-mediated mechanism in systemic viral infection (6,7). A dozen of case reports and case series appeared in the literature from the beginning of pandemic suggesting that SARS-CoV-2 virus could also lead to hearing loss or balance disorders via several previously well described pathways: vascular, metabolic, autoimmune, or inflammatory (6). While it was of utmost importance to draw attention of the physicians to any new possible symptoms or organ damage in this disease, uncritical publishing, and

puteva: vaskularnog, metaboličkog, autoimunskog, ili inflamatornog (6). Dok je od najveće važnosti bilo skrenuti pažnju lekarima na nove moguće simptome ili oštećenja organa u ovoj bolesti, nekritičko objavljivanje i nedostatak pažljivog procesa stručne recenzije tokom pandemije doveli su do ozbiljnih pristrasnosti i netačnih povezanosti.

Do sada je objavljeno nekoliko sistematskih prikaza i dve meta-analiza o ovoj temi, ali zbog ozbiljnih nedostataka objavljene literature (nekontrolisane studije, nedostajući podaci, pristrasnost prisećanja, informativna pristrasnost i pridruženi faktori), veza između KOVID-19 oboljenja i oštećenja sluha ili vrtoglavice ostaje nesigurna. Takođe, većina objavljenih studija ne potvrđuje i ne prikazuje činjenice da je tokom pandemije većina pacijenata lečena ototoksičnim lekovima: hidroksihlorokin, azitromicin, i lopinavir-ritonavir, da nabrojimo samo nekoliko, koji bi sami po sebi mogli da budu uzrok oštećenja čula sluha i vestibularnog oštećenja (8). Da stvari budu još teže, od januara 2021. godine pojavio se jedan broj izveštaja o oštećenju sluha i nesvestici nakon jedne ili dve doze vakcine protiv KOVID-19. Iako su te informacije već dovedene u pitanje (9), reference za ove tvrdnje se i dalje mogu naći širom Interneta.

Cilj ovog rada bio je da se pretraži relevantna literatura i najnoviji rezultati i da se odgovori na pitanje da li imamo dovoljno dokaza da pretpostavimo uzročno-posledičnu vezu između KOVID-19 oboljenja i oštećenja sluha i vrtoglavice. Takođe, cilj je da se istaknu nedostaci u dostupnoj literaturi i rasvetle audio-vestibularni simptomi nakon vakcinacije.

Metode

Pretraživanje i pregled literature dostupne na engleskom jeziku putem *PubMed* baze podataka bilo je fokusirano na audiološke i vestibularne manifestacije Kovida-19. Ključne reči (MeSH termini) uključivale su sledeće: „COVID-19“, „hearing loss“, „tinnitus“ i „vertigo“. Poslednje pretraživanje literature je bilo 1. aprila 2022. Pretraživanje je iznedrilo 460 radova koji su filtrirani pregledom naslova i sažetaka, a zatim uz pomoć sledećih kriterijuma: (1) rad utvrđuje direktnu temporalnu vezu između novo dijagnostifikovanog oštećenja sluha i infekcije Kovidom-19 (do 4 nedelje), (2) infekcija se dokazuje testom lančane reakcije polimeraze (PCR) ili detekcijom specifičnih antitela uz pomoć serološkog testa (poželjno IgM) po preporukama (10) ili prijavom prijema u bolnicu zbog virusne pneumonije, (3) i drugi razlozi, koji mogu da izazovu senzorno oštećenje čula sluha, su isključeni ili barem navedeni. Od svih radova koji su prvobitno identifikovani, samo 16 radova je zadovoljilo sva tri gore navedena kriterijuma. Metodologija je bila u skladu sa preporučenim PRISMA protokolom (eng. P*referred Reporting Items for Systematic Reviews and Meta-analyses*) (11). Analizirane primarne studije su imale visok rizik od pristrasnosti (pristrasnost izveštavanja, informativna i druge vrste pristrasnosti).

Rezultati

Oštećenje sluha

Izolovani prikazi slučaja su pregledani i kratko razmotreni zbog ozbiljnih ograničenja u prikazivanju. Prvi rad o mogućoj vezi između oštećenja sluha i Kovida-19 je objavljen rano na početku pandemije, u maju 2020 (12). Rad je bio u formi preliminarnog prikaza, dok su mnogi važni detalji o pacijentima nedostajali (bez informacije o testovima, vremenske usklađenosti, pre ili post-audiograma ili mogućim pridruženim faktorima). Usledili su drugi prikazi slučaja, i većina njih je bila zasnovana na pojedinačnim izveštajima nedovoljnog kvaliteta, dok je samo nekoliko ispunilo sve preporučene kriterijume (13-17).

Bilo je drugih prikaza o uticaju Kovida-19, koji su poredili broj pacijenata sa SSNHL sa periodom pre pandemije (18). Iako je primećen ozbiljan pad apsolutnih brojeva pacijenata sa SSNHL, nije primećena promena u procentima pacijenata kod kojih je dijagnostikovan SSNHL u poređenju sa ukupnim brojem pregledanih pacijenata tokom dva perioda istraživanja, tako da nije pronađena veza između Kovida-19 i SSNHL-a.

U većini objavljenih studija o simptomima unutrašnjeg uva se, nažalost, javlja nedostatak odgovarajućih dijagnostičkih podataka i one se zasnivaju na subjektivnim merama koje su korišćene za procenu simptoma oštećenja sluha ili stepena nestabilnosti (19,20). Može se tvrditi da bi u toku visoko zarazne bolesti bilo teško i nepraktično koristiti standardnu opremu zbog teškoća u sanaciji i obzirima kliničkog okruženja. Međutim, bez audiometrije (početna vrednost, pre bolesti, i druga, nakon što je primećeno oštećenje sluha), nemo-

lack of careful peer review process during the pandemic also brought forth serious biases and spurious conjunctions.

Up to date, several systematic reviews and two meta-analyses were published on this topic, but due to serious limitations of the published literature (uncontrolled studies, missing data, recall and information bias, confounding factors, etc.) the association between COVID-19 and hearing loss or vertigo remains uncertain. Furthermore, most of the published studies fail to acknowledge and account to the fact that, during the pandemic, the majority of patients were treated with ototoxic drugs: hydroxychloroquine, azithromycin, and lopinavir-ritonavir, to name just a few, which could also be cause of hearing loss and vestibular dysfunction on their own (8). To aggravate matters further, since January 2021, a number of reports emerged on hearing loss and dizziness after one or two doses of COVID-19 vaccines. Although already questioned, the references to these claims can still be found online (9).

The aim of this paper was to search relevant literature and the latest findings and to answer the question whether we have enough proofs to assume causal association between COVID-19 and hearing impairment and vertigo. Moreover, it will point out the deficiencies in the available literature and hopefully shed a light on post-vaccination audio-vestibular symptoms.

Methods

The search and review of the literature available in English language was performed using PubMed database, with a focus on audiological and vestibular manifestations of COVID-19. The key words (MeSH terms) used were: “COVID-19”, “hearing loss”, “tinnitus” and “vertigo”. The last literature search was performed on April 1, 2022. The search yielded 460 papers which were filtered by screening of title and abstract, and then using the following criteria: (1) the paper establishes a direct temporal link between newly diagnosed hearing loss and COVID-19 infection (up to 4 weeks), (2) the infection was proven by Polymerase Chain Reaction (PCR) test or detection of specific antibodies by serology (preferably IgM) as recommended (10) or report of a hospital admission due to viral pneumonia, (3) and other reasons that can cause a sensory deficit in hearing are ruled out or at least acknowledged. Of all paper initially identified, only 16 fulfilled all three criteria mentioned above. The recommended PRISMA (Preferred Reporting Items for Systematic reviews and Meta-analyses) were followed as recommended (11). The discussed primary studies had high risks of bias (reporting bias, information bias, and other types of bias).

Results

Hearing loss

Isolated case reports were screened and only briefly referred to due to severe deficiencies in the reporting. The first ever paper on possible connection between hearing loss and COVID-19 was published relatively early in the pandemic, in May 2020 (12). It was in a form of a preliminary report, with many important patients’ details lacking (no information on tests, temporal concordance, pre- or post-audiological data, or possible confounding factors). Other case reports followed, and the vast majority of them were anecdotal and of insufficient quality, with only several of them fulfilling all the recommended criteria (13-17).

There are other reports on impact of COVID-19 which compared total numbers of patients with SSNHL with the pre-pandemic period (18). Although serious decline in absolute number of patients who presented with SSNHL was observed, no change in percentages of patients diagnosed with SSNHL compared to the total number of examined patients was observed in the two examined periods, so no association between COVID-19 and SSNHL could be found.

Most of the published studies on inner ear symptoms unfortunately lack the proper diagnostics data and are based only on subjective measures used to assess the symptoms of hearing loss or degree of instability (19,20). It could be argued that in times of highly infectious disease, it would be hard and impractical to use standardized equipment due to difficulties in sanitation and clinical environment concerns. But without audiometry (baseline reference, pre-disease, and second, after hearing loss was noticed) it is impossible to make the diagnosis of SSNHL (≥30 dB on three contiguous frequencies in 72 h) (6). Furthermore, making the diagnosis of hearing loss

guće je postaviti dijagnozu SSNHL (>30 dB na tri uzastopne frekvencije tokom 72h) (6). Takođe, postavljanje dijagnoze oštećenja sluha koja se zasniva samo na subjektivno prijavljenim merama ili upitnicima nije čvrsto naučno utemeljeno i dokazano je, ne samo da audiometrijski pragovi nisu u korelaciji sa subjektivno prijavljenim stepenom oštećenja sluha (21), već da subjektivno oštećenje sluha zavisi od stepena neuroticizma i sposobnosti pojedinaca da se izbore (22).

U jedinoj meta-analizi koja je objavljena do sada, prijavljena stopa pojave (eng. *event rate* – ER, pojavna učestalost događaja) oštećenja sluha bila je 3,1% (Interval povrenja - IP: 0,01-0,09) na osnovu ukupnog broja pacijenata koji je iznosio 560 (23). Ovu stopu pojave bi trebalo interpretirati uz oprez zbog niskog nivoa dokaza u primarnim studijama i velike heterogenosti.

Zujanje u uvu - tinitus

Ukupno 90 objavljenih radova opisuje tinitus ili kao prateći simptom tokom infekcije ili retko, kao izolovan simptom. Ne postoje studije koje prijavljuju psihometrijsku evaluaciju zujanja u ušima, ili njegovo trajanje, simptom skor i uticaj; samo u jednom radu je upoređena frekvencija i intenzitet (24). Prijavljena zbirna procenjena prevalencija tinitusa nakon infekcije Kovidom-19 je 8% (IP: 5 do 13%), a ER je 4,5% (IP: 0,012-0,153) (22-24). Treba navesti da emocionalni faktori, poput anksioznosti, straha, stresa i lošeg kvaliteta sna tokom pandemije takođe mogu da igraju važnu ulogu u percepciji i pogoršanju tinitusa, pogotovo kod osoba koje su bile u karantinu (25).

Vrtoglavica

Vrtoglavica je opšti termin i medicinska dijagnoza i obično označava iskustvo rotacione senzacije i kretanja zbog nistagmusa. S druge strane, kada govorimo o nesvestici (eng. *dizziness*), obično se to odnosi na osećaj gubitka svesti, osećaj lakoće u glavi, osećaj slabosti, ili nestabilnosti. Nažalost, zbog nedostatka jedinstvenog sistema klasifikacije, ova dva različita termina se često koriste naizmenično u literaturi, bez pravljenja razlike. U većini objavljenih studija o subjektivnim prikazima simptoma tokom Kovida-19, autori prijavljuju osećaj nesvestice, a ne vrtoglavice. Pored toga, evidentan je visok rizik pristrasnosti izveštavanja, kao i nedostajući podaci; samo jedna studija je opisala jednog pacijenta sa kompletnim objektivnim vestibularnim testiranjem i opisom nistagmusa (26). U jednom objavljenom sistematskom prikazu i meta-analizi, stopa pojave (ER) nesvestice bila je 12,2% (IP: 0,070-0,204) (23), dok je zbirna procena prevalencije rotatorne vrtoglavice bila mnogo niža 3,4% (IP: 1,1-6,9), kao što je i bilo očekivano (27).

Veza između simptoma unutrašnjeg uha i vakcinacije protiv KOVID-19

Nekoliko anegdotalnih prikaza slučaja zasnovanih na pojedinačnim izveštajima se pojavilo u literaturi o SSNHL nakon vakcinacije protiv Kovida-19. Preporučeni dijagnostički kriterijumi ili nisu bili praćeni ili nisu prikazani u objavljenim radovima, tako da ozbiljnost slučajeva nije mogla biti utvrđena. U jednoj studiji preseka koja je uključila ukupno 555 izveštaja o verovatnom SSNHL u okviru Sistema za prijavljivanje neželjenih efekata vakcine (engl. V*accine Adverse Events Reporting System* – VAERS) Centra za prevenciju i kontrolu bolesti Sjedinjenih Američkih Država, rezultati nisu ukazali na vezu između vakcinacije protiv KOVID-19 i povećane incidencije oštećenja sluha u poređenju sa očekivanom incidencijom u opštoj populaciji (9).

Diskusija

Oštećenje audio-vestibularnog sistema u Kovid-19 infekciji ostaje mogućnost. Oštećenje osetljivih struktura unutrašnjeg uha može se povezati sa direktnim uticajem virusa, ali se takođe može dovesti u vezu sa posredovanim imunskim odgovorom. Krvni sudovi, limfni sistem, nervi i u nekim slučajevima meninge, predloženi su kao putevi ulaska virusa (14,19). Tačna anamneza i objektivno testiranje ključni su za pravilnu identifikaciju oštećenja i moguću etiopatogenezu. Druge mogućnosti i potvrđeni slučajevi oštećenja unutrašnjeg uha moraju biti isključeni pre postavljanja konačne dijagnoze ili bilo kakvih tvrdnji o uzročnoj vezi. Naime, kod svih Kovid-19 pacijenata bi trebalo istražiti i isključiti prethodnu istoriju izloženosti buci, traume glave, autoimune bolesti, i druge bolesti unutrašnjeg uva, poput Menierove bolesti. Takođe je postavljena hipoteza da sinergijski audio-vestibularni neželjeni efekti mogu da se jave kod primene mnogih ototoksičnih Kovid-19 lekova (hidroksihlorokin, azitromicin, lopinavir-ritonavir, remdesivir, favipiravir, furosemid, itd.) (8). Drugi faktori, kao što su starija životna dob, neprepoznato oštećenje čula sluha, genetska predispozicija i smanjena eliminacija lekova mogu takođe odi-

based only on subjective reported measures or questionnaires is not scientifically sound and has been proven not only that audiometric thresholds do not correlate well with subjective reported hearing level (21), but also that subjective hearing disability depends on the degree of neuroticism and coping expectancy of the individuals (22).

In the only one meta-analysis published so far, the reported event rate (ER, the occurrence frequency of the event) in case of hearing loss was 3,1% (CI: 0.01-0.09) based on total population of 560 patients (23). This ER should be interpreted with caution due to low level of evidence in primary studies and high heterogeneity.

Tinnitus

A total of 90 published papers are describing tinnitus either as concomitant symptom during the infection or rarely, as isolated one. There are no studies which report psychometric evaluation of the tinnitus, or its duration, resolution of symptom and impact; only one reported its matching frequency and intensity (24). The reported pooled estimated prevalence of tinnitus after COVID-19 infection is 8% (CI: 5 to 13%) and ER is 4.5% (CI: 0.012-0.153) (23-25). It should be noted that other emotional factors, such anxiety, fear, stress, and poor sleep quality during the pandemic can also play a significant role in perceiving and aggravation of tinnitus, especially in persons who were quarantined (25).

Vertigo

Vertigo is a general term and medical diagnosis and usually implies experiencing rotatory sensation and movement due to nystagmus. On the other hand, when we talk about dizziness, we usually refer to feeling faint, light-headed, weak, or unsteady. Unfortunately, due to lack of uniform classification systems, these two different terms are commonly used interchangeably in literature. In most of the published studies with subjective reports on symptoms experienced during COVID-19, authors report the feeling of dizziness, not vertigo. Furthermore, the high risk of reporting bias and missing data is evident; only one study described one patient with complete objective vestibular testing and description of nystagmus (26). In published systematic review and meta-analysis, ER of dizziness was reported to be 12.2% (CI: 0.070-0.204) (23), while pooled estimate of the prevalence of rotatory vertigo (without dizziness) was much lower 3,4% (CI: 1,1-6,9), as expected (27).

Association of inner ear symptoms and COVID-19 vaccination

Several anecdotal case reports have also occurred in literature on SSNHL after COVID-19 vaccination. The recommended diagnostic criteria for SSNHL were either not followed or not reported in the published papers, so the severity of cases could not be established. In the cross-sectional study of all 555 incident reports of probable SSNHL in the Centers for Disease Control and Prevention Vaccine Adverse Events Reporting System (VAERS), findings did not suggest an association between COVID-19 vaccination and increased incidence of hearing loss compared with the expected incidence in the general population (9).

Discussion

The damage of audio-vestibular system in COVID-19 infection remains a possibility. While damage of the sensitive inner ear structures can be related to direct effect of the virus, it could also be related to the virus-mediated immune response. Blood vessels, lymphatics, nerves and in some cases, meninges have all been proposed as entry routes for the virus (14,19). An accurate anamnesis and objective testing are crucial for correct identification of damage and possible etiopathogenesis. Other possibilities and confirmed causes of inner ear damage must be ruled out before making the final diagnosis or any claims of causal relationship. Namely, previous history of noise exposure, head trauma, autoimmune diseases, and other inner ear diseases such as Meniere disease should always be actively searched for and ruled out in all COVID-19 patients. It has also been postulated that synergistic adverse audio-vestibular effects may occur with coadministration of multiple ototoxic COVID-19 drugs (hydroxychloroquine, azithromycin, lopinavir-ritonavir, remdesivir, favipiravir, furosemide, etc.) (8). Other factors, such as old age, unrecognized hearing impairment, genetic susceptibility and reduced drug elimination can also play significant role in the inner ear damage. Unfortunately, this is not acknowledged in most of the published studies to date.

The uncritical and mass publishing from the beginning of the pandemic has led to hundreds

grati važnu ulogu u oštećenju unutrašnjeg uva. Nažalost, to nije potvrđeno u većini studija koje su do danas objavljene.

Nekritičko i masovno objavljivanje od početka pandemije dovelo je do objavljivanja na stotine studija lošeg kvaliteta izveštavanja. Kvalitet skoro svih ocenjenih studija u ovom rukopisu smatra se slabim, zbog nekontrolisanog dizajna i sklonosti ka pristrasnosti informacija i selekcije, grešaka merenja i pridruženih faktora. U drugoj godini pandemije, mnogi objavljeni radovi su unapredili kvalitet prikazanih dokaza, a potpuni audiometrijski podaci, vestibularno testiranje i neuro snimanje su bili dostupni. Osim ovih prikaza slučaja, studija preseka sa jednom grupom i kohortnih studija, do sada su objavljena četiri sistematska pregleda i dve meta-analize o ovoj temi (23,25,27-29). Međutim, zbog velike heterogenosti podataka, visokog rizika pristrasnosti u primarnim studijama i faktora zabune, rezultate svih ovih studija bi trebalo interpretirati uz oprez. U jednoj od meta-analiza koje su primenjivale konzervativnije kriterijume, procene zbirne prevalencije i intervali poverenja za oštećenje sluha, zujanje u ušima i vrtoglavicu bili su 3,86% (95% IP: 0,84-8,42), 9,62% (95% IP: 3,60-18,13), i 2,4% (95% IP: 0,47-6,01) (29). Iako su procene revidirane i stope su pale za skoro 50% za oštećenje sluha i vrtoglavicu i više od 98% uključenih pacijenata je imalo pozitivan PCR test, autori i dalje insistiraju na oprezu prilikom tumačenja podataka. I dalje postoji puno faktora koji mogu da igraju ulogu u patofiziologiji oštećenja unutrašnjeg uha, a koji nisu mogli biti kontrolisani.

Autori nedavno objavljene studije su pokušali da razjasne da li postoji prava veza između Kovida-19 i auditornih simptoma poredeći ih sa drugim tipovima simptoma: oni sa dokazanom vezom sa Kovidom-19 (gubitak čula mirisa i ukusa) i oni koji nemaju utvrđenu vezu (zubobolja) (30). S obzirom da je više od 60% učesnika sa potvrđenim ili verovatnim Kovidom-19, osim simptoma unutrašnjeg uha, prijavilo da je bolest uticala na zubobolju, autor nije mogao odrediti da li su ovi simptomi zaista povezani sa Kovidom-19 ili odražavaju nocebo efekat (novi ili pogoršanje postojećih simptoma koji se razvijaju zbog negativnih informacija ili uverenja povezanih sa zdravljem). Oni su takođe postavili hipotezu da to može biti zbog mogućnosti da su učesnici bili visoko osetljivi na somatske senzacije ili zbog zdravstvene anksioznosti (31).

Zaključak

U ovom preglednom radu je detaljno ispitano šesnaest studija. Objavljeni sistematski pregledi i meta-analize su pokazali da je prevalencija oštećenja sluha, zujanja u ušima i nesvestice značajno viša kod pacijenata sa Kovidom-19. Međutim, imajući u vidu loš kvalitet primarnih studija i visok rizik od pristrasnosti, ove nalaze bi treba interpretirati uz oprez. Na osnovu dostupnih dokaza, i dalje nije moguće ustanoviti uzročnu vezu između Kovida-19 i oštećenja unutrašnjeg uha. Potrebno je više studija dobrog dizajna i dodatnih procena sa standardnim objektivnim testovima da bi se u potpunosti razjasnila incidencija i ozbiljnost simptoma unutrašnjeg uha kod Kovid-19 bolesti.

Konflikt interesa

Autor je izjavio da nema konflikta interesa.

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of published studies with poor reporting quality. The quality of almost all assessed studies in this manuscript were regarded as weak because of uncontrolled design and proneness to selection and information bias, measurement errors and confounding factors. In the second year of pandemic, many published papers improved the quality of reported evidence, and full audiometry data, vestibular testing and neuroimaging was available. Apart from case reports, single-arm cross-sectional and cohort studies, up to date four systematic reviews and two meta-analyses were also published on this subject (23,25,27-29). However, due to high heterogeneity of the data, high risk of bias in primary studies and confounding factors, results of all these studies should be interpreted with caution. In one of the meta-analyses which applied more conservative criteria, pooled prevalence estimates and confidence intervals for hearing loss, tinnitus and vertigo were 3.68% (95% CI: 0.84 – 8.42), 9.62% (95% CI: 3.60 – 18.13), and 2.4% (95% CI: 0.47 – 6.01), respectively (29). Although the estimate was revised and rates fell for almost 50% for hearing loss and vertigo and more than 98% of included patients had positive PCR test, authors still urge caution in interpreting the data. There are still many contributing uncontrolled factors that can still play a role in pathophysiology of inner ear damage.

The authors of recently published study tried to elucidate if there is true association between COVID-19 and auditory symptoms by comparing them with other types of symptoms: those with proven association with COVID-19 (loss of smell and taste) and those with no established association(toothache) (30). Since over 60% of participants with confirmed or probable COVID-19, apart from inner ear symptoms, reported that their toothache had also been affected by the disease, the authors could not determine whether these symptoms are indeed associated with COVID-19 or they reflect nocebo effect (new or worsening of the existing symptoms that develop due to negative health/related information or belief). They also postulated that it could be due to a possibility that participants were highly sensitive to somatic sensations or health anxiety (31).

Conclusion

This review examined sixteen studies in detail. Published systematic reviews and meta-analyses have demonstrated that the prevalence of hearing loss, tinnitus and dizziness is significantly higher in patients with COVID-19. However, given the poor quality of primary studies and high risk of bias, these findings should be interpreted with caution. Based on the available evidence, it is still not possible to ascertain the casual relationship between COVID-19 and inner ear damage. More well-designed studies and follow-up assessments with standard objective tests are needed to fully elucidate incidence and severity of inner ear symptoms in COVID-19 disease.

Competing interests

The author declares no competing interests.

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POREKLO ĆELIJA ENTERIČKOG NERVNOG SISTEMA I PUTEVI MIGRACIJE TOKOM EMBRIONALNOG RAZVOJA

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SAŽETAK

Enterički nervni sistem (ENS) predstavljen je kompleksnom mrežom neurona, glijalnih i drugih ćelija unutar zida digestivne cevi. ENS ostvaruje brojne, vitalno važne funkcije u našem organizmu. Tako, ENS reguliše motilitet digestivnog trakta, sekreciju u lumen creva, razmenu tečnosti i elektrolita kroz sluznicu, kao i perfuziju sluznice. Da bi pravilno funkcionisao i ostvarivao ove važne funkcije, neophodan je pravilan embrionalni razvoj ENS. Ćelije ENS nastaju od prekursorskih ćelija poreklom od nervnog grebena. Dve populacije koje doprinose najvećem broju budućih ćelija ENS jesu ćelije vagalnog i sakralnog dela nervnog grebena. Prekursorske ćelije vagalnog dela nervnog grebena ulaze u primitivno crevo u regionu budućeg jednjaka (prednje crevo) i započinju svoju migraciju, preko srednjeg, ka zadnjem crevu, odnosno ka budućem analnom otvoru. Ćelije sakralnog dela nervnog grebena ulaze u region zadnjeg creva prateći ekstrinzička nervna vlakna i nastavljaju svoju migraciju rostralno, ka ćelijama poreklom od vagalnog dela nervnog grebena. Uporedo sa procesom migracije, prekursorske ćelije prolaze i kroz druge važne procese, kao što su proliferacija, neuro-glijalna diferencijacija, gangliogeneza i stvaranje aksonskih puteva, kao i sinaptogeneza. Svi ovi procesi strogo su regulisani brojnim signalnim putevima, od kojih se mnogi još uvek aktivno istražuju. Savremena dostignuća u nauci omogućila su praćenja pojedinačnih ćelija na razvojnom putu i druge metode u istraživanju koje će značajno doprineti razumevanju embrionalnog razvoja ENS. Ovo može imati reperkusije u poboljšanju dijagnostike i terapije razvojnih (npr. Hiršprungova bolest) i drugih poremećaja ENS, ali i oboljenja u kojima disfunkcija ENS značajno doprinosi patogenezi.

Ključne reči: enterički nervni sistem, embrionalni razvoj, ćelijska migracija, Hiršprungova bolest

Uvod

Enterički nervni sistem (ENS) predstavljen je kompleksnom mrežom ćelija unutar digestivnog trakta. U njegovoj izgradnji učestvuje oko 400-600 miliona neurona i, pretpostavlja se, još veći broj glijalnih i drugih ćelija, što ga čini najvećim delom perifernog nervnog sistema (1). Neuroni i glija ćelije ENS formiraju sitne ganglije koje su međusobno povezane gradeći nervne pleksuse. Dva glavna nervna pleksusa, submukozni (Majsnerov) i mijenterički (Auerbahov), smešteni su u samom zidu digestivne cevi. Submukozni pleksus, kao što mu ime govori, nalazi se u submukozi, dok se mijenterički pleksus nalazi između longitudinalnog i cirkularnog mišićnog sloja zida digestivne cevi (slika 1) (2). Za razliku od submukoznog pleksusa, koji nedostaje u jednjaku, mijenterički pleksus prisutan je čitavom dužinom digestivnog trakta (2,3). Svi neuroni ENS, tokom međusobnih interakcija i primanja spoljašnjih signala, pre svega od drugih delova nervnog sistema, ostvaruju brojne, vitalno važne funkcije u našem organizmu. Tako ENS reguliše motilitet digestivnog trakta, sekreciju u lumen creva, razmenu tečnosti i elektrolita kroz sluznicu, kao i perfuziju sluznice (1,2,4). ENS je sposoban da neke od ovih funkcija obavlja potpuno autonomno, refleksnim lukovima koji su u celini

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ORIGIN OF ENTERIC NERVOUS SYSTEM CELLS AND MIGRATION PATHWAYS DURING EMBRYONIC DEVELOPMENT

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SUMMARY

The enteric nervous system (ENS) is represented by a complex network of neurons, glial and other cells within the wall of the digestive tract. ENS is responsible for numerous, vital functions in our body. Thus, ENS regulates motility of the digestive tract, secretion into the intestinal lumen, exchange of fluid and electrolytes through the mucosa, as well as mucosal perfusion. In order to perform these important functions, proper embryonic development of ENS is necessary. ENS cells are derived from precursor cells of the neural crest (NCCs – neural crest cells). Two cell populations that contribute to the largest number of future ENS cells are the vagal and sacral NCCs. Vagal NCCs enter the primitive gut tube in the region of the future esophagus (foregut), and begin their migration, through the midgut towards the hindgut and the future anal region. Sacral NCCs enter the hindgut region following the extrinsic nerve fibers and continue their migration rostrally, towards vagal NCCs. Along with the migration process, these cells undergo other important processes, such as proliferation, neuro-glial differentiation, gangliogenesis, axonal pathway formation and synaptogenesis. All these processes are strictly regulated by numerous signaling pathways, which are still being actively researched. Modern lineage tracing and other technologies, that enabledfollowing of individual precursor cells through their development pathways, will significantly contribute to the better understanding of development of ENS. This may have repercussions in improving the diagnosis and treatment of some developmental (Hirschsprung disease) and other ENS disorders.

Keywords: enteric nervous system, embryonic development, cell migration, Hirschsprung disease

Introduction

The enteric nervous system (ENS) is represented by a complex network of cells within the digestive tract. Around 400-600 million neurons take part in its development, and even a greater number of glial and other cells, as it is assumed, which makes it the largest part of the peripheral nervous system (1). Neurons and glial cells of the ENS form tiny ganglia that are interconnected, thus creating nerve plexuses. Two main nerve plexuses, the submucous plexus (Meissner's plexus) and myenteric plexus (Auerbach's), are located within the wall of the digestive tract. The submucous plexus, as its name implies, is located in the submucosa while the myenteric plexus is situated between the longitudinal and circular muscle layer in the digestive tract (Figure 1) (2). In contrast to the submucous plexus, which is not found in the esophagus, the myenteric plexus extends the entire length of the digestive tract (2,3). All neurons of the ENS, with their mutual interactions and extrinsic inputs, primarily from other parts of the nervous system, regulate numerous, vital functions in our body. Thus, the ENS regulates the motility of the digestive tract, the secretion into the intestinal lumen, the exchange of fluids and electrolytes through the mucosa, as well as mucosal perfusion (1,2,4). The ENS is able to perform some of these functions completely independently by reflex arcs,

unutar ENS (5). Zbog svoje kompleksne građe i autonomnog ostvarivanja svojih funkcija, ENS se često naziva i „drugim mozgom“ (6).

Da bi pravilno funkcionisao i ostvarivao životno važne funkcije za naš organizam, neophodan je pravilan embrionalni razvoj ENS. Tokom razvoja, prekursorske ćelije ENS različitog porekla se mobilišu i precizno utvrđenim putanjama migriraju ka ciljnom mestu gde će se diferenovati u neurone ili glijalne ćelije (2). Svi procesi tokom embrionalnog razvoja ENS strogo su kontrolisani brojnim signalnim putevima koji se još uvek aktivno izučavaju, pre svega sa ciljem poboljšanja dijagnostike (7) i eventualne modifikacije njihove aktivnosti kod različitih razvojnih poremećaja ENS, kao što je Hiršprungova bolest (8).

Cilj ovog istraživanja je sveobuhvatni prikaz dosadašnjeg znanja o pravilnom embrionalnom razvoju ENS i potencijalnim kritičnim tačkama za razvoj nekih patoloških stanja. Nekoliko skorašnjih preglednih radova se, takođe, sveobuhvatno i detaljno bavilo ovom tematikom (9-13).

