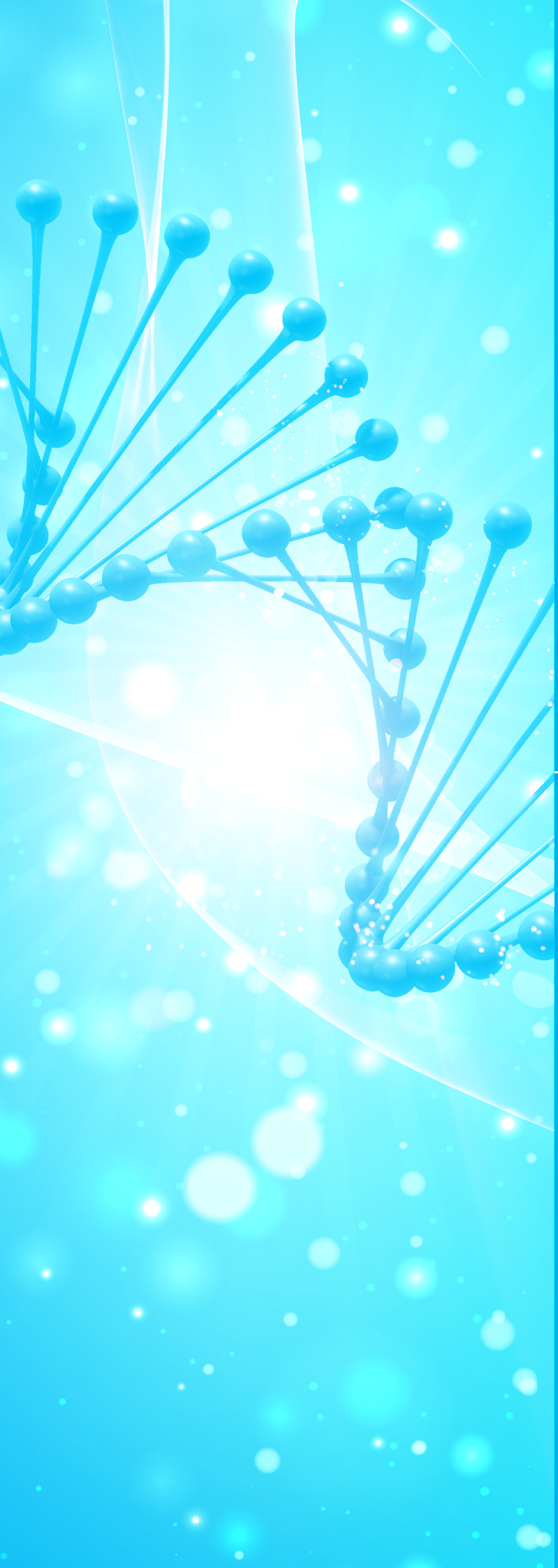


ISSN 0350-3208
eISSN 2683-4286

KOMORA ZDRAVSTVENIH USTANOVA SRBIJE - BEOGRAD

GODIŠTE 50 · SVESKA 3 · OKTOBAR 2021

ZDRAVSTVENA ZAŠTITA

HEALTH CARE

VOLUME 50 · ISSUE 3 · OCTOBER 2021

THE CHAMBER OF HEALTHCARE INSTITUTIONS OF SERBIA - BELGRADE

Stručni časopis **Zdravstvena zaštita**, koji Komora zdravstvenih ustanova Srbije izdaje od 1972. godine, objavljuje neobjavljene originalne stručne i naučne radove, pregledne članke, kratka saopštenja, uvodnike, pisma uredniku, meta-analize, prikaze bolesnika, aktuelne teme, prikaze stručnih knjiga i skupova, i drugo, iz oblasti medicine, farmacije, biohemije, stomatologije i menadžmenta u zdravstvu, čime doprinosi promociji i razvoju nauke, struke i naučno-istraživačkog rada.

Naučne i stručne radove u časopisu objavljuju vrhunski stručnjaci različitih profila sa naučnim i drugim zvanjima, akademici. Prenoseći u proteklom periodu odabrane tekstove do svojih čitalaca, stranice **Zdravstvene zaštite** istovremeno predstavljaju svedočanstvo, kako pionirskog rada Komore, tako i svojevrsne istorije zdravstva Srbije.

Od januara 2019. godine sprovodi se elektronsko uređivanje časopisa **Zdravstvena zaštita** (engl. *Health Care*), a od septembra 2019. dostupan je u režimu otvorenog pristupa (engl. *Open Access*). Otvoren pristup obezbeđuje besplatno čitanje, preuzimanje, čuvanje, štampanje i korišćenje objavljenih rukopisa u časopisu svakom korisniku koji ima pristup internetu. Svi članci objavljeni u časopisu **Zdravstvena zaštita** mogu se besplatno preuzeti sa sajta časopisa: http://scindeks.ceon.rs/journaldetails.aspx?issn=0350-3208. Časopis se objavljuje četiri puta godišnje.

Svi rukopisi pre objavljivanja u časopisu **Zdravstvena zaštita** prolaze internu (preliminarni pregled rukopisa od strane urednika i/ili uređivačkog odbora) i eksternu recenziju (dve nezavisne recenzije od strane stručnjaka u datoj oblasti pri čemu se poštuje anonimnost i autora recenzije i autora rukopisa). Konačna odluka o objavljivanju rukopisa donosi se na osnovu stručne, etičke i statističke recenzije. Pre objavljivanja rukopisa, vrši se kontrola plagijarizma, odnosno rukopis se upoređuje sa svim originalnim tekstovima u dostupnim bazama podataka.

Radovi mogu biti napisani na srpskom ili engleskom jeziku, sa rezimeima na srpskom i engleskom jeziku.

Radove objavljene u časopisu **Zdravstvena zaštita** indeksiraju: SCIndeks - *Serbian Citation Index*, COBISS.SR – ID 3033858 i doiSerbia.

Scientific journal **Zdravstvena zaštita** (The Journal of **Health Care**), which has been published by the Chamber of health institutions of Serbia since 1972, publishes unpublished original expert and scientific papers, review articles, short communications, introductions, letters to the editor, meta – analyses, case reports of the patients, actual topics, depictions of scientific books and conferences, among other things related to the field of medicine, stomatology, pharmacology, biochemistry and health management. In this way, the magazine contributes to the promotion and development of science, as well as expertise and scientific research work.

Preeminent experts of different profiles and with different scientific titles publish their scientific and research papers in the journal. By means of conveying the selected texts, the pages of **Health Care** represent the testimony, of both the pioneering work of the Chamber, and a kind of history of health care in Serbia alike.

The Journal of **Health Care** has been edited electronically since January 2019, and it has been in Open Access Mode since September 2019. Open access enables reading free of charge, downloading, saving, printing and using writings published in the journal to any user that has an internet access. All papers published in **Health Care** can be downloaded for free on the journal's website: http://scindeks.ceon.rs/journaldetails.aspx?issn=0350-3208. The journal is published four times a year.

Prior to publishing in **Health Care**, all writings go through an internal review (preliminary review of the manuscript by the editor and/or editorial board) as well as an external review (independent reviews by the experts form the given field, while respecting the anonymity of the author of the review and the author of the paper). Final decision on whether the paper is going to be published is reached on the basis of expertly, ethical, and statistical review. Control for plagiarism is performed before the paper is published, which implies comparing the manuscript to all original texts in the available databases.

Papers could be written in the Serbian or English language, with summaries in both Serbian and English.

Papers published in The Journal **Health Care** are indexed by: SCIndeks - Serbian Citation Index, COBISS. SR – ID 3033858 and doiSerbia.

ZDRAVSTVENA ZAŠTITA

ISSN 0350-3208
eISSN 2683-4286
COBISS.SR-ID 3033858
UDK 613/614

Zvanični časopis Komore zdravstvenih ustanova Srbije za medicinu, farmaciju, biohemiju, stomatologiju i menadžment u zdravstvu

GODINA 50 **BROJ 3** **OKTOBAR** **2021. GODINA**

VLASNIK I IZDAVAČ:
Komora zdravstvenih ustanova Srbija

ZA IZDAVAČA:
Snežana Rašić Đorđević

ADRESA UREDNIŠTVA:
11000 Beograd, Hajduk Veljkov venac 4-6
Tel/faks: +381 11 3622 523 ili +381 11 3622 524
E-mail: urednik@komorazus.org.rs
Žiro račun: 205-4707-32

Menadžer časopisa:
Đorđe Nikodinović

Tehnički urednik i lektor za srpski jezik:
Asist. dr Aleksandra Nikolić

Prevodilac i lektor za engleski jezik:
Prof. Milica Matić

Štampa:
Cakum Pakum, Beograd

Tiraž: 50 primeraka

Članci u celosti dostupni su na internet stranici: http://scindeks.ceon.rs/journaldetails.aspx?issn=0350-3208

HEALTH CARE

ISSN 0350-3208
eISSN 2683-4286
COBISS.SR-ID 3033858
UDK 613/614

Official journal of the Chamber of Healthcare Institutions of Serbia for medicine, pharmacy, biochemistry, stomatology and healthcare management

YEAR 50 **ISSUE NO. 3** **OCTOBER** **2021**

EDITORIAL BOARD ADDRESS:
11000 Beograd, Hajduk Veljkov venac 4-6
Tel/fax: +381 11 3622 523 ili +381 11 3622 524
E-mail: urednik@komorazus.org.rs
Account number: 205-4707-32

Journal manager:
Đorđe Nikodinović

Technical editor and Serbian language editor :
Asst. prof. Aleksandra Nikolic, MD

Translator and English language editor:
Milica Matic, PhD

Press:
Cakum Pakum, Beograd

Circulation: 50 copies

Articles are available in their integral versions on the webpage:
http://scindeks.ceon.rs/journaldetails.aspx?issn=0350-3208

SADRŽAJ

ORIGINALNI RADOVI

PRIKAZI SLUČAJA

PREGLEDNI RAD

CONTENTS

ORIGINAL ARTICLES

CASE REPORT

REVIEW ARTICLE

ORIGINALNI RAD

UDK BROJEVI:
616.995.132-036.22(497.16)"2010/2019"
DOI: https://doi.org/ 10.5937/zdravzast50-34151

OBOLIJEVANJE OD ENTEROBIJAZE U CRNOJ GORI U PERIODU 2010-2019. GODINE

Jelena Bojanić[1], Sandra Grujičić[2]

[1] Opšta bolnica Kotor, Crna Gora
[2] Institut za epidemiologiju, Medicinski fakultet Univerziteta u Beogradu, Republika Srbija

SAŽETAK

Uvod/Cilj: Procjenjuje se da više od milijardu ljudi u svijetu godišnje oboli od enterobijaze. Enterobijaza predstavlja najčešću helmintozu u razvijenim zemljama sveta kao što su severo-zapadna Evropa i Sjeverna Amerika. Cilj ove deskriptivne studije je da se analizira kretanje obolevanja od enterobijaze u Republici Crnoj Gori za period od 2010. do 2019. godine.
Metode: Podaci o obolijevanju od enterobijaze su preuzeti iz Godišnjeg izvještaja o zaraznim bolestima na teritoriji Crne Gore, Institita za javno zdravlje Crne Gore. Podaci o broju stanovnika Crne Gore za 2011. godinu su iz popisa stanovništva, a za ostale godine korišćen je procenjen broj stanovnika iz publikacija Republičkog zavoda za statistiku. U analizi podataka primenjene su sirove, uzrasno-specifične i standardizovane stope incidencije.
Rezultati: U periodu od 2010. do 2019. godine u Crnoj Gori je prijavljeno 1308 osoba oboljelih od enterobijaze. Odnos oboljelih muškaraca i žena iznosio je 1:1,3. Smrtni ishodi od ove bolesti nisu registrovani. Najviše sirove stope incidencije za oba pola su u uzrasnoj grupi 0-4 godine i iznose 172,9/100.000 za muškarce i 175/100.000 za žene. Prosječna godišnja sirova stopa incidencije enterobijaze za ovaj desetogodišnji period je iznosila 21,1/100.000 stanovnika (21,1/100.000 za žene i 21,0/100.000 za muškarce), a standardizovana (prema populaciji Evrope) 29,3/100.000 (30,7/100.000 za žene i 27,9/100.000 za muškarce). Trend sirovih stopa incidencije pokazuje blagu tendenciju porasta, ali bez statističke značajnosti.
Zaključak: Higijena ruku je najbolja mjera prevencije enterobijaze. U domaćinstvima gde je zaraženo više od jednog člana ili se ponavljaju simptomatske infekcije, preporučuje se da se svi članovi domaćinstva liječe istovremeno, bez obzira da li imaju simptome ili ne. Neohodno je ponoviti liječenje kroz dvije nedjelje jer antihelmintici djeluju samo na odrasle parazite, a ne i na jaja/larve iz kojih se razvijaju nove jedinke.

Ključne reči: enterobijaza, incidencija, trend obolevanja

Uvod

Enterobijaza je parazitarno oboljenje intestinalnog trakta čovjeka, prvenstveno djece. Uzrokuje ga nematoda *Enterobius vermicularis*, u narodu poznata kao „mala dječja glista“ ili pundravac (1,2). *Enterobius vermicularis* je po izgledu mala, tanka, obla glista, bijele boje (3). Pripada klasi nematoda (valjkasti crvi). Odvojenih je polova i ženke su veće od mužjaka (3).

Enterobijaza je kosmopolitska infekcija. Za razliku od drugih crijevnih nematoda, predstavlja najčešću helmintozu u razvijenim zemljama (severo-zapadna Evropa i Sjeverna Amerika) (4). Češće se sreće u zemljama sa kontinentalnom i hladnom klimom, za razliku od ostalih parazita, koje češće srećemo u tropskim krajevima (5). Procjenjuje se da više od milijardu ljudi u svijetu godišnje oboli od enterobijaze (1,2).

U tankom crijevu se iz jaja oslobađaju larve (3,6). Larve sazrijevaju u duodenumu, dok odrastao parazit sazrijeva u cekumu i gornjim dijelovima kolona, gdje se dešava i oplodnja (3,7). Nakon parenja, mužjak ugine (6). Oplođene ženke noću migriraju iz debelog crijeva do perianalnih i perinealnih kožnih nabora, i tu na granici kože i sluzokože, polože 15-20.000 jaja, nakon čega uginu. Vremenski interval od gutanja infektivnih jaja do ovipozicije od strane odraslih ženki je oko mjesec dana (2-6 nedelja) (6,8). Životni vijek odraslih paraz-

ORIGINAL ARTICLE

UDK NUMBERS:
616.995.132-036.22(497.16)"2010/2019"
DOI: https://doi.org/ 10.5937/zdravzast50-34151

INCIDENCE OF ENTEROBIASIS IN MONTENEGRO IN THE PERIOD 2010-2019

Jelena Bojanic[1], Sandra Grujicic[2]

[1] General Hospital Kotor, Montenegro
[2] Institute of Epidemiology, Faculty of Medicine Belgrade University, Republic of Serbia

SUMMARY

Introduction / Aim: It is estimated that more than one billion people worldwide suffer from enterobiasis each year. Enterobiasis is the most common helminthiasis in developed countries such as northwestern Europe and North America. The aim of this descriptive study is to analyze the trends in enterobiasis in the Republic of Montenegro for the period from 2010 to 2019.
Methods: Data on enterobiasis were taken from the Annual Report on Infectious Diseases in Montenegro, the Institute of Public Health of Montenegro. Data on the number of inhabitants of Montenegro for 2011 were taken from the census, and for other years the estimated number of inhabitants from the publications of the Republic Statistical Office was used. Crude, age-specific and standardized incidence rates were applied in the data analysis.
Results: In the period from 2010 to 2019, 1308 people with enterobiasis were reported in Montenegro. The ratio of affected men and women was 1: 1.3. No deaths from this disease have been reported. The highest crude incidence rates for both sexes are in the 0-4 age group and are 172.9/100,000 for men and 175/100,000 for women. The average annual crude incidence rate of enterobiasis for this ten-year period was 21.1/100,000 inhabitants (21.1/100,000 for women and 21.0/100,000 for men), and the standardized (according to the population of Europe) 29.3/100,000 (30.7/100,000 for women and 27.9 / 100,000 for men). The trend of crude incidence rates shows a slight upward trend, but without statistical significance.
Conclusion: Hand hygiene is the best measure to prevent enterobiasis. In households where more than one member is infected or symptomatic infections recur, it is recommended that all household members should be treated at the same time, regardless of whether they have symptoms or not. It is necessary to repeat the treatment in two weeks, because antihelmintics act only on adult parasites, and not on eggs / larvae from which new individuals develop.

Key words: enterobiasis, incidence, trend of incidence

Introduction

Enterobiasis is a parasitic disease of human intestinal tract, which primarily occurs in children. It is caused by a nematode *Enterobius vermicularis* that is known as a pinworm or "seatworm" (1,2). *Enterobius vermicularis* is a small, thin, white roundworm (3). It belongs to the class of nematodes (roundworms). It can be male or female, while the female nematode is bigger than the male (3).

Enterobiasis is a cosmopolitan infection. In contrast to other intestinal nematodes, it represents the most common helminthiasis in developed countries (Northwestern Europe and North America) (4). It is encountered more frequently in countries with continental and cold climate, in contrast to other parasites, which are met more frequently in tropical climates (5). It has been estimated that more than a billion people worldwide is affected by enterobiasis annually (1,2).

Larvae are released from eggs in the small intestine (3,6). Larvae mature in the duodenum, while the adult parasite matures in the cecum and in upper parts of the colon, where fertilization happens (3,7). After copulation, the male pinworm dies (6). Gravid females migrate at night from the anus to perianal and perineal folds, and there, on the border of skin and mucosa they lay 15-20.000 eggs, and after that they die. The time interval

ita je oko dva mjeseca (ženke prežive 37-93, a mužjaci oko 50 dana) (6,9). Jaja postaju zarazna nekoliko sati nakon polaganja, a izvan domaćina preživljavaju oko dvije nedjelje. Gravidne ženke obično aktivno migriraju iz rektuma i mogu da pređu u bliske otvore i tako izazovu komplikacije (10).

Kod oko 40% osoba infekcija je asimptomatska (11). Od simptoma su najčešći perianalni ili perinealni pruritus, koji se pogotovo intenzivira noću (9). Smatra se da su migratorni pokreti ženke i ljepljiva supstanca u koju polažu svoja jaja, odgovorni za pojavu svraba (3,9). Istraživanja pokazuju da se svrab češće javlja kod onih koji već pate od nekog kožnog oboljenja (atopijskog dermatitisa, psorijaze, kontaktnog dermatitisa, seboroičnog dermatitisa, neurodermatitisa) (12). Usled češanja, mogu nastati perianalni eritem i sekundarna bakterijska infekcija i folikulitis (9). Ostali simptomi uključuju nesanicu (zbog isprekidanog sna usled svraba i češanja), nemir i umor. Djeca mogu postati anoreksična, izgubiti na težini (pogotovo kod prisustva velikog broja parazita u crijevima) ili patiti od oslabljene koncentracije, razdražljivosti i emocionalne nestabilnosti (3,9,10,13). Može se javiti i enureza, bruksizam, pa čak i konvulzije.

Ponekad, ženke mogu ektopično migrirati i ući u ženski genito-urinarni trakt, a kod djevojčica mogu i da polažu jaja na sluznicu vagine i usminama i tako dovedu do zapaljenskih promjena tih regija (urinarnih infekcija, kolpitisa, vulvovaginitisa) (14,15,16). U uterusu i adneksima, paraziti mogu da se inakpsuliraju i daju simptome salpingitisa, a veoma rijetko tim putem mogu da dospiju i u peritonealnu šupljinu i dovedu do stvaranja eozinofilnih granuloma i hroničnog pelvičnog peritonitisa. Mogući su i granulomi jetre (17). Za stvaranje granuloma su odgovorne mrtve odrasle jedinke i jaja deponovana na ektopičnim mjestima. Rijetko, enterobijus može da izazove apendicitis tako što blokira lumen apendiksa ili dovede do upalnog procesa oko njega (18,19). Moguće su i intestinalne opstrukcije, perforacija crijeva, enterokolitis koji oponaša Kronovu bolest i pojava eozinofilnog ileokolitisa (20,21).

Infekcija se prenosi najčešće prljavim rukama, ali može da se prenese putem kontaminiranih predmeta, hranom i rjeđe vodom (22). Jaja se, iako rijetko, mogu i udahnuti (udisanjem prašine u kojoj se ona nalaze), a zatim progutati (6). Ovo se dešava kod npr. rastresanja posteljine, odjeće ili u jako kontaminiranim domaćinstvima i institucijama (9,11).

Osjetljivost na ovu infekciju je opšta (10). Najčešće obolijevaju mlađa školska i predškolska djeca, uzrasta 5-10 godina, osobe koje borave u institucijma (vrtići, domovi, kampovi i sl.), kolektivima, porodice sa školskom djecom i oni koji brinu o zaraženoj djeci (3,9,23). U ovim grupama prevalencija može biti 50% (6). Česta je pojava infekcije kod većeg broja članova istog domaćinstva (10,24). Prevalencija je najniža kod odraslih, izuzev majki zaražene djece (10). Veća prevalencija obolijevanja je zabilježena kod muškaraca koji imaju seksualne odnose sa muškarcima. Ne postoje podaci koji sugerišu da je ovo „oportunistička“ infekcija kod HIV-om inficiranih osoba (9). Pošto se imunitet ne razvija, moguće je da dođe do infekcije više puta u toku života (10).

Cilj ove deskriptivne studije je da se analizira kretanje obolevanja od enterobijaze u Republici Crnoj Gori za period od 2010. do 2019. godine.

Metode

U okviru ove deskriptivne studije analizirano je kretanje učestalosti obolevanja od enterobijaze u Crnoj Gori za period 2010-2019. godine. Istraživanjem su obuhvaćeni svi prijavljeni slučajevi obolijevanja od enterobijaze na području Crne Gore u vremenskom intervalu od 2010. do 2019. godine. Podaci o obolelima od enterobijaze preuzeti su iz Godišnjih izvještaja o zaraznim bolestima na teritoriji Crne Gore, Institita za javno zdravlje Crne Gore, po kalendarskim godinama od 2010. do 2019. godine.

U radu su prikazane opšte, specifične i standardizovane stope incidencije, trend stopa incidencije, procentualni udio enterobijaze u ukupnoj strukturi obolijevanja od parazitarnih bolesti, kao i topografska raspodjela oboljelih po regionima. Specifične stope incidencije izračunate su po polu i uzrastu, a standardizovane stope su računate metodom direktne standardizacije, u odnosu na evropsku populaciju (25).

Brojilac za izračunavanje stopa incidencije enterobijaze činile su obolele osobe, a imenilac broj stanovnika Crne Gore. Za 2011. godinu broj stanovnika je dobijen iz popisa stanovništva, a za ostale godine korišćen je procenjen broj stanovnika Crne Gore, preuzet iz publikacija Republičkog zavoda za statistiku (Monstat) (26).

Za izračunavanje specifičnih stopa incidencije po uzrastu, formirano je devet uzrasnih grupa. Za prve četiri uzrasne grupe interval je iznosio pet

from the ingestion of infective eggs to oviposition by the adult females is about one month (2-6 weeks) (6,8). The adult life span is about two months (females survive for 37-93, while males survive for about 50 days) (6,9). The eggs become infective a few hours after being laid, and they survive for about two weeks outside their host. Gravid females usually migrate actively from the rectum, and they can pass into other close areas and cause complications (10).

In about 40% of persons, the infection is asymptomatic (11). The most common symptoms are perianal and perineal pruritus, especially at night (9). It is believed that the migratory movements of females and the adhesive substance, where they lay their eggs, are responsible for the appearance of pruritus (3,9). Studies have shown that pruritus is more common in those people who already suffer from some skin diseases (atopic dermatitis, psoriasis, contact dermatitis, seborrheic dermatitis, neurodermatitis) (12). Perianal erythema, secondary bacterial infection, and folliculitis can be caused by scratching (9). The other symptoms include insomnia (due to sleep disturbance caused by itching and scratching), restlessness, and fatigue. Children may become anorexic, lose weight (especially due to a large number of parasites in their intestines) or suffer from poor concentration, irritability and emotional instability (3,9,10,13). Enuresis, bruxism and even convulsions may appear.

Females may sometimes migrate ectopically into genitourinary tract, while in girls they can sometimes lay their eggs on vaginal mucosa and vulva, and cause inflammatory changes of that area (urinary infections, colpitis, vulvovaginitis) (14,15,16). Parasites can encapsulate in the uterus and the adnexa and give the symptoms of salpingitis, and very rarely they can reach the peritoneal cavity and lead to the appearance of eosinophilic granulomas and chronic pelvic peritonitis. Hepatic granulomas are also possible (17). Dead adult parasites and eggs deposited on the ectopic places are responsible for the appearance of granulomas. Enterobius may rarely cause appendicitis by blocking the appendiceal lumen or lead to the inflammatory process around it (18,19). Intestinal obstructions are also possible, perforation of intestines, enterocolitis, Crohn's disease and the appearance of eosinophilic ileocolitis (20,21).

The infection is usually transmitted via dirty hands, but also via contaminated things, food and water (22). Eggs can be, but very rarely, inhaled (by inhaling the dust in which they exist), and then ingested (6). This happens after shaking bedding and clothes or in very contaminated households and institutions (9,11).

Susceptibility to this infection is general (10). Younger pre-school and school children aged 5-10 are most commonly affected, as well as persons who stay at institutions (kindergartens, dormitories, camps, etc.), in collective settings, in families with school children and in those who take care about the infected persons (3,9,23). In these groups, prevalence can be 50% (6). It often happens that more members of the same household get this infection (10,24). The prevalence is the lowest in adults, except in case of mothers of infected children (10). Higher prevalence is noted in men who have sexual relationships with men. There are no data that suggest that this is an "opportunist infection" in HIV positive persons (9). Since immunity does not develop, this infection can recur several times during lifetime (10).

The aim of this descriptive study was to analyze the trends in enterobiasis in the Republic of Montenegro during the period 2010 to 2019.

Methods

Within this descriptive study, the trends in the incidence of enterobiasis in Montenegro from 2010 to 2019 were analyzed. The study included all reported cases of enterobiasis in the territory of Montenegro during the time period 2010 to 2019. Data on enterobiasis were taken from the Annual Report on Infectious Diseases in Montenegro, the Institute of Public Health of Montenegro per each year from 2010 to 2019.

General, specific and standardized incidence rates, the trends in incidence rates, the percentage share of enterobiasis in the whole structure of incidence of parasitic diseases, as well as the topographic share of affected persons according to different regions were presented in this study. Specific incidence rates were calculated according to sex and age, while standardized rates were calculated with the help of direct standardization method, in relation to the European population (25).

The numerator for the calculation of incidence rates of enterobiasis included affected persons,

godina (0-4, 5-9, 10-14, 15-19 godina), za sledeće četiri 10 godina (20-29, 30-39, 40-49, 50-59 godina), a poslednja uzrasna grupa obuhvatila je osobe starije od 60 godina.

Pri analizi kretanja stopa incidencije, u posmatranom vremenskom periodu, korišćena je jednačina linearnog trenda.

Rezultati

U periodu od 2010. do 2019. godine u Crnoj Gori je prijavljeno 1308 osoba oboljelih od enterobijaze. Najveći broj obolelih (197) je zabežen 2012. godine, a najmanji 2010. godine (Tabela 1). Prosječan godišnji broj oboljelih za dati desetogodišnji period iznosi 131. Najveća stopa incidencije enterobijaze (31,8/100.000 stanovnika), zabilježena je 2012. godine, a najmanja 2011. godine (5,2/100.000) (Grafikon 2). Prosječna godišnja stopa incidencije za ovaj period je iznosila 21,1/100.000 stanovnika. Trend opštih stopa incidencije pokazuje blagu tendenciju porasta, ali bez statističke značajnosti (y = 1,7048x+11,673; p = 0,3015). Najveće učešće enterobijaze u obolijevanju od svih parazitarnih bolesti, koje se obavezno prijavljuju u Crnoj Gori, bilježi se 2018. godine, gdje čini više od četvrtinu obolijevanja od svih parazitarnih bolesti, odnosno 25,4%. Najmanje učešće enterobijaze registruje se 2013. godine, gdje čini 11,1% u strukturi obolijevanja od parazitarnih bolesti. Inače, prosječno godišnje učešće enterobijaze u obolijevanju od parazitarnih zaraznih bolesti u Crnoj Gori u periodu 2010-2019. godine iznosi 18,5%.

Najveća stopa incidencije za oba pola registrovana je 2012. godine i iznosila je 33,3/100.000 muškaraca i 30,3/100.000 žena. Najmanje stope incidencije su zabilježene 2011. godine (za muškarce 4,2/100.000, a za žene 6,1/100.000). Najveća razlika u obolijevanju među polovima je bila u 2015. godini sa dominacijom muškog pola (32,2/100.000 za muškarce i 24,5/100.000 za žene). Prosječna godišnja stopa incidencije u desetogodišnjem periodu je bila 21,1/100.000 za žene i 21/100.000 za muškarace. Trendovi stopa incidencije i kod žena i kod muškaraca pokazuju blagu tendenciju porasta, koji nisu statistički značajni (muškarci: y = 1,5721x+12.373; p = 0,2255; žene: y = 1,8267x+11,013, p = 0,3739).

Najveća prosječna uzrasno-specifična stopa incidencije od enterobijaze je zabilježena u uzrasnoj grupi 0-4 godine (173,9/100.000), a najmanja u uzrasnoj grupi 60 i više godina (1/100.000) (Grafikon 2).

Tabela 1. Ukupan broj oboljelih i opšte stope incidencije obolijevanja od parazitarnih bolesti i enterobijaze na 100.000 stanovnika i procentualni udio enterobijaze u parazitarnim bolestima u Crnoj Gori po kalendarskim godinama 2010-2019. godine

	Parazitarne bolesti		Enterobijaza		Udio obolelih od enterobijaze među obolelima od parazitarnih bolesti
Godina	**Br. oboljelih**	**Incidencija/ 100.000**	**Br. oboljelih**	**Incidencija/ 100.000**	**%**
2010	270	43,6	46	7,4	17,0%
2011	222	35,8	32	5,2	14,4%
2012	942	151,8	197	31,8	20,9%
2013	666	107,2	74	11,9	11,1%
2014	825	132,7	171	27,5	20,7%
2015	1093	175,7	176	28,3	16,1%
2016	1073	172,4	158	25,4	14,7%
2017	769	123,6	161	25,9	20,9%
2018	629	101,1	160	25,7	25,4%
2019	574	92,3	133	21,4	23,3%
Prosjek	706,3	113,6	130,8	21,1	18,5%

while the denominator was the population of Montenegro. Data on the number of inhabitants were taken from the census for 2011, while for other years the estimated number of inhabitants of Montenegro was taken from the publications of the Republic Statistical Office (Monstat) (26).

Nine age groups were formed in order to calculate the specific incidence rates. The interval amounted to five years for the first four groups (0-4, 5-9, 10-14, 15-19), while for the next for groups it was 10 years (20-29, 30-39, 40-49, 50-59 years), and the last group included people older than 60 years.

The equation of linear trend was used for the analysis of trends in incidence rates during the observed time period.