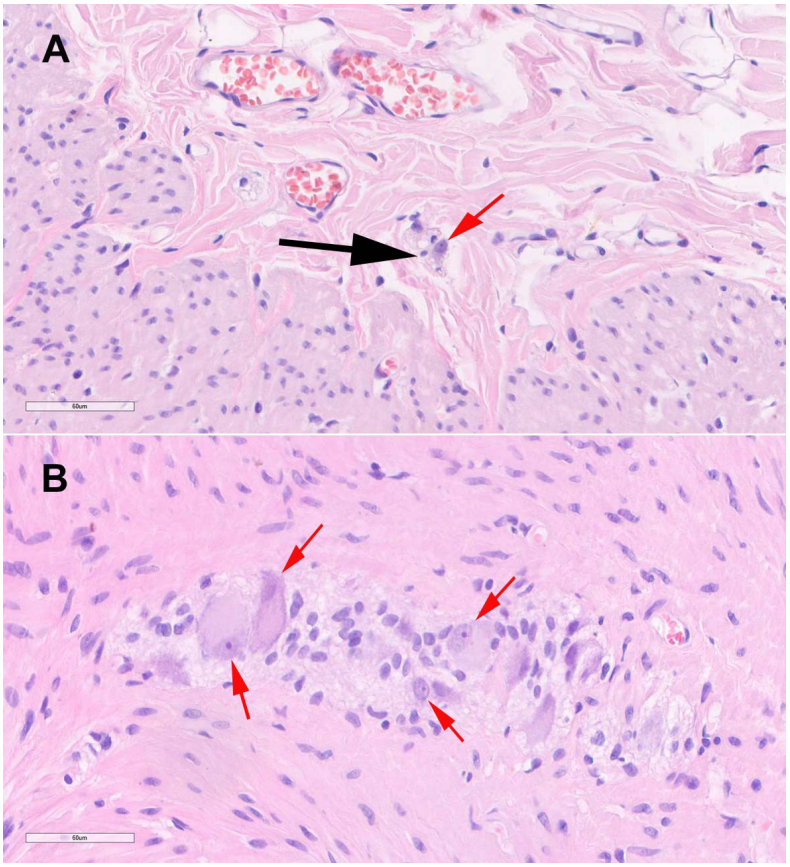
Metode

U ovom preglednom radu, radi što preciznijeg i sveobuhvatnijeg prikaza embrionalnog razvoja ENS, korišćena je literatura dobijena pretraživanjem MEDLINE baze podataka uz pomoć servisa PUBMED. Literatura objavljena na engleskom jeziku, u poslednjih 10 godina, dobijena je pretraživanjem sledećih ključnih reči: enterički nervni sistem, embrionalni razvoj, ćelijska migracija, Hiršprungova bolest.

Poreklo ćelija ENS

Tradicionalno se za proučavanje embrionalnog razvoja ENS koriste animalni modeli, i to mišji model, pileći embrion, zebrice i dr. U poslednje vreme sve više se istražuje i na humanom materijalu, a razvijaju se i različiti *in silico* modeli ENS.

Kao i gotovo svi drugi delovi perifernog nervnog sistema, i ćelije ENS nastaju od prekursorskih ćelija nervnog grebena (NCC – *neural crest cells*). Ovo je potvrđeno studijom Inteme i Hamonda još 1954. godine, i sve buduće studije se oslanjaju



Slika 1. Mikroskopski izgled ganglija enteričkih nervnih pleksusa (submukoznog i mijenteričkog) (HE, x1000).

Submukozne ganglije su sitne (crna strelica), sadrže mali broj ganglijskih ćelija (crvena strelica) (A), dok su mijenteričke ganglije krupnije i sadrže veći broj krupnijih ganglijskih ćelija sa vezikularnim jedrom i uočljivim jedarcetom (crvene strelice) (B).

which are completely within the ENS (5). Due to its complex structure and autonomy of its functions, the ENS is often called the "second brain" (6).

In order to function properly and perform these vital functions, the proper embryonic development of ENS is necessary. During its development, the precursor cells of the ENS, which have different origin, are mobilized and they migrate along the precisely established pathways to the target place where they will differentiate into neurons or glial cells (2). All these processes during the embryonic development are strictly controlled by numerous signaling pathways, which are still being actively researched, mostly in order to improve diagnostics (7) and possible modifications of their activities in different developmental disorders of the ENS, such as Hirschsprung disease (8).

The aim of this research was to present the comprehensive review of current knowledge about the proper embryonic development of ENS and potentially critical points for the development of some pathological conditions. Several recent review articles have dealt with this subject topic in a comprehensive and detailed way. (9-13).

Methods

In this review article, in order to provide a comprehensive and precise review of the embryonic development of ENS, we used literature from the MEDLINE database and the search was performed using the PubMed service. The literature in the English language that has been published in the last 10 years was obtained by searching the following key words: enteric nervous system, embryonic development, cell migration, Hirschsprung disease.

The origin of ENS cells

Traditionally, animal models are used to study the embryonic development of ENS, particularly mice, chicken embryo, zebrafish etc. The human material is being researched more and more, and different *in silico* models of ENS are being developed.

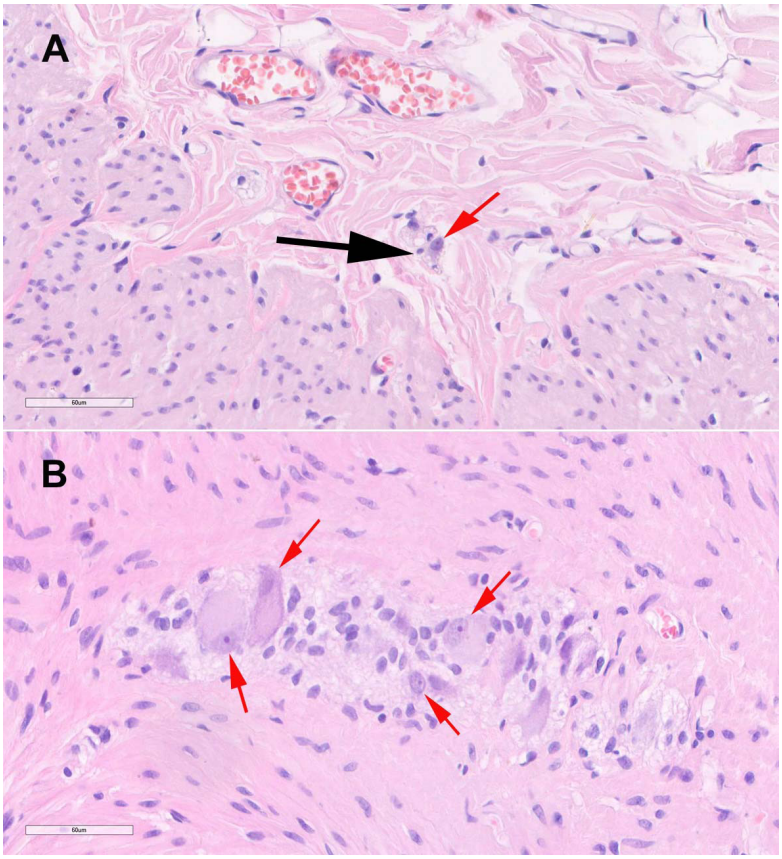
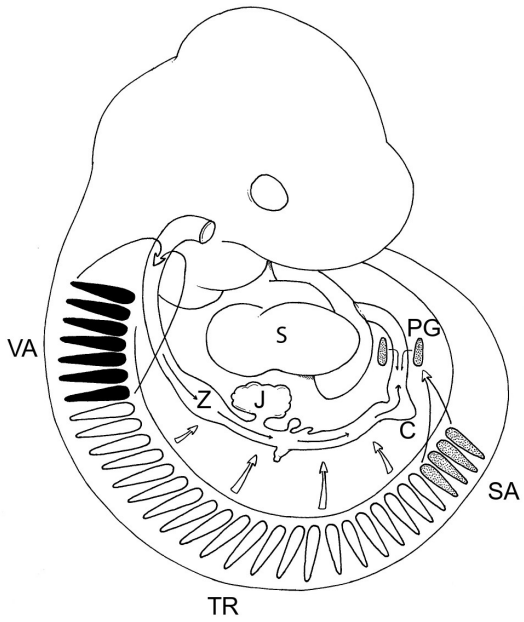


Figure 1. The microscopic view of ganglia of enteric nerve plexuses (submucosal and myenteric) (H&E, x1000).

Submucosal ganglia are small (black arrow), and contain a small number of ganglion cells (red arrow) (A), while myenteric ganglia are larger and contain larger number of larger ganglion cells with vesicular nuclei and prominent nucleoli (red arrows) (B).

na nju kada je u pitanju pomenuta hipoteza (14). Savremene studije, u kojima je vršeno praćenje ćelija poreklom nervnog grebena duž njihove migratorne putanje (*lineage tracing*), potvrdile su da ove ćelije učestvuju u izgradnji ENS (15). Nervni greben je privremena embrionalna struktura, koja se odvaja od nervne ploče prilikom zatvaranja u nervnu cev i pozicionira dorzalno u odnosu na nju. Pored perifernog nervnog sistema, tokom razvoja, ona daje i druge važne strukture kao što su vezivna tkiva glave i vrata, melanociti, srž nadbubrežnih žlezda, itd (16). U pomenutim istraživanjima (14,15) pokazano je, takođe, da najveći broj ćelija ENS vodi poreklo od vagalnog dela nervnog grebena, u nivou od 1-7. somita. Međutim, ne doprinose sve ćelije vagalnog dela nervnog grebena podjednako formiranju ENS. Smatra se da ćelije u nivou trećeg i četvrtog somita daju najveći broj neurona (17). Ove ćelije ulaze u primitivno crevo u regionu budućeg jednjaka i započinju svoju migraciju kaudalno ka budućem analnom otvoru. Ona se odvija uporedo sa rastom creva, tako da migratorna putanja postaje sve duža tokom same migracije, pa se smatra da ove ćelije prelaze najveću putanju od svih embrionalnih ćelija našeg organizma (17,18). Druga važna populacija prekursorskih ćelija ENS jesu ćelije sakralnog dela nervnog grebena, kaudalno od 28. somita kod pilećeg embriona. Ove ćelije migriraju ventromedijalno, daju prekursore budućih ganglija pelvičnog pleksusa, a zatim ulaze u region zadnjeg creva prateći ekstrinzička nervna vlakna i nastavljaju svoju migraciju rostralno, ka ćelijama poreklom od vagalnog dela nervnog grebena (slika 2) (19). Ovakva tradicionalna slika porekla ENS danas se dopunjuje novim saznanjima koja ukazuju da jedan deo ćelija ENS vodi poreklo od prekursorskih Švanovih ćelija, koje migriraju u zid creva duž nervosa vagusa (region jednjaka i želuca), odnosno drugih ekstrinzičkih nervnih vlakana (ostatak digestivnog trakta) (20), kao i da prekursorske ćelije u regionu pankreasa (endodermalnog porekla) malim delom doprinose raznovrsnosti porekla ENS (21).



Slika 2. Poreklo prekursorskih ćelija enteričkog nervnog sistema i njihovi putevi migracije tokom embrionalnog razvoja.

Najveći broj ćelija enteričkog nervnog sistema potiče od ćelija vagalnog dela nervnog grebena (VA). Preostale ćelije potiču iz sakralnog dela nervnog grebena (SA), dok mali broj potiče od ćelija nervnog grebena regiona trupa (TR), kao i prekursorskih ćelija regiona pankreasa. Ćelije vagalnog dela nervnog grebena nakon ulaska u region prednjeg creva započinju migraciju u kaudalnom smeru. Suprotno od njih, ćelije sakralnog dela nervnog grebena prvenstveno učestvuju u izgradnji prekursorapelvičnih ganglija (PG), a zatim ulaze u region zadnjeg creva i započinju migraciju u rostralnom smeru. Prekursorske ćelije regiona trupa, kao prekursoriŠvanovih ćelija, migriraju u zid creva duž ekstrinzičkih nervnih vlakana. Smerovi migracije prikazani su strelicama. S – srce, Z – želudac, J – jetra, C – cekum.

Migracija, proliferacija, diferencijacija

Da bi se pravilno formirao ENS, ćelije prekursori prolaze kroz važne procese kao što su migracija, proliferacija, neuro-glijalna diferencijacija, stvaranje ganglija i aksonskih puteva, kao i sinaptogeneza (11). Ovi procesi se odigravaju u različitom periodu embrionalnog razvoja kod različitih vrsta, međutim, ono što je zajedničko, jeste da se ove faze međusobno u velikoj meri preklapaju. Na primer, kada prekursorske ćelije poreklom od nervnog grebena uđu u region prednjeg creva i započnu kaudalnu migraciju, jedan deo ćelija će se zaustaviti ranije i započeti diferencijaciju, dok će ostale ćelije, koje neprestano proliferišu, nastaviti migraciju kaudalno kako bi naselile čitavo crevo (13).

Ćelije vagalnog dela nervnog grebena u nivou prvog i drugog somita započinju svoj drugi talas migracije ka mezenhimu ventralnog dela somita (sklerotom). Prethodno se u toku prvog talasa migracije odvajaju ćelije koje naseljavaju škržne lukove i region izlaznog trakta srca (17). Migracija ka sklerotomu somita je delom posledica odbojnog dejstva semaforina-3F koji eksprimiraju ćelije zad-

As all other parts of the peripheral nervous system, ENS cells are derived from precursor cells of the neural crest (NCCs – neural crest cells). This was confirmed by the study of Yntema and Hammond back in 1954 and all future studies relied on it in regard to the above-mentioned hypothesis (14). Contemporary studies, in which NCCs have been observed along their migratory pathway (*lineage tracing*), have confirmed that these cells take part in the formation of ENS (15). The neural crest is a temporary embryonic structure, which arises from the neural plate when the neural tube closes and it is positioned along the dorsal side of the neural tube. Besides the peripheral nervous system, during its development, it gives rise to other important structures such as connective tissues of head and neck, melanocytes, and the marrow of adrenal glands, etc (16). In the mentioned research (14,15), it has been shown that the majority of ENS cells originate from the vagal part of the neural crest, region adjacent to somite 1-7. However, not all vagal NCCs contribute equally to the formation of ENS. It is deemed that cells at the level of somites three and four give the largest number of neurons (17). These cells enter the primitive gut tube in the region of future esophagus and begin their migration caudally towards the future anal region. This occurs along with the intestine's growth, and therefore the migratory path becomes longer during migration. Therefore, it is thought that these cells pass the longest path of all embryonic cells of our organism (17,18). Another important population of ENS precursor cells are sacral NCCs, caudal from the 28th somite in the chicken embryo. These cells migrate ventromedially, give precursors of future ganglia of the pelvic plexus, and then enter the hindgut region following the extrinsic nerve fibers and continue their migration rostrally towards vagal NCCs (Figure 2) (19). This traditional picture of the origin of ENS is complemented by new knowledge that indicates that one part of the ENS cells originate from Schwann cells precursors (SCP), which migrate into the intestinal wall along vagus nerve (the region of esophagus and stomach), and other extrinsic nerve fibers (the rest of the digestive tract) (20), as well as that precursor cells in the pancreatic region (endodermal origin) contribute, to a small extent, to the heterogeneity of ENS origin (21).

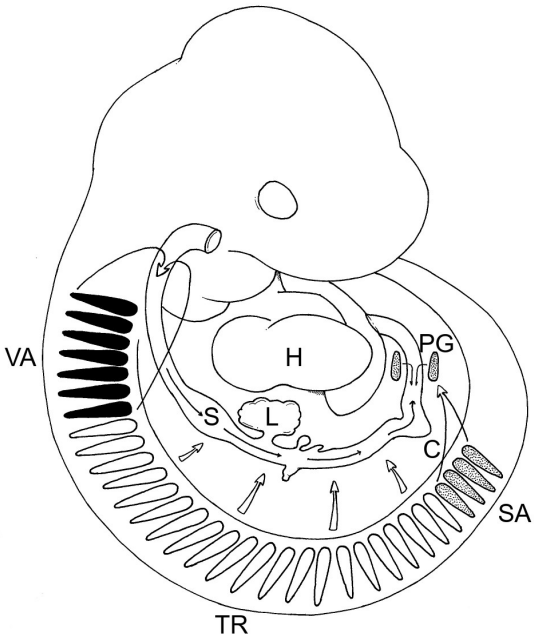


Figure 2. The origin of precursor cells of the enteric nervous system and their migration pathways during embryonic development.

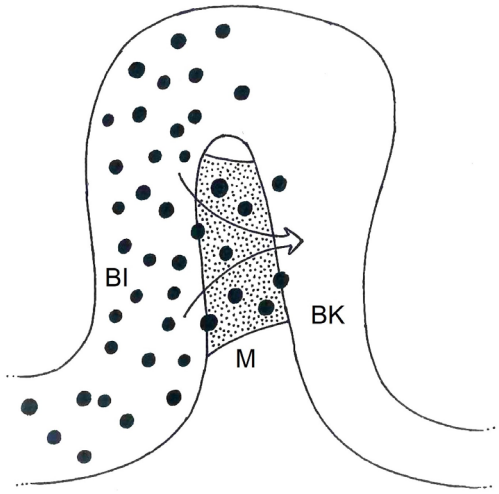
The majority of enteric nervous system precursor cells are derived from the vagal neural crest (VA). Remaining cells originate from the sacral neural crest (SA), the trunk neural crest (TR) and the pancreatic precursor cells. Vagal neural crest cells enter the foregut region and continue their migration caudally. In contrast, sacral neural crest cells primarily form the precursors of the pelvic ganglia (PG), then enter the hindgut region and begin to migrate in the rostral direction. Trunk neural crest cells, as Schwann cell precursors, migrate into the gut wall along extrinsic nerve fibers. The migration pathways are shown by arrows. H – heart, S – stomach, L – liver, C- cecum.

Migration, proliferation and differentiation

Precursor cells pass through important processes such as migration, proliferation, neuro-glial differentiation, creation of ganglia and axonal pathways, as well as synaptogenesis so that the ENS would develop properly (11). These processes unfold in different periods of embryonic development in different species, however, what they have in common is the fact that these stages are mutually interconnected. For example, when NCCs enter the foregut region and begin caudal migration, one part of these cells will be stopped earlier and start differentiation, while other cells, which proliferate all the time, will continue migration caudally in order to colonize the whole gut (13).

njeg dela somita (dermatomiotom), a koji se vezuje za neuropilin-2 receptor na ćelijama nervnog grebena (22). Jedna podgrupa ovih ćelija odvaja se i putuje ka budućem srcu, učestvujući dodatno u izgradnji izlaznog trakta. Smatra se da ovu grupu ćelija koja eksprimira CXCR4 (*CXC chemokine receptor 4*) privlači ligand SDF1 (*stromal cell-derived factor 1*) koji eksprimiraju embrionalne ćelije srca (23). Druga podgrupa daje prekursorske Švanove ćelije koje putuju duž vagusnih nervnih vlakana do jednjaka i želuca i učestvuju u izgradnji ENS ovih delova digestivnog trakta (20).

Ćelije vagalnog dela nervnog grebena u nivou 3-7. somita, koje će dati najveći deo ENS započinju migraciju ka proksimalnom delu prednjeg creva i ulaze u region budućeg jednjaka oko četvrte nedelje gestacije kod čoveka (24), a u mišjem modelu između devetog i desetog dana embrionalnog razvoja (25). Od ovog trenutka, smatra se da su opredeljene za izgradnju ENS pa se nazivaju enteričke ćelije poreklom od nervnog grebena (ENCC – *enteric neural crest-derived cells*) (9). Ove ćelije svoj put nastavljaju u kaudalnom smeru, ka budućem analnom otvoru. Prvenstveno migriraju duž prednjeg (od jednjaka do nishodnog dela dvanaestopalačnog creva u nivou buduće Vaterove papile) i srednjeg creva (od Vaterove papile sve do kraja ⅔ transverzalnog dela debelog creva) (10,11). Primećeno je da jedna subpopulacija migrirajućih ćelija zaobilazi slepo crevo, prelazeći direktno iz proksimalnog u distalni deo srednjeg creva i zadnje crevo preko mezenterijuma, koji prolazno povezuje ove delove creva, odnosno, dovodi ih u neposrednu blizinu. Transmezenterična migracija, kako je nazvana, doprinosi značajnom broju ćelija ENS budućeg debelog creva (slika 3) (26). Na daljem putu kroz zadnje crevo vagalne ENCC susrešće ćelije poreklom od sakralnog dela nervnog grebena, koje migriraju rostralno, i ubrzo završiti svoju longitudinalnu migraciju. U mišjem modelu, čitavo crevo kolonizovano je do 14. embrionalnog dana (25), dok kod čoveka, najverovatnije, do 7. nedelje embrionalnog razvoja (24).



Slika 3. Transmezenterična migracija prekursorskih ćelija enteričkog nervnog sistema.

Prilikom migracije, jedan deo prekursorskih ćelija enteričkog nervnog sistema, prelazi direktno iz regiona budućeg ileuma u region budućeg kolona preko mezenterijuma, zaobilazeći region slepog creva. Crne tačke označavaju prekursorske ćelije poreklom od nervnog grebena koje migriraju. Strelica pokazuje pravac i smer transmezenterične migracije.
BI – budući ileum, M – mezenterijum, BK – budući kolon.

Ćelije nervnog grebena ispod nivoa 7. somita (region trupa) ne ulaze u region prednjeg creva. Pretpostavlja se da mezenhimne ćelije prednjeg creva produkuju određene molekule, kao što je Slit1, koji, vezujući se za Robo receptor eksprimiran na NCC regiona trupa, sprečava migraciju ovih ćelija ka prednjem crevu. Robo receptor nije eksprimiran na vagalnim NCC (27). Stvarajući prekursore Švanovih ćelija, ove ćelije migriraju duž ekstrinzičkih nervnih vlakana, i u manjoj meri doprinose ukupnom broju ćelija ENS (20).

Grupa sakralnih ćelija nervnog grebena koja učestvuje u izgradnji ENS, kao što je rečeno, migrira ventralno ka distalnom delu zadnjeg creva. Nakon što izgrade mrežu ćelija od koje će se formirati pelvični pleksus, ove prekursorske ćelije ulaze u region zadnjeg creva duž ekstrinzičkih nervnih vlakana, gde će dati neurone i glija ćelije ENS ovog regiona. Njihova kratka migracija usmerena je rostralno (19). Iako daju morfološki i funkcionalno identične nervne i glijalne ćelije, vagalne i sakralne ENCC se u mnogo čemu razlikuju (28). Pored suprotnog smera migracije i različite lokalizacije primećene su još neke značajne razlike. Kada se vagalne ENCC implantiraju u sakralni region, one migriraju znatno ranije, u većem broju i proksimalnije od sakralnih ENCC. Pri tome pravac migracije je rostralni, kao i kod sakralnih ENCC. Zaključuje se da vagalne ENCC imaju znatno veći migratorni potencijal od sakralnih, kao i to da smer migracije

Vagal NCCs at the level of the first and the second somites begin their second wave of migration towards the mesenchyme of the ventral part of somites (sclerotome). Previously, during the first wave of migration, cells proliferate and colonize the gill's arches and region of the cardiac outflow tract (17). Migration towards the somite sclerotome is partly the consequence of the inhibiting effect of semaphorin-3F which is expressed in the posterior part of the somites (dermomyotome), and which is bound to the neuropilin-2 receptor on NCCs (22). One subgroup of these cells separates and travels towards the future heart, taking part in the development of outflow tract. It is deemed that this group of cells, which expresses CXCR4 (*CXC chemokine receptor 4*), attracts the ligand SDF1 (*stromal cell derived factor*) expressed in the embryonic heart cells (23). The second subgroup gives the SCPs that travel along vagal nerve fibers to the esophagus and stomach and participate in the development of ENS in these regions of digestive tract (20).

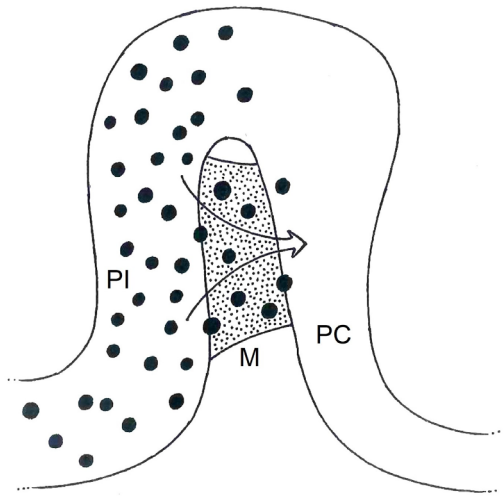


Figure 3. Transmesenteric migration of precursor cells of the enteric nervous system.

During migration, a number of precursor cells of the enteric nervous system pass directly from the region of the future ileum to the region of the future colon through the mesentery, bypassing the region of the cecum. Migrating precursor cells are shown by black dots. The arrow indicate the direction of transmesenteric migration. PI – presumptive ileum, M – mesentery, PC – presumptive colon.

Vagal NCCs at the level of somites 3-7, which would give the largest part of ENS, start their migration towards the proximal part of foregut and enter the region of future esophagus around the fourth gestational week in humans (24), and between the ninth and tenth day of embryonic development in the murine model (25). Since that moment, it is deemed that these cells are responsible for the ENS development, and therefore, they are called enteric neural crest-derived cells (ENCCs) (9). These cells continue their way caudally towards the future anal region. Primarily, they migrate along the foregut (from the esophagus to the descending part of duodenum at the level of future papilla of Vater) and the midgut (from papilla of Vater to the end of 2/3 of the transversal part of colon) (10,11). It has been noticed that one subpopulation of migratory cells avoids the appendix, passing directly from the proximal part to the distal part of midgut and to the hindgut through mesentery, which connects these parts of intestines, bringing them to the immediate vicinity. The transmesenteric migration, as it has been named, contributes to the significant number of ENS cells of the future colon (Figure 3) (26). On their way through the hindgut of the vagal ENCCs meet sacral ENCCs, which migrate rostrally, and they would soon finish their longitudinal migration. In the murine model, the whole gut is colonized until the 14th embryonic day (25), while in humans, most probably, until the 7th week of embryonic development (24).

NCCs below the level of the seventh somite (the trunk region) do not enter the foregut region. It is assumed that mesenchymal cells of the foregut produce certain molecules, such as Slit 1, which attaches to the Robo receptor, expressed on the NCCs of the trunk region, and prevents the migration of these cells towards the foregut. Robo receptor is not expressed on vagal NCCs (27). By creating the SCPs, these cells migrate along extrinsic nerve fibers, less contributing to the total number of ENS cells (20).

A group of sacral NCCs, as it has been mentioned, migrates ventrally towards a distal part of hindgut. After they build a network of cells, which the pelvic plexus will be formed from, these precursor cells enter the region of hindgut along the extrinsic nerve fibers, where they will give rise to neurons and glial

nije unapred određen već zavisi od interakcije sa okolnim ćelijama (28). Zanimljivo je da u modelu zebrica, sakralne NCC ne učestvuju u izgradnji ćelija ENS (29).

Da bi buduće ćelije ENS kolonizovale čitav digestivni trakt neophodna je kontinuirana proliferacija progenitorskih ćelija i održavanje tzv. progenitorskog pula. Pored broja inicijalnih NCC, za pravilnu migraciju i diferencijaciju od velikog značaja je gustina ENCC na tzv. migratornom frontu. Ova gustina održava se proliferacijom prekursorskih ćelija (30). Velika migratorna sposobnost ćelija migratornog fronta, koje vode migraciju u nekolonizovane delove creva, delimično je posledica međućelijske interakcije. Naime, smatra se da ćelijski kontakti posredovani kadherinima, integrinima i nekim proteinima imunoglobulinske superfamilije odbijaju ćelije jedne od drugih i primoravaju ih da pronađu put u nenaseljene delove creva (fenomen kontaktne inhibicije). Manjak ćelijske adhezije doveo bi do nemogućnosti trakcije i samog kretanja ćelija, dok bi prevelika adhezija izazvala usporenje ili prevremeni prekid migracije (31). Nesumjivo je i to da mezenhimne ćelije creva eksprimiraju molekule koji privlače ove ćelije i doprinose usmeravanju migracije. Primer ovakvog molekula je GDNF (*Glial-Derived Neurotrophic Factor*), koji se vezuje za RET (*Rearranged during Transfection*) receptor na ENCC (32).

Uporedo sa napredovanjem migracije, jedan broj ćelija lociranih iza migratornog fronta, započinje diferencijaciju u neurone ili glijalne ćelije dok druge nastavljaju ćelijski ciklus kao progenitorske ćelije, kako bi obezbedile dovoljan broj prekursorskih ćelija u rastućem crevu. Iz ovoga je jasno da je neophodna veoma precizna kontrola i balans između procesa proliferacije i diferencijacije kako bi se razvoj ENS do kraja nesmetano odigrao. Transkripcioni faktori SOX10 (*SRY-box transcription factor 10)* i PHOX2B (*Paired-like Homeobox 2B*), koji su koeksprimirani na prekursorskim ćelijama poreklom od nervnog grebena, doprinose balansu broja neurona odnosno glija ćelija. Prekursorske ćelije ENS koje su opredeljene za diferencijaciju u neurone smanjuju ekspresiju SOX10, a održavaju ekspresiju PHOX2B, dok se u ćelijama opredeljenim za glijalnu liniju dešava suprotno (9). Mutacije koje dovode do prevremene diferencijacije prekursorskih ćelija, manifestovaće se distalnom aganglionozom (Hiršprungova bolest) zbog nemogućnosti diferentovanih ćelija da značajnije migriraju. Takođe, ni kasna diferencijacija nije povoljna zbog prevelikog broja prekursorskih ćelija čija je pravilna diferencijacija potencijalno ograničena novim mikrosredinskim uslovima u razvijajućem crevu. Tokom normalnog embrionalnog razvoja, neurogeneza započinje pre gliogeneze (33). Sa eksprimiranjem neuron-specifičnih markera (pre svega specifičnih neurotransmitera), budući neuroni završavaju svoju migraciju i gube proliferativnu sposobnost (34). Specifične subpopulacije neurona koje se prve javljaju tokom razvoja su serotonergički neuroni (5-HT), holinergički neuroni (ChAT), kao i neuroni koji sadrže enkefalin. Nešto kasnije formiraju se i ostale subpopulacije neurona, kao što su neuroni pozitivni na vazoaktivni intestinalni peptid (VIP), NADPH i drugi (9).

Neophodno je da se neuroni i glija ćelije međusobno organizuju u ganglije, kako bi se pravilno formirali pleksusi ENS. Iako je pokazano postojanje više ganglijskih i neganglijskih pleksusa (2), najčešće se opisuje formiranje dva najveća i najznačajnija: mijenteričkog i submukoznog. Mijenterički pleksus razvija se pre submukoznog. Ovo navodi na zaključak da se uporedo sa longitudinalnom, odigrava i radijalna migracija prekursorskih ćelija ENS. U studiji *Memic i sar*. pokazano je postojanje velikog broja različitih signalnih molekula, odnosno transkripcionih faktora, koji, između ostalog, učestvuju i u procesu formiranja submukoznog pleksusa (35). Jedan od faktora koji doprinosi radijalnoj migraciji prekursorskih ćelija ENS ka mukozi jeste grupa molekula označenih kao netrini (*netrin-1*, *netrin-3* u mišjem modelu). Ovi molekuli ekprimirani su na epitelnim ćelijama mukoze creva, u najvećoj meri upravo u trenutku razvoja submukoznog pleksusa. Sa druge strane, prekursorske ćelije ENS eksprimiraju netrinski receptor DCC (*Detected in Colorectal Cancer*) za koji se netrini vezuju i ostvaruju svoju funkciju (36). Međutim, postavlja se pitanje šta utiče na to da se ove ćelije zaustave u submukozi gde formiraju pleksus, a ne nastave migraciju ka mukozi? Jedno od mogućih objašnjenja jeste da laminin-111 koga produkuju ćelije mukoze deluje suprotno delovanju netrina, i zaustavlja migrirajuće ćelije u blizini mukoze, odnosno u submukozi, gde će se formirati Majsnerov pleksus (37). Ovo je polje intenzivnog istraživanja i očekuje se da će u budućnosti biti otkriven veliki broj drugih molekula odnosno sig-

cells of ENS of this region. Their short migration is directed rostrally (19). Although they give morphologically and functionally identical nerve and glial cells, vagal and sacral ENCCs are different in many respects (28). In addition to the opposite direction of migration and different localization, some other significant differences have been noticed. When vagal ENCCs are implanted into the sacral region, they migrate earlier, in larger number and more proximally than sacral ENCCs. Also, the direction of migration is rostral. It is concluded that vagal ENCCs have significantly greater migratory potential than the sacral ENCCs, as well as that the direction of migration is not determined in advance, but depends on the interaction with surrounding cells (28). It is interesting that in zebrafish model, sacral NCCs do not take part in the development of ENS cells (29).

A continuous proliferation of progenitor cells and maintenance of the progenitor pool are necessary so that the future ENS cells would colonize the entire digestive tract. Besides the number of initial NCCs, the density of ENCCs on the migratory front is of great importance for the proper migration and differentiation. This density is maintained by the proliferation of precursor cells (30). A great migratory capacity of migratory front cells, which lead migration to the non-colonized parts of intestines, is partly a consequence of cell-cell interaction. Namely, it is deemed that cellular contacts, which are mediated by cadherins, integrins and some proteins of immunoglobulin superfamily, repel cells one from another and make them find their way to non-colonized parts of intestines (the phenomenon of contact inhibition). The lack of cell adhesion would lead to the impossibility of traction and movement of cells, while excessive adhesion would cause slowness or premature interruption of migration (31). Undoubtedly, mesenchymal cells in the intestines express molecules that attract these cells and determine the direction of migration. An example of such a molecule is GDNF (*Glial-Derived Neurotrophic Factor*), which is attached to RET (*Rearranged during Transfection*) receptor on ENCCs (32).

Along with the advancement of migration, a number of cells located behind the migratory front begin differentiation into neurons or glial cells, while other cells continue their cellular cycle as progenitor cells, in order to provide a sufficient number of precursor cells in the growing gut. Thus, it is clear that a precise control and balance between proliferation and differentiation processes is necessary so that the ENS development would unfold in an undisturbed way. Transcription factors SOX10 (*SRY-box transcription factor 10*) and PHOX2B (*Paired-like Homeobox 2B*), which are co-expressed on NCCs contribute to the balance of the number of neurons and glial cells. Precursor ENS cells that differentiate into neurons decrease the expression of SOX10, and maintain expression of PHOX2B, while opposite is the case in glial line precursor cells (9). Mutations that lead to the premature differentiation of precursor cells will be manifested with distal aganglionosis (Hirschsprung disease) due to the impossibility of differentiated cells to migrate more significantly. Also, late differentiation is not favorable due to the excess number of precursor cells, whose proper differentiation is potentially limited by new micro-environmental conditions in developing gut. During the normal embryonic development, neurogenesis begins before gliogenesis (33). With the expression of neuron-specific markers (primarily specific neurotransmitters), future neurons end their migration and lose their proliferative capacity (34). Specific sub-populations of neurons, which appear first during the development, are serotonergic neurons (5-HT), cholinergic neurons (ChAT), as well as neurons that contain enkephalin. Later, other subpopulations of neurons are formed, such as neurons positive to vasoactive intestinal peptide (VIP), NADPH and others (9).

It is necessary that neurons and glial cells firstly organize into ganglia, in order to form ENS plexuses properly. Although the existence of more ganglionated and non-ganglionated plexuses has been shown (2), the formation of two largest and most significant ones is described: myenteric and submucosal. The myenteric plexus develops before the submucosal. This instigates the conclusion that along with the longitudinal, radial migration of precursor ENS cells also unfolds. In the study of Memic at al. the existence of a large number of different signaling molecules and transcription factors has been shown which, among other things, take part in the process of formation of submucosal plexus (35). One of the factors, which contributes to radial migration of ENS precursor cells towards mucosa, is a group of molecules marked as netrins (*netrin-1*, *netrin-3* in the

nalnih puteva koji učestvuju ne samo u radijalnoj migraciji, već i u diferencijaciji pojedinih podtipova neurona, uspostavljanju aksonskih puteva i sinaptogenezi.

Za pravilnu funkciju ENS neophodan je pravilan embrionalni razvoj i drugih ćelija, pre svega intersticijskih (Kahalovih) ćelija i glatko-mišićnih ćelija. Kahalove ćelije su sposobne da generišu i propagiraju spore talase, zbog čega su bitne u održavanju fiziološke kontraktilnosti creva. Označene su i kao pejsmejker ćelije (8). Kahalove, kao i glatko-mišićne ćelije, nastaju od mezenhima creva, a smatra se da prekursorske ćelije ENS utiču na njihovu diferencijaciju (38,39).

Zaključak

Dve glavne populacije ćelija, poreklom od vagalnog i sakralnog dela nervnog grebena, prolazeći kroz kompleksne procese migracije, proliferacije, diferencijacije, učestvuju u izgradnji najvećeg dela ENS. Iako različite u mnogim aspektima embrionalnog razvoja, ove dve grupe ćelija daju identične tipove neurona i glijalnih ćelija koji zajednički uspostavljaju i održavaju funkciju ENS, koja je, nesumnjivo, od vitalne važnosti za naš organizam. Veliki broj različitih subtipova neurona, koji nisu organizovani u jasne formacije, kao što je to slučaj u centralnom nervnom sistemu, interakcije sa različitim ćelijama unutar i van ENS samo su neki od razloga zbog kojih su mnoge nejasnoće u strukturi i funkciji ENS još uvek prisutne. Praćenje pojedinačnih ćelija na njihovom razvojnom putu, odnosno sve bolje upoznavanje embrionalnog razvoja ENS uopšte, u velikoj meri može pomoći u razjašnjavanju ovih nedoumica.

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Autori se zahvaljuju Savu Gudžuliću, studentu Medicinskog fakulteta u Beogradu, na urađenim crtežima.

Konflikt interesa

Autori su izjavili da nema konflikta interesa.

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murine model). These molecules are expressed in epithelial cells of intestinal mucosa mostly at the moment of development of submucosal plexus. On the other hand, precursor cells of ENS express netrin receptor DCC (Detected in Colorectal Cancer), which netrins bind to and perform their function (36). However, the question is what causes these cells to stop their migration in submucosa, where they form plexus? One of the possible explanations is that laminin-111 which is produced by mucosal cells acts opposite to netrin, and stops migratory in submucosa, where Meissner's plexus will be formed (37). This is the field of intense research and it is expected that a large number of other molecules or signaling pathways, which take part not only in the radial migration, but also in the differentiation of certain subtypes of neurons, establishing of axonal pathways and synaptogenesis, will be found in the future.

Proper embryonic development of other cells, like interstitial cells of Cajal and smooth muscle cells, is necessary for proper functioning of ENS. Interstitial cells of Cajal can generate and propagate slow waves, and therefore they are important for the maintenance of physiological contractility of intestines. They were marked as pacemaker cells (8). Interstitial cells of Cajal, as well as smooth muscle cells, are derived from the intestinal mesenchyme, and it is deemed that ENS precursor cells influence their differentiation (38,39).

Conclusion

Two main populations of cells, which are derived from the vagal and sacral part of the neural crest, take part in the development of the largest part of ENS. They pass through complex processes of migration, proliferation, differentiation. Although they are different in regard to many aspects of embryonic development, these two groups of cells give identical types of neurons and glial cells which establish and maintain the function of ENS, which is, undoubtedly, vital for our organism. A great number of different subtypes of neurons, not organized in clear formations, as is the case in central nervous system, interactions with different cells within and out of the ENS are just some of the reasons why many uncertainties about the structure and function of ENS are still present. Following individual cells through their development pathways, as well as better understanding of embryonic development of ENS generally, may help to elucidate some of these uncertainties.

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Competing interests

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EFEKTI KADMIJUMA NA TRANSPORTNE PROCESE U PROKSIMALNIM TUBULSKIM ĆELIJAMA BUBREGA

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SAŽETAK

Kadmijum (Cd) je ekstremno toksičan metal koji je u prirodi široko rasprostranjen. Zbog svojih povoljnih osobina masovno se upotrebljavao u industriji za izradu alkalnih baterija, akumulatora, pigmenata, bojenih legura. Ipak, pokazano je da izlaganje kadmijumu u malim koncentracijama dovodi do oštećenja brojnih organa i organskih sistema te se upotreba ovog metala u industriji smanjuje, a zamenjuju ga drugi, manje štetni materijali. Danas je važan izvor izloženosti kadmijumu sagorevanje fosilnih goriva i konzumiranje cigareta. Brojne studije ispitivale su štetne efekte kadmijuma i one ističu bubrege, jetru i gonade kao organe koji trpe najveća oštećenja. Bubrezi, kao glavno mesto deponovanja kadmijuma u organizmu, u najvećoj meri izloženi su njegovim toksičnim efektima. U proksimalnim tubulskim ćelijama bubrega izlaganje kadmijumu remeti transportne procese. Iako se smatra da je jonizovani kadmijum (Cd^{2+}) u najvećoj meri odgovoran za oštećenja koja nastaju, ne može se zanemariti ni uloga kompleksa kadmijuma i metalotioneina (Cd-MT). Peritubularno izlaganje jonizovanom kadmijumu dovodi indirektno do smanjenja aktivnosti Na^+/L-alanin kotransportera i smanjenja brzine spore repolarizacije luminalne membrane, dok kompleks Cd-MT dovodi i do direktne i do indirektne inhibicije ovog transportera. Takođe, Cd-MT kompleks inhibira aktivnost Na^+/glukoza kotransportera. Izlaganje kadmijumu dovodi i do smanjenja preuzimanja niskomolekularnih proteina putem olakšane endocitoze što je praćeno mikroalbuminurijom.

Ključne reči: kadmijum, proksimalni tubuli, transport, nefrotoksičnost.

Uvod

Kadmijum (Cd) je teški metal koga su otkrili 1817. godine, nemački hemičari *Friedrich Strohmeyer* i *Karl Hermann* kao nečistoću u cink-karbonatu. U prirodi ovaj element se može naći u sastavu Zemljine kore, emituje se pri vulkanskim erupcijama, šumskim požarima, eroziji stena u reke i mora (1,2). Značajna količina kadmijuma nalazi se u fosilnim gorivima pa se njihovim sagorevanjem emituje u atmosferu. U prošlosti kadmijum je imao različite namene – u obliku kadmijum jodida koristio se za lečenje otoka zglobova, promrzlina, a zbog svoje povoljne osobine da teško korodira koristio se za premazivanje legure čelika i gvozđa. Široko se primenjivao u industriji: za izradu alkalnih baterija, akumulatora, pigmenata, bojenih legura, plastike (1-3). Danas se prema podacima Američke agencije za registrovanje toksičnih supstanci i bolesti (engl. A*gency for Toxic Substances and Disease Registry* - ATSDR) kadmijum nalazi na sedmom mestu prioritetne liste opasnih supstanci zbog čega se njegova upotreba smanjuje. Ova agencija kao glavni izvor izloženosti kadmijumu ističe konzumiranje cigareta kod pušača, dok se nepušači najčešće izlažu konzumacijom hrane zagađene kadmijumom (4). Voda u blizini industrijskih objekata koji koriste kadmijum sadrži povećane koncentracije ovog metala, a izloženost putem vazduha je takođe značajna samo u ovim područjima. Do profesionalne izloženost može doći u rudnicima ili tokom proizvodnje i prerade sirovina koje sadrže kadmijum (4).

Brojne studije pokazale su da kadmijum uzrokuje oštećenja različitih organa, a svoju toksičnost ispoljava i pri izlaganju niskim koncentracijama

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THE EFFECTS OF CADMIUM ON THE TRANSPORT PROCESSES IN PROXIMAL TUBULAR CELLS OF KIDNEYS

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SUMMARY

Cadmium (Cd) is an extremely toxic metal that is widespread in nature. Due to its favorable properties, it was widely used in the industry for the production of alkaline batteries, accumulators, pigments, and colored alloys. However, it has been shown that exposure to low concentrations of cadmium leads to damage to many organs and organ systems, and the use of this metal in industry is reduced, and it is replaced by other, less harmful materials. Today, fossil fuel combustion and cigarette consumption are important sources of cadmium exposure. Numerous studies have examined the toxic effects of cadmium and they highlight the kidneys, liver, gonads as the organs that suffer the most damage. The kidneys, as the main place of cadmium storage in the body, are mostly exposed to its toxic effects. In the proximal tubular cells of the kidney, exposure to cadmium disrupts transport processes. Although ionized cadmium (Cd^{2+}) is thought to be largely responsible for the damage that occurs, the role of the cadmium and metallothionein complex (Cd-MT) cannot be ignored. Peritubular exposure to ionized cadmium indirectly leads to a decrease in the activity of the Na^+/L-alanine cotransporter and a decrease in the rate of slow repolarization of the luminal membrane, while the Cd-MT complex leads to both direct and indirect inhibition of this transporter. Also, the Cd-MT complex inhibits Na^+/Glucosa cotransporter activity. Exposure to cadmium also leads to a decrease in the endocytic uptake of low molecular weight proteins, which is accompanied by microalbuminuria.

Key words: cadmium, proximal tubules, transport, nephrotoxicity

Introduction

Cadmium (Cd) is a heavy metal that was discovered in 1817 by German chemists *Friedrich Strohmeyer* and *Karl Hermann* as an impurity in zinc-carbonate. In nature, this element can be found in the Earth's crust, and it is released during volcanic eruptions, forest fires, coastal erosion (1,2). Significant quantities of cadmium are present in fossil fuels, and therefore, emissions of cadmium are caused by their combustion. In the past, cadmium had different uses – it was used as cadmium iodide for the treatment of swollen joints, frostbite, and due to its favorable properties regarding corrosion, cadmium coatings were used to protect alloys of steel and iron against corrosion. It was widely applied in industry: for the production of alkaline batteries, accumulators, pigments, colored alloys, plastics (1-3). Today, according to the US Agency for Toxic Substances and Disease Registry (ATSDR), cadmium ranks seven on the priority list of hazardous substances, and therefore, its usage has been reduced. This agency stresses cigarette consumption as the main source of cadmium exposure in smokers, while non-smokers are most frequently exposed to cadmium via food that is contaminated by cadmium (4). Water that is near industrial facilities that use cadmium contains higher concentrations of this metal, while exposure via air is also significant in these regions. Occupational exposure may happen in mines or during the production and processing of raw materials that contain cadmium (4).

(5). Mehanizmi kojima ispoljava štetno dejstvo su višestruki – vezujući se za sulfhidrilne grupe antioksidativnih enzima inhibira njihovu aktivnost, nagomilavanjem u ćeliji može izazvati disfunkciju mitohondrijalnog transportnog lanca elektrona i stvaranje reaktivnih kiseoničnih radikala što posledično dovodi do oksidativnog stresa, smanjuje koncentraciju selena u organizmu potrebnog za formiranje glutation peroksidaze, indukuje inflamaciju, apoptozu ćelija (5-7). U najvećoj meri kadmijum oštećuje bubrege, jetru i gonade ali oštećenjem su zahvaćeni i drugi organski sistemi – respiratorni, hematopoezni, kardiovaskularni, koštano zglobni (5).

Bubrezi predstavljaju glavni organ za deponovanje i toksične efekte kadmijuma. Od ukupne količine kadmijuma u organizmu, u bubrezima se nalazi 30-50%, pri čemu je glavno mesto deponovanja u ćelijama proksimalnih tubula (8). Pokazano je da u ćelijama proksimalnih tubula bubrega deponovani kadmijum remeti procese reapsorpcije različitih supstanci iz primarnog urina. Stoga, cilj ovog istraživanja je da što preciznije sumira dosadašnja znanja o uticaju kadmijuma na transportne procese u ćelijama proksimalnih tubula bubrega.

Metode

U ovom preglednom radu, radi što preciznijeg i sveobuhvatnijeg prikaza efekata kadmijuma na ćelije proksimalnih tubula, korišćena je literatura dobijena pretraživanjem MEDLINE baze podataka uz pomoć servisa PUBMED. Literatura objavljena na engleskom jeziku, u poslednjih 10 godina, dobijena je pretraživanjem sledećih ključnih reči: kadmijum, nefrotoksičnost, proksimalni tubuli.

Kadmijum – toksikokinetika i toksikodinamika

Dominantan put ulaska kadmijuma u organizam kod osoba koje nisu profesionalno izložene ovom elementu je oralni, kod profesionalno izloženih dominantan je inhalatorni put unosa, a ukupnoj ekspoziciji doprinose i apsorpcija kadmijuma preko kože i gastrointestinalnog trakta (8). Iz lumena creva kadmijum se transportuje u enterocite preko transportera za dvovalentne metale tip 1 (DMT-1, *divalent metal ion transporter 1*) i proteina za transport metala tip 1 (MTP-1, *metal transport protein 1*), a apsorpcija je moguća i vezivanjem za sulfhidrilne grupe cisteina i glutationa (9,10). Kada dospe u krv, kadmijum se transportuje eritrocitima i vezan za proteine plazme (8). *Yiling Li* i saradnici su u nedavno rađenoj studiji pokazali da je jedan od proteina ljudske plazme za koji se kadmijum vezuje u krvi apolipoprotein A-I (11). Dalje se kadmijum transportuje do svojih depoa u organizmu – bubrega, jetre i mišića (8). U akutnoj toksičnosti primarno oštećenje izazvano kadmijumom događa se na nivou jetre, dok se u hroničnoj toksičnosti dominantno oštećuju bubrezi (12). U hepatocitima kadmijum stimuliše sintezu metalotioneina (MT) sa kojima formira kompleks koji štiti ćelije od oksidativnog stresa. Smatra se da oštećenje hepatocita nastaje kada se prevaziđe kapacitet metalotioneina za puferovanje jona kadmijuma (13,14). Po odumiranju hepatocita kompleks Cd-MT se ciruklacijom transportuje do bubrega. Kadmijum je delom vezan i za druge tiolne grupe – glutation (GSH), L-cistein (L-Cis), a delom se transportuje do bubrega i kao slobodan, jonizovani kadmijum (Cd^{2+}). U bubrezima se Cd-MT, Cd-GSH, Cd-Cis i slobodan Cd^{2+} lako filtriraju kroz glomerule bubrega, a zatim se na različite načine reapsorbuju (15,16). Cd-MT na nivou proksimalnih tubula se preuzima endocitozom, Cd-Cis i Cd-GSH se preuzimaju preko apikalne i bazolateralne membrane proksimalnih tubula, a Cd^{2+} se različitim transportnim sistemima može apsorbovati (15,16). Eliminacija kadmijuma u najvećoj meri vrši se preko gastrointestinalanog trakta, a u manjoj meri se u kompleksu sa metalotioneinima eliminiše preko bubrega (6,8).

Proksimalni tubuli bubrega

Osnovna strukturna i funkcionalna jedinica bubrega je nefron koji se sastoji od glomerula i pratećih kanalica. Glomerul je izgrađen od spleta kapilara smeštenih unutar Boumanove (*Bowman's*) kapsule. Na nivou glomerula vrši se filtracija krvi i stvara se primarni urin (17). Dalje se na urinarni pol glomerula nastavlja proksimalni tubul koji ima svoj izvijugani (*pars convoluta*) i pravi deo (*pars recta*) (18). Na nivou proksimalnog tubula vrši se reapsorpcija najvećeg dela ogranskih supstanci poput aminokiselina, glukoze, albumina i niskomolekularnih proteina ali i velikog dela neorganskih supstanci: natrijuma, hlorida, fosfata, bikarbonata. Većina ovih supstanci transportuje se kotransportom sa natrijumom. Naime, na bazolateralnom polu epitelnih ćelija proksimalnog

Numerous studies have shown that cadmium causes damage of different organs and that it shows toxicity even during exposure to low concentrations (5). The mechanisms, with which it shows its harmful effects, are numerous – by binding to the sulfhydryl groups of antioxidative enzymes, it inhibits their activity, by accumulating in the cell, it can cause the dysfunction of mitochondrial electron transport chain and generation of reactive oxygen radicals resulting in oxidative stress; it reduces the concentration of selenium in the body necessary for the formation of glutathione peroxidase; it induces inflammation, cell apoptosis (5-7). It mostly damages kidneys, liver and gonads, however, other organ systems are damaged, as well – respiratory, hematopoietic, cardiovascular, musculoskeletal (5).

Kidneys present the main organ for depositing and toxic effects of cadmium. Of the total quantity of cadmium in the body, 30-50% is in the kidneys, while the main place of depositing is in proximal tubular cells (8). It has been shown that in the proximal renal tubules, deposited cadmium disturbs the processes of reabsorption of different substances from primary urine. Therefore, the aim of this study is to summarize the current knowledge about the effects of cadmium on the transport processes in proximal tubular cells of kidneys.

Methods

In this review article, we used literature that was searched in the MEDLINE database with the help of PUBMED service in order to present the effects of cadmium on proximal tubular cells in a precise and comprehensive way. The literature, which has been published in the English language during the last ten years, was obtained by searching the following key words: cadmium, nephrotoxicity, proximal tubules.

Cadmium – toxicokinetics and toxicodynamics

Oral ingestion is the predominant pathway of exposure in persons, who are not professionally exposed to this element, while inhalation is a major route of occupational exposure, and the absorption of cadmium via skin and gastrointestinal tract contribute to the total exposure (8). Cadmium is transported from the intestinal lumen into enterocytes via divalent metal ion transporter type 1 (DMT-1) and metal transport protein 1 (MTP-1), while the absorption is possible by binding to the sulfhydryl groups of cysteine and glutathione (9,10). When it enters the bloodstream, cadmium is transported via erythrocytes and it is bound to plasma proteins (8). *Yiling* and associates have shown in a recent study that one of the proteins of human plasma, which cadmium is bound to in blood, is apolipoprotein A-I (11). Furthermore, cadmium is transported to its depots in the body – kidneys, liver and muscles (8). In its acute toxicity, primary damage caused by cadmium happens in liver, while in chronic toxicity, kidneys are dominantly damaged (12). In hepatocytes, cadmium stimulates the synthesis of metallothionein (MT), with which it forms the complex that protects cells from oxidative stress. It is deemed that the damage of hepatocytes occurs when the capacity of metallothionein for buffering cadmium ions is exhausted (13,14). When hepatocytes die off, Cd-MT complex is transported to kidneys through circulation. Cadmium is partly bound to other thiol groups – glutathione (GSH), L-cysteine (L-cys), and partly it is transported to kidneys as free, ionized cadmium (Cd^{2+}). In kidneys, Cd-MT, Cd-GSH, Cd-Cys and free Cd^{2+} are easily filtered through renal glomeruli, and then they are reabsorbed in different ways (15,16). Cd-MT at the level of proximal tubules is taken up through endocytosis, Cd-Cys and Cd-GSH are taken up through apical and basolateral membrane of proximal tubules, while Cd^{2+} can be absorbed by different transport systems (15,16). Cadmium is to the greatest extent eliminated through gastrointestinal tract, while it is eliminated to the lesser extent in the complex of metallothionein via kidneys (6,8).

Proximal tubules of kidneys

Basic structural and functional unit of kidneys is a nephron which consists of glomerulus and tubules. The glomerulus is composed of a network of capillaries within the Bowman's capsule. Blood is filtered at the level of glomerulus and primary urine is produced (17). The proximal tubule begins at the urinary pole of the glomerulus and it has *pars convoluta* and *pars recta* (18). The proximal tubule is the site of reabsorption of most organic substances such as amino acids, glucose, albumin and low-molecular-weight proteins, as well as

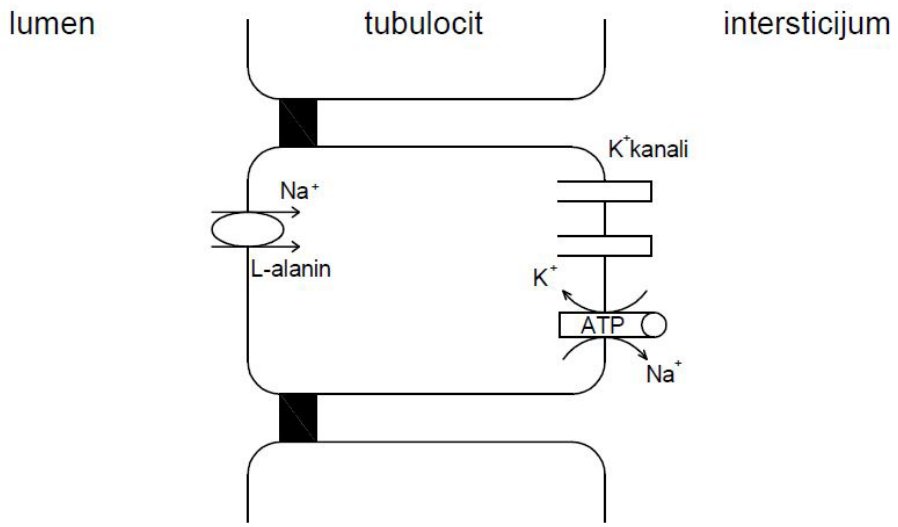
tubula postoji Na^+/K^+ ATP-azna pumpa koja izbacuje natrijum iz ćelije, a ubacuje kalijum i tako stvara hemijski gradijent za apsorpciju natrijuma na luminalnom polu ćelije. Na luminalnom polu postoje kotransporteri: Na^+/glukoza kotransporter, Na^+/amino-kiselinski kotransporter, Na^+/Cl^- kotransporter, Na^+/HCO_3^- kotransporter, preko kojih dolazi do reapsorpcije ovih supstanci, a zahvaljujući elektrohemijskom gradijentu natrijuma (19) (slika 1).

Efekti kadmijuma na proksimalne tubule bubrega

Kao što je napred spomenuto, glavno mesto deponovanja kadmijuma su ćelije proksimalnih tubula bubrega (8). Smatra se da glavnu ulogu u oštećenju ima slobodan Cd^{2+}, a da metalotioneini imaju zaštitnu ulogu i da do oštećenja dolazi kada se prevaziđe kapacitet bubrega da stvara metalotioneine. Studije rađene na miševima koji ne mogu da sintetišu metalotioneine i miševima koji mogu da ih sintetišu, pokazuju da su prvi znatno osetljiviji na toksično dejstvo kadmijuma, a kako postoje velike varijacije u ekspresiji metalotioneina kod ljudi, neki bi mogli biti predisponirani na toksična oštećenje indukovana kadmijumom (13,20). Ipak, pojedine studije sugerišu da metalotioneini u većoj meri imaju protektivno dejstvo na ćelije jetre nego na ćelije bubrega (20).

U ćelijama proksimalnih tubula bubrega vrši se reapsorpcija najvećeg dela supstanci iz primarnog urina i to uglavnom sekundarnim aktivnim transportom – kotransportom sa natrijumom (19). Stoga, nije iznenađujuće što se veliki broj studija bavio ispitivanjem uticaja kadmijuma na transportne procese u proksimalnim tubulskim ćelijama bubrega. Pošto se većina supstanci transportuje na osnovu elektrohemijskog gradijenta natrijuma, promene membranskog potencijala u velikoj meri utiču na transportne procese (21). Na bazolateralnoj membrani ćelija proksimalnih tubula nalaze se kalijumski kanali kojima se K^+ transportuje iz ćelije u intersticijum, niz gradijent koncentracije, čime se obezbeđuje hiperpolarizacija bazolateralne membrane. Bazolateralna i luminalna membrana su električno spojene, pa se ova hiperpolarizacija prenosi i na luminalnu membranu. Hiperpolarizacija luminalne membrane je važna jer obezbeđuje električni gradijent – pokretačku snagu za ulazak natrijuma u ćeliju (22,23).

Peritubulska perfuzija mikromolarnim koncentracijama kadmijuma (Cd^{2+}) dovodi do održive, reverzibilne hiperpolarizacije bazolateralne, a potom i luminalne membrane. Do hiperpolarizacije membrane najverovatnije dolazi zbog vezivanja kadmijuma za kalijumske kanale na bazolateralnoj membrani što dovodi do konformacionih promena samih kanala pa kalijum pojačano izlazi iz ćelije (24-26) (slika 2). Da hiperpolarizacija zaista nastaje kao rezultat povećane propustljivosti bazolateralnih kalijumskih kanala pokazano je njihovom blokadom nespecifičnim blokatorom barijumom, kada hiperpolarizacija izostaje (25,26). Međutim, ova hiperpolarizacija je praćena smanjenjem visine depolarizacije pokrenute aktivacijom Na^+/L^-alanin kotransportera na luminalnoj membrani (27), što bi moglo da ukaže na smanjenje reapsorpcije L-al-



Slika 1. Shematski prikaz transportnih procesa u proksimalnim tubulskim ćelijama

the great part of inorganic substances: sodium, chloride, phosphate, bicarbonate. The majority of these substances are transported by the co-transport with sodium. Namely, at the basolateral pole of epithelial cells of proximal tubule, there is Na^+/K^+ ATPase pump which extrudes sodium from the cell and imports potassium into the cell, thus creating the chemical gradient for the absorption of sodium at the luminal pole of the cell. At the luminal pole, there are co-transporters: Na^+/glucose co-transporter, Na^+/amino-acid co-transporter, Na^+/Cl- co-transporter, $Na^+/HCO3^-$ co-transporter, through which the reabsorption of these substances occurs thanks to the electrochemical gradient of sodium (19) (Figure 1).

The effects of cadmium on the proximal renal tubules

As it has already been mentioned, proximal tubular cells are the main site of cadmium deposition (8). It is deemed that free Cd^{2+} has the main role in the damage, while metallothioneins have the protective role and that it comes to the damage when the capacity of kidneys to produce metallothioneins is exhausted. Studies conducted on mice that cannot synthesize metallothioneins and mice that can synthesize them show that the first group of mice is more sensitive to the toxic effect of cadmium, and since there are great variations regarding the expression of metallothionein in people, some could be predisposed to the toxic effect induced by cadmium (13,20). However, certain studies suggest that metallothioneins to the greatest extent have the protective effect on the liver cells in comparison to the renal cells (20).

Most substances from primary urine are reabsorbed in proximal tubular cells in kidneys mainly through secondary active transport – co-transport with sodium (19). Therefore, it is not surprising that great number of studies examine the influence of cadmium on the transport processes in renal proximal tubular cells. Since the majority of substances are transported according to the electrochemical gradient of sodium, changes of membrane potential influence, to the greatest extent, transport processes (21). At the basolateral membrane of proximal tubular cells, there are potassium channels, through which K^+ is transported from the cell into interstitium, down the concentration gradient, thus enabling the hyperpolarization of basolateral membrane. Basolateral and luminal membranes are electrically connected, and therefore, this polarization is transferred to luminal membrane, as well. Hyperpolarization of luminal membrane is important because it provides electrical gradient – driving force for sodium to enter the cell.

Peritubular perfusion with micromolecular concentrations of cadmium (Cd^{2+}) leads to the sustained, reversible hyperpolarization of basolateral, and later luminal membrane. The hyperpolarization of membrane occurs most probably due to the binding of cadmium to potassium channels on the basolateral membrane, which leads to the conformation changes of channels and therefore, potassium moves out

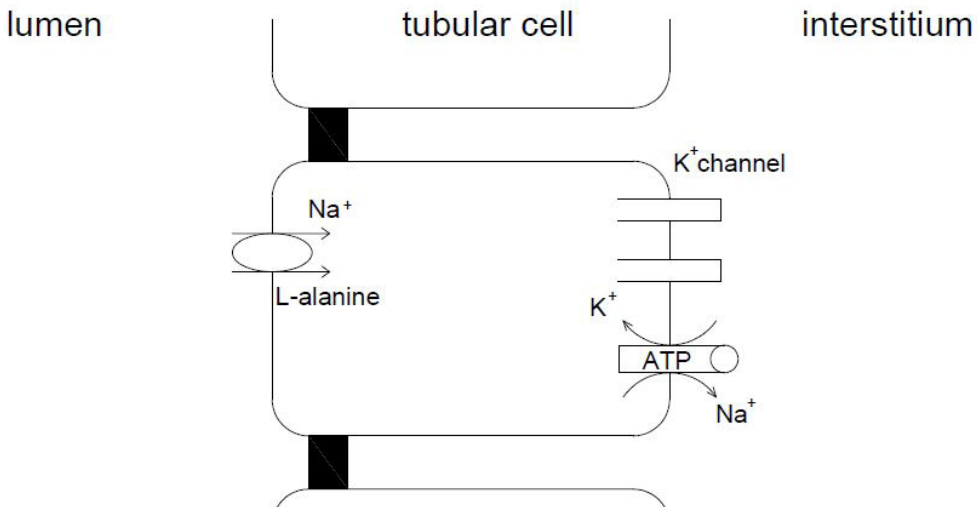
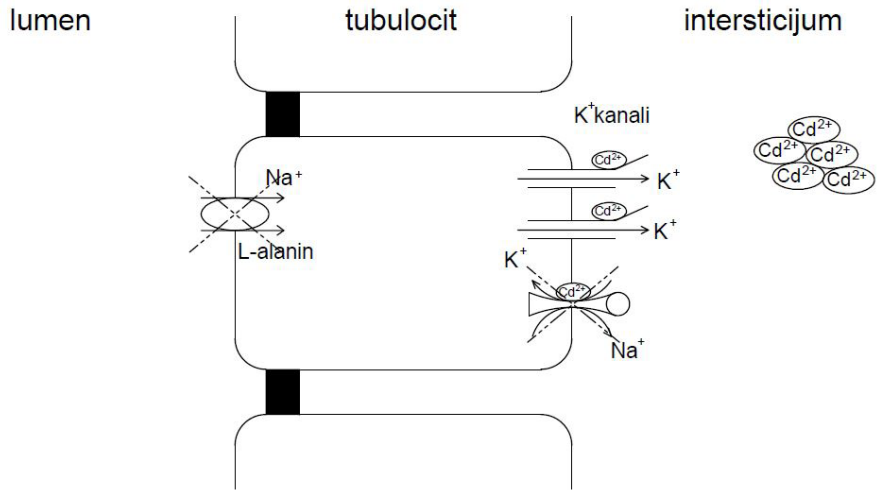


Figure 1. Scheme of transport processes in proximal tubular cells



Slika 2. Shematski prikaz efekata kadmijuma na transportne procese u proksimalnim tubulskim ćelijama

anina putem ovog transportera. Sa druge strane, izlaganje luminalne membrane mikromolarnim koncentracijama kadmijuma ne dovodi do smanjenja ovog kotransporta, odnosno, kadmijum nema direktno inhibitorno dejstvo na rad Na^+/L-alanin kotransportera (25). Ovakvi rezultati sugerišu da kadmijum pored uticaja na kalijumske kanale na bazolateralnoj membrani, verovatno stupa u interakciju i sa sulfhidrilnim grupama drugih proteina bazolateralne membrane. Moguće objašnjenje je da se kadmijum vezuje za sulfhidrilne grupe Na^+/K^+ ATP-aze, dovodi do njenih konformacionih promena usled čega se smanjuje njena aktivnost. Natrijum se zadržava u ćeliji, smanjuje se njegov koncentracioni gradijent te se posledično smanjuje transport preko Na^+/L-alanin kotransportera (27) (slika 2).