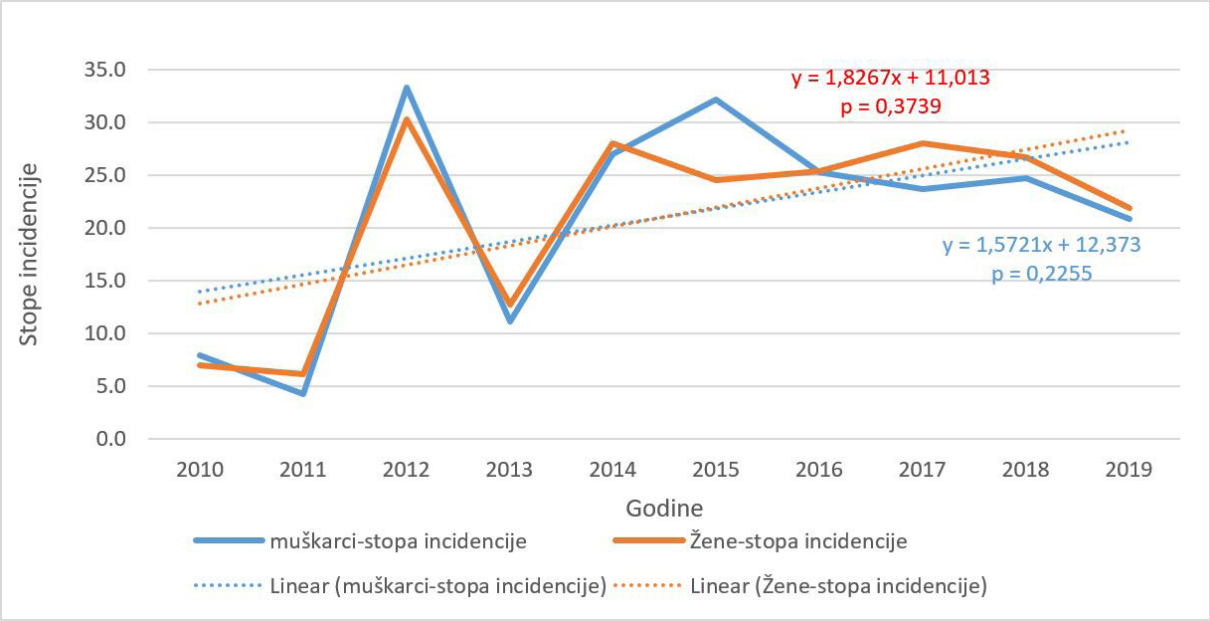
Results

In the period 2010-2019, 1308 people with eneterobiasis were reported in Montenegro. The largest number of people with enterobiasis (197) was noted in 2012, while the smallest number in 2010 (Table 1). The average annual number of affected people for the ten-year period amounted to 131. The highest incidence rate (31.8/100.000) was noted in 2012, while the lowest incidence rate was noted in 2011 (5.2/100,000) (Figure 2). The average annual incidence rate for this period amounted to 21.1/100,000 inhabitants. The trend in general incidence rates showed a slight tendency of rise, but with no statistical significance ($y = 1.7048x+11.673$; $p = 0.3015$). The largest share of enterobiasis in the incidence of all parasitic diseases, which are necessarily reported in Montenegro, was noted in 2018, when that share was 25.4%, that is, one fourth of all parasitic diseases. The smallest share of enterobiasis was registered in 2013, with the share of 11.1% in the structure of parasitic diseases incidence. However, the average annual share of enterobiasis in the incidence of parasitic diseases in Montenegro amounted to 18.5% from 2010 to 2019.

The highest incidence rate for both males and females was registered in 2012 and it amounted to 33.3/100,000 men and 30.3/100,000 women. The lowest incidence rates were registered in 2011 (for men 4.2/100.000, and for women 6.1/100,000). The biggest difference in incidence among men and women was in 2015, when men were dominant (32.2/100,000 for men and 24.5/100,000 for women). The average annual incidence rate in the ten-year period was 21.1/100,000 for women and 21/100,000 for men. Incidence rates showed a slight upward trend in both men and women,

Table 1. Total number of cases and incidence rates of parasitic diseases and enterobiases per 100,000 inhabitants and the percentage of enterobiases in all parasitic diseases in Montenegro by calendar years from 2010 till 2019

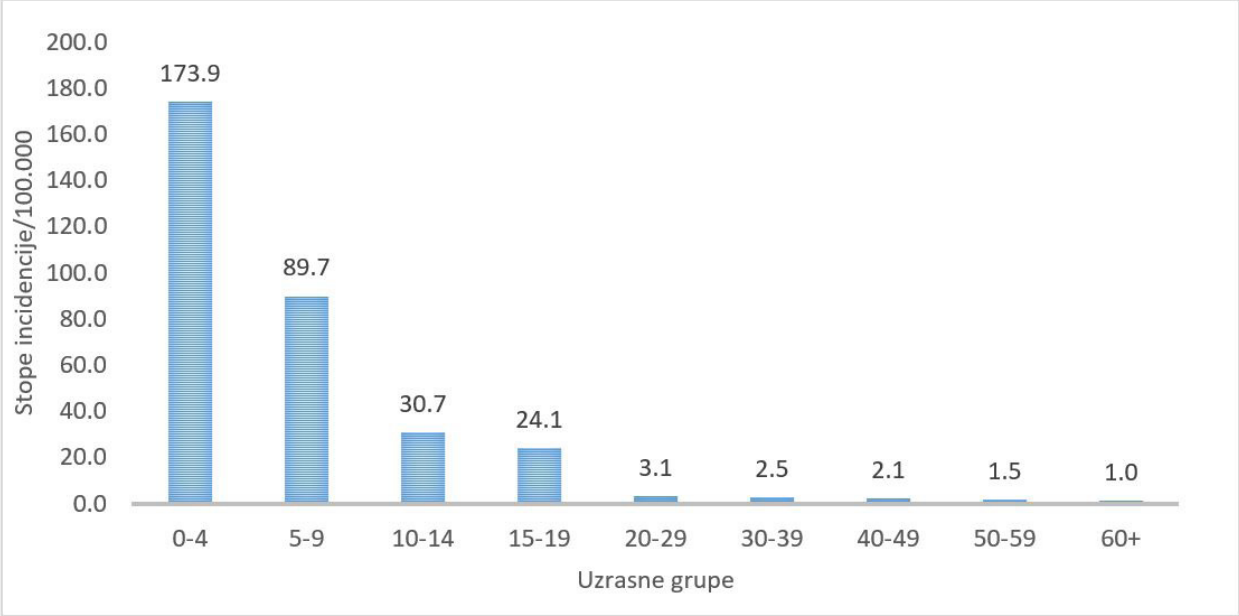
	Parasitic diseases		Enterobiases		The percentage of enterobiases in all parasitic diseases
Years	**No of cases**	**Incidence rate per 100,000**	**No of cases**	**Incidence rate per 100,000**	**%**
2010	270	43.6	46	7.4	17.0%
2011	222	35.8	32	5.2	14.4%
2012	942	151.8	197	31.8	20.9%
2013	666	107.2	74	11.9	11.1%
2014	825	132.7	171	27.5	20.7%
2015	1093	175.7	176	28.3	16.1%
2016	1073	172.4	158	25.4	14.7%
2017	769	123.6	161	25.9	20.9%
2018	629	101.1	160	25.7	25.4%
2019	574	92.3	133	21.4	23.3%
Average	706.3	113.6	130.8	21.1	18.5%



Grafikon 1. Specifične stope incidencije (na 100.000 stanovnika) od enterobijaze i trend stopa incidencija po polu, Crna Gora 2010-2019. godine

Najviša prosječna stopa incidencije od enterobijaze kod muškaraca je bila 172,9/100.000 u uzrastu 0-4 godine, dok je kod osoba ženskog pola 175/100.000 u istom uzrastu (Grafikon 3). Najmanja stopa incidencije od enterobijaze je bila kod muškaraca u uzrastu 30-39 godina (1,1/100.000), a kod žena u uzrastu 60 i više godina (0,9/100.000).

Prosečna standardizovana stopa incidencije enterobijaze prema evropskoj populaciji iznosi 18,1/100.000 stanovnika, a prema svjetskoj populaciji 29,3/100.000 stanovnika. Kod muškaraca najviša standardizovna stopa incidencije (dobijena korišćenjem evropske standardne populacije) registrovana je 2015. godine i iznosila je 27,2/100.000, a kod žena 2012. godine i iznosila je 26,8/100.000 (Grafikon 4). Najniža standardizovana stopa incidencije je bila 2011. godine (3,3/100.000 za muškarce i 5,3/100.000 za žene). Trend stopa incidencije kod oba pola pokazuje tendenciju porasta, ali bez statističke značajnosti (za muškarce y = 1,3939x-2790,9; p = 0,2604; za žene y = 1,7364x-3478,8; p =0,4108). Prosječna stopa incidencije kod muškaraca je bila 17,2/100.000, a kod žena 19,1/100.000.



Grafikon 2. Prosječne uzrasno-specifične stope incidencije (na 100.000) od enterobijaze, Crna Gora, 2010-2019. godine

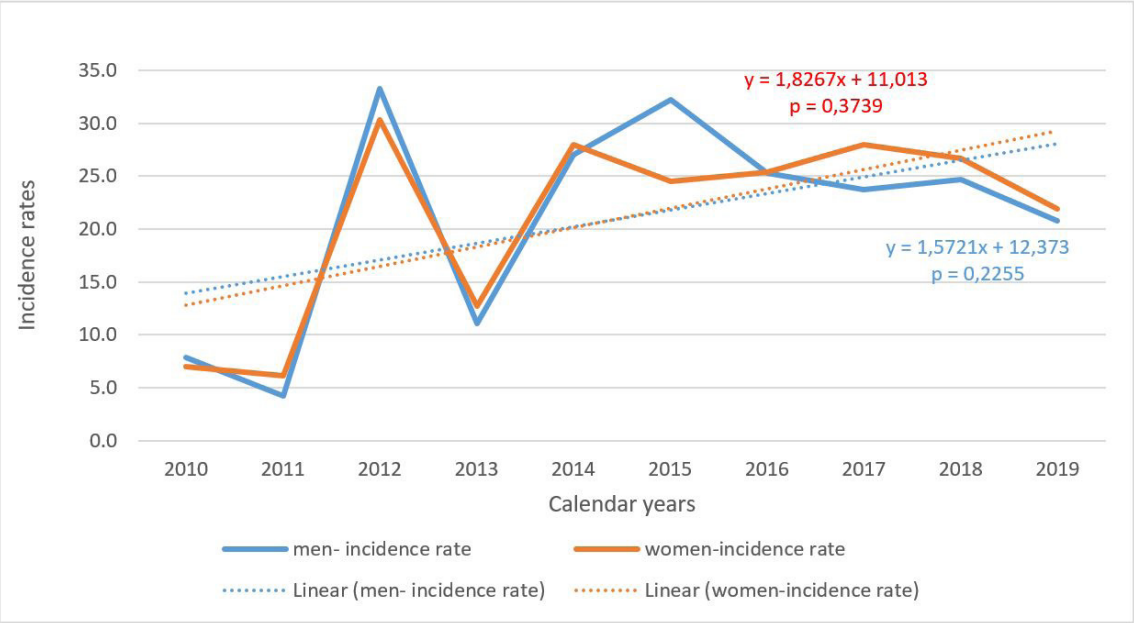


Figure 1. Sex-specific incidence rates (per 100,000) and trend in incidence of enterobiasis by gender, Montenegro 2010-201

but they were not statistically significant (men: y = 1.572x+12.373; p = 0.2255; women: y = 1.8267x+11.013, p = 0.3739).

The highest average age-specific incidence rate of enterobiasis was registered in the age group 0-4 years (173.9/100,000), while the lowest was in the age group 60 years and older (1/100,000) (Figure 2).

The highest average incidence rate of enterobiasis in men was 172.9/100,000 in the age group 0-4 years, while in women it was 175/100,000 in the same age group (Figure 3). The lowest incidence rate was in men aged 30-39 (1.1/100,000), while in women it was in the age group 60 years and older (0.9/100,000).

The average standardized incidence rate of enterobiasis in the European population amounts to 18.1/100,000, while in the world population this rate amounts to 29.3/100,000. In men, the highest standardized incidence rate (obtained using the European standard population) was registered in 2015 and it amounted to 27.2/100,000, while in women, the highest standardized incidence rate was registered in 2012 and it amounted to

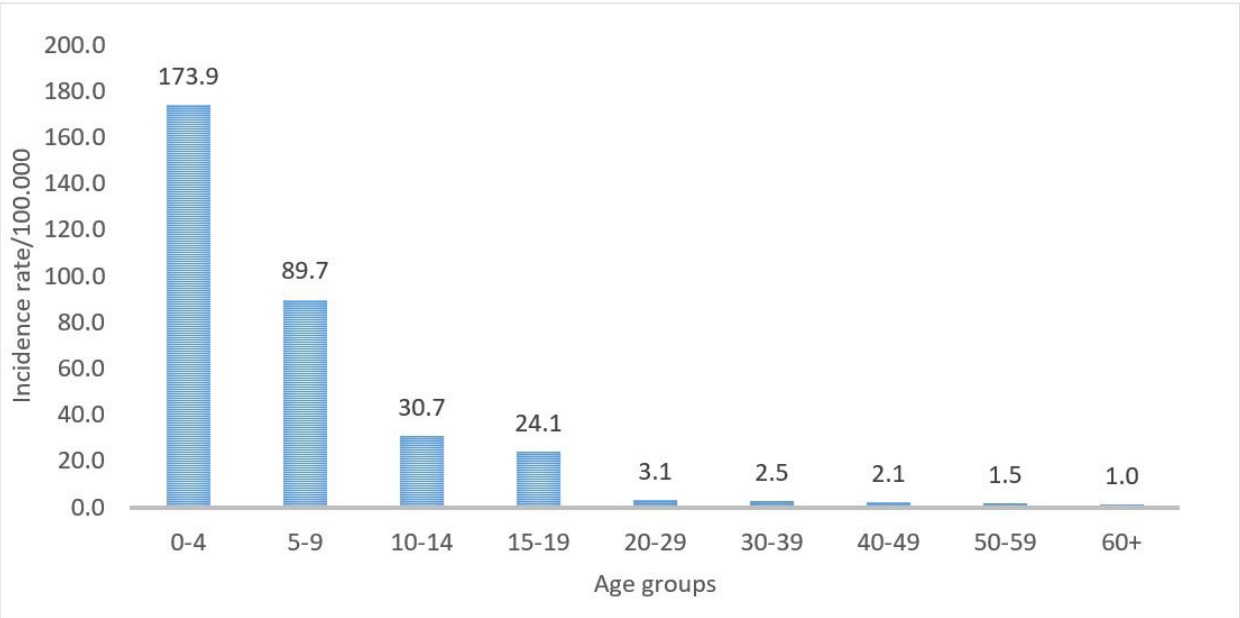
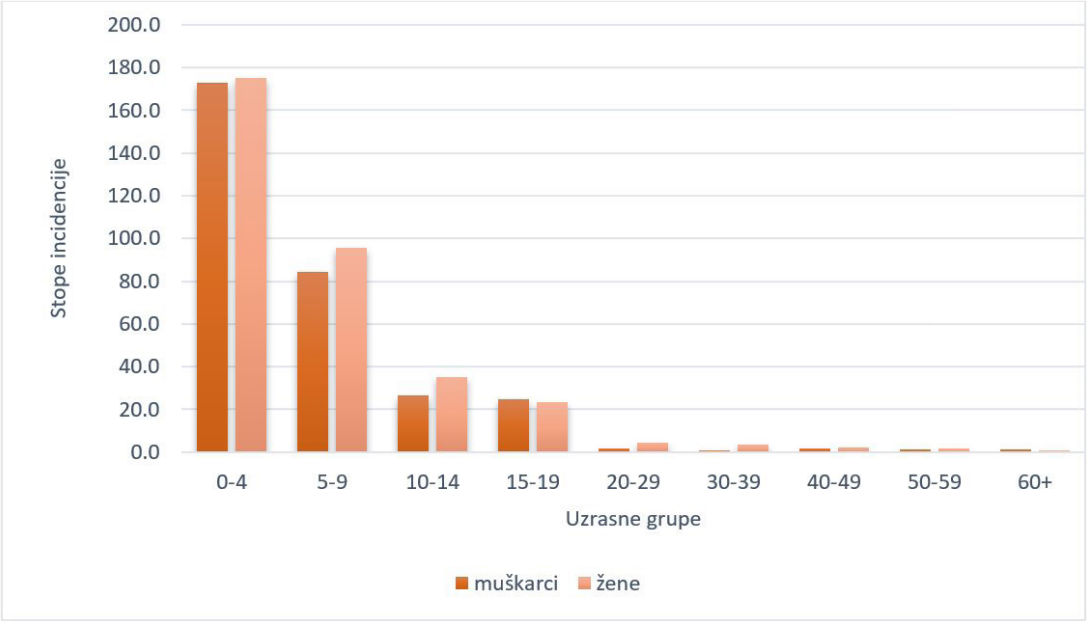


Figure 2. Average age-specific incidence rates of enterobiasis (per 100,000), Montenegro 2010-2019

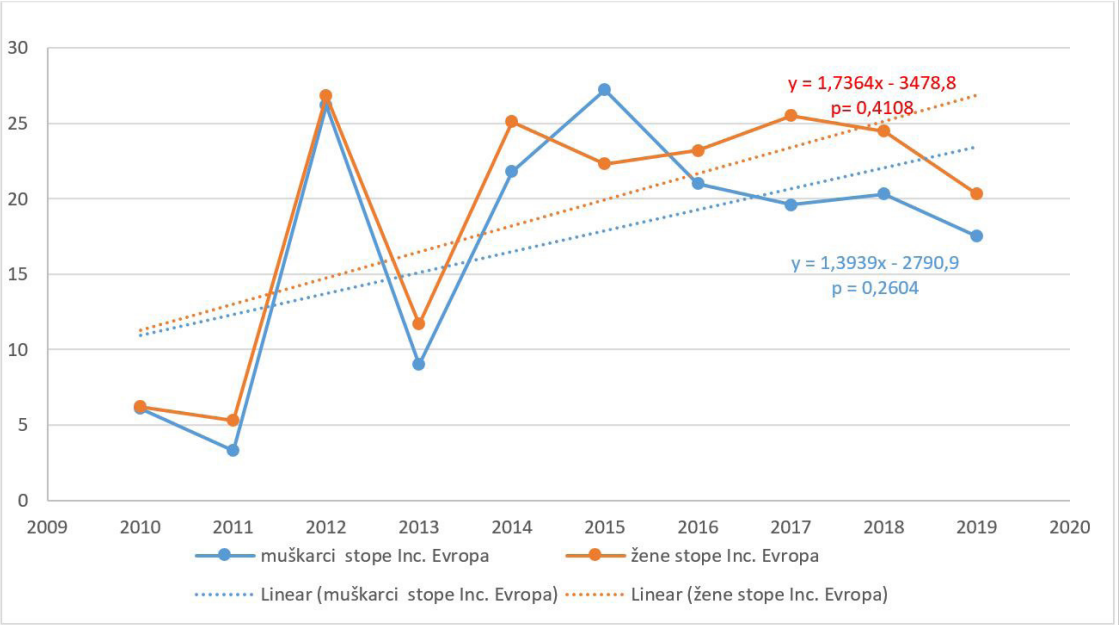


Grafikon 3. Prosječne uzrasno-specifične stope incidencije od enterobijaze u odnosu na pol, Crna Gora, 2010-2019. godine

U navedenom periodu više od 2/3 (tačnije 69%) registrovanih slučajeva, prijavljeno je u Primorskom regionu, 23% u Središnjem, a najmanji broj slučajeva u Sjevernom regionu (8%) (Grafikon 5). Odnos oboljelih od enterobijaze u Primorskom, Središnjem i Sjevernom regionu je bio 8,6: 2,9: 1.

Diskusija

Enterobijaza je helmintijaza koja se javlja širom svijeta, i za razliku od drugih iz ove grupe bolesti, javlja se podjednako i u razvijenim i u zemljama u razvoju. Češće se javlja u zemljama umjerenog pojasa, što je još jedna njena osobenost (1,2,5). U većini slučajeva infekcija protiče asimptomatski, tako da se samo mali broj dijagnostikuje, što predstavlja tzv. vrh ledenog brijega. Osim toga, ova parazitarna bolest u većini zemalja Evrope, Amerike i svijeta ne podliježe obaveznom prijavljivanju, tako da ostajemo uskraćeni za podatke o njenoj incidenciji i kretanju tokom vremena. Enterobijaza se ne prijavljuje ni u Republici Srbiji.



Grafikon 4. Standardiziovane stope incidencije (prema Evropskoj populaciji, na 100.000) po polu, kao i trend stopa incidencije, Crna Gora, 2010-2019. godine

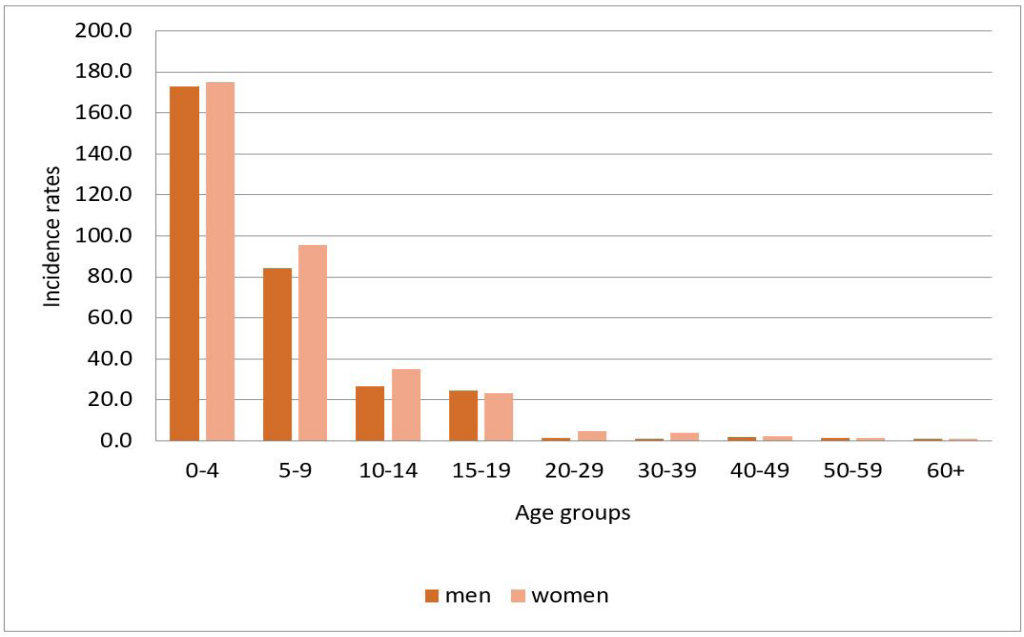


Figure 3. Average age-specific incidence rates of enterobiasis (per 100,000) in relation to gender, Montenegro, 2010-2019

26.8/100,000 (Figure 4). The lowest standardized incidence rate was in 2011 (3.3/100,000 in men, and 5.3/100,000 in women). The incidence rates showed an upward trend in both females and males, but without statistical significance (for men y = 1.3939x-2790.9; p = 0.2604; for women y = 1.7364x-3478.8; p = 0.4108). The average incidence rate in men was 17.2/100,000, while in women, it was 19.1/100,000.

In the given time period, more than 2/3 (more precisely 69%) of registered cases were reported in the coastal region, 23% in the central region, while the smallest number of cases was in the northern region (8%) (Figure 5). The ratio of people with enterobiasis in the coastal, central and northern region was 8.6: 2.9: 1.

Discussion

Enterobiasis is a helminthiasis that occurs worldwide, and in contrast to other diseases from this group of diseases, it occurs equally in developed, as well as in developing countries. It appears more frequently in countries

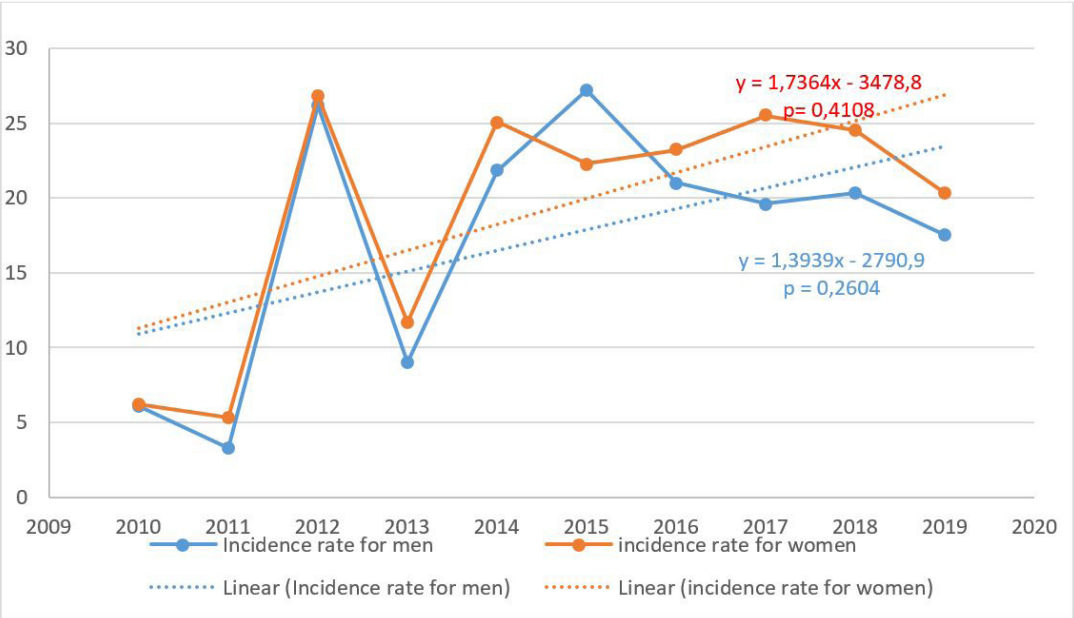
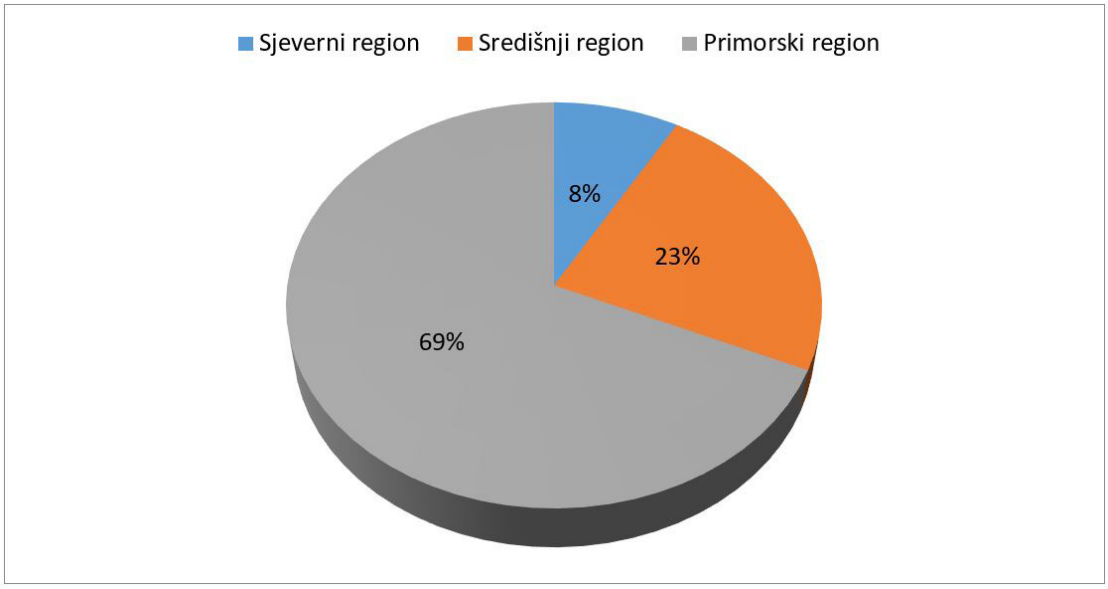


Figure 4. Standardized incidence rates (per 100,000) of enterobiasis (according to the population of Europe) and trend in incidence rate, by gender, Montenegro 2010-2019



Grafikon 5. Distribucija oboljelih od enterobijaze po regionima u Republici Crnoj Gori za period 2010-2019. godine

Ipak, u mnogim zemljama Evrope i i Sjedinjenjih Američkih Država ispitivala se učestalost ovog oboljenja (prevalencija) kroz različita sprovedena istraživanja. Tako se u radu iz 2018. godine, u Istočnoj Slovačkoj ispitavala učestalost ove helmintijaze kod predškolske i školske djece (27). Uzorak je obuhvatao 390 djece, (tačnije 218 devojčica i 172 dječaka), a prevalencija *E. vermicularis* je iznosila 3,59%. Nešto je bila viša kod dječaka (4,07%) nego kod djevojčica (3,21%) (14). Jaja *E. vermicularis*-a su najčešće izolovana u grupi djece uzrasta od 3 do 6 godina, a među njima najviše je bilo oboljelih u uzrastu između 4 i 5 godina.

Slični su bili rezultati epidemiološke studije kod djece i adolescenata iz sjeveroistočnog regiona Poljske sprovedene u periodu 2008-2009., koji su pokazali prevalenciju enterobijaze od 3%, a istraživanje sprovedeno par godina kasnije ukazalo je da je ukupna prevalencija ove helmintijaze u Poljskoj 10,1% (28). U studiji u Italiji, jaja *E. vermicularis* pronađena su kod 13,4% dece (29). Prevalencija među djecom u nekim zajednicama ide i do 61% u Indiji, 50% u Engleskoj, 39% na Tajlandu, 37% u Švedskoj i 29% u Danskoj (30). Učestalost ove parazitoze u Turskoj kod djece koja pohađaju osnovne škole varira između 5,4% i 67% (31).

Što se tiče istraživanja iz Sjedinjenih Američkih Država, ona ukazuju da je i kod njih *E. vermicularis* najčešća helmintijaza sa opštom prevalencijom kod djece 0,2-20%, dok se kod onih koje žive u institucijama prevalencija kreće 50-100% (6,32). Od svih starosnih grupa, djeca školskog uzrasta su najviše izložena riziku od infekcije. Kod odraslih, enterobijaza je najčešća kod roditelja (starih 30-39 godina) djece uzrasta 5-9 godina (6,32). Generalno, kod njih se pokazalo da muškarci obolijevaju dvostruko češće nego žene, osim kod osoba starih od 5 do 14 godina, gdje su uglavnom obolijevale žene (6,32).

U malom broju Izvještaja o zaraznim bolestima u Republici Hrvatskoj, koji su dostupni na sajtu hrvatskog Zavoda za javno zdravstvo, uočavamo da se ni u ovoj zemlji, enterobijaza ne prijavljuje kao posebno oboljenje, već od skora u sklopu helmintijaza (33). Međutim, kako navode, poslednjih godina u njihovom informacionom sistemu postoji mogućnost uvida u uzročnike iste, tako da je 2016. godine od svih helmintijaza, enterobijaza učestvovala sa 46%, a u 2017. godini (poslednjoj objavljenoj na sajtu) sa čak 70% (19). Ovdje moramo dati napomenu da je u 2016. godini u Hrvatskoj čak 37% helmintijaza prijavljeno kao nedefinisano, a 10% kao nespecifično (33), te se vjerovatno u ovome dobrim dijelom krije objašnjenje zbog čega je ovolika razlika u učešću enterobijaze. Može se zaključiti da je u Hrvatskoj *E. vermicularis* najčešći iz grupe svih helminata, bar što se tiče navedenih godina. Ako ove dvije godine uporedimo sa našim, u Crnoj Gori je u 2016. godini enterobijaza činila 84%, a 2017. godine 85% svih helmintijaza i 21% svih parazitarnih bolesti koje se obavezno prijavljuju. U izvještaju hrvatskog Zavoda za javno zdravstvo iz 2017. godine, jasno se uočava porast broja prijavljenih slučajeva helmintijaze u

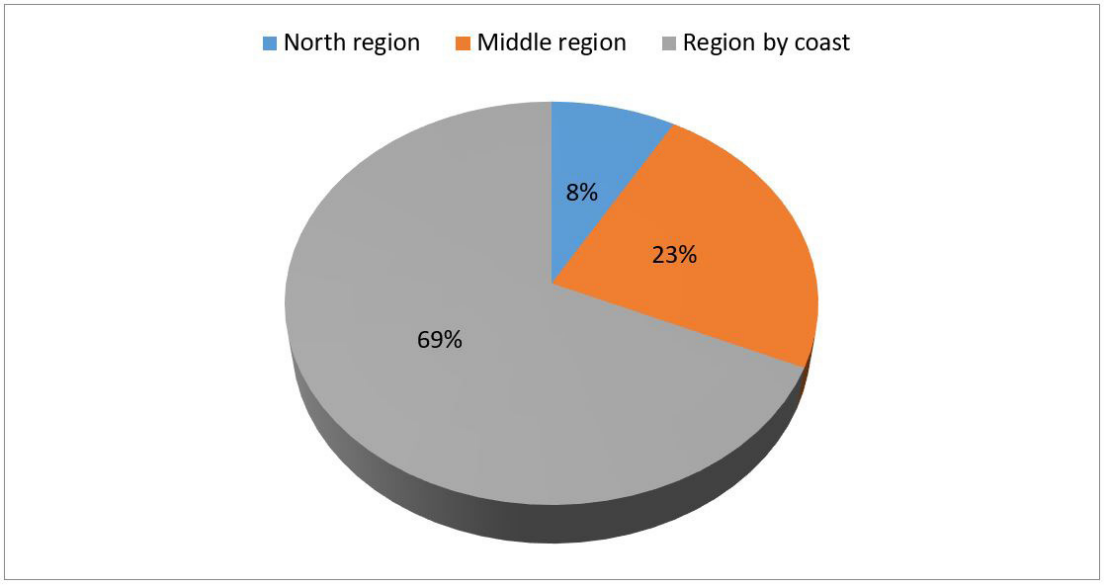


Figure 5. Distribution of enterobiasis patients by regions, Montenegro , 2010-2019

with moderate climate, which is one of its characteristics (1,2,5). In most cases, the infection passes as an asymptomatic infection, and only the small number is diagnosed, which represents the tip of the iceberg. In addition, this parasitic disease is not subject to mandatory registration in many European, American countries and across the world. Therefore, we remain deprived of data about its incidence and trends over time. Enterobiasis is nor reported in the Republic of Serbia, as well.