Iako glavno mesto u oštećenju transportnih procesa u bubrezima zauzima jonizovani kadmijum, postoje studije koje su pokazale da i kompleks Cd-MT dovodi do smanjenja transporta preko Na^+/L-alanin transportera i to direktnom inhibicijom samog transportera, ali i indirektno smanjenjem aktivnosti Na^+/K^+ ATP-aze na bazolateralnoj membrani. Ovaj kompleks takođe direktno deluje i na Na^+/Glu kotransporter smanjujući njegovu aktivnost (28).

Osim što dovodi do hiperpolarizacije bazolateralne membrane, peritubularna izloženost mikromolarnim koncentracijama kadmijuma (Cd^{2+}) smanjuje brzinu spore repolarizacije luminalne membrane. Do faze spore repolarizacije dolazi usled aktivacije kalijumskih kanala i kretanja kalijuma iz ćelije niz gradijent koncentracije. Moguće objašnjenje nalazi se u tome da kadmijum izaziva konformacione promene kalijumskih kanala na bazolateralnoj membrani usled čega kalijum pojačano izlazi iz ćelije i peritubularna koncentracija kalijuma se povećava (25,26). Zbog postojanja kružnog kola, kalijum prolazi kroz paracelularni šant te se njegova koncentracija povećava i sa luminalne strane (29). Ovo dovodi do smanjenja koncentracionog gradijenta i posledično smanjenja brzine protoka kalijuma iz ćelije što konačno rezultuje smanjenjem brzine spore repolarizacije (25,26).

Mali broj studija bavio se ispitivanjem direktnog efekta kadmijuma na endocitozno preuzimanje proteina u ćelijama proksimalnih tubula. U nedavnoj studiji, *Fujishiro* i saradnici su koristeći kultivisane bubrežne ćelije ispitivali efekte kadmijuma na endocitozno preuzimanje fluorescentno obeleženih albumina, β2 mikroglobulina, transferina i metalotioneina u proksimalne ćelije bubrega. Rezultati njihove studije pokazali su da se unos β2 mikroglobulina i metalotioneina smanjio nakon trodnevnog izlaganja subletalnim dozama kadmijuma, dok ovo izlaganje nije imalo uticaj na preuzimanje albumina i transferina. Jedno od objašnjenja ovakvih rezultata moglo bi biti *in vivo* zapažanje da je β2 mikroglobulin najosetljiviji marker oštećenja bubrežne tubularne reapsorpcije (30).

Zaključak

Brojne studije sprovedene u okviru humane populacije, ali i na različitim animalnim modelima imale su za cilj ispitivanje toksičnih efekata kadmijuma. U tim istraživanjima korišćene su različite doze izlaganja kadmijumu, od mikromolarnih do

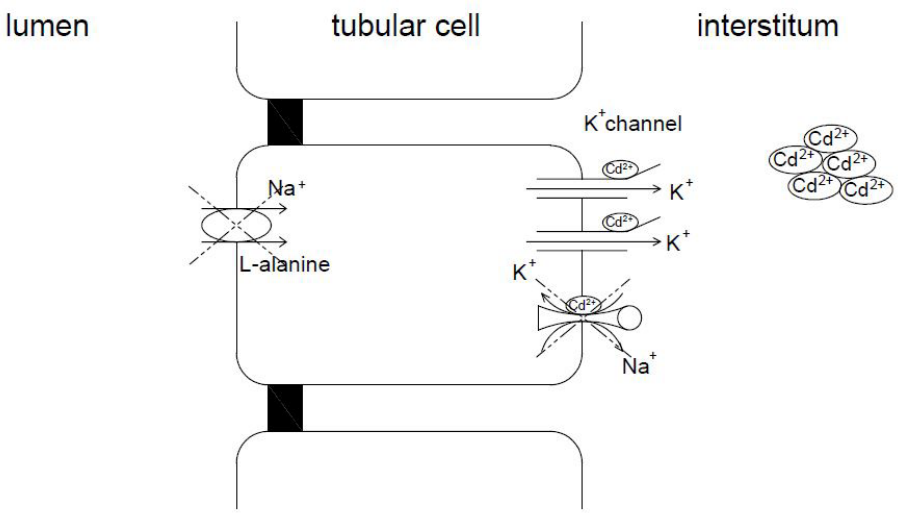


Figure 2. Scheme of the cadmium effects on transport processes in proximal tubular cells

of the cell increasingly (24-26) (Figure 2). It was shown that hyperpolarization occurred as a result of increased conductance of basolateral potassium channels when they were inhibited by non-specific barium that abolished hyperpolarization (25,26). However, this hyperpolarization was followed by the reduction in the amplitude of depolarization induced by the activation of Na^+/L-alanine co-transporter on the luminal membrane (27), which could point to the reduction in reabsorption of L-alanine with the help of this transporter. On the other hand, exposure of luminal membrane to micromolar concentrations of cadmium does not lead to the reduction in this co-transporter, that is, cadmium does not have a direct inhibiting effect on Na^+/L-alanine co-transporter (25). Such results suggest that cadmium, in addition to the potassium channels on the basolateral membrane, probably interacts with sulfhydryl groups of other proteins of basolateral membrane. A possible explanation is that cadmium is bound to sulfhydryl groups Na^+/K^+ ATPase, which leads to conformation changes due to which its activity is reduced. Sodium is maintained in the cell, its concentration gradient is reduced which consequentially reduces the transport through Na^+/L-alanine co-transporters (27) (Figure 2).

Although ionized cadmium takes the main place in the damage of transport processes in kidneys, there are studies which have shown that Cd-Mt complex leads to the reduction in transport through Na^+/L-alanine, that is, with the direct inhibition of the transporter, and indirectly by reducing the activities of Na^+/K^+ ATPase on the basolateral membrane. This complex also directly influences Na^+/GLU co-transporter by reducing its activity (28).

In addition to the fact that it leads to the hyperpolarization of basolateral membrane, peritubular exposure to micromolar concentrations of cadmium (Cd^+) reduces the speed of slow repolarization of luminal membrane. The phase of slow repolarization is induced by activation of potassium channels and movement of potassium out of the cell down the concentration gradient. A possible explanation may be that cadmium causes conformation changes of potassium channels on the basolateral membrane, due to which potassium moves out of the cell increasingly and therefore, peritubular concentration of potassium is increased (25,26). Due to the existence of circuit, potassium passes through the paracellular shunt and therefore, its concentration increases at the luminal side (29). This causes the reduction in the concentration gradient resulting in the decrease of the speed of potassium flow from the cell, which finally causes the reduction in the speed of slow repolarization (25,26).

Few studies have examined the direct effects of cadmium on endocytic uptake of proteins into proximal tubular cells. In a recent study, by using the cultivated renal cells, *Fujishiro* and associates have examined the effects of cadmium on endocytic uptakes of fluorescently marked albumin, ß2 microglobulin, transferrin and metallothionein into proximal renal cells. The results of their study have shown that the intake of ß2 microglobulin and metallothionein decreased after a three-day

milimolarnih. Studije izvedene na animalnim modelima po vrednosti dobijenih podataka, ne zaostaju za istraživanjima u humanoj populaciji, s obzirom da su korišćene životinjske vrste koje odlikuje velika strukturna i funkcionalna sličnost organa, u prvom redu bubrega, sa humanim bubregom, koji je jedan od glavnih meta deponovanja kadmijuma nakon izlaganja.

Kadmijum se deponuje u organizmu u najvećem procentu u ćelijama proksimalnih tubula bubrega gde direktno ili indirektno menja aktivnost ćelijskih transportnih sistema smanjujući reapsorpciju aminokiselina, glukoze, niskomolekularnih proteina. Peritubulska perfuzija ovih ćelija mikromolarnim koncentracijama Cd^{2+}, u *in vitro* uslovima, dovodi do održavane, reverzibilne hiperpolarizacije peritubulskog membranskog potencijala (PD) i povećava kalijumsku selektivnost njihove bazolateralne membrane. Takođe, akutno peritubulsko izlaganje, proksimalnih tubulskih ćelija, mikromolarnim koncentracijama Cd^{2+} izaziva smanjenje visine brze depolarizacije i brzine spore repolarizacije (PD), prilikom istovremene luminalne aplikacije L-alanina, u poređenju sa ovim parametrima pri izlaganju L-alanina u odsustvu Cd^{2+}. Obzirom da se radi o reverzibilnim efektima, oni se mogu smatrati posledicom vezivanja Cd^{2+} za sulfhidrilne (-SH) grupe proteina koji se nalaze u bazolateralnoj membrani ovih ćelija. Ovo smanjenje visine brze depolarizacije i brzine spore repolarizacije (PD), za posledicu može imati smanjenu reapsorpciju L-alanina, a to bi mogao biti rani znak oštećenja proksimalne tubulske ćelije prilikom akutnog izlaganja mikromolarnim koncentracijama kadmijuma.

Konflikt interesa

Autori su izjavili da nema konflikta interesa.

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exposure to sublethal doses of cadmium, while this exposure did not have effects on the uptake of albumin and transferrin. One of the explanations of such results could be in vivo observation that ß2 microglobulin is the most sensitive marker of damage of renal tubular reabsorption (30).

Conclusion

The aim of numerous studies conducted within human populations and on different animal models was to examine the toxic effects of cadmium. In these studies, different doses of exposure to cadmium were used, from micromolar to millimolar. Studies conducted on animal models according to the values of obtained data do not lag behind studies of human populations, considering the fact that they used animal species characterized by great structural and functional similarity between organs, first of all, kidneys and human kidneys, which is one of the main targets of cadmium deposits after exposure.

Cadmium is deposited in the body to the greatest extent in proximal tubular cells, where it directly or indirectly changes the activity of transport systems of cells by reducing the reabsorption of amino acids, glucose and low-molecular-weight proteins. Peritubular perfusion of these cells with micromolar concentrations of Cd^{2+} in in vitro conditions leads to a sustained, reversible hyperpolarization of peritubular membrane potential (PD) and increases potassium selectivity of their basolateral membrane. Also, acute peritubular exposure of proximal tubular cells to micromolar concentrations of Cd^{2+} causes the reduction in the amplitude of fast depolarization and speed of slow repolarization (PD), during the simultaneous luminal application of L-alanine in comparison to these parameters during the exposure to L-alanine in the absence of Cd^{2+}. Considering the fact that these effects are reversible, they may be deemed to be the result of binding of Cd^{2+} to sulfhydryl groups of proteins in the basolateral membrane of these cells. This reduction in the amplitude of fast depolarization and the speed of slow repolarization (PD) can cause the decreased reabsorption of L-alanine, and that could be the early sign of damage of proximal tubular cells during acute exposure to micromolar concentrations of cadmium.

Competing interests

The author declares no competing interests.

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PREVALENCIJA SIFILISA I HIV INFEKCIJE MEĐU MUŠKARCIMA KOJI IMAJU SEKSUALNE ODNOSE SA MUŠKARCIMA TESTIRANIM U JEDNOJ NEVLADINOJ ORGANIZACIJI U BEOGRADU

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SAŽETAK

Uvod/Cilj: U Beogradu sifilis i infekcija izazvana virusom humane imunodeficijencije (HIV) se najčešće javljaju među muškarcima koji imaju seksualne odnose sa muškarcima, koji su neretko stigmatizovani i diskriminisani, te se ne usuđuju da rade testiranja na ove bolesti u zdravstvenim ustanovama. Cilj ovog istraživanja je bio da se utvrde prevalencija sifilisa i HIV infekcije među muškarcima koji imaju seksualne odnose sa muškarcima koji su testirani u „*check-point*" centru udruženja „Potent" u Beogradu.
Metode: U ovu studiju preseka uključene su 993 osobe testirane na HIV i 860 osoba testiranih na sifilis brzim skrining testovima HEXAGON SYPHILIS i HEXAGON HIV. Rezultati brzih testova na HIV su potvrđeni pozitivnim Western-Blot testom na Infektivnoj klinici Kliničkog centra Srbije, a na sifilis pozitivnim serološkim testovima (VDRL – *Venereal Disease Research Laboratory*; laboratorijski test za istraživanje veneričnih bolesti i TPHA – *Treponema Pallidum Haemagglutination Assay; Treponema Pallidum* hemaglutacioni test) u Gradskom zavodu za kožne i venerične bolesti u Beogradu. U statističkoj analizi podataka korišćene su proporcije, procenti i χ^2 test.
Rezultati: Pozitivan test na HIV je zabeležen kod 11 (1,1%), a pozitivan test na sifilis kod 34 ispitanika (3,9%). Najmlađi novootkriveni HIV pozitivni ispitanik imao je 22 godine, a najstariji 46 godina, dok je najmlađa osoba sa pozitivnim testom na sifilis imala 19 godina, a najstarija 68.
Zaključak: Strategije testiranja na sifilis i HIV među muškarcima koji imaju seksualne odnose sa muškarcima bi trebalo da budu raznolike uključujući i testiranje u zajednici koje sprovode nevladine organizacije u saradnji sa zdravstvenim ustanovama.

Ključne reči: muškarci koji imaju seksualne odnose sa muškarcima, sifilis, HIV, brzi testovi, prevalencija

Uvod

Prema podacima Svetske zdravstvene organizacije za 2016. godinu u svetu je od neke od 4 izlečive infekcije koje se prenose seksualnim putem (hlamidijaza, trihomonijaza, gonoreja i sifilis) inficirano 376 miliona osoba, od toga procenjen broj obolelih od sifilisa je bio 6 miliona (1). Najveća prevalencija sifilisa je zabeležena među ključnim populacijama za širenje ove bolesti: seksualnim radnicama - 3,2% i muškarcima koji imaju seksualni odnos sa muškarcima (MSM) - 6% (2). Podaci iz naše zemlje ukazuju da je s početkom novog milenijuma došlo do značajnog porasta broja obolelih od sifilisa, a najveći broj inficiranih pripadao je upravo MSM populaciji (3). Najviše stope obolevanja od sifilisa u poslednjoj deceniji zabeležene su u 2018. godini kada je incidencija bila 2,93 na 100.000 stanovnika (4).

U svetu je krajem 2018. godine 38 miliona osoba živelo sa HIV infekcijom, a podaci iz Centralne Evrope, gde se nalazi i Republika Srbija ukazuju da je stopa novodijagnostikovanih osoba inficiranih HIV-om porasla u periodu od 2008. do 2017. godine za 129% (sa 1,4/100.000 u 2008. na 3,2/100.000 u 2017. godini) (5). Podaci iz naše zemlje pokazuju da su od prvog registrovanog slučaja HIV infekcije u 1984. godini pa do kraja 2018. godine registrovane 3852 osobe inficirane HIV-om, od kojih je 1967 obolelo od side (4). U toku 2018. godine u Republici Srbiji novootkriveno je 179 nosilaca anti-HIV antitela, i stopa novodi-

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PREVALENCE OF SYPHILIS AND HIV INFECTION AMONG MEN WHO HAVE SEX WITH MEN TESTED IN A NON-GOVERNMENTAL ORGANIZATION IN BELGRADE

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SUMMARY

Introduction/Aim: In Belgrade, syphilis and human immunodeficiency virus (HIV) infection most often occur among men who have sex with men who are often stigmatized and discriminated and hesitate to come to health institutions for testing for these diseases. The aim of this study was to assess prevalence of syphilis and HIV infection among men who have sex with men who were tested in the "check-point" center of the association "Potent" in Belgrade.
Methods: This cross-sectional study included 993 participants tested for HIV and 860 participans tested for syphilis by rapid screening tests HEXAGON SYPHILIS and HEXAGON HIV. The results of rapid HIV tests were confirmed by a positive Western-Blot test at the Infectious Diseases Clinic of the Clinical Center of Serbia, and for syphilis by positive serological tests (VDRL - Venereal Disease Research Laboratory and TPHA - Treponema Pallidum Haemagglutination Assay) at the City Institute for Skin and Venereal Diseases in Belgrade. Proportions, percentages and the χ^2 test were used in the statistical analysis of the data.
Results: A positive test for HIV was recorded in 11 (1.1%), and a positive test for syphilis in 34 participants (3.9%). The youngest newly diagnosed HIV positive case was 22 years old and the oldest 46 years old, while the youngest case with a positive test for syphilis was 19 years old and the oldest 68.
Conclusion: Strategies for testing for syphilis and HIV among men who have sex with men should be diverse, including community testing conducted by non-government organization in cooperation with health facilities.

Key words: men who have sex with men, syphilis, HIV, rapid tests, prevalence

Introduction

According to the data of the World Health Organization for 2016, 376 million people were infected with one of four curable sexually transmitted infections (chlamydia, trichomoniasis, gonorrhea, and syphilis), while the estimated number of new cases of syphilis was 6 million (1). The highest prevalence of syphilis was noted among key populations for the spread of this disease: female sex workers – 3.2% and men who had sex with men (MSM) – 6% (2). Data from our country indicate that at the beginning of new millennium there came to the significant increase in new cases of syphilis, while the largest number of infected belonged to the MSM population (3). The highest incidence rates of syphilis in the last decade were reported in 2018, when the incidence was 2.93 per 100,000 people (4).

Globally, 38 million people were living with HIV at the end of 2018, while data from Central Europe, where Serbia is located, indicate that the rate of new HIV diagnoses increased for 129% from 2008 to 2017 (from 1.4/100,000 in 2008 to 3.2/100,000 in 2017) (5). Data from our country show that since the first registered case of HIV infection in 1984, 3852 new cases of HIV were registered until the end of 2018, and of these 1967 people were affected by AIDS (4). During 2018, in the Republic of Serbia, there were 179 newly discovered carriers of anti-HIV antibodies, while the rate of newly diagnosed cases of HIV infection was 2.25 per 100,000 people. About 30% of patients became aware of their HIV status at the stage when AIDS was clinically manifested, while 76% of newly diagnosed cases of HIV infection

jagnostikovanih slučajeva HIV infekcije bila je 2,25 na 100.000 stanovnika. Oko 30% pacijenata je svoj HIV status saznalo u stadijumu klinički manifestne side, dok je 76% novootkrivenih slučajeva HIV infekcije pripadalo MSM populaciji (4).

U našoj sredini i sifilis i HIV infekcija se najčešće javljaju među muškarcima koji imaju seksualni odnos sa muškarcima koji su neretko stigmatizovani i diskriminisani te se ne usuđuju da se testiraju na ove bolesti u zdravstvenim ustanovama. Stoga se javlja potreba da se u okviru terenskih aktivnosti (*outreach*) i u *drop-in* centrima za MSM populaciju, a u saradnji sa zdravstvenim ustanovama, testiranja obave na njima „prihvatljivijem" terenu (6).

Cilj ovog istraživanja je bio da se utvrde prevalencija sifilisa i HIV infekcije među testiranim muškarcima koji imaju seksualni odnos sa muškarcima u „*check-point*" centru nevladinog udruženja „Potent" u Beogradu.

Metode

Istraživanje je sprovedeno u okviru projekta Ministarstva zdravlja broj 4013: „Podrška aktivnostima udruženja građana u oblasti prevencije i kontrole HIV infekcije u 2020. godini" i projekta Nacionalnog centra za seksualno i reproduktivno zdravlje „Potent" pod nazivom „Održivost usluga testiranja u zajednici i sveobuhvatna parnjačka podrška za osobe koje žive sa HIV-om u Republici Srbiji". U istraživanje su bili uključeni muškarci koji imaju seksualne odnose sa muškarcima koji su došli na dobrovoljno i poverljivo savetovanje i testiranje (DPST) na HIV i na sifilis u centar za testiranje udruženja „Potent" u Beogradu u periodu od 1. maja do 31. decembra 2020. godine.

U laboratorijskoj dijagnostici ovih oboljenja rađeni su imunohromatografski brzi skrining testovi treće generacije HEXAGON SYPHILIS i HEXAGON HIV (HUMAN GmbH) za detektovanja IgG, IgM i IgA antitela na *Treponema pallidum* za sifilis i detekciju antitela na HIV-1 i HIV-2. Senzitivnost ovog testa na HIV je 100% a specifičnost 99,9%, dok je senzitivnost testa na sifilis 99,3%, a specifičnost 99%. Testiranje je bilo sprovedeno prema preporukama proizvođača testova (7). Licencirani zdravstveni radnik je svim učesnicima istraživanja uzeo uzorak krvi iz prsta i nakon 20 minuta interpretirao rezultate. Svim ispitanicima je pruženo dobrovoljno i poverljivo savetovanje na HIV pre testiranja i nakon preuzimanja rezultata od strane tri savetnika za DPST. Svi rezultati testiranja uvođeni su u dnevne laboratorijske protokole. Rezultati brzih testova na HIV su potvrđeni pozitivnim *Western-Blot* testom na Infektivnoj klinici Kliničkog centra Srbije, a na sifilis pozitivnim serološkim testovima (VDRL – *Venereal Disease Research Laboratory* i TPHA – *Treponema Pallidum Haemagglutination Assay*) u Gradskom zavodu za kožne i venerične bolesti u Beogradu. U statističkoj analizi podataka korišćene su proporcije, procenti i χ^2 test.

Rezultati

U okviru istraživanja testirano je 993 MSM osoba na HIV i 860 na sifilis (Tabela 1). Pozitivan test na HIV je zabeležen kod 11 (1,1%), a na sifilis kod 34 ispitanika (3,9%). Najmlađi novootkriveni HIV pozitivni ispitanik imao je 22 godine a najstariji 46 godina, dok je najmlađa osoba sa pozitivnim testom na sifilis imala 19 godina, a najstarija 68.

Testirani na HIV i testirani na sifilis se nisu značajno razlikovali po uzrastu ($p > 0,05$). Značajnih razlika po uzrastu nije bilo ni između HIV pozitivnih i HIV negativnih, kao ni između onih sa pozitivnim i negativnim testom na sifilis ($p > 0,05$). Pozitivnih na sifilis je u poređenju sa pozitivnima na HIV bilo značajno više u uzrastu ≤ 29 godina ($p = 0,003$), kao i u uzrastu 30-39 godina ($p = 0,016$).

Tabela 1. Distribucija ispitanika testiranih na HIV i sifilis prema rezultatima testova i uzrastu

Uzrasne grupe	Testirani na HIV (N=993)		Testirani na sifilis (N=860)	
	Pozitivni Broj (%)	Negativni Broj (%)	Pozitivni Broj (%)	Negativni Broj (%)
≤ 29	2 (18,2)	459 (46,7)	14 (41,2)	411 (49,8)
30-39	6 (54,5)	380 (38,7)	16 (47,1)	305 (36,9)
40+	3 (27,3)	143 (14,6)	4 (11,7)	110 (13,3)
Ukupno	11 (1,1)	982 (98,9)	34 (3,9)	826 (96,1)

belonged to the MSM population (4).

In our environment, both syphilis and HIV infection appear most commonly among men who have sex with men, who are often stigmatized and discriminated, and therefore, they hesitate to come to health institutions for testing for these diseases. Therefore, the need arises to conduct testing in places which are "more acceptable" for them, within outreach activities and in drop-in centers for the MSM population (6).

The aim of this research was to determine the prevalence of syphilis and HIV infection among men who had sex with men who were tested at the check-point center of the non-governmental association "Potent" in Belgrade.

Methods

The study was conducted within the project number 4013 of the Ministry of Health: "Support to the activities of citizens' associations in the field of HIV prevention and control in 2020", and also the project of the National center for sexual and reproductive health "Potent", under the name "Sustainability of testing services in the community and the comprehensive peer support to people living with HIV in the Republic of Serbia". The study included men who had sex with men, who came for voluntary and confidential counseling and testing (VCCT) for HIV and syphilis to the center for testing of the association "Potent" in Belgrade in the period from 1st May to 31st December 2020.

Immunochromatographic 3rd generation rapid screening tests HEXAGON SYPHILIS and HEXAGON HIV (HUMAN GmbH) were done in the laboratory diagnostics of these diseases for IgG, IgM and IgA antibodies to *Treponema pallidum* for syphilis and for antibodies to HIV-1 and HIV-2. The sensitivity of this test for HIV is 100%, while its specificity is 99%, whereas the sensitivity of the test for syphilis is 99.3% and the specificity is 99%. Testing was conducted according to the recommendations of tests' manufacturers (7). A licensed healthcare worker collected a finger-stick blood sample from all participants of the study and interpreted the results after 20 minutes. All participants were offered a voluntary and confidential HIV counseling before testing and after the results were taken by three counselors for VCCT. All results of testing were introduced into daily laboratory protocols. The results of rapid HIV tests were confirmed by a positive Western-Blot test at the Infectious Diseases Clinic of the Clinical Center of Serbia, and for syphilis by positive serological tests (VDRL – Venereal Disease Research Laboratory and TPHA – Treponema Pallidum Haemagglutination Assay) at the City Institute for Skin and Venereal Diseases in Belgrade. Proportions, percentages and the χ^2 test were used in the statistical analysis of data.

Results

Within this study, 993 MSM participants were tested for HIV and 860 for syphilis (Table 1). A positive test for HIV was recorded in 11 (1.1%), and a positive test for syphilis in 34 participants (3.9%). The youngest newly diagnosed HIV positive participant was 22 years old and the oldest 46 years old, while the youngest person with a positive test for syphilis was 19 years old and the oldest 68 years old.

There was no significant difference between people tested for HIV and people tested for syphilis regarding age ($p > 0.05$). Also, there was no significant difference regarding age between HIV positive and HIV negative persons, as well as between those who had a positive and negative test for syphilis ($p > 0.05$). There were significantly more people who had a positive test for syphilis in comparison to those who had a positive test for

Table 1. Distribution of participants tested for HIV and syphilis according to testing results and age

Age groups	Tested for HIV (N=993)		Tested for syphilis (N=860)	
	Positive Number (%)	Negative Number (%)	Positive Number (%)	Negative Number (%)
≤ 29	2 (18.2)	459 (46.7)	14 (41.2)	411 (49.8)
30-39	6 (54.5)	380 (38.7)	16 (47.1)	305 (36.9)
40+	3 (27.3)	143 (14.6)	4 (11.7)	110 (13.3)
Total	11 (1.1)	982 (98.9)	34 (3.9)	826 (96.1)

Diskusija

Na globalnom nivou, procenjeno je da je kod muškaraca koji imaju seksualne odnose sa muškarcima 19 puta češća infekcija HIV-om u odnosu na opštu populaciju (8). Prevalencija sifilisa je najveća među MSM osobama koje žive sa HIV-om (9), a podaci Centara za prevenciju i kontrolu bolesti (engl. *Centers for Disease Control and Prevention* - CDC) su pokazali da čak 47% muškaraca koji praktikuju seksualne odnose sa muškarcima imaju koinfekciju sifilisa i HIV-a (10). Kombinacija ove dve bolesti je veoma opasna, jer kliničke i biološke karakteristike sifilisa olakšavaju transmisiju HIV infekcije, a nova infekcija sifilisom povećava koncentraciju HIV-a u telesnim tečnostima i smanjuje broj CD4 limfocita kod HIV-pozitivnih osoba, što povećava rizik da ljudi koji žive sa HIV-om lakše prenose virus (11). Stoga su rana identifikacija i lečenje sifilisa veoma važni i u sprečavanju prenošenja HIV infekcije.

U poslednjoj deceniji u Evropskim zemljama, kao i u našoj zemlji, sifilis se najčešće registrovao među MSM populacijom (12,13), a faktori koji doprinose pojavi ove infekcije bili su: promiskuitetno ponašanje, upotreba društvenih mreža i mobilnih aplikacija za brže nalaženje seksualnih partnera, zloupotreba psihoaktivnih supstanci, upotreba pre ekspozicione profilakse (PreP) za HIV i posledično nebezbedno seksualno ponašanje, naročito nekorišćenje kondoma za oralne seksualne odnose (14).

S obzirom na to da je MSM populacija teško dostupna javnom zdravstvenom sektoru, testiranja na sifilis i HIV u svetu se neretko sprovode u okviru „*outreach*" aktivnosti na terenu primenom brzih testova. Istraživanje sprovedeno u Nepalu među 167 muškaraca koji imaju seksualne odnose sa muškarcima je utvrdilo da je prevalencija HIV infekcije bila 5%, a novootkrivenog sifilisa 4% (15), dok je studija sprovedena u *Solt Lake County* u SAD utvrdila da je među 405 testiranih MSM osoba prevalencija sifilisa bila 3% (16).

Ovi rezultati su slični rezultatima našeg istraživanja. Naime, u Beogradu je prevalencija sifilisa među testiranim osobama bila 3,9%, a HIV infekcije 1,1%. Ako uporedimo broj novootkrivenih osoba sa sifilisom tokom ovog istraživanja sa ukupnim brojem novootkrivenih slučajeva sifilisa u Gradskom zavodu za kožne i venerične bolesti u Beogradu tokom 2020. godine (125 pacijenata), videćemo da je čak 27,2% novoobolelih pacijenata detektovano u „*check-point*" centru. Imajući u vidu da zbog COVID-19 pandemije veliki broj pacijenata nije imao pristup zdravstvenom sistemu, došlo je do značajnog porasta obolevanja od sifilisa u odnosu na prethodne dve godine (17), ⅓ novoobolelih je preko Centra za testiranje udruženja „Potent" došla do dijagnoze, a potom upućena referentnoj službi u Gradskom zavodu za kožne i venerične bolesti u Beogradu na lečenje. Prema podacima Gradskog zavoda za javno zdravlje, tokom 2020. godine je novootkriveno 33 slučaja HIV infekcije u Beogradu (18). Od tog broja 31 osoba je pripadala MSM populaciji, a jedna trećina novootkrivenih slučajeva HIV infekcije je detektovana tokom DPST-a u udruženju „Potent".

Rezultati ovog istraživanja su ohrabrujući i ukazuju na potrebu daljeg nastavka prakse testiranja posebno vulnerabilnih grupa, poput muškaraca koji imaju seksualne odnose sa muškarcima, na HIV i sifilis, na mestima gde se oni ne osećaju stigmatizovano i koja su im lako dostupna, a u okviru udruženja koja sarađuju sa zdravstvenim ustanovama.

Zaključak

Strategije testiranja na sifilis i HIV bi trebalo da budu raznolike, uključujući testiranje u zajednici u okviru projekata koje sprovode nevladine organizacije koje se bave prevencijom polno prenosivih infekcija među vulnerabilnim grupama u saradnji sa javnim zdravstvenim ustanovama, kao i testiranje na HIV infekciju u zatvorima, u ustanovama koje se bave lečenjem bolesti zavisnosti i zaštitom reproduktivnog zdravlja.

Konflikt interesa

Autor je izjavio da nema konflikta interesa.

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HIV in the age group < 29 years (p = 0.003), as well as in the age group 30-39 years (p = 0.016).

Discussion

Globally, it has been estimated that in men who have sex with men, the HIV infection is 19 times more frequent in comparison to the general population (8). The highest prevalence of syphilis is in MSM persons living with HIV (9), while data from the Centers for Disease Control and Prevention (CDC) have shown that even 47% of men, who have sex with men, have HIV and syphilis co-infection (10). The combination of these two diseases is very dangerous, because clinical and biological characteristics of syphilis facilitate the transmission of HIV infection, while the new infection with syphilis increases the concentration of HIV in body fluids and diminishes the number of CD4 lymphocytes in HIV positive persons, which increases the risk of easier transmission of virus in people living with HIV (11). Therefore, the early identification and treatment of syphilis is very important for the prevention of transmission of HIV infection, as well.

In the last decade, in European countries, as well as in our country, syphilis was most frequently registered in MSM population (12,13), while the factors that contribute to the appearance of this infection were the following: promiscuous behavior, use of social networks and phone applications to find sexual partners, abuse of psychoactive substances, use of pre-exposure prophylaxis (PrEP) for HIV and consequential unsafe sexual practices, especially non-use of condoms for oral intercourse (14).

Considering the fact that MSM population is hardly available for the public health sector, testing for syphilis and HIV are often conducted in the world within outreach activities with the help of rapid tests. In a study that was conducted in Nepal, it was found that among 167 men who had sex with men, the prevalence of HIV infection was 5%, and of syphilis 4% (15), while a study, which was conducted in the Salt Lake County in the USA, found that among 405 tested MSM persons, the prevalence of syphilis was 3% (16).

These results are in accordance with the results of our study. Namely, in Belgrade, the prevalence of syphilis among the tested persons was 3.9%, while the prevalence of HIV was 1.1%. If we compare the number of newly discovered persons with syphilis during this research with the total number of newly discovered syphilis cases at the City Institute for Skin and Venereal Diseases in Belgrade during 2020 (125 patients), we will see that even 27.2% of new cases were detected at the check-point center. Having in mind the fact that due to the COVID-19 pandemic, a large number of patients did not have access to the healthcare system, there came to the significant increase in syphilis cases in comparison to previous two years (17). 1/3 of new cases were diagnosed at the Center for testing of the association “Potent”, and then they were referred to the City Institute for Skin and Venereal Diseases in Belgrade for treatment. According to the data of the Public Health Institute, during 2020, 33 new cases of HIV infection were discovered in Belgrade (18). Of that number, 31 persons belonged to the MSM population, while ⅓ of the newly discovered cases of HIV infection were detected during VCCT in “Potent” association.