However, in many countries of Europe and United States of America, the frequency of this disease (prevalence) has been examined in different studies. Thus, in a study from 2008, conducted in Eastern Slovakia, the prevalence of this helminthiasis was examined in pre-school and school children (27). The sample included 390 children, that is, 218 girls and 72 boys, while the prevalence of *E. vermicularis* amounted to 3.59%. It was slightly higher in boys (4.07%) than in girls (3.21%) (14). The eggs of *E. vermicularis* were most frequently isolated in the group of children aged 3 to 6 years, while the majority of children with this disease were in the age group 4 to 5 years.

Similar results were obtained in the epidemiological study that was conducted in 2008-2009 and that included children and adolescents from the northeastern region of Poland. The results showed the prevalence of enterobiasis of 3%, while one study that was conducted a few years later indicated that the prevalence of this helminthiasis was 10.1% in Poland (28). In one study conducted in Italy, the eggs of *E. vermicularis* were found in 13.4% of children (29). The prevalence among children in some communities amounts to 61% in India, 50% in England, 39% in Thailand, 37% in Sweden, 29% in Denmark (30). The prevalence of this parasitic disease in primary school children in Turkey varies between 5.4% and 67% (31).

As far as studies from the United States of America are concerned, they point to the fact that *E. vermicularis* is the most common helminthiasis in their population, with the general prevalence in children from 0.2-20%, while the prevalence varies from 50-100% in people living in institutions (6,32). Of all age groups, school children are most exposed to the risk of this infection. In adults, enterobiasis is most frequent in parents (aged 30-39 years) of children aged 5 to 9 years (6,32). Generally, it was shown that men got this disease two times more frequently than women, except in case of persons aged 5 to 14 years, where females usually got the disease (6,32).

In a small number of reports on infectious diseases in the Republic of Croatia, which are available on the website of the Public Health Institute, we notice that in this country, enterobiasis is not reported as a separate disease, as well, but as a share within helminthiases (33). However, as they claim, in their information system, the cause of disease could be seen during the last few years, so in 2016, the share of enterobiasis was 46% of all helminthic diseases, while in 2017 (the last year published on the site), this share was even 70% (19). We have to note that in Croatia in 2016, even

periodu 2010-2017. godine (33). U 2017. godini, malo više od polovine oboljelih (52%) su osobe ženskog pola, dok su u Crnoj Gori, u tom periodu, žene činile 56% registrovanih. Među oboljelima u Hrvatskoj, najviše je bilo djece: više od polovine činila su djeca uzrasta 0-7 godina, (podaci za 2016. i 2017. godinu) sa najvećom učestalošću kod djece 4-6 godina (19). Podaci za Crnu Goru u ovom periodu nam govore da je i u 2016. i 2017.godini više od polovine oboljele djece od svih helmintijaza u uzrastu do 4. godine (52%), a od 74-78% u dobnoj grupi 0-9 godina. Incidencija helmintijaza u Hrvatskoj 2017. godine iznosi 45,4 na 100.000 stanovnika (33), dok u istoj godini u Crnoj Gori iznosila 30,4/100.000.

Što se našeg istraživanja tiče, prosječno učešće enterobijaze u grupi parazitarnih bolesti, koje se obavezno prijavljuju u Crnoj Gori, u periodu 2010-2019. godine iznosi 18,5%; najčešća bolest iz ove grupe je definitivno šuga. Najveći udio enterobijaze u parazitarnim bolestima zabilježen je 2018. godine gdje je činila četvrtinu, a najmanji 2013. godine sa učešćem od 11,1%. Najveća incidencija enterobijaze zabilježena je 2012. (31,8/100.000) i 2015. godine (28,3/100.000), a najmanja 2011. godine (5,2/100.000). Udio enterobijaze u ukupnim helmintijazama koje se prijavljuju u Crnoj Gori u ovom periodu prelazi 80%, tako da i u našem slučaju opravdava naziv „najčešća helmintijaza“.

Enterobijazu odlikuje visoka kontagioznost, što posebno dolazi do izražaja u gusto naseljenim objektima/ustanovama. Osjetljivost na ovu bolest je opšta, ali je zbog ekspozicije ona najčešća među predškolskom i školskom djecom (1,2,5). Takođe, lična i higijena prostora igraju bitnu ulogu u nastanku bolesti, te se iz ovoga može naslutiti zašto se ova parazitoza najčešće javlja među ovom populacijom. U našem periodu posmatranja, djeca do 9. godine starosti činila su čak preko 80% svih prijavljenih slučajeva obolijevanja. Među njima, najčešće su obolijevala ona uzrasta 0-4 godine (stopa incidencije 173,9/100.000 ili ukupno 53% registrovanih), dok su ona 5-9 godina sa stopom incidencije 89,7/100.000, skoro dvostruko rjeđe bila inficirana (činila su 27,3% ukupno prijavljenih osoba). Rezultati koje smo dobili, a koji se slaže da podacima drugih istraživanja, mogu se objasniti navikama i ponašanjem djece određenog uzrasta. Mala djeca, predškolskog uzrasta, često stavljaju prste u usta, kao i kontaminirane predmete (igračke), sisaju palac, nemaju izgrađene higijenske navike u vidu čestog i pravilnog pranja ruku, pogotovo prije jela i nakon upotrebe toaleta. Dalje, najčešće borave u kolektivu (vrtići i druge predškolske ustanove), što značajno povećava rizik obolijevanja, olakšavajući transmisiju. Školska djeca, iako i ona veliki dio vremena borave u kolektivima, što su starija, praktikuju bolju higijenu ruku i imaju drugačije navike i aktivnosti.

Što se odraslih osoba tiče, (ako uzmemo da su odrasle osobe one iznad 18 godina) u našem istraživanju, najčešće su obolijevale osobe uzrasne grupe 20-29 (stopa incidencije 3,1/100.000), odnosno činile su 0,9% svih oboljelih od enterobijaze. Odmah iza njih su obolevale osobe 30-39 godina (sa 0,8% učešća), a najmanje osobe uzrasta 60 i više godina (0,3% učešća među ukupno registrovanim slučajevima obolelim od enterobijaze u Crnoj Gori u desetogodišnjem periodu praćenja). Podaci iz literature nam kazuju da od odraslih osoba najčešće obolijevaju oni koji se nalaze u ustanovama kolektivnog smještaja ili se brinu o zaraženima (roditelji, staratelji, vaspitačice, njegovateljice).

Kao što je već navedeno, osjetljivost na ovu infekciju je opšta, tako da ni polne predilekcije nema. To nam potvrđuje i prosječna stopa incidencije u posmatranom periodu koja iznosi 21/100.000 za muškarce i 21,1/100.000 za žene. Ipak, prosječne specifične stope incidencije u odnosu na uzrast i pol, bile su veće kod žena u skoro svim starosnim grupama, izuzev u onoj 15-19 i 60 i više godina, gdje su češće obolijevali muškarci.

Što se teritorijalne distribucije tiče, više od 2/3 slučajeva (69%) je prijavljeno u primorskom regionu. Zabilježeno je, npr. i u susjednoj Hrvatskoj, da je najveća učestalost enterobijaze u županijama južnijeg dijela ove zemlje (19). Najmanje slučajeva u Crnoj Gori, registrovano je u sjevernom regionu (8%).

U cilju prevencije ove bolesti treba naglasiti da ne postoje specifične mjere. Od opštih mjera najvažnija je higijena ruku. Takođe, neophodno je edukovati stanovništvo o neophodnosti održavanja lične higijene, posebno o važnosti redovnog pranja ruku, naročito nakon toaleta, prije jela ili pripreme hrane, nakon mijenjanja pelena (6,10).

Zaključak

Higijena ruku je najbolja mjera prevencije eneterobijaze. Lična higijena i higijena prostora, takođe igraju bitnu ulogu. U domaćinstvima gde

37% of helmintiases were reported as undefined, while 10% were reported as non-specific (33), and therefore, the difference in the share of eneterobiasis can be explained by this fact. It can be concluded that in Croatia *E. vermicularis* is the most common form the group of all helminthes, as far as the above mentioned years are concerned. If we compare these two years with ours, in Montenegro in 2016, enterobiasis made 84%, while in 2017, 85% of all helminthiases, and 21% of all parasitic diseases that are necessarily reported. In the Report of the Institute of Public Health of Croatia from 2107, the rise in the number of reported cases of helminthiasis was noticed in the period 2010-2017 (33). In 2017, slightly more than a half of infected persons (52%) were females, while in Montenegro, in the same period, women made 56% of all registered persons. The majority of persons with enterobiasis in Croatia were children: more than a half were children aged 0-7 years (data for 2016 and 2017) with the highest prevalence in children aged 4-6 years (19). Data for Montenegro in this period indicate that in 2016 and 2017, more than a half of children with all helminthic diseases were in the age group 0 to 4 years (52%), while 74-78% in the age group 0-9 years. The incidence of helminthiases in Croatia in 2017 amounted to 45.4 per 100,000 inhabitants (33), while in the same year in Montenegro it amounted to 30.4/100,000.

As far as our study is concerned, the average share of enterobiasis in the group of parasitic diseases, which are mandatorily reported in Montenegro, in the period 2010-2019, amounted to 18.5%. The most common disease in this group is scabies. The largest share of enterobiasis in parasitic diseases was registered in 2018, when it made one fourth, while the smallest was in 2013 with the share of 11.1%. The highest incidence of enterobiasis was registered in 2012 (31.8/100,000) and in 2015 (28.3/100,000), while the smallest was in 2011 (5.2/100,000). The share of enterobiasis in all helminthic diseases that are reported in Montenegro in this period was more than 80%, and therefore, it is also "the most common helminthiasis".

Enterobiasis is characterized by high contagiousness, which is particularly pronounced in densely populated settings/institutions. The susceptibility to this disease is general, but due to exposure it is most common in pre-school and school children (1,2,5). Also, personal hygiene and room hygiene have an important role in disease occurrence, and therefore, one may conclude why this parasitosis occurs in this population. In the period of our observation, children aged 0-9 years made 80% of all reported cases. Among them, the incidence was the highest in the age group 0-4 years (incidence rate 173.9/100,000 or 53% of registered persons), while the incidence was 89.7/100,000 in children aged 5-9 years, who were two times more rarely infected (27.3% of all registered persons). The obtained results, which are in accordance with the data from other studies, may be explained by children's behavior and habits in specific age groups. Younger, pre-school children often put their fingers into their mouths, as well as contaminated items (toys), they suck the thumb, do not have developed hygiene habits regarding regular and proper handwashing, especially before eating and after going to the toilet. Furthermore, they often stay in collective settings (kindergartens and other pre-school institutions), which significantly increases the risk of getting the disease, which can be transmitted more easily. Although school children spend a lot of time in collectives, when they get older, they practice better hand hygiene and have different habits and activities.

As far as adults are concerned (persons older than 18), in our study, persons aged 20-29 got the disease most frequently (incidence rate was 3.1/100,000), that is, they made 0.9 of all persons with enterobiasis. Soon after them were persons aged 30-30 (with 0.8% share), while the smallest share was in persons older than 60 (0.3% share among all registered cases of enterobiasis in Montenegro in the ten-year observation period). Literature data indicate that persons who are accommodated at collective institutions or who take care of infected persons (parents, guardians, pre-school teachers, care providers) get this disease most frequently.

As it has already been mentioned, susceptibility to this infection is general, and therefore, there is no sex predilection. It is confirmed by the average incidence rate in the observed period which amounted to 21/100,000 for men and 21.1/100,000 for women. However, average specific incidence rates regarding sex and age were higher in females in almost all age groups, except in the age groups 15-19 and 60 and older, where men were affected more frequently.

je zaraženo više od jednog člana ili se ponavljaju simptomatske infekcije, preporučuje se da se svi članovi domaćinstva liječe istovremeno, bez obzira da li imaju simptome ili ne. Neohodno je ponoviti liječenje kroz dvije nedjelje, jer antihelmintici djeluju samo na odrasle parazite, a ne i na jaja/larve iz kojih se razvijaju nove jedinke. Potrebno je pružiti dodatnu edukaciju i kontrolisati sprovođenje prije svega higijene ruku, djeci predškolskog i školskog uzrasta, kao i svim osobama koje žive i rade u ustanovama kolektivnog smještaja, budući da su oni u povećanom riziku od infekcije uzrokovane *Enterobius Vermicularis*-om.

Literatura

1. Norhayati M, Fatmah MS, Yusof S, Edariah AB: Intestinal parasitic infections in man: a review. Med J Malaysia 2003; 58: 296–305.
2. Li HM, Zhou CH, Li ZS, et al. Risk factors for Enterobius vermicularis infection in children in Gaozhou, Guangdong, China. Infect Dis Poverty 2015; 4:28.
3. Wendt S, Trawinski H, Schubert S, Rodloff AC, Mössner J, Lübbert C: The diagnosis and treatment of pinworm infection. Dtsch Arztebl Int 2019; 116:213–9.
4. Cook GC. Parasitic disease in clinical practice. London: Springer-Verlag, 1990; 114-65.
5. Moore TA, McCarthy JS. Enterobiasis. In: Tropical Infectious Diseases: Principles, Pathogens and Practice. 3rd ed. Guerrant R, Walker DH, Weller PF (Eds). Saunders Elsevier: Philadelphia; 2011. p.788.
6. Centers for Disease Control and Prevention. Parasites-Enterobiasis (also known as Pinworm Infection). Parasitic Diseases Information. Available at: https://www.cdc.gov/parasites/pinworm/ Accessed: September 18, 2020.
7. Arsentijević Arsić V. Medicinska mikologija i parazitologija. Centar za inovacije u mikologiji Beograd. Društvo medicinskih mikologa Srbije:Beograd; 2012.
8. Chiodini PL, Moody AH, Manser DW. Atlas of medical helminthology and protozoology. 4th edition. Edinburgh: Churchill Livingstone, 2019.
9. Cook GC. Enterobius vermicularis infection. Gut 1994; 35:1159-1162.
10. Heymann LD. Control of communicable diseases Manual, 20th ed. American Public Health Association, Washington: United Book Press, 2015.
11. Kubiak K, Dzika E, Paukszto L: Enterobiasis epidemiology and molec - ular characterization of Enterobius vermicularis in healthy children in north-eastern Poland. Helminthologia 2017; 54: 284–91.
12. Chaudhry AZ, Longworth DL. Cutaneous manifestations of intestinal helminthic infections. System Mycoses Parasit Dis 1989; 7:275-90.
13. Rawla P, Sharma S. Enterobius Vermicularis. [Updated 2021 Aug 3]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing, 2021. Available at: https://www.ncbi.nlm.nih.gov/books/NBK536974/
14. Kollias G, Kyriakopoulos M, Tiniakos G. Epididymitis from Enterobius vermicularis: Case Report. J Urol 1992; 147:1114-6.
15. Arsenault PS, Gerbie AB. Vulvovaginitis in the pre-adolescent girl. Pediat Ann 1986; 15: 577-9, 583-5.
16. McKay T. Enterobius vermicularis infection causing endometritis and persistant vaginal discharge in three siblings. N Z Med J 1989; 102(861):56.
17. Little MD, Cuello CJ, D'Alessandro A. Granuloma of the liver due to Enterobius vermicularis: Report of a case. Am J Trop Med Hyg 1973; 22:567-9.
18. Baharkaran CS, Devi ES, Rao KV. Enterobius vermicularis of vermiform appendix. J India Med Assoc 1975; 64:334-46.
19. Sarmast MH, Maraghi S, Elahi A, Mostofi NE. Appendicitis and Enterobius vermicularis. Pak J Med Sci 2005; 21(2):202-5.
20. Liu LX, Chi J, Upton MP, Ash LR: Eosinophilic colitis associated with larvae of the pinworm Enterobius vermicularis. Lancet 1995; 346:410–2.
21. Al-Saffar F, Najjar N, Ibrahim S, Clark M. Pin worms presenting as suspected Crohn's disease. Am J Case Rep 2015; 16: 737–9.
22. Cranston I, Potgieter N, Mathebula S, Ensink JHJ. Transmission of Enterobius vermicularis eggs through hands of school children in rural South Africa. Acta Trop 2015; 150: 94–6
23. Kucik CJ, Martin GL, Sortor BV. Common intestinal parasites. Am Fam Physician. 2004; 69:1161–8.
24. Chen KY, Yen CM, Hwang KP, Wang LC. Enterobius vermicularis infection and its risk factors among pre-school children in Taipei, Taiwan. J Microbiol Immunol Infect. 2018; 51:559–64.
25. Standardna populacija Evrope (EU i EFTA projekcije populacije za period 2011.-30.). Dostupno na: https://ec.europa.eu/eurostat/cache/metadata/Annexes/hlth_cdeath_ esms_an1.pdf
26. Uprava za statistiku Monstat. Procijenjeni broj stanovnika po opštinama sredinom godine. Dostupno na: https://www.monstat.org/cg/page.php?id=273&pageid=48
27. Dudlova A, Juriš P, Jarčuška P, Vasilkova Z, Vargova V, Sumkova M, Krčmery V. The incidence of pinworm (Enterobius vermicularis) in preschool and school aged children in Eastern Slovakia. Helminthologia 2018; 55(4):275-280.
28. Kubiak K, Dzika E, Paukszto L. Enterobiasis epidemiology and molecular characterization of Enterobius vermicularis in healthy children in north-eastern Poland. Helminthologia 2017; 54(4):284–291.
29. Crotti D, D'annibale ML. Enterobiasis during 2002 – 2003 in Perugia province: beyond diagnostics. Infez Med 2006; 14(2):92–98.
30. Burkhart CN, Burkhart CG. Assessment of frequency, transmission, and genitourinary complications of enterobiasis (pinworms). Int J Dermatol 2005; 44(10):837–840.