The results of this study are encouraging and they point to the need of further continuation of testing for HIV and syphilis, especially in vulnerable groups such as men who have sex with men, in places where they do not feel stigmatized and which are easily available to them, within associations that cooperate with healthcare institutions.

Conclusion

Strategies for testing for syphilis and HIV should be diverse, including testing in the community within projects conducted by non-governmental organizations, which deal with the prevention of sexually transmitted infections among vulnerable groups in cooperation with public healthcare institutions, as well as testing for HIV infection in prisons, in institutions that deal with the treatment of addiction and protection of reproductive health.

Competing interests

The author declares no competing interests.

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ANTIBIOTSKA REZISTENCIJA UZROČNIKA INFEKCIJA URINARNOG TRAKTA KOD PACIJENATA INSTITUTA ZA JAVNO ZDRAVLJE KRAGUJEVAC

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SAŽETAK

Uvod/Cilj: Urinarne infekcije su među najčešćim u primarnoj zdravstvenoj zaštiti. Cilj ovog rada je bila mikrobiološka analiza urina ambulantnih pacijenata u Institutu za javno zdravlje u Kragujevcu u periodu od šest meseci (01.11.2020. - 30.04.2021.).
Metod: Retrospektivno, na osnovu prikupljenih rezultata, vršena je identifikacija uzročnika urinarnih infekcija i određivanje njihove osetljivosti na antibiotike metodom antibiograma.
Rezultati: Šestomesečna analiza uzoraka urina ambulantnih pacijenata obuhvatila je 4809 uzoraka urina. Od svih testiranih uzoraka pozitivno na patogene mikroorganizme je bilo 1218 (25,3%). Najzastupljeniji patogeni urinarnog trakta u ispitivanom periodu bili su: *Escherichia coli* 842 (69,1%), *Klebsiella spp*. 252 (20,7%), *Pseudomonas aeruginosa* 71 (5,8%) i *Enterococcus grupa* 53 (4,4%). Bakterija *E. coli* je bila češća kod žena (82,7%), a *Pseudomonas aeruginosa* kod muškaraca (76,1%). *Klebsiella spp*. i *Enterococcus spp*. su se skoro podjednako javljale kod oba pola. Lekovi prvog izbora u terapiji urinarnih infekcija bi bili sledeći: za *E. coli* amikacin (aminoglikozid) i fosfomicin, a za *Klebsiella spp*. meropenem (karbapenem) i takođe amikacin. Što se tiče *P. aeruginosa*, najefikasnijim su se pokazali takođe meropenem, kao i penicilinski antibiotik piperacilin-tazobaktam.
Zaključak: S obzirom na to da uropatogeni poseduju mehanizme za razvoj rezistencije, potrebno je u terapiji koristiti antibiotike u zavisnosti od rezultata antibiograma.

Ključne reči: uropatogeni, antibiotska rezistencija, antibiogram

Uvod

Infekcija urinarnog trakta je jedna od najčešćih infekcija u primarnoj medicinskoj praksi. Svake godine, širom sveta, se dijagnostikuje oko 150 miliona ljudi sa infekcijom urinarnog trakta (bilo da se radi o komplikovanoj ili nekomplikovanoj infekciji). Nekomplikovana urinarna infekcija je češća kod seksualno aktivnih mlađih žena sa anatomsko-fiziološki normalnim urinarnim traktom, dok se komplikovana infekcija češće sreće kod osoba koje imaju neku drugu bolest (prolongirana upotreba antibiotika, dijabetes), abnormalnost urinarnog trakta, prisustvo stranog tela (kateter, kalkulus) i drugo (1).

Antimikrobni agensi su sve hemijske supstance (prirodne i veštačke) i fizički agensi (toplota, UV zračenje, radioaktivno zračenje) koji ubijaju mikroorganizme ili inhibiraju njihov rast. Antimikrobna aktivnost predstavlja najmanju količinu agensa potrebnu za inhibiciju rasta test organizma i naziva se minimalna inhibitorna koncentracija (MIK). Parametri za podelu antimikrobnih lekova su molekularna struktura, mehanizam delovanja i spektar antimikrobne aktivnosti. Grubo se mogu podeliti na: sintetičke antimikrobne lekove i antibiotike (4).

Neki oblici rezistencije (otpornosti) na antimikrobne lekove su urođene osobine određenih grupa mikroorganizama (4). Na primer, mikroorganizmi koji proizvode antibiotike su i otporni na sopstveni proizvod. Ćelijski zid gram-negativnih bakterija nepropustljiv je za penicilin G pa je većina

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ANTIBIOTIC RESISTANCE OF CAUSES OF URINARY TRACT INFECTIONS IN PATIENTS AT THE PUBLIC HEALTH INSTITUTE KRAGUJEVAC

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SUMMARY

Introduction/Aim: Urinary tract infections are one of the most common infections in primary healthcare. The aim of this study was the microbiological analysis of urine of infirmary patients at the Institute of Public Health in Kragujevac during the six-month period (1st November 2020 – 30th April 2021).
Methods: Causes of urinary tract infections were identified retrospectively, according to the collected data, and their sensitivity to antibiotics was determined with the help of antibiogram method.
Results: A six-month analysis of urine samples of infirmary patients included 4809 urine samples. Of all analyzed samples, 1218 (25.3%) were positive to pathogens. The commonest urinary tract pathogens in the examined period were the following: *Escherichia coli* 842 (69.1%), *Klebsiella spp*. 252 (20.7%), *Pseudomonas aeruginosa* 71 (5.8%) and *Enterococcus group* 53 (4.4%). *E. coli* was more frequent in women (82.7%), while *Pseudomonas aeruginosa* was more frequent in men (76.1%). *Klebsiella spp*. and *Enterococcus spp*. were almost equally present in men and women. First-choice drugs in the treatment of urinary infections would be the following: for *E. coli* amikacin (aminoglycoside) and fosfomycin, and for *Klebsiella spp*. meropenem (carbapenem) and also amikacin. As far as *P. aeruginosa* is concerned, meropenem was shown to be the most efficient, as well as the penicillin antibiotic piperacillin-tazobactam.
Conclusion: Considering that uropathogens possess mechanisms for the development of resistance, the treatment should include antibiotics depending on the results of antibiogram.

Key words: uropathogens, antibiotic resistance, antibiogram

Introduction

Urinary tract infections are one of the most common infections in the primary healthcare practice. Every year, 150 million people are diagnosed with urinary tract infections worldwide (complicated or uncomplicated infections). Uncomplicated urinary tract infections are more frequent in sexually active younger women, who have a normal urinary tract in terms of anatomy and physiology, while complicated infections are more common in persons with other diseases (prolonged usage of antibiotics, diabetes), the abnormality of urinary tract, the presence of foreign bodies (catheter, calculus) etc (1).

Antimicrobial agents are all chemical substances (natural and artificial) and physical agents (warmth, UV radiation, radioactive radiation) that kill microorganisms or inhibit their growth. Antimicrobial activity presents the smallest quantity of agents necessary for the inhibition of growth of test organisms and it is called the minimal inhibitory concentration (MIC). Parameters for the classification of antimicrobial drugs are the molecular structure, mechanisms of action and wide spectrum of antimicrobial activity. They can be approximately classified into: synthetic antimicrobial drugs and antibiotics (4).

Some forms of resistance to antimicrobial drugs are innate characteristics of certain groups of microorganisms (4). For example, microorganisms that produce antibiotics are resistant to their own

predstavnika prirodno otporna. Ali kada se govori o rezistenciji na antimikrobne lekove misli se na stečenu otpornost mikroorganizama prema antimikrobnim agensima na koje su oni do tada bili osetljivi.

Antibiotska rezistencija može biti kodirana genima na bakterijskom hromozomu ili na plazmidu koji se naziva R (engl. *resistance*) plazmid (4). Hromozomski kodirana rezistencija je rezultat mutacije u genima za ciljne strukture (kao što su ribozomi, RNK polimeraza, giraza) ili komponente membrane koje učestvuju u izbacivanju antibiotika iz ćelije (efluks pumpe). Široko rasprostranjeni oblici rezistencije kodirani su R plazmidima i mogu se razmenjivati horizontalnim transferom gena. Na R plazmidima se nalaze geni čiji produkti modifikuju (acetilacija, fosforilacija, adenilacija) ili razgrađuju lekove, ili geni koji kodiraju enzime koji mogu da spreče usvajanje antibiotika ili da ih aktivno izbacuju (pumpaju) napolje. Na primer, neki R plazmidi kodiraju β-laktamazu, enzim koji razara β-laktamski prsten, indukujući rezistenciju na peniciline i cefalosporine.

Na svaki antibiotik vremenom će se razviti rezistencija. Konzervativna i svrsishodna upotreba antibiotika može produžiti ili čak povratiti efikasnu kliničku upotrebu nekog antibiotika. Dugotrajno rešenje za antibiotsku rezistenciju zahteva konstantno otkrivanje i razvijanje novih lekova. Nakon pronalaska novog antimikrobnog agensa i testiranja efikasnosti i toksičnosti, potrebne su i dugotrajne kliničke probe na ljudima, što znači da je potrebno oko 10-25 godina da bi se antibiotik mogao upotrebljavati (4).

Cilj ovog rada je bila mikrobiološka analiza urina ambulantnih pacijenata u Institutu za javno zdravlje u Kragujevcu u periodu od šest meseci (01.11.2020. godine - 30.04.2021. godine).

Metode

Istraživanje je sprovedeno retrospektivno na osnovu prikupljenih rezultata šestomesečnih (01.11.2020. godine - 30.04.2021. godine) mikrobioloških analiza koje su obuhvatale izolaciju i identifikaciju bakterijskih uzročnika urinarnih infekcija i određivanje njihove osetljivosti na antibiotike metodom antibiograma. Analiza je vršena na Odeljenju za bakteriologiju i parazitologiju, Centra za mikrobiologiju Instituta za javno zdravlje Kragujevac, na osnovu uputa iz ustanove primarnog nivoa zdravstvene zaštite – Doma zdravlja Kragujevac.

Pozitivan rezultat znači da su patogene bakterijske vrste nađene u signifikantnom (značajnom) broju u uzorku urina pacijenta. Signifikantan broj bakterija u urinu je broj bakterija ≥ 100.000 CFU/ml (engl. *colony forming units per milliliter*) u uzorku srednjeg mlaza urina, što se smatra kriterijumom za potvrdu bakterijske infekcije. Manji broj od toga se smatra kontaminacijom. Nakon adekvatnog uzimanja uzorka, korišćeno je najmanje 10 µl uzorka za analizu.

Uzorci su zasejavani na hromogeni agar UTI (engl. *urinary tract infections*) i inkubirani na 35 ± 2°C tokom 24 sata. To je podloga za identifikaciju, diferencijaciju i potvrdu kako gram-negativnih, tako i gram-pozitivnih bakterija, kao i kvasnica iz uzoraka urina. Ta podloga omogućava brzu identifikaciju gram-negativnih bakterija i nekih gram-pozitivnih bakterija na osnovu različitih boja kolonija proizvedenih reakcijom enzima specifičnih za rodove ili vrste sa dva hromogena supstrata. Karakteristične boje kolonija za svaku bakteriju su: *E. coli* - ružičaste, *Klebsiella spp*. - krupne zelene, *Proteus spp*. - mrke, roje se, *Psedomonas aeruginosa* - zelene, fluorescentne, *Enterococcus* grupa - sitne svetlo zelene boje.

Izolacija *Streptococcus spp*. i E*nterococcus spp*. vršena je na krvnom agru. Nakon zasejavanja uzoraka podloge su gajene aerobno, na tempetaruri 35-37˚C, 16-24 sata. Kolonije su sitne, sivkaste ili prozračne, konveksne i kompaktne. Na krvnom agaru streptokoki pokazuju jedan od tri tipa hemolize. Alfa – zelenkasta zona hemolize oko kolonija zbog delimične destrukcije eritrocita i parcijalne redukcije hemoglobina (do methemoglobina) u eritrocitima (*Streptococcus pneumoniae* i većina *viridans* streptokoka). Beta - prozirna zona oko kolonija zbog destrukcije eritrocita i potpune dekolorizacije hemoglobina (*Streptococcus β* hemolitičke bakterije gr. A, B, C, G). *Streptococcus agalactiae* (streptokok gr. B) ima usku zonu hemolize oko kolonije, kolonije su nešto sjajnije i nisu kompaktne. Kolonije *Enterococcus spp*. su sivkaste, sjajne i nisu kompaktne (razmazuju se ezom).

Još jedan test koji je korišćen za identifikaciji urinarnih patogena je hidroliza eskulina u 40% žuči. Zasejava se kosi eskulin-žučni agar ispitivanim sojem i inkubira preko noći na 37˚C. Ukoliko dođe do hidrolize eskulina, podloga menja boju u crnu (reakcija nastalog eskuletina sa feri-citratom u podlozi). Ovaj test je pozitivan kod enterokoka i beta-hemolitičkog streptokoka grupe D.

product. The cell wall of gram-negative bacteria is impermeable to penicillin G, so the majority of representatives are naturally resistant. However, when we speak of resistance to antimicrobial drugs, this resistance refers to the acquired resistance of microorganisms to antimicrobial agents, which they had been susceptible to before.

Antibiotic resistance may be coded by genes on the bacterial chromosome or on plasmid which is called R plasmid (4). Chromosomally encoded resistance is the result of mutation in genes for target structures (such as ribosomes, RNK polymerase, gyrase) or components of membrane that take part in the expulsion of antibiotics from the pump (pump efflux). Wide-spread forms of resistance are encoded in R plasmids and they can be exchanged by the horizontal gene transfer. Genes, whose products modify (acetylation, phosphorylation, adenylation) or dissolve drugs, or genes which encode enzymes that can prevent the adoption of antibiotics or actively expel (pump) them out, are on R plasmids. For example, some R plasmids encode ß-lactamase, enzyme that destroys ß-lactam ring, thus inducing the resistance to penicillin and cephalosporins.

Resistance to each antibiotic will develop over time. Conservative and useful application of antibiotics can prolong or even bring back the efficient clinical use of an antibiotic. A longstanding solution for antibiotic resistance requires a constant discovery and development of new drugs. After new antimicrobial agents are found and efficacy and toxicity are tested, longstanding clinical trials on people are necessary, which means that 10-25 years are needed so that an antibiotic could be used (4).

The aim of this study was the microbiological analysis of urine of infirmary patients at the Institute of Public Health during six months (1st November 2020 – 30th April 2021).

Methods

A retrospective study was conducted on the basis of obtained results of microbiological analyses that included the isolation and identification of bacterial causes of urinary tract infections and determination of their sensitivity to antibiotics with the help of antibiogram method during a six-month period (1st November 2020 – 30th April 2021). The analysis was done at the Department for Bacteriology and Parasitology at the Center for Microbiology of the Public Health Institute Kragujevac, based on the doctor's referral from the primary healthcare – Health Center Kragujevac.

A positive result means that bacterial pathogens were found in significant numbers in the patient's urine sample. A significant number of bacteria in urine is the number of bacteria > 100,000 CFU/ml (colony forming units per milliliter) in the midstream sample of urine, which is deemed to be the criterion necessary for the confirmation of bacterial infection. A smaller number than that one is deemed to be contamination. After an adequate sample collection, at least 10 µl of the sample was used for the analysis.

The samples were inoculated on chromogenic UTI agar and incubated at 35 ± 2˚C during 24 hours. That was the medium for the identification, differentiation and confirmation of gram-negative and gram-positive bacteria, as well as of fungi from urine samples. That medium enabled the fast identification of gram-negative bacteria and some gram-positive bacteria according to different colors of colonies produced by the reaction of enzymes specific for the species with two chromogenic substrates. Characteristic colors of colonies for each bacterium are the following: *E. coli* – pink, *Klebsiella spp.* – big green, *Proteus spp.* – dark, swarming, *Psedomonas aeruginosa* – green, fluorescent, *Enterococcus* group – tiny light green.

Streptococcus spp. and *Enterococcus spp.* were isolated on blood agar. After samples were inoculated, media were grown aerobically at 35-37˚C, 16-24 hours. Colonies were small, grayish or translucent, convex and compact. *Streptococci* show one of three types of hemolysis on blood agar. Alpha – green zone of hemolysis around colonies due to the partial reduction of erythrocytes and partial reduction of hemoglobin (to methemoglobin) in erythrocytes (*Streptococcus pneumoniae* and most of *viridians streptococci*). Beta - translucent zone around colonies due to the destruction of erythrocytes and complete decolorization of hemoglobin (*Streptococcus ß* hemolytic bacteria gr. A, B, C, G). *Streptococcus agalactiae* (*streptococcus group B*) had a narrow zone of hemolysis around the colony, colonies were a little bit shinier and not compact. Colonies *Enterococcus spp.* were grayish, shiny and not compact (they were stained using esis).

Za izolaciju i identifikaciju enterobakterija se koristila diferencijalna podloga endo agar. Zasejani uzorci se inkubiraju aerobno, na temperaturi 35-37˚C, 16-24 sata. Identifikacija enterobakterija primarno se zasniva na ispitivanju biohemijskih osobina. U rutinskoj dijagnostici koristi se klasični biohemijski niz za ispitivanje biohemijskih osobina: *Kligler*-ov trostruki ili dvostruki šećer (ispitivanje sposobnosti fermentacije šećera laktoze i dekstroze, kao i redukcije sumpora, tj. stvaranja vodonik sulfida); peptonska voda za dokazivanje indola (ispitivanje sposobnosti razgradnje triptofana); metil red - *Voges Proskauer* (ispitivanje produkata fermentacije glukoze); *Simons* citratni agar (ispitivanje sposobnosti korišćenja citrata); ureaza test (ispitivanje sposobnosti razgradnje uree).

Za identifikaciju gram negativnih nefermentujućih bacila (*Pseudomonas spp*., *Acinetobacter spp*. i dr.) uglavnom se koristi CHROM agar, na kome daju karakterističnu boju kolonije: *Pseudomonas aeruginosa* – zelene, fluorescentne; *Acinetobacter* - zelene, sitnije, sjajne kolonije. Inače, bakterije rastu aerobno, na temperaturi 35-37˚C, 16-24 sata. Za identifikaciju koriste se sledeće osobine: nereaktivnost na *Kligler*-ovom dvostrukom/trostrukom šećeru; preparat sa kulture - gram negativni bacili, kokobacili; oksidaza reakcija (5).

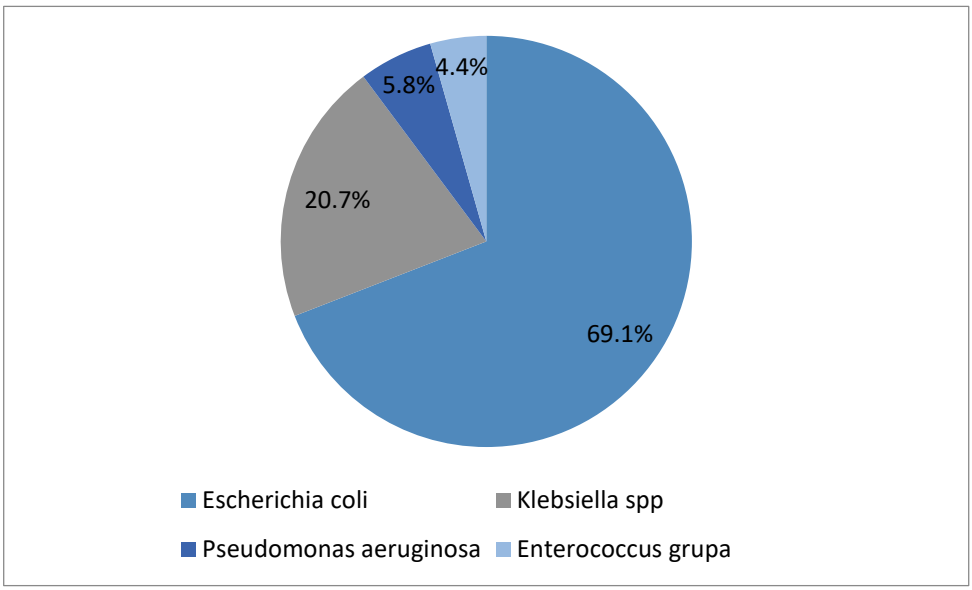
Ispitivanje osetljivosti na antibiotike je sprovedeno disk-difuzionom metodom na *Mueller-Hinton* agaru (antibiogram metoda). Bakterijski inokulum se priprema razblaživanjem 18-24 časovne čiste bakterijske kulture. Ezom se pikira 4-5 kolonija i prenese u 3 ml fiziološkog rastvora. Suspenzija se vorteksira, a potom se gustina suspenzije podešava poređenjem sa *McFarland* standardom 0,5 za turbiditet. Inokulum se zasejava ravnomerno na površinu podloge. Posle zasejavanja inokuluma poželjno je da podloga stoji 10-15 minuta na sobnoj temperaturi da bi se površina osušila (5,6).

Postavljanje antibiogram tableta ili diskova se izvodi dispenzerom ili sterilnom pincetom. Razmak između diskova je najmanje 1,7-2 cm, a rastojanje od ivice petri šolje najmanje 1 cm. Na ploču prečnika 9 cm nanosi se maksimalno 6 diskova, a na ploču prečnika 12 cm maksimalno 8 diskova. Zone inhibicije rasta očitavane su prema preporukama *Eucast*-a (engl. *European Committee on Antimicrobial Susceptibility Testing*). Ispitivana je osetljivost na: cefaleksin, ceftriakson, amikacin, gentamicin, ciprofloksacin, fosfomicin, meropenem, ceftazidim, piperacilin tazobaktam, ampicilin i norfloksacin. Test osetljivosti je interpretiran prema kriterijumima koje je objavio *Eucast* (7).

Analiza svih prikupljenih uzoraka urina u navedenom šestomesečnom period vršena je primenom deskriptivne statistike.

Rezultati

Od svih testiranih uzoraka pozitivno na patogene mikroorganizme je bilo 1.218 (25,3%). Najzastupljeniji patogeni urinarnog trakta u ispitivanom periodu bili su: *Escherichia coli* 842 (69,1%), *Klebsiella spp*. 252 (20,7%), *Pseudomonas aeruginosa* 71 (5,8%) i *Enterococcus* grupa 53 (4,4%) (grafikon 1).



Grafikon 1. Procentualno učešće pojedinih uzročnika urinarnih infekcija među svim identifikovanim prouzrokovačima u urinu ambulantnih pacijenata Instituta za javno zdravlje Kragujevac

One more test that was used for the identification of urinary pathogens was hydrolysis of esculin in 40% bile. Bile esculin agar is inoculated with the examined strain and incubated at 37˚C at night. If it comes to esculin hydrolysis, the medium changes its color into black (reaction of esculin with ferric-citrate in the medium). This test is positive in *enterococcus* and beta-hemolytic *streptococcus* group D.

A differential medium endo agar was used for the isolation and identification of *Enterobacteriaceae*. Inoculated samples are incubated aerobically, at 35-37˚C, 16 to 24 hours. The identification of *Enterobacteriaceae* is primarily based on the examination of biochemical properties. Classical biochemical series is used in routine diagnostics for the examination of biochemical properties: Kligler's triple or double sugar test (examination of the ability of fermentation of lactose and dextrose , as well as the reduction of sulfur, that is, the creation of hydrogen-sulfide); peptone water for the detection of indole (test the ability of tryptophan dissolution); methyl red – *Voges-Proskauer* (test the products of glucose fermentation); Simmons citrate agar (test the ability to utilize citrate); urease test (test the ability to split urea).

CHROM agar is mainly used for the identification of gram-negative non-fermenting germs (*Pseudomonas spp.*, *Acinetobacter spp.*, etc.), and characteristic color is shown for the following colonies: *Pseudomonas aeruginosa* – green, fluorescent; *Acinetobacter* – green, smaller, shiny colonies. However, bacteria grow aerobically, at 35-37˚C, 16-24 hours. The following characteristics are used for the identification: non-fermentation on Kligler double/triple sugar; preparation of the culture – gram-negative bacteria, coccobacilli; oxidase reaction (5).

Disc-diffusion method on Mueller-Hinton agar (antibiogram method) is used to test resistance to antibiotics. Bacterial inoculum is prepared by diluting the clean bacterial culture. Four-five colonies are picked with esis and transferred to 3 ml of physiological solution. The suspension is vortexed, and then density of the suspension is adjusted by comparing it with McFarland standard 0.5 for turbidity. Inoculum is inoculated equally on the surface of the medium. After it is inoculated, the medium should remain 10-15 minutes at room temperature so that the surface would dry (5,6).

Antibiogram tablets or discs are placed with the help of dispenser or sterile tweezers. The distance between discs is at least 1.7-2 cm, while the distance from the edge of Petri mug is at least 1 cm. Maximum 6 discs can be put on a 9 cm diameter Petri dish plate, and maximum 8 discs on 12 cm diameter plate. Zones of the inhibition of growth were read according to the recommendations of the European Committee on Antimicrobial Susceptibility Testing (Eucast). Susceptibility to following drugs was tested: cephalexin, ceftriaxone, amikacin, gentamicin, ciprofloxacin, fosfomycin, meropenem, ceftazidime, piperacillin tazobactam,

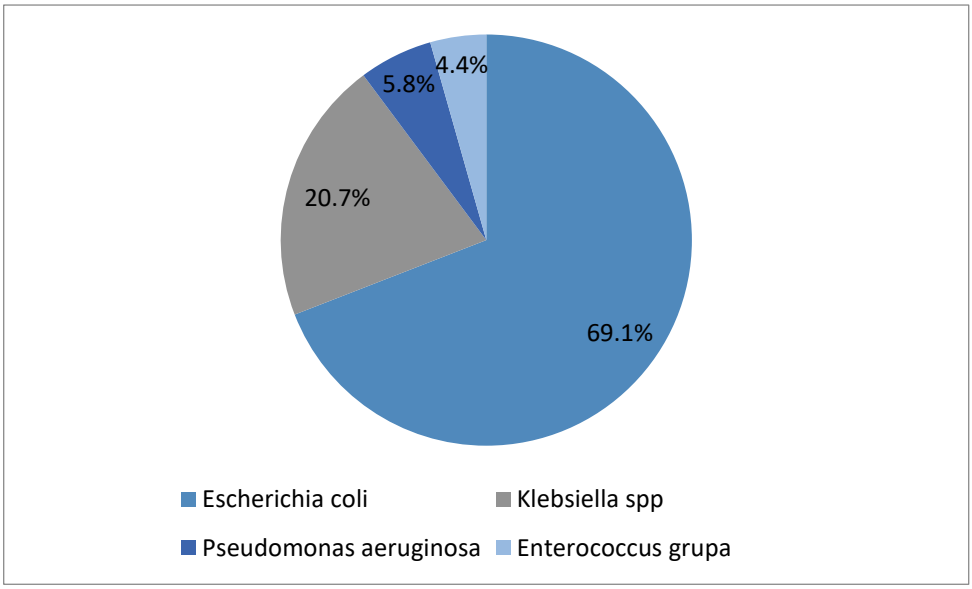


Figure 1. Percentage share of individual causes of urinary tract infections among all identified causative agents in the urine of the Institute of Public Health Kragujevac

Tabela 1. Distribucija izolovanih uzročnika urinarnih infekcija prema polu ispitanika, Institut za javno zdravlje Kragujevac

Vrsta uzročnika	*Escherichia coli*		*Klebsiella spp.*		*Pseudomonas aeruginosa*		*Enterococcus* grupa	
Ukupno	**842 (100%)**		**252 (100%)**		**71 (100%)**		**53 (100%)**	
Pol	Muškarci	Žene	Muškarci	Žene	Muškarci	Žene	Muškarci	Žene
Broj (%)	146 (17,3)	696 (82,7)	130 (51,6)	122 (48,4)	54 (76,1)	17 (23,9)	26 (49,1)	27 (50,9)

Bakterija *E. coli* je bila češća kod žena (82,7%), a *Pseudomonas aeruginosa* kod muškaraca (76,1%). *Klebsiella spp*. i *Enterococcus spp*. su se skoro podjednako javljale kod oba pola (tabela 1).

Osetljivost je testirana na antibiotike iz grupa cefalosporina, aminoglikozida, karbapenema, penicilina, kao i na hemioterapeutike iz grupe fluorohinolona i na fosfomicin (ukoliko je broj izolovanih patogena bio manji od 100.000 nije rađena antibiogram metoda).

Izolovani patogeni (osim predstavnika *Enterococcus spp*.) su testirani na cefaleksin, ceftriakson i ceftazidim koji spadaju u grupu cefalosporina, a dobijeni rezultati su prikazani u tabeli 2. Kada se analizira grupna osetljivost prema svim testiranim antibioticima iz grupe cefalosporina, uočava se da je najosetljivija bakterija bila E. coli, a najrezistentnija *P. aeruginosa*. Dok se ozbiljna analiza rezistencije na ceftazidim ne može vršiti zbog veoma malog testiranog uzorka, preostala dva antibiotika su delovala prema sledećem obrascu: (1) Ceftriakson je bio najdelotvorniji prema *E. coli* (čak 88,3% senzitivnih izolata), dok je njegova delotvornost bila značajno manja prema *Klebsiella spp.* (51,8% osetljivih sojeva) i naročito prema *P. aeruginosa* (20,3% osetljivosti). Cefaleksin je bio nešto manje aktivan, ali je osetljivost izolata i u ovom slučaju mogla da se poređa u sledeći opadajući niz: E. coli (75,5%), *Klebsiella spp*. (33,1%) i *P. aeruginosa* (samo 4,3% osetljivosti).

Kada je testirana efikasnost aminoglikozida, uočena je takođe velika osetljivost *E. coli*, za kojom slede *Klebsiella spp*., *P. aeruginosa* i *Enterococcus spp.* (tabela 3). Iz grupe aminoglikozida na amikacin su bile senzitivne, u najvećem broju slučajeva bakterije *E. coli* (čak 96%), zatim slede *Klebsiella*

Tabela 2. Osetljivost izolovanih patogena na antibiotike iz grupe cefalosporina

Patogeni uzročnici	*Escherichia coli*		*Klebsiella spp.*		*Pseudomonas aeruginosa*	
Vrsta antibiotika	**S Broj (%)**	**R Broj (%)**	**S Broj (%)**	**R Broj (%)**	**S Broj (%)**	**R Broj (%)**
Cefaleksin	628 (75,5)	204 (24,5)	84 (33,1)	168 (66,6)	3 (4,3)	67 (95,7)
Ceftriakson	743 (88,3)	98 (11,7)	130 (51,8)	121 (48,2)	14 (20,3)	55 (79,7)
Ceftazidim	1 (50)	1 (50)	5 (55,6)	4 (44,4)	25 (44,6)	31 (55,4)

S-senzitivni, R-rezistentni izolati

Tabela 3. Osetljivost izolovanih patogena na antibiotike iz grupe aminoglikozida

Patogeni uzročnici	*Escherichia coli*		*Klebsiella spp.*		*Pseudomonas aeruginosa*		*Enterococcus* grupa	
Vrsta antibiotika	**S Broj (%)**	**R Broj (%)**	**S Broj (%)**	**R Broj (%)**	**S Broj (%)**	**R Broj (%)**	**S Broj (%)**	**R Broj (%)**
Gentamicin	665 (79,3)	174 (20,7)	133 (52,8)	119 (47,2)	28 (40,0)	42 (60,0)	13 (25,5)	38 (74,5)
Amikacin	806 (96,0)	33 (4,0)	187 (74,8)	63 (25,2)	46 (69,7)	20 (30,0)	nt	nt

S-senzitivni, R-rezistentni izolati, nt-nije testirano

Table 1. Sex-specific distribution of isolated causes of urinary tract infections, Institute of Public Health Kragujevac

Type of causative agent	*Escherichia coli*		*Klebsiella spp.*		*Pseudomonas aeruginosa*		*Enterococcus* grupa	
Total	**842 (100%)**		**252 (100%)**		**71 (100%)**		**53 (100%)**	
Gender	Men	Women	Men	Women	Men	Women	Men	Women
Number (%)	146 (17.3)	696 (82.7)	130 (51.6)	122 (48.4)	54 (76.1)	17 (23.9)	26 (49.1)	27 (50.9)

ampicillin and norfloxacin. Susceptibility test was interpreted according to the criteria published by Eucast (7).

The analysis of all collected urine samples in the given time period was done with the help of descriptive statistics.

Results

Of all tested samples, 1218 were positive to pathogenic microorganisms (25.3%). The most common urinary tract pathogens in the examined period were the following: *Escherichia coli* 842 (69.1%), *Klebsiella spp.* 252 (20.7%), *Pseudomonas aeruginosa* 71 (5.8%) and *Enterococcus* group 53 (4.4%) (Figure 1).

E. coli was more frequent in women (82.7%), while *Pseudomonas aeruginosa* was more frequent in men (76.1%). *Klebsiella spp.* and *Enterococcus spp.* appeared almost equally in both men and women (Table 1).

Susceptibility was tested for antibiotics from the group of cephalosporins, aminoglycosides, carbapenem, penicillin, as well as for chemotherapeutics from the group of fluoroquinolones and fosfomycin (if the number of isolated pathogens was less than 100,000, antibiogram method was not done). Isolated pathogens (except the representatives of *Enterococcus spp.*) were tested for susceptibility to cephalexin, ceftriaxone and ceftazidime that belong to the group of cephalosporins, and the obtained results were presented in Table 2.

When group susceptibility to all tested antibiotics from the group of cephalosporins is analyzed, bacterium *E. coli* is found to be the most susceptible, while *P. aeruginosa* is the most

Table 2. Susceptibility of isolated pathogens to cephalosporin antibiotics

Pathogenic causes	*Escherichia coli*		*Klebsiella spp.*		*Pseudomonas aeruginosa*	
Types of antibiotics	**S Number (%)**	**R Number (%)**	**S Number (%)**	**R Number (%)**	**S Number (%)**	**R Number (%)**
Cefalexin	628 (75.5)	204 (24.5)	84 (33.1)	168 (66.6)	3 (4.3)	67 (95.7)
Ceftriaxon	743 (88.3)	98 (11.7)	130 (51.8)	121 (48.2)	14 (20.3)	55 (79.7)
Ceftazidim	1 (50)	1 (50)	5 (55.6)	4 (44.4)	25 (44.6)	31 (55.4)

S-sensitive, R-resistant isolates

Table 3. Susceptibility of isolated pathogens to antibiotics from the group of aminoglycosides

Pathogenic causes	*Escherichia coli*		*Klebsiella spp.*		*Pseudomonas aeruginosa*		*Enterococcus* group	
Types of antibiotics	**S N (%)**	**R N (%)**	**S N (%)**	**R N (%)**	**S N (%)**	**R N (%)**	**S N (%)**	**R N (%)**
Gentamycin	665 (79.3)	174 (20.7)	133 (52.8)	119 (47.2)	28 (40.0)	42 (60.0)	13 (25.5)	38 (74.5)
Amikacin	806 (96.0)	33 (4.0)	187 (74.8)	63 (25.2)	46 (69.7)	20 (30.0)	nt	nt

S-sensitive, R-resistant isolates, nt- not tested

Tabela 4. Osetljivost izolovanih patogena na antibiotike iz grupe fluorohinolona

Patogeni uzročnici	*Escherichia coli*		*Klebsiella spp.*		*Pseudomonas aeruginosa*		*Enterococcus* grupa	
Vrsta antibiotika	S Broj (%)	R Broj (%)	S Broj (%)	R Broj (%)	S Broj (%)	R Broj (%)	S Broj (%)	R Broj (%)
Ciprofloksacin	542 (64,5)	298 (35,5)	115 (45,8)	136 (54,2)	24 (36,4)	42 (63,6)	28 (53,8)	24 (46,2)
Norfloksacin	nt	nt	nt	nt	nt	nt	3 (33,3)	6 (66,7)

S-senzitivni, R-rezistentni izolati, nt-nije testirano

Tabela 5. Osetljivost izolovanih patogena na antibiotike iz grupe karbapanema

Patogeni uzročnici	*Escherichia coli*		*Klebsiella spp.*		*Pseudomonas aeruginosa*		*Enterococcus* grupa	
Vrsta antibiotika	S Broj (%)	R Broj (%)	S Broj (%)	R Broj (%)	S Broj (%)	R Broj (%)	S Broj (%)	R Broj (%)
Meropenem	8 (88,9)	1(11,1)	39 (100,0)	nt	45 (78,9)	12(21,1)	28 (53,8)	24 (46,2)

S-senzitivni, R-rezistentni izolati, nt-nije testirano

spp. i *P. aeruginosa*, sa približnim procentom osetljivih sojeva (74,8% i 69,7%), dok bakterije iz *Enterococcus* grupe nisu testirane na ovaj antibiotik. Slična je situacija i sa gentamicinom, s tim da su na njega testirani i izolati *Enterococcus spp*., ali je samo manji procenat (25,5%) bio senzitivan.

Iz grupe fluorohinolona svi izolati su testirani na ciprofloksacin (tabela 4). Određeni broj svih izolovanih patogena je bio senzitivan na ciprofloksacin, pri čemu je najveći procenat bio zastupljen kod bakterije *E. coli* (64,5%). Slede bakterije *Enterococcus* grupe (53,8%), *Klebsiella spp*. (45,8%) i *P. aeruginosa* (36,4%). Na norfloksacin su testirane jedino bakterije iz *Enterococcus* grupe i mali broj nalaza je bio senzitivan na ovaj antibiotik, s tim da je i ukupan broj testiranih izolata bio mali.

Iz grupe karbapenema izolovani patogeni, sem bakterija iz *Enterococcus* grupe, su testirani na antibiotik meropenem (tabela 5). Ovde su svi testirani izolati *Klebsiella spp*. (ukupno 39) svi bili osetljivi, dok je procenat osetljivosti bio nešto manji u slučaju *E. coli* (88,9%) i *P. aeruginosa* (78,9%). Treba samo istaći da je senzitivnost *E. coli* testirana prema malom broju izolata.

Tabela 6. Osetljivost izolovanih patogena na antibiotike iz grupe penicilina

Patogeni uzročnici	*Escherichia coli*		*Klebsiella spp.*		*Pseudomonas aeruginosa*		*Enterococcus* grupa	
Vrsta antibiotika	S Broj (%)	R Broj (%)	S Broj (%)	R Broj (%)	S Broj (%)	R Broj (%)	S Broj (%)	R Broj (%)
Piperacilin tazobaktam	1 (50,0)	1 (50,0)	6 (85,7)	1 (14,3)	43 (81,1)	10 (18,9)	nt	nt
Ampicilin	nt	nt	nt	nt	nt	nt	6 (11,8)	45 (88,2)

S-senzitivni, R-rezistentni izolati, nt-nije testirano

Tabela 7. Osetljivost izolovanih patogena na antibiotike iz grupe uroantiseptika

Patogeni uzročnici	*Escherichia coli*		*Klebsiella spp.*		*Pseudomonas aeruginosa*		*Enterococcus* grupa	
Vrsta antibiotika	S Broj (%)	R Broj (%)	S Broj (%)	R Broj (%)	S Broj (%)	R Broj (%)	S Broj (%)	R Broj (%)
Fosfomicin	243 (93,8)	16 (6,2)	54 (65,8)	28 (34,2)	2 (16,7)	10 (83,3)	6 (11,8)	45 (88,2)

S-senzitivni, R-rezistentni izolati

Table 4. Susceptibility of isolated pathogens to fluoroquinolone antibiotics

Pathogenic causes	*Escherichia coli*		*Klebsiella spp.*		*Pseudomonas aeruginosa*		*Enterococcus* group	
Types of antibiotics	S N (%)	R N (%)	S N (%)	R N (%)	S N (%)	R N (%)	S N (%)	R N (%)
Ciprofloxacin	542 (64.5)	298 (35.5)	115 (45.8)	136 (54.2)	24 (36.4)	42 (63.6)	28 (53.8)	24 (46.2)
Norfloxacin	nt	nt	nt	nt	nt	nt	3 (33.3)	6 (66.7)

S-sensitive, R-resistant isolates, nt- not tested

Table 5. Susceptibility of isolated pathogens to carbapanem antibiotics

Pathogenic causes	*Escherichia coli*		*Klebsiella spp.*		*Pseudomonas aeruginosa*		*Enterococcus* group	
Types of antibiotics	S N (%)	R N (%)	S N (%)	R N (%)	S N (%)	R N (%)	S N (%)	R N (%)
Meropenem	8 (88.9)	1(11.1)	39 (100.0)	nt	45 (78.9)	12(21.1)	28 (53.8)	24 (46.2)

S-sensitive, R-resistant isolates, nt- not tested

resistant. While a serious analysis of resistance to ceftazidime cannot be done due to a very small tested sample, the remaining two antibiotics acted according to the following pattern: (1) Ceftriaxone is the most efficient for *E. coli* (even 88.3% of sensitive isolates), while its efficacy is significantly smaller for *Klebsiella spp.* (51.8% of sensitive strains) and especially for *P. aeruginosa* (20.3% of sensitivity). Cephalexin was somewhat less active, but the sensitivity of the isolate in this case could be placed in the following decreasing sequence: *E. coli* (75.5%), *Klebsiella spp.* (33.1%) and *P. aeruginosa* (only 4.3% of sensitivity).

When the efficiency of aminoglycosides was tested, high sensitivity of *E. coli* was observed, and then of *Klebsiella spp.*, *P. aeruginosa* and *Enterococcus spp.* (Table 3). From the group of aminoglycosides, in most cases bacteria *E. coli* was sensitive to amikacin (even 96%), followed by *Klebsiella spp.* and *P. aeruginosa*, with the similar percentage of sensitive strains (74.8% and 69.7%), while bacteria from the *Enterococcus* group were not tested for susceptibility to these antibiotics. Similar results were obtained for gentamicin,

Table 6. Susceptibility of isolated pathogens to penicillin antibiotics

Patogeni uzročnici	*Escherichia coli*		*Klebsiella spp.*		*Pseudomonas aeruginosa*		*Enterococcus* group	
Vrsta antibiotika	S N (%)	R N (%)	S N (%)	R N (%)	S N (%)	R N (%)	S N (%)	R N (%)
Piperacillin tazobactam	1 (50.0)	1 (50.0)	6 (85.7)	1 (14.3)	43 (81.1)	10 (18.9)	nt	nt
Ampicillin	nt	nt	nt	nt	nt	nt	6 (11.8)	45 (88.2)

S-sensitive, R-resistant isolates, nt- not tested

Table 7. Susceptibility of isolated pathogens to uroantiseptic

Pathogenic causes	*Escherichia coli*		*Klebsiella spp.*		*Pseudomonas aeruginosa*		*Enterococcus* group	
Types of antibiotics	S N (%)	R N (%)	S N (%)	R N (%)	S N (%)	R N (%)	S N (%)	R N (%)
Fosfomycin	243 (93.8)	16 (6.2)	54 (65.8)	28 (34.2)	2 (16.7)	10 (83.3)	6 (11.8)	45 (88.2)

S-sensitive, R-resistant isolates

Iz grupe penicilina testirani antibiotici su: piperacilin tazobaktam (prema malom broju *E. coli* i *Klebsiella spp*. izolata, i nešto većem broju izolata *P. aeruginosa*) i ampicilin (prema *Enterococcus spp*.) (tabela 6). Procentualno gledano, *Klebsiella spp*. je bila najsenzitivnija (85,7%), a zatim bakterije *P. aeruginosa* (81,1%), na piperacilin tazobaktam. S druge strane, bakterije iz *Enterococcus* grupe, testirane samo na ampicilin, bile su senzitivne u malom broju uzoraka (11,8%).

Iz grupe uroantiseptika primenjen je fosfomicin, a testirani su svi patogeni sem bakterija iz *Enterococcus* grupe. Bakterija *E. coli* i u manjem procentu *Klebsiella spp*. su pokazale osetljivost, dok je bakterija *P. aeruginosa* bila pretežno rezistentna (Tabela 7).

Diskusija

Urinarna infekcija je čest problem svuda u svetu, češće kod osoba ženskog pola u svim uzrastima (1). S obzirom na to da uropatogeni poseduju mehanizme za razvoj rezistencije, potrebno je u terapiji koristiti antibiotike u zavisnosti od rezultata antibiograma. Uglavnom, preko 90% svih izolata su gram negativni patogeni (8-10). Kao što se i očekivalo, u našem istraživanju *E. coli* je bila najčešći izolat i češće je izazivala urinarne tegobe kod osoba ženskog pola. To je ujedno bio i najčešći patogen povezan sa infekcijama urogenitalnog trakta detektovan i u drugim studijama (1-4). *E. coli* generalno pripada normalnoj flori debelog creva čoveka i zbog toga može lako kolonizovati urinarni trakt. Ostali gram-negativni patogeni koji se mogu naći u urinu, iako su prisutni u manjim procentima, igraju značajnu ulogu u urinarnim infekcijama zbog svoje patogenosti i visoke otpornosti na antibiotike. Zapravo, šestomesečni presek je pokazao uropatološki status populacije na nivou jednog grada. Pokazali smo da su, pored *E. coli*, ostali uropatogeni bili *Klebsiella spp.*, *Enterococcus spp.* i *Pseudomonas aeruginosa*.

Naši rezultati su uporedivi sa rezultatima studije Đorđević i sar., koji su analizirali učestalost vanbolničkih infekcija urinarnog trakta (2). Najčešće izolovani uzročnici urinarnih infekcija kod njih su bili *E. coli* (56,6%), zatim redom vrste *Klebsiella spp.* (16,2%), *Proteus spp.* (14,68%), *Enterococcus spp.* (5,3%) i *P. aeruginosa* (3,7%). Drugim rečima, procentualna zastupljenost patogena je opadala u sličnom nizu kao i u našoj studiji (*E. coli*, *Klebsiella spp.*, *P. aeruginosa*, *Enterococcus spp.*), s tim da su oni uočili i značajno prisustvo *Proteus spp.* Što se tiče distribucije patogena po polovima, sličnost pomenute i naše studije se uočava u slučaju *E. coli*, koja je mnogo češće izazivala infekcije kod žena svih starosnih grupa (oko 70%) (2).

Afinitet uropatogena prema polu određen je pre svega anatomsko-fiziološkim karakteristikama pola. Ovo bi mogli objasniti činjenicom da osobe ženskog pola se češće javljaju doktoru kada sumnjaju na urinarnu infekciju, mada se to sa sigurnošću ne može potvrditi. Podaci prikazani u ovom radu govore da je kod ženskog pola znatno zastupljeniji uropatogen *E. coli.* Sa druge strane, *P. aeruginosa* je prisutnija kod muškog pola, dok su se *Klebsiella spp.* i *Enterococcus spp.* pojavljivale u urinu oba pola u približno jednakom broju. U skladu sa ovim su i literaturni podaci koji ukazuju da su učestaliji uzročnici infekcija urinarnog trakta kod muškog pola bile *Klebsiella spp.*, *Proteus spp.*, *Pseudomonas spp.* i *Acinetobacter spp.* (1,12).

U prošlosti, porast rezistencije uropatogena bio je mnogo veći problem bolničkih sredina. Međutim, novije studije pokazuju da je porast rezistentnih sojeva prisutan i u opštoj populaciji (3). Iz ovog razloga treba biti obazriv prilikom propisivanja terapije, naročito ako se radi o starijim osobama, osobama s hroničnim oboljenjima, ili osobama s malformacijama urinarnog trakta. Tada se naročito preporučuje propisivanje terapije po antibiogramu (4). Podaci naše studije pokazuju da je rezistencija bila najmanje zastupljena kod izolata *E. coli*, a najčešća kod *P. aeruginosa* i *Enterococcus spp.*. Imajući u vidu da obe bakterije nose R plazmide koji im pružaju multiplu rezistenciju prema većem broju antibiotika (4,11), kao i činjenicu da je multipla rezistencija kod enterokoka u značajnom porastu (13), ovaj rezultat ne iznenađuje.

Iako je studija obuhvatila relativno mali uzorak izolata urogenitalnih patogena, naročito *Enterococcus spp.* i *P. aeruginosa*, ako se uzmu u obzir i veličina testiranog uzorka i procenti rezistencije, lekovi prvog izbora u terapiji urinarnih infekcija bi bili sledeći: za *E. coli* amikacin (aminoglikozid) i fosfomicin, donekle i ceftriakson (cefalosporin), a za *Klebsiella spp.* meropenem (karbapenem) i takođe amikacin. Što se tiče *P. aeruginosa*, najefikasnijim su se pokazali takođe meropenem, kao i penicilinski antibiotik piperacilin-tazobaktam. Konačno, na osnovu dobijenih rezultata ne može se preporučiti nijedan od testiranih antibiotika kao terapija infekcija bakterijama *Enterococcus spp.*, s

however, isolates of *Enterococcus spp.* were also tested, and only a small percentage (25.5%) was sensitive.

From the group of fluoroquinolones, all isolates were tested for susceptibility to ciprofloxacin (Table 4). A certain number of isolated pathogens were sensitive to ciprofloxacin, while the greatest percentage was in bacteria *E. coli* (64.5%), followed by *Enterococci* (53.8%), *Klebsiella spp.* (45.8%) and *P. aeruginosa* (36.4%). Bacteria from the *Enterococcus* group were tested for susceptibility to norfloxacin, and a small number of results were sensitive to it, although the total number of tested isolates was small.

From the group of carbapenem, all isolated pathogens, except bacteria from *Enterococcus* group, were tested for susceptibility to meropenem (Table 5). Here of all tested *Klebsiella spp.* isolates (total 39), all were sensitive, while the percentage of susceptibility was a little bit smaller in case of *E. coli* (88.9%) and *P. aeruginosa* (78.9%). It should be emphasized that the sensitivity of *E. coli* was tested according to a small number of isolates.

As far as penicillin is concerned, the following antibiotics were tested: piperacillin tazobactam (according to the small number of *E. coli* and *Klebsiella spp.* isolates, and somewhat larger number of *P. aeruginosa* isolates) and ampicillin (*Enterococcus spp.*) (Table 6). In percentages, *Klebsiella spp.* was the most sensitive (85.7%), and then bacteria *P. aeruginosa* (81.1%) to piperacillin tazobactam. On the other hand, bacteria from *Enterococcus* group were tested only for susceptibility to ampicillin, and they were sensitive in a small number of samples (11.8%).

From the group of uroantiseptics, fosfomycin was applied, and all pathogens were tested except bacteria from *Enterococcus* group. Bacteria *E. coli* and *Klebsiella spp.* in smaller percentages showed sensitivity, while *P. aeruginosa* was dominantly resistant (Table 7).

Discussion

Urinary tract infections are a common problem worldwide, and they appear more frequently in women of all ages (1). Considering the fact that urinary pathogens possess mechanisms for the development of resistance, antibiotics should be used according to the antibiogram. More than 90% of all isolates are mainly gram-negative pathogens (8-10). As it had been expected, in our study *E. coli* was the most frequent isolate and it caused urinary tract problems more frequently in females. At the same time, it was the most frequent pathogen connected with the infections of urogenital tract that was detected in other studies, as well (1-4). *E. coli* generally belongs to the normal intestinal microflora and therefore, it can easily colonize the urinary tract. Other gram-negative pathogens that can be found in urine, although they are present in small percentages, have a significant role in urinary tract infections due to their pathogenicity and high resistance to antibiotics. Actually, the six months analysis showed the uropathological status of population at the level of one city. We showed that, in addition to *E.coli*, other uropathogens were *Klebsiella spp.*, *Enterococcus spp.* and *Pseudomonas aeruginosa*.

Our results are comparable to the results of a study of Djordjevic and associates, who analyzed the frequency of out-patient urinary tract infections (2). The most frequently isolated causes of urinary tract infections in their study were *E. coli* (56.6%), and then *Klebsiella spp.* (16.2%), *Proteus spp.* (14.68%), *Enterococcus spp.* (5.3%) and *P. aeruginosa* (3.7%). In other words, percentage of pathogens decreased in a similar sequence as in our study (*E. coli*, *Klebsiella spp.*, *P. aeruginosa*, *Enterococcus spp.*), but they noted a significant presence of *Proteus spp.* As far as distribution of pathogens by gender is concerned, the similarity between the above mentioned and our study is noticed in case of *E. coli*, which caused infections more frequently in women of all ages (about 70%) (2).

The affinity of uropathogens with gender was determined, first of all, by anatomical-physiological characteristics of gender. This could be explained by the fact that women more frequently visit their doctors when they suspect that they have a urinary infection, although this cannot be confirmed with certainty. Data shown in this paper indicate that uropathogen *E. coli* is more present in women. On the other hand, *P. aeruginosa* is more present in males, while *Klebsiella spp.* and *Enterococcus spp.* appeared in urine of both men and women in almost equal numbers. Literature data are in accordance with this, and they indicate that more frequent causes of urinary tract infections in males were *Klebsiella spp.*, *Proteus spp.*, *Pseudomonas spp.* and *Acinetobacter spp.* (1,12).

In the past, the increase in the resistance of uropathogens was a great problem in the hospital

obzirom na to da je i brojnost testiranih izolata bila mala, ali i rezistencija među testiranim izolatima visoka. Interesantno je da su uropatogeni uglavnom bili otporni na antibiotike iz grupe penicilina, što se razlikuje od nekih dostupnih literaturnih podataka. Naime, ranija studija pokazuje da su sojevi enterokoka pokazali izrazitu osetljivost na ampicilin i penicilin, što se razlikuje od rezultata našeg istraživanja (1,14).

Zaključak

Institutu za javno zdravlje Kragujevac, koji predstavlja jedinu zdravstvenu ustanovu tog tipa u Šumadijskom okrugu, u periodu od novembra 2020. do aprila 2021. godine, od 4.809 uzoraka urina, 1218 je bilo pozitivno na uropatogene mikroorganizme. Češće su bili izolovani Gram-negativni uropatogeni od Gram-pozitivnih, a bakterija *Escherichia coli* je bila najčešća među njima. Žene su bile češće sa urinarnom infekcijom uzrokovanom *E. coli*, a osobe muškog pola bakterijom P. aeruginosa. Infekcije *E. coli* su se u najvećem procentu mogu lečiti amikacinom i fosfomicinom, a *Klebsiella spp.* amikacinom i meropenemom. Najpouzdaniji način definisanja pravilne antibiotske terapije upravo predstavlja izolacija i identifikacija uzročnika, a zatim testiranje osetljivosti konkretnog izolata metodom antibiograma.

Konflikt interesa

Autori su izjavili da nema konflikta interesa.

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environment. However, recent studies have shown that the increase in resistant strains is present in general population, as well (3). Due to this reason, one should be careful when prescribing therapy, especially in older persons, persons with chronic diseases, or persons with urinary tract malformations. Then, therapy is prescribed according to the antibiogram (4). Data from our study show that resistance was least present in *E. coli* isolates, while it was most frequent in *P. aeruginosa* and *Enterococcus spp.* Having in mind that both bacteria have R plasmids that provide multiple resistance to a great number of antibiotics (4,11), as well as the fact that multiple resistance in *Enterococci* is significantly increasing (13), this result is not surprising.

Although the study included a relatively small number of isolates of urogenital pathogens, especially *Enterococcus spp.* and *P. aeruginosa*, and if we take into account the size of the tested sample and the percentages of resistance, first choice drugs for the treatment of urinary tract infections would be the following: for *E. coli* amikacin (aminoglycoside) and fosfomycin, partly ceftriaxone (cephalosporin), and for *Klebsiella spp.* meropenem (carbapenem) and also amikacin. As far as *P. aeruginosa* is concerned, meropenem was shown to be the most efficient, as well as penicillin antibiotic piperacillin-tazobactam. Finally, according to the obtained results, there are no antibiotics that can be recommended as the therapy for infections caused by bacteria *Enterococcus spp.*, considering that the number of tested isolates was small, and resistance among the tested isolates high. It is interesting that uropathogens are mainly resistant to penicillin antibiotics, which is contrary to some available literature data. Namely, the previous study has shown that enterococci strains show high sensitivity to ampicillin and penicillin, which is contrary to the results of our research.

Conclusion

Of 4809 urine samples, 1219 were positive to uropathogenic microorganisms at the Institute of Public Health Kragujevac, which is the only health care institution of that kind in Sumadija county from November 2020 to April 2021. Gram-negative pathogens were isolated more frequently than gram-positive, while *Escherichia coli* was the most frequent among them. Women had more frequently the urinary tract infections caused by *E. coli*, while men had more frequently infections caused by *P. aeruginosa*. *E. coli* infections can be treated with amikacin and fosfomycin in most cases, while *Klebsiella spp.* with amikacin and meropenem. The most reliable way of defining the correct antibiotic therapy is precisely the isolation and identification of causative agents, and then testing the sensitivity of particular isolate with the help of antibiogram method.

Competing interests

The author declares no competing interests.

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STAVOVI ZDRAVSTVENIH RADNIKA O AKADEMSKOM OBRAZOVANJU MEDICINSKIH SESTARA U BOSNI I HERCEGOVINI

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SAŽETAK

Uvod/Cilj: Problemi sa kojima se suočava zdravstveni sektor u zemljama Balkana, ali i u u većini zemalja Evropske unije (naročito visoko razvijenim zemljama) je nedostatak fakultetski obrazovanih medicinskih sestara na tržištu rada. Cilj rada je bio da se ispitaju stavovi zdravstvenih radnika o značaju i mogućnostima akademskog obrazovanja medicinskih sestara.
Metode: Studija preseka je provedena na 233 zdravstvena radnika u periodu od 10.07.2019. do 31.08.2019. godine u Javnoj zdravstvenoj ustanovi (JZU) Dom zdravlja Zvornik, JZU Dom zdravlja Foča, JZU Opšta bolnica Zvornik, JZU Univerzitetska bolnica Foča. Svi ispitanici su popunili upitnik, koji je prethodno kulturološki adaptiran. U statističkoj analizi podataka korišćen je χ^2.
Rezultati: Većina zdravstvenih radnika (73,8%) činile su žene. Žena je bilo nešto više u uzrastu do 50 godina, a muškaraca starijih od 50 godina. Lekara je bilo 41,3%, medicinskih sestara sa srednjom stručnom spremom (SSS) 42,2%, a medicinskih sestara sa višom (VŠS) i visokom stručnom spremom (VSS) 16,4%. Najveći procenat zdravstvenih radnika (91,4%) navodi da je upoznat sa mogućnostima sestrinskog obrazovanja, dok 69,5% ima stav da je za medicinske sestre dovoljno temeljno srednjoškolsko obrazovanje i specijalizacija. Svaki drugi zdravstveni radnik (50,2%), nezavisno od nivoa obrazovanja, navodi da ne postoji potreba za obrazovanjem medicinskih sestara na nivou doktorskih studija, a ¼ je suzdržana po ovom pitanju.Lekari i medicinske sestre sa SSS su značajno češće (χ^2 =10,151; p=0,038) smatrali da ne postoji potreba za obrazovanjem medicinskih sestara na nivou doktorskih studija nego medicinske sestre sa VŠS i VSS. Najveći procenat zdravstvenih radnika (66,6%) ima stav da medicinska sestra ima jednak autoritet kao i ostali članovi tima, 86,3% se slaže sa tvrdnjom da će razvoj sestrinske profesije uticati na kvalitet zdravstvene zaštite, a 55,8% smatra da medicinske sestre imaju profesionalne kapacitete, znanja i iskustava za inovativna rešenja i upravljanje neminovnim promenama ka putu u savremeno sestrinstvo.
Zaključak: Kako bi se podigao ugled sestrinske profesije, neophodno je definisati obim posla i postaviti jasne granice između kompetencija medicinskih sestara različitog stepena obrazovanja na nivou cele Bosne i Hercegovine. U cilju daljeg razvoja sestrinske profesije neophodna je bolja promocija novih programa poslediplomske nastave.

Ključne reči: sestrinstvo, obrazovanje, autonomija, kompetencije

Uvod

Tokom poslednjih decenija uloge medicinske sestre i opisi poslova stalno i značajno se menjaju, a sestrinstvo se razvilo u disciplinu za koju su potrebna znanja i veštine u neprestanom suočavanju sa novim izazovima (1). Poslednjih dvadeset godina sestrinstvo postiže kvalitetan skok u edukaciji i praksi, a posebno u istraživanjima, razvoju standarda, menadžmentu kvaliteta i stvaranju akademskog statusa. Edukacija u sestrinstvu dobija adekvatan značaj pa se brzo razvijaju visokoškolske ustanove za obrazovanje sestara sa akademskim odeljenjima i novim programima edukacije, koji omogućuju implementaciju postdiplomske nastave, magistarske i doktorske studije (2). Kako bi

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ATTITUDES OF HEALTH WORKERS ABOUT THE ACADEMIC EDUCATION OF NURSES IN BOSNIA AND HERZEGOVINA

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SUMMARY

Introduction/Aim: The problems facing the health sector in the Balkans, but also in most European Union countries (especially highly developed countries) is the lack of university-educated nurses in the labor market. The aim of the paper was to examine the attitudes of health professionals about the importance and possibilities of academic education of nurses.
Methods: The cross-sectional study was conducted on 233 health workers in the period from July 10, 2019 to August 31, 2019 in the Public Health Institution (PHI) Health Center Zvornik, PHI Health Center Foca, PHI General Hospital Zvornik, PHI University Hospital Foca. All respondents filled out a questionnaire, which was previously culturally adapted. χ^2 was used in the statistical analysis of the data.
Results: The majority of health workers (73.8%) were women. There were slightly more women under the age of 50, and men over the age of 50. There were 41.3% of doctors/specialists, 42.2% of nurses with secondary education, and 16.4% of nurses with post-secondary and higher education. The largest percentage of health workers (91.4%) state that they are familiar with the possibilities of nursing education, while 69.5% are of the opinion that thorough secondary education and specialization is enough for nurses. About half of health workers (50.2%), regardless of the level of education, states that there is no need for the education of nurses at the level of doctoral studies, and ¼ abstained on this issue. Doctors of medicine and specialist doctors and nurses with secondary education were statistically significantly more likely (χ^2 = 10,151; p = 0.038) to consider that there is no need for education of nurses at the level of doctoral studies than nurses with post-secondary and higher education. The largest percentage of health workers (66.6%) have the opinion that the nurse has the same authority as other team members, 86.3% agree with the statement that the development of the nursing profession will affect the quality of health care, and 55.8% believe that nurses have the professional capacity, knowledge and experience to innovate solutions and manage the inevitable changes on the pathway to modern nursing.
Conclusion: In order to raise the reputation of the nursing profession, it is necessary to define the scope of work and set clear boundaries between the competencies of nurses of different levels of education at the level of the whole of Bosnia and Herzegovina. In order to further develop the nursing profession, better promotion of new postgraduate programs is necessary.

Key words: nursing, education, autonomy, competencies

Introduction

During the last decades, nurse's roles and job description have changed constantly and significantly, and the nursing profession has developed into a discipline, which requires knowledge and skills in the face of constant challenges (1). During the last twenty years, the nursing profession has achieved the improvement of quality regarding education and practice, and especially research, development of standards, management of quality and creation of academic status. Nurses' education has been given proper significance, and therefore, higher educational institutions for nurses with academic departments and new educational programs are developing

karijera medicinskih sestara bila uspešna i planirana potrebna je spoznaja o sebi i svojim potencijalima, vrednostima i preferencijama (3).

Problemi sa kojima se suočava zdravstveni sektor u zemljama Balkana, ali i u većini zemalja Evropske unije (naročito visoko razvijenim zemljama) je nedostatak fakultetski obrazovanih medicinskih sestara na tržištu rada. Ovaj problem je povezan pre svega sa sve manjim brojem studenata koji se upisuju na fakultete za medicinske sestre, zatim s povećanim potrebama za zdravstvenim uslugama medicinskih sestara, relativno manje atraktivnim radnim uslovima, niskim prihodima u odnosu na druga zanimanja, ograničenim mogućnostima napredovanja u poslu, kao i ranijim odlaskom u penziju (4).

Sa druge strane, problem predstavlja i aktuelna društvena slika medicinskih sestara koja je raznolika i neskladna. Ta slika je stvorena od samih medicinskih sestara kroz njihovu nevidljivost i neprepoznatljivost u zdravstvenom sistemu zbog njihove pasivnosti u javnim raspravama. Medicinske sestre su formirale svoj koncept shvatanja samih sebe i koncept profesionalnog identiteta iz javnog mnenja, radne okoline, radnih vrednosti, sistema obrazovanja i kulturnih vrednosti (5).

Cilj rada je bio da se ispitaju stavovi zdravstvenih radnika o značaju i mogućnostima akademskog obrazovanja medicinskih sestara.

Metode

Studija preseka sprovedena je u periodu od 10.7.2019. do 31.08.2019. godine, anonimnim upitnikom koji je testiran pre početka istraživanja, sačinjenim od strane autora za potrebe ovog istraživanja. Kako bi se testirao upitnik, sprovedeno je pilot istraživanje na uzorku od 40 ispitanika u maju 2019. godine. Na temelju rezultata pilot istraživanja, i komentara ispitanika upitnik je kulturološki modifikovan na način da neka pitanja budu jasnija, a neka su isključena iz upitnika. Takođe je napravljena promena redosleda pitanja. Završna verzija upitnika imala je 40 pitanja.

U studiju presekasu uključena233 zdravstvena radnika (lekari i medicinske sestre) koji su zaposleni u JZU Dom zdravlja Zvornik, JZU Dom zdravlja Foča, JZU Opšta bolnica Zvornik i JZU Univerzitetska bolnica Foča. Svi ispitanici su potpisali dobrovoljni pristanak za uključivanje u istraživanje. Kriterijumi za uključivanje u istraživanje su bili dobrovoljni pristanak, da je ispitanik po zanimanju medicinski tehničar ili doktor medicine/specijalista zaposlen na primarnom ili sekundarnom nivou zdravstvene zaštite, a kriterijumi za isključivanje su bili odbijanje učestvovanja u studiji i neadekvatno popunjavanje upitnika. Svi ispitanici su popunjavali svoje uputnike na radnom mestu. Popunjavanje upitnika je trajalo oko 10 minuta.