As far as territorial distribution is concerned, more than 2/3 of cases (69%) were registered in the coastal region. It was registered in the neighboring Croatia that the highest incidence of enterobiasis was in the southern counties of this country (19). The smallest number of cases in Montenegro was registered in the northern region (8%).

Aimed at disease prevention, it should be emphasized that there are no specific measures. Hand hygiene is the most important measure of all general measures. Also, the population should be educated about the necessity of maintaining personal hygiene, especially about the importance of regular hand washing, especially after going to the toilet, before eating and food preparation, after changing diapers (6,10).

Conclusion

Hand hygiene is the best prevention measure regarding enterobiasis. Personal hygiene and room hugiene also have an important role. In households, where more than one family member is infected or symptomatic infections recur, it is recommended that all household members should be treated, no matter whether they have symptoms or not. It is necessary to repeat the treatment in two weeks, because antihelminthics have effect only on adult parasites, but not on eggs/larvae, from which new individuals develop. It is necessary to provide additional education and control prevention measures, primarily hand hygiene in pre-school and school children, as well as in all persons who live and work in institutions of collective accommodation because they are at higher risk of infection caused by *Enterobius Vermicularis*.

Literature

1. Norhayati M, Fatmah MS, Yusof S, Edariah AB: Intestinal parasitic infections in man: a review. Med J Malaysia 2003; 58: 296–305.
2. Li HM, Zhou CH, Li ZS, et al. Risk factors for Enterobius vermicularis infection in children in Gaozhou, Guangdong, China. Infect Dis Poverty 2015; 4:28.
3. Wendt S, Trawinski H, Schubert S, Rodloff AC, Mössner J, Lübbert C: The diagnosis and treatment of pinworm infection. Dtsch Arztebl Int 2019; 116:213–9.
4. Cook GC. Parasitic disease in clinical practice. London: Springer-Verlag, 1990; 114-65.
5. Moore TA, McCarthy JS. Enterobiasis. In: Tropical Infectious Diseases: Principles, Pathogens and Practice. 3rd ed. Guerrant R, Walker DH, Weller PF (Eds). Saunders Elsevier: Philadelphia; 2011. p.788.
6. Centers for Disease Control and Prevention. Parasites-Enterobiasis (also known as Pinworm Infection). Parasitic Diseases Information. Available at: https://www.cdc.gov/parasites/pinworm/ Accessed: September 18, 2020.
7. Arsentijević Arsić V. Medicinska mikologija i parazitologija. Centar za inovacije u mikologiji Beograd. Društvo medicinskih mikologa Srbije:Beograd; 2012.
8. Chiodini PL, Moody AH, Manser DW. Atlas of medical helminthology and protozoology. 4th edition. Edinburgh: Churchill Livingstone, 2019.
9. Cook GC. Enterobius vermicularis infection. Gut 1994; 35:1159-1162.
10. Heymann LD. Control of communicable diseases Manual, 20th ed. American Public Health Association, Washington: United Book Press, 2015.
11. Kubiak K, Dzika E, Paukszto L: Enterobiasis epidemiology and molec - ular characterization of Enterobius vermicularis in healthy children in north-eastern Poland. Helminthologia 2017; 54: 284–91.
12. Chaudhry AZ, Longworth DL. Cutaneous manifestations of intestinal helminthic infections. System Mycoses Parasit Dis 1989; 7:275-90.
13. Rawla P, Sharma S. Enterobius Vermicularis. [Updated 2021 Aug 3]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing, 2021. Available at: https://www.ncbi.nlm.nih.gov/books/NBK536974/
14. Kollias G, Kyriakopoulos M, Tiniakos G. Epididymitis from Enterobius vermicularis: Case Report. J Urol 1992; 147:1114-6.
15. Arsenault PS, Gerbie AB. Vulvovaginitis in the pre-adolescent girl. Pediat Ann 1986; 15: 577-9, 583-5.
16. McKay T. Enterobius vermicularis infection causing endometritis and persistant vaginal discharge in three siblings. N Z Med J 1989; 102(861):56.
17. Little MD, Cuello CJ, D'Alessandro A. Granuloma of the liver due to Enterobius vermicularis: Report of a case. Am J Trop Med Hyg 1973; 22:567-9.
18. Baharkaran CS, Devi ES, Rao KV. Enterobius vermicularis of vermiform appendix. J India Med Assoc 1975; 64:334-46.
19. Sarmast MH, Maraghi S, Elahi A, Mostofi NE. Appendicitis and Enterobius vermicularis. Pak J Med Sci 2005; 21(2):202-5.
20. Liu LX, Chi J, Upton MP, Ash LR: Eosinophilic colitis associated with larvae of the pinworm Enterobius vermicularis. Lancet 1995; 346:410–2.
21. Al-Saffar F, Najjar N, Ibrahim S, Clark M. Pin worms presenting as suspected Crohn's disease. Am J Case Rep 2015; 16: 737–9.
22. Cranston I, Potgieter N, Mathebula S, Ensink JHJ. Transmission of Enterobius vermicularis eggs through hands of school children in rural South Africa. Acta Trop 2015; 150: 94–6
23. Kucik CJ, Martin GL, Sortor BV. Common intestinal parasites. Am Fam Physician. 2004; 69:1161–8.
24. Chen KY, Yen CM, Hwang KP, Wang LC. Enterobius vermicularis infection and its risk factors among pre-school

31. Celiksöz A, Aciöz M, Değerli S, Alim A, Aygan C. Egg positive rate of Enterobius vermicularis and Taenia spp. by cellophane tape method in Primary School Children in Sivas, Turkey. Korean J. Parasitol 2005; 43(2):61–64.
32. Huh S. Pinworm (Enterobiasis). Emedicine. medscape, 2019. Available at: https://emedicine.medscape.com/article/225652-overview
33. Hrvatski zavod za javno zdravstvo. Izvještaj o zaraznim bolestima u Hrvatskoj za 2016. i 2017. god. Dostupno na: https://www.hzjz.hr/sluzba-epidemiologija-zarazne-bolesti/zarazne-bolesti-u-hrvatskoj-2017-godine

Sukob interesa: Nije prijavljen.
Primljen: 27.09.2021.
Revizija: 28.09.2021.
Prihvaćen: 03.10.2021.

Autor za korespondenciju: Jelena Bojanić, Opšta bolnica Kotor, Škaljari b.b., 85330 Kotor, Crna Gora; e-mail: jelena.b.8@gmail.com

children in Taipei, Taiwan. J Microbiol Immunol Infect. 2018; 51:559–64.

25. Standardna populacija Evrope (EU i EFTA projekcije populacije za period 2011.-30.). Dostupno na: https://ec.europa.eu/eurostat/cache/metadata/Annexes/hlth_cdeath_ esms_an1.pdf
26. Uprava za statistiku Monstat. Procijenjeni broj stanovnika po opštinama sredinom godine. Dostupno na: https://www.monstat.org/cg/page.php?id=273&pageid=48
27. Dudlova A, Juriš P, Jarčuška P, Vasilkova Z, Vargova V, Sumkova M, Krčmery V. The incidence of pinworm (Enterobius vermicularis) in preschool and school aged children in Eastern Slovakia. Helminthologia 2018; 55(4):275-280.
28. Kubiak K, Dzika E, Paukszto L. Enterobiasis epidemiology and molecular characterization of Enterobius vermicularis in healthy children in north-eastern Poland. Helminthologia 2017; 54(4):284–291.
29. Crotti D, D'annibale ML. Enterobiasis during 2002 – 2003 in Perugia province: beyond diagnostics. Infez Med 2006; 14(2):92–98.
30. Burkhart CN, Burkhart CG. Assessment of frequency, transmission, and genitourinary complications of enterobiasis (pinworms). Int J Dermatol 2005; 44(10):837–840.
31. Celiksöz A, Aciöz M, Değerli S, Alim A, Aygan C. Egg positive rate of Enterobius vermicularis and Taenia spp. by cellophane tape method in Primary School Children in Sivas, Turkey. Korean J. Parasitol 2005; 43(2):61–64.
32. Huh S. Pinworm (Enterobiasis). Emedicine. medscape, 2019. Available at: https://emedicine.medscape.com/article/225652-overview
33. Hrvatski zavod za javno zdravstvo. Izvještaj o zaraznim bolestima u Hrvatskoj za 2016. i 2017. god. Dostupno na: https://www.hzjz.hr/sluzba-epidemiologija-zarazne-bolesti/zarazne-bolesti-u-hrvatskoj-2017-godine

Conflict of interest: None declared.
Received: 09/27/2021
Revised: 09/28/2021
Accepted: 10/03/2021

Corresponding author: Jelena Bojanić, General Hospital Kotor, Škaljari bb, 85330 Kotor, Montenegro; e-mail: jelena.b.8@gmail.com

ORIGINALNI RAD

UDK BROJEVI:
618.146-006.6-07/-084
DOI: https://doi.org/10.5937/zdravzast50-33910

PROCENA UTICAJA STAVOVA ŽENA O BENEFITU SKRININGA I MOTIVACIONIH FAKTORA NA NJIHOVO UČEŠĆE U PAPANIKOLAU SKRINING TESTU

Vesna Jovanović[1], Vanja Pažun[1], Vesna Maksimović[1], Vesna Vuković[1]

[1] Akademija strukovnih studija Beograd, Odsek Visoka zdravstvena škola, Beograd, Republika Srbija

SAŽETAK

Uvod/Cilj: U Srbiji karcinom grlića materice (KGM) je drugi vodeći uzrok obolevanja, odmah posle karcinoma dojke. U cilju ranog otkrivanja KGM koristi se Papanikolau test (Papa test). Motivacioni faktori i stavovi žena o benefitu Papanikolau skrining testa mogu uticati na stopu participacije u njemu. Cilj istraživanja je bio da se ispita da li postoji veza između stavova žena o benefitu skrininga i motivacionih faktora sa njihovim učešćem u Papanikolau skrining testu KGM-a.

Metode: Ovom studijom preseka bilo je obuhvaćeno 300 žena uzrasta od 21 do 69 godina, koje su došle na ginekološki pregled u Ginekološko-akušersku kliniku „Narodni front"u Beogradu, u periodu od juna do decembra 2015. godine. Od svih ispitanica prikupljeni su podaci o njihovim demografskim karakteristikam, razlogu dolaska na kliniku, koliko su se često podvrgavale skriningu (Papanikolau testu), kao i podaci o njihovim stavovima o benefitu skrininga i motivaciji za podvrgavanje Papanikolau skrining testu korišćenjem jednog dela upitnika pod nazivom CPC-28 upitnik (na španskom *Creencias, Papanicolaou, Cancer -28*).

Rezultati: Od 300 žena koje su učestvovale u istraživanju, 37,3% žena je redovno participiralo u Papanikolau testu, 43,7% neredovno, a 19,0% nikada. Žene koje su redovno participirale u skriningu su značajno više verovale da je važno da se redovno radi Papa test da bi znale da su zdrave ($F = 13,59$; $p < 0,001$) i da je Papanikolau test najbolji način da se utvrdi rani stadijum KGM-a ($F = 12,29$; $p < 0,001$) u odnosu na žene koje su neredovno i koje nikada nisu participirale u skriningu. Žene koje su neredovno ili nikada nisu učestvovale u skrining programu značajno bi više bile motivisane na skrining ukoliko bi neko u njihovoj porodici ili okolini oboleo od KGM-a ($F = 5,11$; $p = 0,007$), a žene koje su redovno učestvovale u skriningu značajno bi više bile motivisane preporukom od strane ginekologa ($F=5,58$; $p=0,004$) i željom da brinu o svom zdravlju ($F = 6,93$; $p = 0,001$). Visok stepen motivacije je značajano bio povezan sa donošenjem odluke žena da redovno participiraju u skriningu ($\chi^2 = 13,05$; $p = 0,001$) u odnosu na žene koje su neredovno ili koje nikada nisu učestvovale u skriningu.

Zaključak: U okviru ovog istraživanja uočeno je da se tek svaka treća ispitanica redovno podvrgavala Papanikolau skrining testu, što je daleko niže od postavljenih nacionalnih ciljeva da najmanje 75% ciljne populacije žena uzrasta od 25 do 64 godine treba da bude obuhvaćeno organizovanim skriningom. Neophodno je podizanje svesti žena o značaju Papanikolau skrininga za rano otkrivanje KGM-a, kao i o svim drugim preventivnim merama, a posebno o značaju HPV vakcine.

Ključne reči: karcinom grlića materice, Papanikolau test, skrining test, stavovi, motivacija

Uvod

U svetu, karcinom grlića materice (KGM) je četvrti vodeći uzrok umiranja (posle karcinoma dojke, pluća i kolorektuma) sa tedencijom rasta smrtnih slučajeva u regionima kao što su Istočna, Severna i Srednja Afrika. Kada govorimo o Evropi najviše stope mortaliteta (na 100,000) KGM-a, u 2016. godini,su bile u Rumuniji (14,2), Moldaviji (10,3) i Srbiji (10,3), za razliku od Malte, Finske i Švajcarske gde su se kretale od 1,4 do 1,6 na 100.000 (1). Skrining i pravovremeno lečenje su od ključnog značaja za smanjenje stope mortaliteta od KGM-a. Najvažnija metoda za rano otkrivanje (skrining) KGM-a je konvencionalni Papanikolau (Pap) test (2). Ministarstvo zdravlja Republike Srbije je, 2008. godine, sprovelo Nacionalni program za rano otkrivanje (skrining) KGM-a koji se još uvek primenjuje (3). Međutim, organizovani skrining u Srbiji je započeo u decembru 2012. godine, a ciljnu grupu

ORIGINAL PAPER

UDK NUMBERS:
618.146-006.6-07/-084
DOI: https://doi.org/10.5937/zdravzast50-33910

THE ASSESSMENT OF THE INFLUENCE OF WOMEN'S ATTITUDES ABOUT SCREENING BENEFITS AND MOTIVATIONAL FACTORS ON THEIR PARTICIPATION IN THE PAPANICOLAU SCREENING TEST

Vesna Jovanovic[1], Vanja Pazun[1], Vesna Maksimovic[1], Vesna Vukovic[1]

[1] Academy for Applied Studies Belgrade,The College of Health Sciences, Belgrade, Republic of Serbia

SUMMARY

Introduction: In Serbia, cervical cancer (CC) is the second most common cancer, after breast cancer, The Papanicolaou test is used for the early detection of cervical cancer. Motivational factors and attitudes of women about the benefits of the Papanicolaou screening test can affect the rate of participation in it. The aim of the research was to examine whether there is a connection between women's attitudes about the benefits of screening and motivational factors and their participation in the Papanicolaou screening test of CC.