Kompjuterska obrada podataka sprovedena je pomoću SPSS 20 softverskog statističkog paketa. U obradi podataka primenjene su metode deskriptivne statistike. Značajnost razlika u frekvencijama određena je primjenom Hi kvadrat testa, a kao nivo statističke značajnosti uzeta je vrednost $p < 0,05$. Tokom prikupljanja i analiziranja podataka, kao i rada, poštovao se Zakon o zaštiti ličnih podataka („Službeni glasnik BiH“ br 49/2006, 76/2011 – ispr).

Rezultati

U ovoj studiji preseka bilo je uključeno 233 ispitanika, od kojih su 73,8% činile žene, a 26,2% muškarci (Tabela 1). Žena je bilo nešto više u uzrastu do 50 godina, a muškaraca starijih od 50 godina. Najveći deo ispitanika (26,2%) su činile osobe uzrasta 30 i manje godina, a zatim osobe uzrasta 31-40 godina (25,8%) i 41-50 godina (25,3%). U primarnoj zdravstvenoj zaštiti (PZZ) i sekundarnoj zdravstvenoj zaštiti (SZZ) je bilo više žena nego muškaraca. U PZZ bilo je više žena mlađih od 50 godina, a muškaraca starijih od 50 godina. U SZZ bilo je najviše muškaraca uzrasta 31-40 godina, a žena mlađih od 31 godine. Nije uočena statistički značajna razlika između muškaraca i žena u odnosu na uzrast zdravstvenih radnika.

Većina zdravstvenih radnika 213 od 233, što je 91,4%, navodi da su upoznati sa mogućnostima sestrinskog obrazovanja. U SZZ je zaposlen značajno veći broj sestara sa srednjim (45,3%), višim i fakultetskim obrazovanjem (22,2%) koje su upoznate sa mogućnostima sestrinskog obrazovanja nego u PZZ (38,5% i 9,4%) (Tabela 2). Doktori medicine/specijalisti PZZ značajno su više bili upoznati sa mogućnostima sestrinskog obrazovanja nego doktori medicine/specijalisti SZZ.

Doktora medicine/specijalista je bilo 41,3%, medicinskih sestara sa srednjom stručnom spremom 42,2%, a medicinskih sestara sa višom i visokom stručnom spremom 16,4%.

Najveći broj zdravstvenih radnika (69,5%) smatra da je u školovanju medicinskih sestara dovoljno temeljno obrazovanje (SSS) i dodatna specijalizacija na području delovanja (Tabela 3). Nije uočena statistički značajna razlika između

quickly, thus enabling the implementation of postgraduate courses, master and doctoral studies (2). In order to have a successful and well-planned nursing career, one needs to realize their own potentials, values and preferences (3).

The lack of nurses with a university degree in the labor market is the problem, which the health sector in the Balkans, as well as in most European Union countries (particularly highly developed countries), is faced with. This problem is connected, first of all, with a small number of students who enroll at faculties for nurses, then with the increased need for health services that nurses provide, relatively less attractive work conditions, lower income in comparison to other professions, limited possibilities of career advancement, as well as with the earlier retirement (4).

On the other hand, the problem also refers to the current social image of nurses, which is diverse and disharmonious. That image was created by nurses themselves because they are invisible and unrecognizable in the healthcare sector due to their passivity in public hearings. Nurses have formed their own concept of self-realization and the concept of professional identity based on the public opinion, work environment, professional values, educational system, and cultural values (5).

The aim of this paper was to examine the attitudes of health professionals about the importance and possibilities of academic education of nurses.

Methods

A cross-sectional study was conducted from 10th April 2019 to 31st August 2019 with the help of an anonymous questionnaire that had been tested before the beginning of research and that had been made by authors for the needs of this research. In order to test the questionnaire, a pilot research was conducted in May 2019 and it included the sample of 40 participants. Based on the results of this pilot research and participants' comments, the questionnaire was culturally modified so that some questions became clearer and some were excluded from the questionnaire. Also, the order of questions was changed. The final version of the questionnaire had 40 questions.

A cross-sectional study included 233 health workers (doctors and nurses), who were employed at the Health Center Zvornik, Health Center Foca, General Hospital Zvornik and University Hospital Foca. All participants have signed a written consent to be included in the study. Inclusion criteria were the following: voluntary informed consent; participants were medical technicians or medical doctors/specialists employed in primary or secondary healthcare, while exclusion criteria were the following: participants denied to take part in the study or filled in the questionnaire inadequately. All participants filled out their questionnaires in their work place. Filling out the questionnaire lasted 10 minutes.

Computer analysis of data was done with the help SPSS 20 software statistical package. Descriptive statistics was used for the analysis of data. The significance of difference regarding frequency was obtained with the help of chi-squared test, while p value $p < 0.05$ was deemed to be statistically significant. During the collection and analysis of data, as well as during work, the Law on personal data protection was obeyed ("Official Gazette of BiH" no. 49/2006, 76/2011).

Results

The cross-sectional study included 233 participants, that is, 73.8% of them were women, and 26.2% were men (Table 1). There were slightly more women under the age of 50, and men older than 50. The largest number of participants (26.2%) was persons aged 30 and younger, and then persons aged 31-40 years (25.8%) and 41-50 years (25.3%). There were more women than men in the primary and secondary healthcare. In the primary healthcare, there were more women younger than 50 years, and men older than 50. In the secondary healthcare, the largest number of men was in the age group 31-40 years, while the largest number of women was in the age group younger than 31. There was no statistically significant difference between men and women regarding the age of healthcare workers.

The majority of healthcare workers, that is, 213 of 233, which presents 91.4%, indicate that they are familiar with the possibilities of nursing education. In the secondary healthcare, there are significantly more nurses with secondary education (45.3%), post-secondary and higher education (22.2%) who were familiar with the possibilities of nursing education than in primary healthcare (38.5% and 9.4%) (Table 2). Medical doctors and specialists in the primary healthcare were significantly more often acquainted with the

Tabela 1. Distribucija zdravstvenih radnika primarne i sekundarne zdravstvene zaštite prema polu i uzrasnim grupama

Uzrasne grupe (godine)	Primarna zdravstvena zaštita		Sekundarna zdravstvena zaštita		Ukupno*		Total
	Muškarci Broj (%)	Žene Broj (%)	Muškarci Broj (%)	Žene Broj (%)	Muškarci Broj (%)	Žene Broj (%)	Broj (%)
<31	4 (19,0)	19 (22,1)	12(30,0)	26(30,2)	16 (21,1)	45 (26,2)	61 (26,2)
31-40	5 (23,8)	23 (26,7)	13(32,5)	19(22,1)	18 (23,7)	42 (24,4)	60 (25,8)
41-50	5 (23,8)	23 (26,7)	9(22,5)	22(25,6)	14 (18,4)	45 (26,2)	59 (25,3)
>50	7 (33,3)	21 (24,4)	6(15,0)	19(22,1)	28 (36,8)	40 (23,2)	53 (22,8)
Total	21 (100)	86 (100)	40(100)	86(100)	61 (26,2)	172 (73,8)	233 (100)

*χ^2 = 5,4488; p = 0,142

ispitanika različitog nivoa obrazovanja u odnosu na tvrdnju da je u školovanju medicinskih sestara dovoljno temeljno obrazovanje i specijalizacija na području delovanja. Potrebu za obrazovanjem medicinskih sestara, koje rade na poslovima koji zahtevaju samostalni rad, na nivou master studija je iskazalo 62,7% ispitanika. Nije uočena statistički značajna razlika između ispitanika različitog nivoa obrazovanja u stavu po pitanju potrebe obrazovanja medicinskih sestara, koje rade na poslovima koji zahtevaju samostalni rad, na nivou master studija. Većina ispitanika (50,2%), nezavisno od nivoa obrazovanja, navodi da ne postoji potreba za obrazovanjem medicinskih sestara na nivou doktorskih studija, a 24% je suzdržano. Doktori medicine/specijalisti i medicinske sestre sa SSS su statistički značajno češće (χ^2 =10,151; p=0,038) smatrali da ne postoji potreba za obrazovanjem medicinskih sestara na nivou doktorskih studija nego medicinske sestre sa VŠS/VSS.

Najveći procenat zdravstvenih radnika (68,2%) navodi da u svakoj zdravstvenoj ustanovi koja zapošljava veći broj sestara, treba da budu zaposlene medicinske sestre obrazovane na nivou diplomskih studija (Tabela 4). Između zdravstvenih radnika različitog nivoa obrazovanja nije postojala značajna razlika u odnosu na ovaj stav.

Između zdravstvenih radnika različitog nivoa obrazovanja nije utvrđena statistički značajna razlika (χ^2=5,515; p=0,238) u pogledu njihovih stavova da medicinske sestre imaju pravo da ospore autoritet lekara, ukoliko on u određenim okolnostima ne deluje u najboljem interesu bolesnika (Tabela 5). Skoro svaki drugi zdravstveni radnik se slaže se da u određenim okolnostima kada je očigledno da lekar ne deluje u najboljem interesu bolesnika sestra ima pravo da spori lekarov autoritet.

Najveći procenat zdravstvenih radnika (66,6%) se slaže sa stavom da medicinska sestra ima jednak autoritet kao i ostali članovi tima, 86,3% se slaže sa

Tabela 2. Distribucija zdravstvenih radnika primarne i sekundarne zdravstvene zaštite koji su upoznati sa mogućnostima sestrinskog obrazovanja prema nivou obrazovanja

Upoznati sa mogućnostima sestrinskog obrazovanja	Primarna zdravstvena zaštita N=96	Sekundarna zdravstvena zaštita N=117	Ukupno* N=233
Nivo obrazovanja	**Broj (%)**	**Broj (%)**	**Broj (%)**
Doktor medicine/specijalista	50 (52,1)	38 (32,5)	117 (54,9)
Medicinska sestra sa SSS*	37 (38,5)	53 (45,3)	90 (42,2)
Medicinska sestra sa VŠS/VSS**	9 (9,4)	26 (22,2)	35 (16,4)
Ukupno	96 (45,1)	117 (54,9)	213 (100,0)

SSS - srednja stručna sprema; VŠS/VSS - viša stručna sprema/visoka stručna sprema; χ^2 = 10,772; p = 0,005

Table 1. Distribution of health workers in primary and secondary healthcare by gender and age

Age groups (years)	Primary healthcare		Secondary healthcare		Total*		Total
	Men No. (%)	Women No. (%)	Men No. (%)	Women No. (%)	Men No (%)	Women No. (%)	No. (%)
<31	4 (19.0)	19 (22.1)	12(30.0)	26(30.2)	16 (21.1)	45 (26.2)	61 (26.2)
31-40	5 (23.8)	23 (26.7)	13(32.5)	19(22.1)	18 (23.7)	42 (24.4)	60 (25.8)
41-50	5 (23.8)	23 (26.7)	9(22.5)	22(25.6)	14 (18.4)	45 (26.2)	59 (25.3)
>50	7 (33.3)	21 (24.4)	6(15.0)	19(22.1)	28 (36.8)	40 (23.2)	53 (22.8)
Total	21 (100)	86 (100)	40(100)	86(100)	61 (26.2)	172 (73.8)	233 (100)

*χ^2 = 5.4488; p = 0.142

possibilities of nursing education than medical doctors/specialists in secondary healthcare.

The majority of healthcare workers, that is, 213 of 233, which presents 91.4%, indicate that they are familiar with the possibilities of nursing education. In the secondary healthcare, there are significantly more nurses with secondary education (45.3%), post-secondary and higher education (22.2%) who were familiar with the possibilities of nursing education than in primary healthcare (38.5% and 9.4%) (Table 2). Medical doctors and specialists in the primary healthcare were significantly more often acquainted with the possibilities of nursing education than medical doctors/specialists in secondary healthcare.

The largest number of healthcare workers (68.2%) stated that in each healthcare institution, which employed large number of nurses, nurses with BA studies should be employed (Table 4). There was no statistical difference regarding this attitude between healthcare workers with different levels of education.

There was no statistical difference between healthcare workers with different levels of education (χ^2=5.515; p=0.238) regarding their opinion that nurses had the right to deny doctor's authority, if he in certain circumstances did not act patients' best interests (Table 5). Almost half of healthcare workers agreed that in certain circumstances, when it was obvious that a doctor did not act in the patient's interest, a nurse had the right to deny doctor's authority.

The largest percentage of healthcare workers (66.6%) agreed that nurses had equal authority as other team members; 86.3% agreed that the development of nursing profession would influence the quality of healthcare, while 55.8% thought that nurses possess professional capacities, knowledge and experience for innovative solutions and management of unavoidable changes that are part of the pathway to modern nursing profession (Table 6). There was no statistically significant difference between healthcare workers with different levels of education regarding their

Table 2. Distribution of healthcare workers in primary healthcare and secondary healthcare who were familiar with the possibilities of nursing education according to the level of education

Familiar with the possibilities of nursing education	Primary healthcare N=96	Secondary healthcare N=117	Total* N=233
Level of education	**No. (%)**	**Broj (%)**	**Broj (%)**
Doctor/specialist	50 (52.1)	38 (32.5)	117 (54.9)
Nurse with SE*	37 (38.5)	53 (45.3)	90 (42.2)
Nurse with PSE/HE**	9 (9.4)	26 (22.2)	35 (16.4)
Total	96 (45.1)	117 (54.9)	213 (100.0)

SE – secondary education; PSE/HE – post-secondary education/higher education; χ^2 = 10.772; p = 0.005

Tabela 3. Distribucija zdravstvenih radnika različitog nivoa obrazovanja prema stavovima o potrebi medicinskih sestara za različitim nivoima obrazovanja

Stavovi o različitim nivoima obrazovanja medicinskih sestara		Doktor medicine/ specijalista N=96 Broj (%)	Medicinska sestra sa SSS N=101 Broj (%)	Medicinska sestra sa VŠS/ VSS N=36 Broj (%)	Ukupno N=233 Broj (%)
U školovanju medicinskih sestara dovoljno je temeljno obrazovanje (SSS) + specijalizacija na području delovanja.*	Uopšte/uglavnom se ne slažem	18 (18,8)	12 (11,9)	5 (13,9)	35 (15,0)
	Niti se slažem, niti se ne slažem	14 (14,6)	14 (13,9)	8 (22,2)	36 (15,4)
	Uglavnom/u potpunosti se slažem	64 (66,7)	75 (74,2)	23 (63,9)	162 (69,5)
Postoji potreba za obrazovanjem na nivou 4+1 (diplomske i master studije) za određeni broj sestara na poslovima koji zahtevaju samostalni rad.**	Uopšte/uglavnom se ne slažem	23 (24,0)	16 (15,8)	6 (16,7)	45 (19,3)
	Niti se slažem, niti se ne slažem	19 (19,8)	17 (16,8)	6 (16,7)	42 (18,0)
	Uglavnom/u potpunosti se slažem	54 (56,2)	68 (67,3)	24 (66,6)	146 (62,7)
Postoji potreba za obrazovanjem sestara i na nivou doktorskih studija.***	Uopšte/uglavnom se ne slažem	56 (58,3)	49 (48,5)	12 (33,3)	117 (50,2)
	Niti se slažem, niti se ne slažem	21 (21,9)	27 (26,7)	8 (22,2)	56 (24,0)
	Uglavnom/u potpunosti se slažem	19 (19,8)	25 (24,8)	16 (44,4)	60 (25,8)

SSS - srednja stručna sprema; VŠS/VSS - viša stručna sprema/visoka stručna sprema; *χ^2 = 3,465; p = 0,483; **χ^2 = 3,189; p = 0,527; ***χ^2 = 10,151; p = 0,038

tvrdnjom da će razvoj sestrinske profesije uticati na kvalitet zdravstvene zaštite, a 55,8% smatra da medicinske sestre poseduju profesionalne kapacitete, znanja i iskustava za inovativna rešenja i upravljanje neminovnim promenama ka putu u savremeno sestrinstvo (Tabela 6). Između zdravstvenih radnika različitog nivoa obrazovanja nije bilo značajne razlike u odnosu na stav da medicinska sestra ima jednak autoritet kao i ostali članovi tima i da će razvoj sestrinske profesije uticati na kvalitet zdravstvene zaštite. Međutim, medicinske sestre sa VŠS/VSS su značajno češće nego doktori medicine/specijalisti i medicinske sestre sa SSS smatrale da medicinske sestre poseduju profesionalne kapacitete, znanja i iskustava za inovativna rešenja i upravljanje neminovnim promenama ka putu u savremeno sestrinstvo.

Tabela 4. Distribucija zdravstvenih radnika različitog nivoa obrazovanja prema stavovima o potrebi zapošljavanja diplomiranih medicinskih sestara u ustanovama koje zapošljavaju veći broj sestara

Stavovi o potrebi zapošljavanja diplomiranih medicinskih sestara		Doktor medicine/ specijalista N=96 Broj (%)	Medicinska sestra sa SSS N=101 Broj (%)	Medicinska sestra sa VŠS/ VSS N=36 Broj (%)	Ukupno N=233 Broj (%)
U svakoj ustanovi koja upošljava veći broj sestara, treba biti zaposlena medicinska sestra obrazovana na nivou diplomskih studija	Uopšte/uglavnom se ne slažem	12 (12,5)	14 (13,9)	2 (5,6)	28 (12,0)
	Niti se slažem, niti se ne slažem	21 (21,9)	22 (21,8)	3 (8,3)	46 (19,7)
	Uglavnom/u potpunosti se slažem	63 (65,6)	65 (64,3)	31 (86,1)	159 (68,2)

SSS - srednja stručna sprema; VŠS/VSS - viša stručna sprema/visoka stručna sprema; χ^2 = 6,367; p = 0,173.

Table 3. Distribution of healthcare workers with different levels of education regarding their attitudes about nurses' needs to get education at different levels

Attitudes about different levels of education of nurses		Medical doctor/ specialist N=96 No. (%)	Nurse with SE N=101 No. (%)	Nurse with PSE/HE N=36 No. (%)	Total N=233 No. (%)
In the education of nurses thorough sec-ondary education (SE)+vocational spe-cialization are suffi-cient.*	Completely disagree/mainly disagree	18 (18.8)	12 (11.9)	5 (13.9)	35 (15.0)
	Neither agree nor disagree	14 (14.6)	14 (13.9)	8 (22.2)	36 (15.4)
	Mainly/completely agree	64 (66.7)	75 (74.2)	23 (63.9)	162 (69.5)
There is a need for education 4+1 (BA and master studies) for certain number of nurses whose jobs require independent work.**	Completely disagree/mainly disagree	23 (24.0)	16 (15.8)	6 (16.7)	45 (19.3)
	Neither agree nor disagree	19 (19.8)	17 (16.8)	6 (16.7)	42 (18.0)
	Mainly/completely agree	54 (56.2)	68 (67.3)	24 (66.6)	146 (62.7)
There is a need to educate nurses at the level of doctoral studies.***	Completely disagree/mainly disagree	56 (58.3)	49 (48.5)	12 (33.3)	117 (50.2)
	Neither agree nor disagree	21 (21.9)	27 (26.7)	8 (22.2)	56 (24.0)
	Mainly/completely agree	19 (19.8)	25 (24.8)	16 (44.4)	60 (25.8)

SE – secondary education; PSE/HE – post-secondary education/higher education; *χ^2 = 3.465; p = 0.483; **χ^2 = 3.189; p = 0.527; ***χ^2 = 10.151; p = 0.038

attitude that a nurse has the same authority as other team members and that the development of nursing profession would influence the quality of healthcare. However, nurses with PSE/HE in comparison to medical doctors/specialists and nurses with SE thought significantly more often that nurses possess professional capacities, knowledge and experience for innovative solutions and management of unavoidable changes that are part of the pathway to contemporary nursing profession.

Discussion

Nurses, as the most numerous staff in the system of healthcare, have a significant role in psychocare, therapeutic aspects of care, immediate care, social care, and health education. The course of patient's recovery depends on the quality of

Table 4. Distribution of healthcare workers with different levels of education regarding the attitudes about the need to employ graduate nurses in institutions which employ large number of nurses

The need to employ graduate nurses in institutions		Medical doctor/ specialist N=96 No. (%)	Nurse with SE N=101 No. (%)	Nurse with PSE/HE N=36 No. (%)	Total N=233 No. (%)
In each institution that employs large number of nurs-es, graduate nurses should be employed	Completely disagree/mainly disagree	12 (12.5)	14 (13.9)	2 (5.6)	28 (12.0)
	Neither agree nor disagree	21 (21.9)	22 (21.8)	3 (8.3)	46 (19.7)
	Mainly/completely agree	63 (65.6)	65 (64.3)	31 (86.1)	159 (68.2)

SE – secondary education; PSE/HE – post-secondary education/higher education; χ^2 = 6.367; p = 0.173.

Tabela 5. Distribucija zdravstvenih radnika različitog nivoa obrazovanja prema stavu o pravu sestara da ospore autoritet lekara u određenim okolnostima

Stavovi o potrebi zapošljavanja diplomiranih medicinskih sestara		Doktor medicine/ specijalista N=96 Broj (%)	Medicinska sestra sa SSS N=101 Broj (%)	Medicinska sestra sa VŠS/ VSS N=36 Broj (%)	Ukupno N=233 Broj (%)
Ako je u određenim okolnostima očigledno da lekar ne deluje u najboljem interesu bolesnika sestra ima pravo osporiti lekarov autoritet.	Uopšte/uglavnom se ne slažem	26 (27,1)	28 (27,7)	8 (22,2)	62 (26,6)
	Niti se slažem, niti se ne slažem	36 (37,5)	24 (23,8)	11 (30,6)	71 (30,5)
	Uglavnom/u potpunosti se slažem	34 (35,4)	49 (48,5)	17 (47,2)	100 (42,9)

SSS - srednja stručna sprema; VŠS/VSS - viša stručna sprema/visoka stručna sprema; $^{*}\chi^2 = 5{,}515$; $p = 0{,}238$.

Diskusija

Medicinske sestre, kao najbrojniji kadar u sistemu zdravstvene zaštite, imaju bitnu ulogu u psihonezi, terapijskim aspektima njege, neposrednoj nezi, socionezi, ali i zdravstvenom vaspitanju. Zavisno od kvaliteta pruženih usluga od strane medicinskih sestara zavisi i sam tok oporavka bolesnika. Razvojem medicinskih nauka, kao i povećanim potrebama stanovništva za zdravstvenim uslugama, kretao se razvoj zdravstvene nege, koja još u prošlom veku, pored zdravstvene zaštite bolesnih, dobija jedan novi aspekt – zdravstvena zaštita zdravih ljudi.

Rezultati ovog istraživanja su pokazali da je većina zdravstvenih radnika i upoznata sa mogućnostima obrazovanja medicinskih sestara u Republici Srpskoj. Stavovi zdravstvenih radnika prema obrazovanju medicinskih sestara se razlikuju između država, ali i ustanovama unutar iste

Tabela 6. Distribucija zdravstvenih radnika različitog nivoa obrazovanja prema stavovima o sestrinskoj profesiji

Stavovi o sestrinskoj profesiji		Doktor medicine/ specijalista N=96 Broj (%)	Medicinska sestra sa SSS N=101 Broj (%)	Medicinska sestra sa VŠS/ VSS N=36 Broj (%)	Ukupno N=233 Broj (%)
Medicinska sestra ima jednak autoritet kao i ostali članovi tima.*	Uopšte/uglavnom se ne slažem	13 (13,5)	15 (14,8)	7 (19,4)	35 (15,0)
	Niti se slažem, niti se ne slažem	24 (25,0)	14 (13,9)	5 (13,9)	43 (18,4)
	Uglavnom/u potpunosti se slažem	59 (61,5)	72 (71,3)	24 (66,7)	155 (66,6)
Razvoj sestrinske profesije će uticati na kvalitet zdravstvene zaštite.**	Uopšte/uglavnom se ne slažem	5 (5,2)	5 (4,9)	1 (2,8)	11 (4,7)
	Niti se slažem, niti se ne slažem	11 (11,5)	9 (8,9)	1 (2,8)	21 (9,0)
	Uglavnom/u potpunosti se slažem	80 (83,3)	87 (86,2)	34 (94,4)	201 (86,3)
Da li medicinske sestre poseduju profesionalne kapacitete, znanja i iskustva za inovativna rešenja i upravljanje neminovnim promenama ka putu u savremeno sestrinstvo?***	Uopšte/uglavnom se ne slažem	20 (20,8)	5 (4,9)	3 (8,3)	28 (12,0)
	Niti se slažem, niti se ne slažem	33 (34,4)	35 (34,7)	7 (19,4)	75 (32,2)
	Uglavnom/u potpunosti se slažem	43 (44,8)	61 (60,4)	26 (72,2)	130 (55,8)

SSS - srednja stručna sprema; VŠS/VSS - viša stručna sprema/visoka stručna sprema; $^{*}\chi^2 = 5{,}115$, $p = 0{,}276$; $^{**}\chi^2 = 2{,}914$, $p = 0{,}572$; $^{***}\chi^2 = 17{,}170$, $p = 0{,}002$

Table 5. Distribution of healthcare workers with different levels of education regarding the attitude about nurses' right to deny doctor's authority

Nurse has the right to deny doctor's authority		Medical doctor/ specialist N=96 No. (%)	Nurse with SE N=101 No. (%)	Nurse with PSE/HE N=36 No. (%)	Total N=233 No. (%)
If in certain circumstances it is obvious that a doctor does not act in patient's best interests, a nurse has the right to deny doctor's authority.	Completely disagree/mainly disagree	26 (27.1)	28 (27.7)	8 (22.2)	62 (26.6)
	Neither agree nor disagree	36 (37.5)	24 (23.8)	11 (30.6)	71 (30.5)
	Mainly/completely agree	34 (35.4)	49 (48.5)	17 (47.2)	100 (42.9)

SE – secondary education; PSE/HE – post-secondary education/higher education; $^{*}\chi^2 = 5.515$; p = 0.238.

services provided by nurses. The development of medical sciences, as well as the increased needs of the population for healthcare services influenced the development of healthcare, which even in the previous century, in addition to the healthcare of ill persons gained one new aspect – healthcare of healthy people.

The results of this research have showed that the majority of healthcare workers are acquainted with the possibilities of education of nurses in the Republic of Srpska. Attitudes of healthcare workers about the education of nurses are different in different countries, but also in different institutions within the same country. Thus, one study that was conducted in 2009 in healthcare institutions in the Republic of Croatia proved that 28% of participants thought that secondary education of nurses was sufficient, while 48% thought that

Table 6. Attitudes of healthcare workers with different levels of education in the nursing profession

Attitudes about nursing profession		Medical doctor/ specialist N=96 No. (%)	Nurse with SE N=101 No. (%)	Nurse with PSE/HE N=36 No. (%)	Total N=233 No. (%)
Nurse has the equal authority as other team members.*	Completely disagree/mainly disagree	13 (13.5)	15 (14.8)	7 (19.4)	35 (15.0)
	Neither agree nor disagree	24 (25.0)	14 (13.9)	5 (13.9)	43 (18.4)
	Mainly/ completely agree	59 (61.5)	72 (71.3)	24 (66.7)	155 (66.6)
Development of nursing profession will influence the quality of life.**	Completely disagree/mainly disagree	5 (5.2)	5 (4.9)	1 (2.8)	11 (4.7)
	Neither agree nor disagree	11 (11.5)	9 (8.9)	1 (2.8)	21 (9.0)
	Mainly/ completely agree	80 (83.3)	87 (86.2)	34 (94.4)	201 (86.3)
Do nurses possess professional capacities, knowledge and experience for innovative solutions and unavoidable changes on the pathway to modern nursing?***	Completely disagree/mainly disagree	20 (20.8)	5 (4.9)	3 (8.3)	28 (12.0)
	Neither agree nor disagree	33 (34.4)	35 (34.7)	7 (19.4)	75 (32.2)
	Mainly/ completely agree	43 (44.8)	61 (60.4)	26 (72.2)	130 (55.8)

SE – secondary education; PSE/HE – post-secondary education/higher education; $^{*}\chi^2 = 5.115$, p =0.276; $^{**}\chi^2 = 2.914$, p = 0.572; $^{***}\chi^2 = 17.170$, p = 0.002

države. Tako istraživanjem sprovedenim 2009. godine u zdravstvenim ustanovama Republike Hrvatske, dokazano je da 28% ispitanika smatra da je dovoljno srednjoškolsko obrazovanje medicinskih sestara, dok48% smatra da postoji potreba za obrazovanjem na nivou diplomskog i master studija. Većina ispitanika (36%), u navedenom istraživanju, smatra da postoji potreba za obrazovanjem sestara na nivou doktorskih studija (6). U istraživanju u Republici Hrvatskoj, koje je sprovedeno 2021. godine, većina ispitanika (40,1%) smatra da je nepotrebno obrazovanje na nivou doktorskih studija, a 39,4% ispitanika je navelo da je doktorat iz sestrinstva potreban (7). U našem istraživanju većina zdravstvenih radnika je bila protiv (50,2%) ili indiferentna (24,0%) prema tvrdnji da postoji potreba za doktorskim studijem iz oblasti sestrinstva, dok se složila da je dodiplomsko i master obrazovanje potrebno. Međutim, možemo videti da preko ⅔ lekara, ali i preko ⅔ medicinskih sestara, smatra da je srednjoškolsko obrazovanje uz određenu subspecijalizaciju dovoljno za obavljanje posla medicinske sestre, što nam ukazuje da u praksi postoji i dalje nerazumevanje razlike između kompetencija sestara različitog stepena obrazovanja.

Razlike između našeg istraživanja i istraživanja sprovedenog u Republici Hrvatskoj su rezultat različitog izbora ispitanika uključenih u istraživanje. U istraživanju sprovedenom u Republici Hrvatskoj, 2009. godine, preko 90% ispitanika je bilo akademski obrazovano (54% doktora medicine), dok u našem istraživanju i istraživanju u Republici Hrvatskoj, 2021. godine, većideouzorka čine ispitanici sa srednjom stručnom spremom.

Medicinska sestra i lekar predstavljaju tim koji udruženim snagama treba da rade na poboljšanju kvaliteta pružanja zdravstvenih usluga. Lekari su dugi niz godina percipirani kao osobe nadređene medicinskim sestrama, odnosno autoriteti. Danas medicinska sestra kao punopravni član zdravstvenog tima deluje kao autonomni ekspert sa moralnom i pravnom odgovornošću, a u skladu sa pravilima struke (8).

Medicinske sestre u Bosni i Hercegovini imaju veći autoritet i autnomiju nego što je to bilo ranije, što potvrđuje naše istraživanje. Naime, većina našihispitanika (66,6%) smatra da medicinska sestra ima jednak autoritet kao i drugi članovi tima. Dokaz tome jeste i činjenjica da većina ispitanika (42,9%) smatra da ako je u određenim okolnostima očigledno da lekar ne deluje u najboljem interesu bolesnika sestra ima pravo osporiti njegov autoritet.

Naše istraživanje nije u saglasnosti sa studijom u Republici Hrvatskoj gde većina studenata sestrinstva (47%) smatra da medicinska sestra nema jednak autoritet kao drugi članovi tima (8). Za razliku od studenata, zaposlenici KBC Rijeka (88%) i SB Medico (92%) u Republici Hrvatskoj smatraju da medicinska sestra ima visok stepen autonomije (9).

Medicinska sestra koja zna da koristiti autoritet na adekvata način je efikasnija u zadovoljavanju personalnih odeljenskih i organizacionih ciljeva. Na ovakav način, ona je u stanju da izgradi visok stepen morala (10). U prilog tome govori i podatak da većina ispitanika smatra da razvoj sestrinske profesije u Bosni i Hercegovini može dovesti do unapređenja kvaliteta zdravstvene zaštite, kao i da medicinske sestre poseduju dovoljno profesionalnih kapaciteta.

Zaključak

Najveći procenat zdravstvenih radnika koji su bili obuhvaćeni ispitivanjem su upoznati sa mogućnostima školovanja medicinskih sestara. Ispitanici su saglasni da su potrebne dodiplomske i master studije iz oblasti sestrinstva, ali navode da ne postoji potreba za obrazovanjem medicinskih sestara na nivou doktorskih studija. Veliki procenat ispitanika smatra da je srednjoškolsko obrazovanje uz specijalizaciju iz oblasti u kojoj će medicinska sestra raditi je sasvim dovoljno za obavljanje posla medicinske sestre. Kako bi se podigao ugled i stanje sestrinske profesije, neophodno je definisati obim posla i postaviti jasne granice između kompetencija medicinskih sestara različitog stepena obrazovanja na nivou cele Bosne i Hercegovine. Takođe, deo strateškog plana za dalji razvoj sestrinske profesije treba biti i njena promocija u medijima i sredstvima javnog informisanja.