Methods: This cross-sectional study included 300 women aged 25 to 64, who came for a gynecological examination to the Gynecology and Obstetrics Clinic "Narodni Front" in Belgrade, in the period from June to December 2015. Data were collected from all respondents on their demographic characteristics, reason for coming to the clinic, how often they underwent screening (Papanicolaou test), as well as data on their attitudes about the benefits of screening and motivation for undergoing the Papanicolaou screening test using a part of the questionnaire called CPC -28 questionnaire (in Spanish *Creencias, Papanicolaou, Cancer - 28*).

Results: Of the 300 women who participated in the study, 37.3% of women regularly participated in the Papanicolaou test, 43.7% irregularly, and 19.0% never. Women who participated regularly in screening were significantly more likely to believe that it was important to have a regular Pap test to know they were healthy ($F = 13.59$; $p < 0.001$) and that the Pap test was the best way to determine early-stage CC ($F = 12.29$; $p < 0.001$) compared to women who were irregular and who had never participated in screening. Women who participated irregularly or who had never participated in the screening program would be motivated to participate in it significantly more often if someone developed CC in their family or environment ($F = 5.11$; $p = 0.007$), while women who regularly participated would be motivated significantly more often by gynecologist's recommendations ($F = 5.58$; $p = 0.004$) and care for their own health ($F = 6.93$; $p = 0.001$). The high level of motivation was significantly related to the decision of women to regularly participate in screening ($\chi^2 = 13.05$; $p = 0.001$) in relation to women who were irregular or who had never participated in screening.

Conclusion: Within this research, it was noticed that only every third respondent regularly underwent the Papanicolaou screening test, which is far lower than the set national goals that at least 75% of the target population of women aged 21 to 69 should be covered by organized screening. It is necessary to raise women's awareness about the importance of Papanicolaou screening for early detection of CC, as well as about all other preventive measures, and especially about the importance of the HPV vaccine.

Key words: cervical cancer, Papanicolaou test, screening test, attitudes, motivation

Introduction

Cervical cancer (CC) is the fourth leading cause of death worldwide (after breast, lung and colorectal cancer), with death rates that tend to rise in regions such as Eastern, North and Central Africa. As far as Europe is concerned, the highest mortality rates of CC (per 100.000) in 2016 were in Romania (14.2), Moldavia (10.3) and Serbia (10.3) in contrast to Malta, Finland and Switzerland, where these rates ranged from 1.4 to 1.6 per 100.000 (1). Screening and timely treatment are of key importance for the decline in cervical cancer mortality rate. The most important method

čine žene starosti od 25 do 64 godine. Uprkos činjenici da se organizovani skrining KGM-a sprovodi u Republici Srbiji bez troškova nadoknade, većina žena ga ipak ne koristi (4). Prepoznavanje faktora koji utiču na stopu incidencije KGM-a je od značaja za povećanje stope participacije u skriningu. Mnoge studije ukazuju da su negativni stavovi prema skriningu, dominirajući faktori rizika za nastanak KGM-a (5,6).

Žene koje su verovale da imaju veći rizik od bolesti koja može uzrokovati ozbiljne posledice po njihovo zdravlje i kvalitet života, imale su pozitivne stavove o benefitu participacije u skriningu, što je dovodilo do promene ponašanja i njihovu spremnost da iskoriste prednosti različitih dostupnih zdravstvenih promocija u cilju prevencije bolesti (7). To je u skladu sa mnogim studijama u kojima se većina žena složila da će im redovna participacija u skriningu dati psihološki mir da ne postoji prisustvo KGM-a ili da će se rano otkriti promene na grliću materice i to pre nastanka invazivnog karcinoma, čak i ako ne postoji pozitivna porodična istorija za ovaj karcinom (8).

Cilj istraživanja je bio da se ispita da li postoji veza između stavova žena o benefitu skrininga i motivacionih faktora sa njihovim učešćem u skriningu KGM-a.

Metode

Ovom studijom preseka obuhvaćeno je 300 žena uzrasta od 21 do 69 godina koje su došle na ginekološki pregled u Ginekološko-akušersku kliniku „Narodni front“ u Beogradu, u periodu od juna do decembra 2015. godine. Kriterijumi za isključivanje ispitanica iz studije su: žene koje nisu imale mesto prebivališta na teritoriji grada Beograda; žene mlađe od 21 godine, kao i starije od 69 godina; trudnice; osobe sa dijagnostikovanim KGM-om; i žene sa histerektomijom.

Od svih ispitanica prikupljeni su podaci o njihovim demografskim karakteristikama (uzrast, mesto stanovanja), kao i razlog dolaska na kliniku i koliko su se često podvrgavale skriningu (Papa testu). Takođe, za percepciju benefita skrininga i motivisanost za participiranje u skriningu korišćen je jedan deo upitnika pod nazivom CPC-28 upitnik (na španskom *Creencias, Papanicolaou, Cancer -28*) (7). Stavovi žena su mereni petostepenom skalom Likertovog tipa koja se sastoji od: skale percepcije benefita skrininga (4 pitanja, ukupan skor skale u opsegu od 4 do 20) i skale motivisanosti (5 pitanja, ukupan skor skale u opsegu od 5 do 25). Za svako pitanje ispitanice su mogle da izaberu stepen saglasnosti prema svojim uverenjima i to od potpunog slaganja (5 bodova) do potpunog neslaganja (1 bod). Ukupan skor glavnih skala je izračunat na osnovu zbirnog skora pitanja uključenih u datu skalu. Ispitanice koje su imale skor iznad 60% maksimalnog ukupanog skora glavne skale za percepciju definisane su kao žene sa pozitivnim stavom (visok nivo percepcije), a žene sa skorom jednakim ili ispod 60% imale su negativan stav (nizak nivo percepcije) (7). Takođe, ispitanice koje su imale skor za motivisanost iznad 60% maksimalnog ukupnog skora glavne skale za motivisanost definisane su kao žene sa visokim stepenom motivisanosti, a žene sa skorom jednakim ili ispod 60% sa nisim nivoom motivisanosti (7).

U analizi podataka ispitanice smo podelili prema učestalosti korišćenja skrininga na tri grupe: žene koje su se redovno podvrgavale skriningu (odnosno podvrgavane su skriningu u periodima ≤ 3 godine), žene koje su se neredovno podvrgavale skriningu (odnosno koje su se podvrgavale skriningu u periodima dužim od 3 godine) i žene koje se nikad nisu podvrgle skriningu.

Studija je razmotrena i odobrena od strane Etičkog odbora GAK „Narodni front” u Beogradu.

Analiza i obrada podataka vršene su pomoću paketa namenjenog statističkoj obradi *SPSS 17.0 for Windows*. U statističkoj analizi podataka korišćena je analiza varijanse (ANOVA) za više od dve populacije, hi-kvadrat (χ^2) test i *Post Hoc* testovi (*Dunnetts T3* i *Bonferroni*) su korišćeni da bi se uočile razlike između određenih grupa. Statistički značajnim su smatrani rezultati gde je verovatnoća greške prvog tipa bila manja od 5% ($p < 0,05$).

Rezultati

Od 300 žena koje su učestvovale u istraživanju, 37,3% žena je redovno participiralo u skriningu KGM-a, 43,7% neredovno, a 19,0% nikada (Tabela 1).

Žene koje su redovno participirale u skriningu su značajno više verovale (tj. imale veće vrednosti skora) da je važno da se redovno radi Papa test da bi znale da su zdrave ($F = 13,59$; $p < 0,001$) i da je Papa test najbolji način da se utvrdi rani stadijum KGM-a pre pojave simptoma ($F = 12,29$; $p < 0,001$) u odnosu na žene koje su neredovno i koje nikada nisu participirale u skriningu (Tabela 2). Kada je

for the early detection (screening) of CC is the conventional Papanicolaou (Pap) test (2). In 2008, The Ministry of Health of The Republic of Serbia conducted the National program for the early detection (screening) of cervical cancer that is still being applied (3). However, organized screening in Serbia began in December 2012, while the target group included women aged 25 to 64 years. Although organized screening is realized without any compensatory costs, the majority of women do not use it (4). Recognizing factors that influence the incidence rate of CC is important for increasing the rates of participation in screening. Numerous studies point to the fact that negative attitudes towards screening are dominant risk factors for the appearance of cervical cancer (5,6).

Women, who believed that they were at higher risk of this disease that could cause serious consequences for their health and quality of life, had positive attitudes to benefits of participation in screening, which led to the change in their behavior and their readiness to use the advantages of different available health promotions aimed at disease prevention (7). This is in accordance with numerous studies, in which women agreed that regular participation in screening would give them psychological peace regarding the absence of CC, and that they would discover early changes on the cervix before the occurrence of invasive cancer, even if there was no positive family history for this cancer (8).

The aim of this study was to examine the relationship between women's attitudes towards benefits of screening and motivational factors and their participation in screening of cervical cancer.

Methods

This cross-sectional study included 300 women, aged 21 to 69, who attended the Gynecology and Obstetrics Clinic "Narodni Front" in Belgrade for a gynecological examination from June to December 2015. Exclusion criteria were the following: women whose place of residence was not in the territory of the city of Belgrade; women younger than 21, as well as older than 69; pregnant women; persons with diagnosed CC; and women with hysterectomy.

Data which were collected from all respondents included their demographic characteristics (age, place of residence), reasons for coming to the clinic, and how often they underwent screening (Papanicolaou test). Also, one part of the questionnaire, called CPC-28 questionnaire (in Spanish *Creencias, Papanicolaou, Cancer-28*) was used for the perception of the benefits of screening and motivation to participate in screening. Women's attitudes were measured by five-point Likert scale that included the following: scale of the perception of screening benefits (4 questions, total score ranged from 4 to 20) and motivation scale (5 questions, total score ranged from 5 to 25). The respondents could choose the level of agreement for each question according to their beliefs, ranging from complete agreement (5 points) to complete disagreement (1 point). The total score of main scales was measured according to the sum total of questions included in the given scale. The respondents who had score above 60% of the maximal total score of the main scale for perception were defined as women with a positive attitude (high level of perception), while those women with the equal score or below 60% had a negative attitude (low level of perception) (7). Also, the respondents with a score for motivation above 60% of the maximal total score of the main scale for motivation were defined as women with a high level of motivation, while women with the equal score or below 60% as women with a low level motivation (7).

In the analysis of data, we classified the respondents according to the frequency of using screening into three groups: who underwent screening regularly (that is, who underwent screening in periods ≤ 3 years), who underwent screening irregularly (that is, who underwent screening in periods longer than three years) and women who had never undergone screening.

The study was considered and approved by the Ethics Committee of the Obstetrics and Gynecology Clinic "Narodni Front" in Belgrade.

The analysis of data was done with the help of package for the statistical analysis SPSS 17.0 for Windows. The analysis of variance (ANOVA) was used for the statistical analysis for more than two populations, as well as chi-squared test (χ^2). Post Hoc tests (Dunnetts T3 and Bonferroni) were used in order to notice differences between certain groups. Statistically significant results were those when the probability of type I error was less than 5% ($p < 0,05$).

Tabela 1. Participacija žena u skriningu za karcinom grlića materice

Poslednji Papa test	Broj ispitanika (N=300)	Procenat
Redovno (≤ 3 godine)	112	37,3
Neredovno (>3 godine)	131	43,7
Nikada	57	19,0

ispitivana međugrupna razlika nije bilo značajne razlike između ispitivanih grupa u odnosu na stav da je prihvatanje skrininga dobro uloženo vreme u sopstveno zdravlje i da je ranim otkrivanjem KGM-a maligno oboljenje 100% izlečivo.

Žene koje su redovno participirale u skriningu su značajno više verovale (tj. imale veće vrednosti skora) da je važno da se redovno radi Papa test da bi znale da su zdrave ($F = 13,59$; $p < 0,001$) i da je Papa test najbolji način da se utvrdi rani stadijum KGM-a pre pojave simptoma ($F = 12,29$; $p < 0,001$) u odnosu na žene koje su neredovno i koje nikada nisu participirale u skriningu (Tabela 2). Kada je ispitivana međugrupna razlika nije bilo značajne razlike između ispitivanih grupa u odnosu na stav da je prihvatanje skrininga dobro uloženo vreme u sopstveno zdravlje i da je ranim otkrivanjem KGM-a maligno oboljenje 100% izlečivo.

Takođe, žene koje su redovno participirale u Papanikolau skrining testu su imale značajno češće pozitivan stav o benifitu participacije u skriningu KGM-a u odnosu na žene koje su neredovno ili koje nikada nisu učestvovale u ovom skriningu ($\chi^2 = 6,48$; $p = 0,039$) (Tabela 3).

Žene koje nikada nisu učestvovale u skrining programu KGM-a značajno su se više slagale (prema većem skoru) da bi se uključile u skrining ako neko oboli od KGM-a u njihovoj porodici ili okolini ($F = 5,11$; $p = 0,007$) u odnosu na žene koje su redovno i neredovno podvrgavane skriningu (Tabela 4). Međutim, žene koje su redovno participirale u skriningu, u odnosu na žene koje su neredovno ili koje se nikada nisu odazvale skriningu, značajno su se više slagale (prema većem skoru) da bi ih razgovor sa ginekologom ($F = 5,58$; $p = 0,004$) i briga o svom zdravlju ($F = 6,93$; $p = 0,001$) motivisali na skrining.

Visok stepen motivacije je značajano češće bio povezan sa donošenjem odluke žena da redovno participiraju u skriningu KGM-a ($\chi^2 = 13,05$; $p = 0,001$) u odnosu na žene koje neredovno ili koje nikada nisu učestvovale u skriningu KGM-a (Tabela 5).

Tabela 2. Stavovi žena o benefitu skrininga u odnosu na participaciju u Papanikolau skrining testu

	Participacija u skriningu						Statistika				
	Redovno I N=112		Neredovno II N=131		Nikada III N=57		I-II	I-III	II-III	I-II-III	
Stavovi o benefitu skrininga KGM	x̄	SD	x̄	SD	x̄	SD	p*	p*	p*	F	p**
Važno je da redovno radim Papa test da bih znala da sam zdrava	4,63	0,60	4,27	0,78	4,05	0,87	0,000	0,000	0,272	13,59	0,000
Papa test je najbolji način da se utvrdi rani stadijum KGM pre pojave simptoma	4,59	0,56	4,12	0,89	4,11	0,92	0,000	0,001	0,998	12,29	0,000
Participacija u skriningu KGM je dobro uloženo vreme u moje sopstveno zdravlje	2,75	1,13	2,81	0,89	2,64	1,03	0,961	0,893	0,636	0,55	0,580
Ako se otkrije rani stadijum KGM onda je maligno oboljenje 100% izlečivo	3,86	0,99	3,65	1,04	3,51	1,12	0,265	0,126	0,806	2,55	0,080

x̄ – srednja vrednost; SD – standardna devijacija; *Bonferroni test; **ANOVA.

Table 1. The participation of women in screening for cervical cancer

The last Pap test	The number of respondents (N=300)	Percentage (%)
Regularly (≤ 3 years)	112	37.3
Irregularly (> 3 years)	131	43.7
Never	57	19.0

Results

Of all the 300 women, who participated in the study, 37.7% regularly participated in screening of cervical cancer, 43.7% irregularly and 19.0% never (Table 1).

Women who regularly participated in screening believed significantly more often (that is, they had higher values of score) that it was important to do the Pap test regularly in order to know that they were healthy ($F = 13.59$; $p < 0.001$) and that the Pap test was the best way to discover the early stage of CC before the appearance of symptoms ($F = 12.29$; $p < 0.001$) in comparison to women who participated irregularly or who had never participated in screening (Table 2). When the inter-group difference was examined, there was no significant difference between the examined groups regarding the attitude that accepting screening means that time is well invested in their own health and that the early detection of CC means that malign disease is 100% curable.

Also, women who participated regularly in the Papanicolaou screening test had significantly more often a positive attitude about the benefit of participation in screening for CC, in comparison to women who irregularly or never participated in this screening ($\chi^2 = 6.48$; $p = 0.039$) (Table 3).

Women that had never participated in the screening program agreed significantly more often (according to a higher score) that they would participate in screening if somebody got CC in their family or environment ($F = 5.11$; $p = 0.007$) in comparison to women who regularly or irregularly underwent screening (Table 4). However, women who regularly participated in screening, in comparison to women who participated irregularly or who had never participated, agreed significantly more often (according to a higher score) that the conversation with a gynecologist ($F = 5.58$; $p = 0.004$) and care for their health ($F = 6.93$; $p = 0.001$) would motivate them to undergo screening.