Konflikt interesa

Autori su izjavili da nema konflikta interesa.

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there was a need for the education at the level of BA or master studies. The majority of participants (36%), in the above mentioned study, thought that there was a need to educate nurses at the level of doctoral studies (6). In a study that was conducted in the Republic of Croatia in 2021, the majority of participants (40.1%) thought that the education at the level of doctoral studies was not necessary, while 39.4% of participants stated that doctorate from the field of nursing was needed (7). In our study, the majority of healthcare workers were against it (50.2%) or indifferent (24.0%) to the statement that there was a need for doctoral studies from the field of nursing, while they agreed that bachelor and master education is necessary. However, we can see that more than ⅔ of doctors, and more than ⅔ of nurses thought that secondary school education with certain subspecialization is sufficient for nursing jobs, which indicates that in practice the difference between competences of nurses with different levels of education is not well-understood.

Differences between our study and the study that was conducted in the Republic of Croatia are the result of different choice of participants included in the research. In the study conducted in the Republic of Croatia in 2009, more than 90% of participants had university degrees (54% medical doctors), while in our study and in the study conducted in the Republic of Croatia in 2021, greater part of the sample were participants with secondary school education.

A nurse and a doctor make a team which with its united forces should work on the improvement of quality of healthcare services. Doctors have been perceived for a lot of time as persons superior to nurses, that is, authorities. Today, a nurse, as a full-fledged team member acts as an autonomous expert with moral and legal accountability, in accordance with the rules of the profession (8).

Nurses in Bosnia and Herzegovina have a greater authority than before, which is confirmed in our study. Namely, the majority of our participants (66.6%) thought that a nurse has an equal authority as other team members. It is supported by the fact that the majority of participants (42.9%) thought that in certain circumstances, when it is obvious that a doctor does not act in patient's best interests, a nurse has the right to deny his authority.

Our study is not in accordance with the study in the Republic of Croatia, where the majority of nursing students (47%) thought that a nurse does not have an equal authority as other team members (8). Contrary to students, employees at the CHC Rijeka (88%) and SH Medico (92%) thought that a nurse had a high degree of autonomy (9).

A nurse, who knows how to use authority in an adequate way, is more efficient when satisfying personal, departmental and organizational goals. Thus, she is able to create a high degree of moral (10). It is proved by the fact that the majority of participants thought that the development of nursing profession in Bosnia and Herzegovina can lead to the improvement of quality of healthcare, as well as that nurses posses enough professional capacities.

Conclusion

The greatest percentage of healthcare workers, who were included in the study, was familiar with the possibilities of education of nurses. The participants agreed that bachelor and master studies were needed from the field of nursing, however, they stated that there was no need for the education of nurses at the level of doctoral studies. A great percentage of participants thought that secondary school education with specialization from the field, where a nurse is going to work in, is sufficient for nursing jobs. In order to raise the reputation and state of the nursing profession, it is necessary to define the scope of work and set clear boundaries between the competencies of nurses of different levels of education at the level of the whole of Bosnia ands Herzegovina. Also, one part of the strategic plan for further development of nursing profession should be its promotion in media and means of informing the public.

Competing interests

The author declares no competing interests.

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HEMOLITIČKA BOLEST NOVOROĐENČETA

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SAŽETAK

Uvod/Cilj: Hemolitička bolest novorođenčeta predstavlja hemoliznu anemiju koja se javlja kao posledica nepodudarnosti krvi roditelja, odnosno nepodudarnosti krvnih elemenata majke i ploda u ABO ili Rh sistemu. Cilj ovog rada je da prikaže slučaj hemolitičke bolesti novorođenčeta nastale kao posledica ABO inkompatibilije kod majke.
Prikaz bolesnika: Ženskom novorođenčetu je u osamnaestom satu života registrovana indirektna hiperbilirubinemija. Krvna grupa novorođenčeta je bila A Rh (D) pozitivna, direktni Coombs test bio je pozitivan, a u krvi novorođenčeta su detektovana anti-A antitela. Ovaj slučaj je uspešno lečen fototerapijom i transfuzijom resuspendovanih eritrocita bez potrebe za eksangvinotransfuzijom.
Zaključak: ABO inkompatibilija može predstavljati veliki problem kako u prenatalnom, tako i u perinatalnom i neonatalnom periodu.Posebnu pažnju treba obratiti na trudnice čija su deca iz prethodnih trudnoća imala hiperbilirubinemiju ili su im se trudnoće završavale spontanim pobačajem.

Ključne reči: ABO inkompatibilija, hemolitička bolest, hiperbilirubinemija

Uvod

Hemolitička bolest novorođenčeta predstavlja hemoliznu anemiju koja se javlja kao posledica nepodudarnosti krvnih elemenata majke i ploda u ABO ili Rh sistemu.

ABO hemolitička bolest novorođenčeta je najčešći uzrok žutice koji se javlja u prvih 24 sata života, sa incidencijom 0,33–2,2% novorođenih (1). Pripada grupi patoloških nekonjugovanih hiperbilirubinemija ispunjavajući sledeće kriterijume: pojava žutice u prvih 24 sata života, porast bilirubina preko 8,5 µmol/h, snižena koncentracija hemoglobina ispod 100 g/l i pozitivan *Coombs*-ov test (2). Nastaje kao rezultat prolaska antitela imunoglobulina G (IgG) kroz placentu kod novorođenčadi žena krvne grupe O koje imaju IgG anti-A ili anti-B antitela (3). Prolazeći kroz posteljicu, antitela iz krvotoka majke oštećuju eritrocite u krvotoku ploda, zbog čega se javljaju znaci hemolize: anemija, žutica, a u težim slučajevima i fetalni hidrops (4). Fetalni hidrops, koji je i najteži oblik hemolitičke bolesti novorođenčeta, je redak i javlja se kod 0,13% novorođenih (5). Usled hemolize eritrocita dolazi do razgradnje hema i hiperprodukcije bilirubina koji je u plazmi delom vezan za albumin, a delom u vidu slobodnog (nevezanog) bilirubina. Nevezani bilirubin prelazi krvno-moždanu barijeru i deponuje se u mozgu novorođenčeta. Nezrela jetra novorođenčeta, a posebno prevremeno rođenog, nije u stanju da metabolišе povećanu količinu bilirubina koja nastaje usled hemolize eritrocita i razvija se nekonjugovana (indirektna) hiperbilirubinemija što rezultira pojavom žutice u prvom danu života (6).

Brzo prepoznavanje i lečenje hiperbilirubinemije i hemolitičke bolesti su od izuzetne važnosti za izbegavanje dugotrajne neurološke disfunkcije kod ove novorođenčadi.

Prikaz slučaja

Ženskom novorođenčetu je u osamnaestom satu života registrovana indirektna hiperbiliru-

CASE REPORT

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HEMOLYTIC DISEASE OF THE NEWBORN

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SUMMARY

Introduction/Aim: Haemolytic disease of the newborn is haemolytic anaemia that occurs as a consequence of mismatched blood of the parents, that is, the mismatch of blood elements between the mother and fetus in the ABO or Rh system. The aim of this study is to report a case of haemolytic disease of the newborn which occurred as a result of ABO incompatibility.
Case report: Indirect hyperbilirubinemia was registered in a female newborn in the 18th hour of life. The blood group of the newborn was A Rh (D) positive, the direct Coombs test was positive and anti-A antibodies were detected in baby's blood. This case was successfully managed with phototherapy and simple red blood cells transfusion without the need for exchange transfusion.
Conclusion: ABO incompatibility can be a major problem in the prenatal, perinatal or neonatal periods. Special attention should be paid to pregnant women whose children from previous pregnancies had hyperbilirubinemia or whose pregnancies were ended in miscarriage.

Keywords: ABO incompatibility, hemolytic disease, hyperbilirubinemia

Introduction

Hemolytic disease of a newborn is haemolytic anaemia that occurs as a consequence of the incompatibility of blood elements between a mother and child in the ABO or Rh system.

ABO hemolytic disease of a newborn is the most common cause of jaundice that appears during the first 24 hours of life, with the incidence of 0.33-2.2% in newborns (1). It belongs to the group of pathological unconjugated hyperbilirubinemias when it meets the following criteria: the occurrence of jaundice during the first 24 hours of life, the increase in bilirubin more than 8.5 µmol/h, lowered concentration of hemoglobin below 100 g/l and the positive Coombs test (2). It occurs when immunoglobulin G antibodies (IgG) pass through the placenta in newborns of women whose blood type is O and who have IgG anti-A or anti-B antibodies (3). When passing through the placenta, antibodies from mother's circulation damage erythrocytes in the circulatory system of a fetus, due to which signs of hemolysis occur: anemia, jaundice and in some cases hydrops fetalis (4). Hydrops fetalis, which is the most severe form of hemolytic disease in newborns, is rare and it occurs in 0.13% of newborns (5). Hemolysis of red blood cells causes the destruction of red blood cells and hyperproduction of bilirubin, which is partly bound to albumin in the plasma and partly it is free (non-albumin bound) bilirubin. Non-albumin bound bilirubin passes the blood-brain barrier and it is deposited in the brain of a newborn. The immature liver of a newborn and especially of prematurely born children cannot metabolize the increased amount of bilirubin resulting from hemolysis of red blood cells and therefore, unconjugated (indirect) hyperbilirubinemia develops, which causes the occurrence of jaundice in the first 24 hours of life (6).

Recognizing and treating hyperbilirubinemia and hemolytic disease on time are of great

binemija. Ukupan bilirubin u prvom danu života bio je 162 µmol/l. Novorođenče je drugo dete iz treće trudnoće sedamnaestogodišnje majke koja je krvne grupe O Rh (D) pozitivna. Porođaj je završen prirodnim putem u 36. gestacijskoj nedelji. Na rođenju, telesna masa novorođenčeta iznosila je 2380 g, telesna dužina 44 cm, Apgar skor 9. Iz anamnestičkih podataka smo saznali da je druga trudnoća majke završena spontanim pobačajem u 11. gestacijskoj nedelji.

Prilikom pregleda novorođenče je bilo budno, snažnog plača, hemodinamski stabilno, ikterične kože očuvanog turgora i elasticiteta. Grudni koš je bio simetrično respiratorno pokretan, sa 56 respiracija u minutu. Srčana radnja je bila ritmična, tonovi jasni, bez šumova, srčana frekvenca 137 otkucaja u minutu. Abdomen je bio iznad ravni grudnog koša, palpatorno bolno neosetljiv, obima 39 cm. Mišićni tonus je bio uredan, a primitivni refleksi su se uredno izazivali.

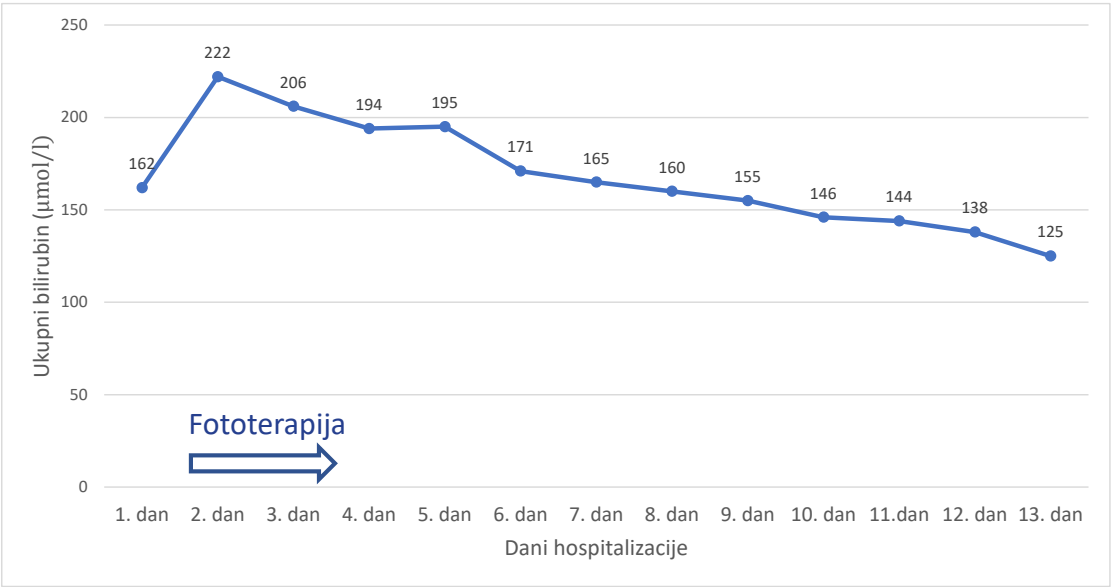
U drugom danu života došlo je do porasta ukupnog bilirubina na 222 µmol/l (direktni 8,1 µmol/l, indirektni 213,9 µmol/l). Hemoglobin je bio 137 g/l, procenat retikulocita 14,8%, haptoglobin < 0,1 g/l, laktat-dehidrogenaza (LDH) 739 U/l. Vrednosti gasnih analiza u krvi, pH i acidobazne ravnoteže kretale su se u normalnom opsegu. Protrombinsko vreme (PT) i aktivirano parcijalno tromboplastinsko vreme (apTT)su bili u granicama referentnih vrednosti čime je isključen poremećaj hemostaze kod novorođenčeta. Krvna grupa novorođenčeta bila je A Rh (D) pozitivna, direktni *Coombs* test bio je pozitivan. U plazmi novorođenčeta registrovano je prisustvo anti-A antitela. Ultrazvučnim pregledom CNS-a isključena je intrakranijalna hemoragija. Na ultrazvučnom pregledu trbuha, jetra, slezina, oba bubrega i nadbubrega, i mokraćna bešika su bili urednog položaja, oblika, veličine i ehostrukture i nije registrovano prisustvo tečnosti u trbušnoj duplji. Bakteriološkom obradom novorođenčeta isključena je sepsa.

Analiziranjem anamnestičkih, kliničkih i laboratorijskih rezultata zaključeno je da je uzrok patološke nekonjugovane žutice kod novorođenčeta bila imunska hemolizna anemija.

Prema protokolu za lečenje hemolizne bolesti novorođenčeta, zbog indirektne hiperbilirubinemije od drugog dana života, sprovođena je fototerapija u trajanju od 36 sati, nakon čega je zabeleženo opadanje vrednosti bilirubina u krvi (grafikon 1). Tokom hospitalizacije, usled hemolize eritrocita beležio se postepeni pad hemoglobina, zbog čega je novorođenčetu šestog dana života ordinirana transfuzija resuspendovanih eritrocita O krvne grupe (grafikon 2). Nije bilo potrebe za primenom eksangvinotransfuzije. Sve vreme hospitalizacije novorođenče je bilo stabilnog opšteg stanja i napredovalo je u telesnoj masi. Otpušteno je kući 13. dana života, telesne mase na otpustu 2440 g i urednog fizikalnog nalaza.

Diskusija

Rutinska upotreba profilakse Rh IgG rezultirala je značajnim padom incidencije RhD aloimuni-



Grafikon 1. Vrednosti ukupnog bilirubina po danima hospitalizacije

importance for the prevention of longstanding neurological dysfunction in these newborns.

Case report

Indirect hyperbilirubinemia was registered in a female newborn in the 18th hour of life. Total bilirubin in the first day of life was 162 µmol/l. The newborn was the second child from the third pregnancy of a seventeen-year old mother whose blood group was O Rh (D). The vaginal delivery was completed at a gestational age of 36 weeks. At birth, the newborn's body weight was 2380 g, body length was 44 cm, while the Apgar score was 9. We found in the medical history that the second pregnancy of this mother ended in miscarriage at 11 weeks of gestation.

During the examination, the newborn was awake, crying intensely, hemodynamically stable and had icteric skin with normal skin turgor and elasticity. The chest wall movement was symmetric with 56 breaths per minute. The heart rhythm was normal, sounds clear with no heart murmur, while heart rate was 137 beats per minute. The abdomen was above the chest line, abdominal palpation was insensitive, while circumference was 39 cm. The muscle tone was normal, and primitive reflexes were normal, as well.

In the second day of life, total bilirubin increased to 222 µmol/l (direct 8.1 µmol/l, indirect 213.9 µmol/l). Hemoglobin was 137 g/l, the percentage of reticulocytes was 14.8%, haptoglobin < 0.1 g/l, lactate-dehydrogenase (LDH) 739 U/l. The values of blood gas tests, the pH and acid-base balance were within the normal range. The prothrombin time (PT) and activated partial thromboplastin time (apt) were within the normal reference values, and therefore, hemostasis abnormality was excluded in the newborn. The newborn's blood group was A Rh positive, while the direct Coombs test was positive. Anti-A antibodies were registered in the plasma. The ultrasound examination of CNS excluded intracranial hemorrhage. The ultrasound examination of abdomen showed that liver, spleen, both kidneys and adrenal glands, and urinary bladder had normal position, shape, size and echostructure and no fluid was registered in the abdominal cavity. Sepsis was excluded by the bacteriological examination of the newborn.

The analysis of anamnestic, clinical and laboratory findings led to the conclusion that the cause of pathological unconjugated jaundice in the newborn was immune hemolytic anemia.

According to the protocol for the treatment of hemolytic disease of the newborn, due to indirect hyperbilirubinemia from the second day of life, phototherapy was applied lasting 36 hours, after which the value of bilirubin in blood decreased (Figure 1). During hospitalization, due to hemolysis of erythrocytes, gradual decrease in hemoglobin was noted and therefore, in the sixth day of life the transfusion of type O resuspended red cells was administered (Figure 2). There was no need for exchange transfusion. The newborn's general state was stable all the time during hospitalization

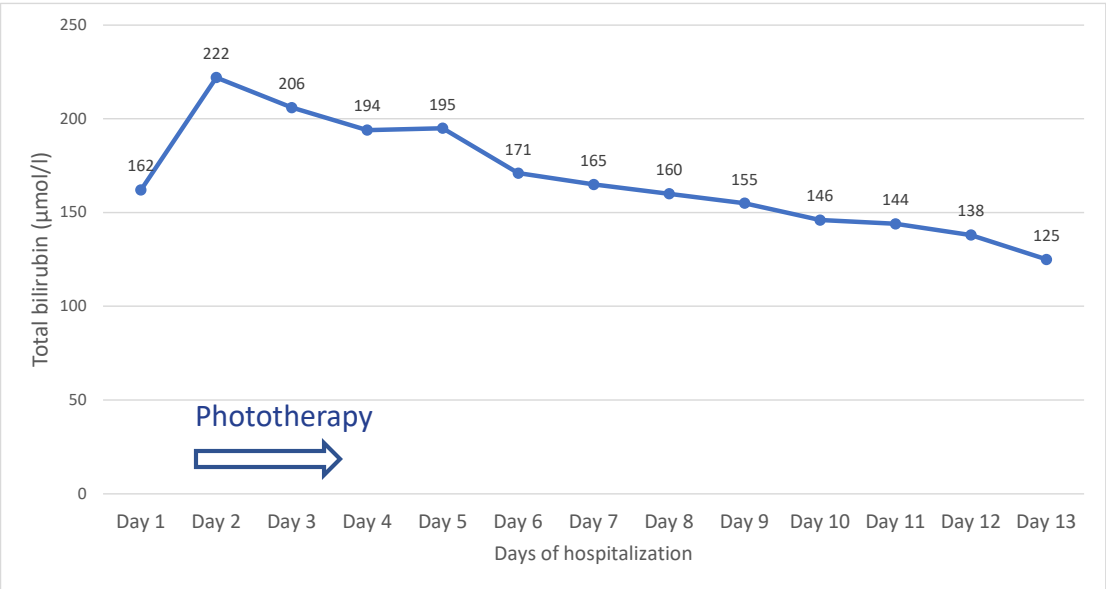
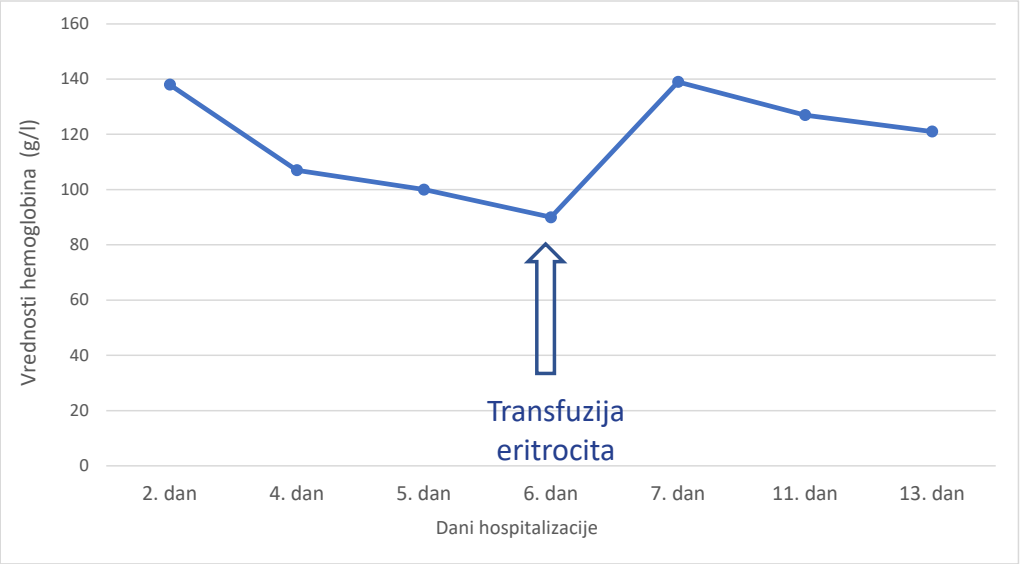


Figure 1. Total bilirubin values by days of hospitalization



Grafikon 2. Vrednosti hemoglobina po danima hospitalizacije

zacije (7). Zbog toga je ABO inkompatibilija postala najčešći uzrok izoimune hemolitičke bolesti novorođenčeta. Iako je hemolitička bolest zbog ABO inkompatibilije klinički blaža od one izazvane RhD inkompatibilijom, povremeno se može javiti teška hemoliza koja zahteva i eksangvinotransfuziju (8). U poslednje vreme ABO inkompatibilija posebno postaje značajna i aktuelna u prenatalnoj dijagnostici i lečenju ženske neplodnosti. Posle neobjašnjenih ili uzastopnih spontanih pobačaja sve više se pridaje značaj upravo imunološkim faktorima.

Literaturni podaci nam ukazuju na to da se ABO inkompatibilija češće javlja kod novorođenčadi koja pripadaju određenim etničkim grupama i imaju B krvnu grupu (9). *Adewuyi* i saradnici su otkrili da je serumska hemolitička aktivnost anti-A i anti-B antitela kod osoba crne rase veća od odgovarajuće aktivnosti nađene kod osoba bele rase. Njihova studija je pokazala da je hemolitička aktivnost anti-B antitela veća nego anti-A antitela kod obe rase (10). *McDonnell* je opisao dva slučaja fetalnog hidropsa kod osoba crne rase uzrokovana ABO-inkompatibilijom (11).

Nekoliko drugih studija je ispitivalo vezu između ABO inkompatibilije i reproduktivnog neuspeha. *Malekasgar* opisuje istraživanje sprovedeno u Britanskoj Kolumbiji u kome su trudnice sa O krvnom grupom i ABO inkompatibilijom imale značajno višu incidenciju spontanih pobačaja između 40. i 135. dana trudnoće od trudnica drugih krvnih grupa (12). *Stiller* i saradnici su u svom članku prikazali trudnoću komplikovanu anti-B izoimunizacijom koja je dovela do fetalnog ascitesa, anemije, hepatomegalije i polihidramniona (13).

S obzirom na to da hemolizna bolest novorođenčadi najčešće nastaje zbog ABO i Rh aloizoimunizacije, postoje jasni klinički vodiči za njihovo zbrinjavanje. Prema protokolima za lečenje, indikacija za fototerapiju kod preterminske novorođenčadi u 36. gestacijskoj nedelji sa hemoliznom bolešću i telesnom masom većom od 2000 g, su vrednosti bilirubina od 170-204 µmol/l u drugom danu života (14). Indikacije za eksangvinotransfuziju u hemoliznoj bolesti podrazumevaju tešku anemiju (Hb < 100 g/l) i porast bilirubina za više od 8,5 µmol/l/h. Prema kliničkim vodičima, manja gestacijska starost i manja telesna masa novorođenčeta sa hemoliznom bolešću podrazumevaju i niže vrednosti ukupnog bilirubina za započinjanje fototerapije i eksangvinotransfuzije.

U našem prikazu slučaja ABO hemolitička bolest novorođenčeta bila je srednje teške kliničke slike. Pošto je ukupni bilirubin u drugom danu života bio 222 µmol/l, novorođenčetu je, prema protokolu za gestacijsku starost i telesnu masu, primenjena fototerapija u trajanju od 36 sati koja je dovela do postepenog pada vrednosti ukupnog bilirubina. Pošto indikacije za eksangvinotrasfuziju podrazumevaju porast vrednosti ukupnog bilirubina za više od 8,5 µmol/l/h, što se kod našeg novorođenčeta nije događalo nego su vrednosti padale, fototerapija je bila dovoljna za lečenje i nije bilo potrebe za eksangvinotransfuzijom. Vrednosti hemoglobina usled hemolize eritrocita pale su ispod 100 g/l šestog dana života zbog čega je novorođenčetu ordinirana jedna transfuzija re-

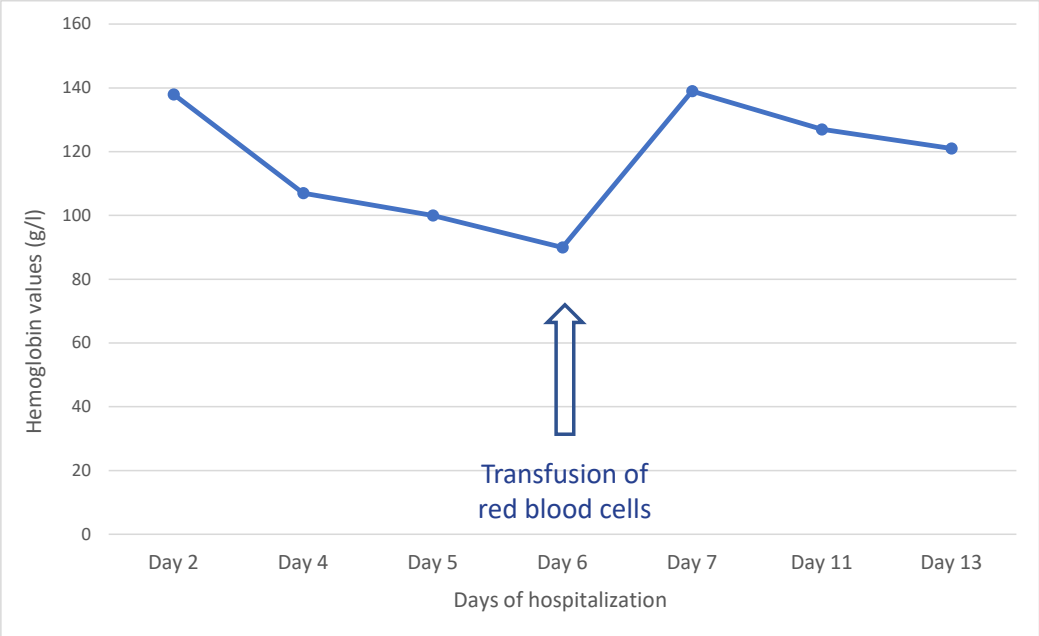


Figure 2. Hemoglobin values by days of hospitalization

and the newborn had healthy weight gain. The baby was discharged during the 13th day of life with body weight 2440 g and normal physical findings.

Discussion

Routine use of Rh IgG as prophylaxis resulted in the significant decrease in the incidence of RhD alloimmunization (7). Therefore, ABO incompatibility became the most frequent cause of isoimmune hemolytic disease of the newborn. Although hemolytic disease due to ABO incompatibility is clinically less severe than hemolytic disease caused by RhD incompatibility, severe hemolisis may occur occasionally, which demands exchange transfusion (8). ABO incompatibility has become particularly significant and actual lately in prenatal diagnostics and the treatment of female infertility. After unexplained or spontaneous recurrent miscarriages, immune factors are given more and more importance.

Literature data indicate that ABO incompatibility is more frequent in neonates that belong to certain ethnic groups and have B blood group (9). Adewuyi and associates found that serum hemolytic activity of anti-A antibodies and anti-B antibodies is greater in black persons than the corresponding activity found in white persons. Their study showed that hemolytic activity of anti-B antibodies is greater than of anti-A antibodies in both racial groups (10). McDonnell described two cases of hydrops fetalis caused by ABO incompatibility in persons belonging to black racial group (11).

Several other studies examined the connection between ABO incompatibility and reproductive failure. Malekasgar described a research that was conducted in British Columbia, in which pregnant women with O blood group and ABO incompatibility had significantly higher incidence of miscarriages between the 40th and 135th day of pregnancy in comparison to pregnant women with other blood groups (12). Stiller and associates reported in their article a pregnancy that was complicated by anti-B isoimmunization which resulted in fetal ascites, anemia, hepatomegaly and polyhydramnios (13).

Considering that hemolytic disease of newborns most frequently occurs due to ABO and Rh alloimmunization, there are clear clinical guidelines for their treatment. According to the treatment protocols, the indications for phototherapy in pre-term newborns with hemolytic disease and body weight higher than 2000 g at 36 weeks of gestation are values of bilirubin 170-204 µmol/l in the second day of life (14). Indications for exchange transfusion in hemolytic disease include severe anemia (Hb < 100 g/l) and the rate of rise of bilirubin that is more than 8.5 µmol/l/h. According to clinical guidelines, younger gestational age and smaller body weight of newborns with hemolytic disease include lower values of total bilirubin necessary to start phototherapy and exchange transfusion.

In our case study, ABO hemolytic disease of the newborn was moderately severe. Since total bilirubin in the second day of life was 222 µmol/l,

suspendovanih eritrocita O krvne grupe. Nasuprot literaturnim podacima koji govore da se ABO inkompatibilija češće javlja kod novorođenčadi koja pripadaju crnoj rasi i imaju B krvnu grupu, naše novorođenče pripada beloj rasi i imalo je A krvnu grupu. U krvi prikazanog novorođenčeta registrovana su anti-A antitela. Mladoj, sedamnaestogodišnjoj majci, je novorođenče dete iz treće trudnoće začete prirodnim putem u kratkom vremenskom intervalu, a druga trudnoća je završena spontanim pobačajem u 11. gestacijskoj nedelji. Na osnovu anamnestičkih podataka, kliničkog nalaza i rezultata učinjenih ispitivanja, prevremeni porođaj u našem slučaju mogao je nastati kao posledica ABO inkompatibilije.

Zaključak

Želeli smo da podsetimo da, iako retka i relativno lakše kliničke slike, sa već usvojenim protokolima za uspešno lečenje, ABO inkompatibilija može predstavljati veliki problem kako u prenatalnom, tako i u perinatalnom i neonatalnom periodu.Ginekolozi bi trebalo da obrate posebnu pažnju na trudnice sa O krvnom grupom, a koje imaju partnere A, B ili AB krvne grupe, da im prate titar anti-A i anti-B antitela i da na vreme obaveste i roditelje i pedijatre o mogućim posledicama po novorođenče. Posebnu pažnju treba obratiti na trudnice čija su deca iz prethodnih trudnoća imala hiperbilirubinemiju ili su im se trudnoće završavale spontanim pobačajem.

Konflikt interesa

Autori su izjavili da nema konflikta interesa.

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phototherapy lasting 36 hours was administered according to the protocol for gestational age and body weight and it resulted in the gradual decrease in values of total bilirubin. Since indications for exchange transfusion include the rise of values of total bilirubin for more than 8.5 µmol/l/h, which did not happen in case of our newborn, whose values of bilirubin decreased, phototherapy was sufficient for the treatment and there was no need for exchange transfusion. The values of hemoglobin due to hemolysis of erythrocytes decreased below 100 g/l, and therefore the transfusion of resuspended type O red blood cells was administered. Opposite to literature data, which indicate that ABO incompatibility is more frequent in newborns who belong to black racial group and have B blood group, our newborn belongs to white racial group and has A blood group. Anti-A antibodies were registered in the blood of the presented newborn. The newborn is the child of a young, seventeen-year old mother who got pregnant naturally at short interval and this was the third pregnancy, while the second pregnancy ended in spontaneous miscarriage at a gestational age of 11 weeks. According to the anamnestic data, clinical findings and results of examinations, pre-term delivery in our case could have been the result of ABO incompatibility.

Conclusion

Although ABO incompatibility is rare and has a mild clinical picture, with already adopted protocols for successful treatment, we wanted to remind that it can present a serious problem in the prenatal, perinatal and neonatal period. Gynecologists should pay special attention to pregnant women with O blood group who have partners with A, B or AB blood group, to observe the ani-A and anti-B antibodies titer and to inform parents and pediatricians about possible consequences for the newborn. Special attention should be paid to pregnant women whose children from previous pregnancies had hyperbilirubinemia or their pregnancies ended in spontaneous miscarriage.

Competing interests

The authors declare no competing interests.

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