Table 2. Women's attitudes about the benefits of screening in relation to the participation in the Papanicolaou screening test

	Participation in screening						Statistics				
	Regularly I N=112		Irregularly II N=131		Never III N=57		I-II	I-III	II-III	I-II-III	
Attitudes about the benefits of screening of CC	x̄	SD	x̄	SD	x̄	SD	p*	p*	p*	F	p**
It is important to do the Pap test regularly in order to know that I am healthy	4.63	0.60	4.27	0.78	4.05	0.87	0.000	0.000	0.272	13.59	0.000
Pap test is the best way to detect the early stage of CC before the appearance of symptoms	4.59	0.56	4.12	0.89	4.11	0.92	0.000	0.001	0.998	12.29	0.000
Participation in screening is time well invested in my own health	2.75	1.13	2.81	0.89	2.64	1.03	0.961	0.893	0.636	0.55	0.580
If the early stage of CC is detected, then the malign disease is 100% curable	3.86	0.99	3.65	1.04	3.51	1.12	0.265	0.126	0.806	2.55	0.080

x̄ – mean value; SD – standard deviation; *Bonferroni test; **ANOVA.

Tabela 3. Uticaj stava žena o benefitu skrininga na njihovu odluku da participiraju u skriningu

	Participacija u skriningu									
	Redovno		Neredovno		Nikada		Ukupno		Statistika	
Stav o benefitu participacije u skriningu	Broj	%	Broj	%	Broj	%	Broj	%	χ^2	p
Pozitivan stav	4,63	0,60	4,27	0,78	4,05	0,87	115	38,33		
Negativan stav	4,59	0,56	4,12	0,89	4,11	0,92	185	61,67	6,48	0,039
Ukupno	2,75	1,13	2,81	0,89	2,64	1,03	300	100		

Pozitivan stav > 60% ukupnog skora stava o benefitu skrininga KGM-a;
Negativan stav ≤ 60% ukupnog skora stava o benefitu skrininga KGM-a

Diskusija

U našoj studiji žene koje veruju da imaju veći rizik od KGM-a i da bolest može uzrokovati ozbiljne posledice po njihovo zdravlje i kvalitet života, značajno su češće spremne da promene ponašanje i iskoriste prednosti participacije u skriningu u cilju ranog otkrivanja KGM-a. Autori mnogobrojnih istraživanja ukazuju da više od 85% žena priznaje da je rano otkrivanje KGM-a pomoću Papanikolau testa važan benefit i zauzimaju stav da su promene na grliću materice usled ranijeg otkrivanja izlečive (9-12). U našoj studiji 38,3% žena prepoznaje korisnost od Papnikolau testa za rano otkrivanje KGM-a što se podudara sa brojem žena koje su redovno participirale u skriningu. *Seow* i sar. su pokazali da žene koje su već imale prethodno iskustvo sa Papanikolau testom imaju pozitivan stav da učestvuju u programima skrininga i sledeći put (13), što povrđuje i naše istraživanje gde isti stav zauzima 44,6% žena koje redovno participiraju u skriningu.

Većina žena u našoj studiji koje su redovno učestovale u skrining programu je značajno više verovala da bi bile motivisane razgovorom sa ginekologom da se odazovu skriningu u odnosu na žene koje neredovno ili nikada nisu participirale u skriningu. U literaturi nalazimo da je značaj dobre komunikacije između ginekologa i pacijenta važan motivator i stimulans za učestvovanje u skriningu (14-16). U dve studije sprovedene u SAD, 59% Latinoamerikanki (14) i 58% Kineskinja veruje da ginekolog treba da im preporuči Papanikolau skrining test, pre nego što isti urade (15).

Tabela 4. Stavovi žena o motivacionim faktorima u odnosu na particpiciju u skriningu

	Participacija u skriningu						Statistika				
	Redovno I N=112		Neredovno II N=131		Nikada III N=57		I-II	I-III	II-III	I-II-III	
Motivacioni faktori za prihvatanje skrininga	x̄	SD	x̄	SD	x̄	SD	p*	p*	p*	F	p**
Radila bih Papa test, jer se tako brinem o svom zdravlju	4,37	1,06	3,94	1,11	3,79	1,13	0,008	0,004	1,000	6,93	0,001
Radila bih Papa test, ako bi moj ginekolog tako zahtevao	4,32	1,08	3,86	1,18	3,88	1,17	0,006	0,052	1,000	5,58	0,004
Radila bih Papa test, ako bi me bliske osobe savetovale da to uradim	3,25	1,05	3,08	1,09	3,12	1,17	0,653	1,000	1,000	0,79	0,457
Radila bih Papa test, ako bi imala simptome	3,88	0,99	3,73	1,04	3,63	1,11	0,793	0,455	1,000	1,19	0,306
Radila bih Papa test, ako bi neko u mojoj porodici ili okolini oboleo od KGM	2,98	1,41	3,11	1,34	3,67	1,21	0,862	0,004	0,017	5,11	0,007

x̄ – srednja vrednost; SD – standardna devijacija; *Bonferroni test; **ANOVA.

Table 3. The influence of women's attitudes about the benefits of screening on their decision to participate in screening

	Participation in screening									
	Regularly		Irregularly		Never		Total		Statistis	
Attitudes about the benefits of screening	No	%	No	%	No	%	Broj	%	χ^2	p
Positive attitude	4.63	0.60	4.27	0.78	4.05	0.87	115	38.33		
Negative attitude	4.59	0.56	4.12	0.89	4.11	0.92	185	61.67	6.48	0.039
Total	2.75	1.13	2.81	0.89	2.64	1.03	300	100		

Positive attitude > 60% of the total score regarding the attitude about the benefits of screening of CC;
Negative attitude < 60% of the total score regarding the attitude about the benefits of screening.

A high level of motivation was significantly more often associated with decisions to participate in screening regularly (χ^2=13.05; p=0.001) in comparison to women who irregularly participated or who had never participated in CC screening (Table 5).

Discussion

In our study, women, who believed that they were at higher risk of developing CC and that this disease could provoke serious consequences for their health and quality of life, were significantly more often ready to change their behavior and to use the advantages of participation in screening aimed at the early discovery of CC. Authors of numerous studies pointed to the fact that more than 85% of women admitted that the early discovery of CC with the help of Papanicolaou test is an important benefit and they claimed that changes on the cervix are curable due to the earlier discovery (9-12). In our study, 38.3% of women recognized the usefulness of Papanicolaou test for the early discovery of CC, which was in accordance with the number of women who participated regularly in screening. Seow and associates showed that women, who had a previous experience with the Pap test, had a positive attitude towards participating in screening programs in the future (13), which was confirmed n our study, where 44.6% of women who regularly participated in screening had the same attitude.

The majority of women in our study, who participated regularly in the screening program,

Table 4. Women's attitudes about motivational factors regarding the participation in screening

	Participation in screening						Statistics				
	Regularly I N=112		Irregularly II N=131		Never III N=57		I-II	I-III	II-III	I-II-III	
Motivational factors for accepting screening	x̄	SD	x̄	SD	x̄	SD	p*	p*	p*	F	p**
I would do the Pap test because in that way I take care of my health	4.37	1.06	3.94	1.11	3.79	1.13	0.008	0.004	1.000	6.93	0.001
I would do the Pap test if my gynecologist demanded	4.32	1.08	3.86	1.18	3.88	1.17	0.006	0.052	1.000	5.58	0.004
I would do the Pap test if close people would advise me to do it	3.25	1.05	3.08	1.09	3.12	1.17	0.653	1.000	1.000	0.79	0.457
I would do the Pap test if I had symptoms	3.88	0.99	3.73	1.04	3.63	1.11	0.793	0.455	1.000	1.19	0.306
I would do the Pap test if somebody in my family or environment developed CC	2.98	1.41	3.11	1.34	3.67	1.21	0.862	0.004	0.017	5.11	0.007

x̄ – mean value; SD – standard deviation; *Bonferroni test; **ANOVA.

Tabela 5. Analiza participacije žena u skriningu KGM-a prema stepenu njihove motivacije

	Participacija u skriningu									
Motivacija	**Redovno**		**Neredovno**		**Nikada**		**Ukupno**		**Statistika**	
Stav o benefitu participacije u skriningu	**Broj**	**%**	**Broj**	**%**	**Broj**	**%**	**Broj**	**%**	χ^2	**p**
Visok stepen*	68	60,7	53	40,5	21	36,8	142	47,33		
Nizak stepen **	44	39,3	78	59,5	36	63,2	158	52,67	13,05	0,001
Ukupno	112	100	131	100	57	100	300	100		

*Visok stepen motivacije - >60% ukupnog skora na skali motivacije preuzimanja zdravstvene akcije;
**Nizak stepen motivacije - ≤60% ukupnog skora na skali motivacije preuzimanja zdravstvene akcije

Studija sprovedena u Velikoj Britaniji pokazala je da su žene koje su smatrale da su izložene većem riziku od KGM-a bile više motivisane da se uključe u redovan skrining (17). Svoj stav su bazirale na prodičnoj istoriji karcinoma i bile su sigurne da kod njih postoji genetska predispozicija. U našoj studiji, žene koje nisu nikada ili koje su neredovno učestovale u skriningu bile bi značajno više pozitivno motivisane da se uključe u skrining program KGM-a ako bi neko u njihovoj porodici ili okolini oboleo od KGM-a, u odnosu na žene koje redovno učestvuju u ovom skriningu. Smatra se da su žene koje su doživele KGM u svojim porodicama svesnije ozbiljnosti bolesti (6).

Brojna istraživanja govore o benefitu i ograničenjima Papanikolau skrining testa. Nijedan skrining test, pa tako ni Papanikolau test, nema 100% senzitivnost i 100% specifičnost. To znači da rezultati skrining testa mogu da budu lažno pozitivni i lažno negativni. Zato se svaka osoba koja je skriningom označena pozitivno podvrgava određenoj dijagnostičkoj proceduri. Upravo ove činjenice treba da bude svesna svaka žena koja se podvrgava skrining testu. Takođe, često se dešava da neke žene sa abnormalnim promenama mogu biti povrgnute intervenciji iako date promene nikada ne bi progredirale i ne bi uzrokovale simptome i smrt tokom njihovog života (engl. *overdiagnosis*) (18). S druge strane, korist od skrining testa je ogromna, jer doprinosi ranom otkrivanju KGM-a i to pre pojave simptoma, što doprinosi redukciji umiranja i unapređenju kvaliteta života.

I pored malog broja ispitanika obuhvaćenih ovom studijom, dobijeni rezultati su od izuzetne važnosti u cilju sagledavanja načina da se unapredi sprovođenje organizovanog skrininga za KGM, odnosno da se žene ohrabre da redovno učestvuju u skrining programima. Neophodno je da se sve žene starosti od 25 do 64 godine podvrgnu Papanikolau testu i to najpre dve godine za redom, a nakon toga, ako su ta dva rezultata bila uredna, da nastave sa pregledima na svake tri godine.

Zaključak

Žene sa pozitivnim stavom o benefitu skrininga značajno češće se podvrgavaju skriningu nego žene sa negativnim stavom. Redovni preventivni pregledi bi doprineli nižoj stopi mortaliteta od KGM-a, a kasnije i incidencije, što je glavni prioritet zdravstvene službe.

Literatura

1. The European Network of Cancer Registries ENCR, 2016.
2. Oranratanaphan S, Amatyakul P, Iramaneerat K, Srithipayawan S. Knowledge, attitudes and practices about the Pap smear among medical workers in Naresuan University Hospital. Thailand. Asian Pac J Cancer Prev 2010; 11:1727-30.
3. Nacionalni program organizovanog skrininga za rak grlića materice. Republička stručna komisija za prevenciju i kontrolu raka grlića materice, 2013.
4. Matejić B, Vuković D, Pekmezović T, Kesić V, Marković M. Determinants of preventive health behavior in relation to cervical cancer screening among the female population of Belgrade. Health Educ Res 2011; 26(2):201-211,
5. Glanz K, Rimer BK, Lewis FM. Health behavior and health education: theory, research, and practice, (3rd ed). New Jersey: Jossey-Bass, 2002.
6. Hoque M, Ibekwe CM, Ntuli-Ngcobo B. Screening and perceived severity of cervical cancer among women attending Mahalapye District Hospital, Botswana, APJPH2009; 10(6):1095-1100.
7. Urrutia MT, Araya A, Rivera S, Viviani SP, Villarroel L. A predictive model for the quality of sexual life in hysterectomized women. Rev Med Chile 2007; 135(3):317-325.

Table 5. The analysis of women's participation in screening of cervical cancer according to the level of their motivation

	Participation in screening									
	Regularly		Irregularly		Never		Total		Statistis	
Motivation	Np	%	No	%	No	%	No	%	χ^2	p
High level*	68	60.7	53	40.5	21	36.8	142	47.33		
Low level **	44	39.3	78	59.5	36	63.2	158	52.67	13.05	0.001
Total	112	100	131	100	57	100	300	100		

*High level of motivation - >60% of the total score on the motivation scale of taking health action;
**Low level of motivation - ≤60% of the total score on the motivation scale of taking health action.

believed significantly more often that the conversation with a gynecologist would motivate them to undergo screening in comparison to women who irregularly participated in screening or who had never participated in it. In literature, we find that the significance of good communication between a gynecologist and patient is an important motivator and stimulant for the participation in screening (14-16). In two studies conducted in the USA, 59% of Latin Americans (14) and 58% of Chinese Americans believed that the gynecologist should recommend them the Papanicolaou screening test, before they undergo it (15).

A study conducted in the UK showed that women who thought that they were exposed to greater risk of CC were more motivated to participate in regular screening (17). Their attitude was based on family history of cancer and they were sure that they had a genetic predisposition. In our study, women who had never participated in screening or who participated irregularly were significantly more positively motivated to take part in the screening program of CC, if somebody from their family or environment got CC, in comparison to women who regularly participated in this screening. It is believed that women who experienced CC in their families were more aware of the seriousness of this disease (6).

Numerous studies speak about benefits and limitations of the Papanicolaou screening test. There are no screening tests which have 100% sensitivity and 100% specificity, including the Papanicolaou test. This means that the results of this screening test can be false positive and false negative. Therefore, each person that is marked as positive should undergo a certain diagnostic procedure. Each woman who undergoes the screening test should be aware of the fact. Also, it often happens that some women with abnormal changes could be subjected to interventions, although these changes would never progress and would not cause symptoms and death during their lifetime (overdiagnosis) (18). On the other hand, the benefit of screening test is huge because it contributes to the early discovery of CC before the appearance of symptoms, which contributes to the reduction of dying and improvement of quality of life.

Although this study included the small number of respondents, the obtained results are of utmost significance aimed at finding ways to improve the organized screening programs, that is, to encourage women to participate regularly in screening programs. All women aged 25 to 64 years should necessarily undergo the Papanicolaou test, at first two years in a row, and after that, if those two results had no abnormality detected, they should continue with examinations every three years.

Conclusion

Women with a positive attitude towards the benefits of screening undergo screening significantly more often than women with a negative attitude. Regular preventive examinations would contribute to lower cervical cancer mortality rate, and later to lower incidence, which is the main priority of healthcare services.

8. Leyva M, Byrd T, Tarwater P. Attitudes towards cervical cancer screening: A study of beliefs among women in Mexico. CJHP 2006; 4(2):13-24,
9. Jirojwong S, Maclennan R, Manderson L, Health beliefs and Pap smears among Thai women in Brisbane, Australia. APJPH 2001; 13(1):20-23.
10. Holroyd E, Taylor Piliae RE, Twinn SF. Investigating Hong Kong's Filipino domestic workers' healthcare behavior, knowledge, beliefs and attitudes towards cervical cancer and cervical screening, Women Health 2003; 38(1):69-82.
11. Moreland S, Engelman K, Greiner KA, Mayo MS. Papanicolaou testing among Native American and Hispanic populations, Ethn Dis 2006; 16(1):223-227.
12. Byrel TL, Peterson SK, Chavez R. Cervical cancer screening beliefs among young Hispanic women. Prev Med 2004; 38:192-197.
13. Seow A, Wong ML, Smith WCS, Lee HP. Beliefs and attitudes as determinants of cervical cancer screening: a community-based study in Singapore. Prev Med 1995; 24:134-41.
14. Fernandez Esquer ME, Cardenas Turanza M. Cervical cancer screening among Latinas recently immigrated to the United States. Prev Med 2004; 38:529-535.
15. Taylor VM, Jackson JC, Tu SP, Yasui Y, Schwartz SM, Kuniyuki A. Cervical cancer screening among Chinese Americans. Cancer Detec Prev 2002; 26(2):139-145.
16. Eunice E, Lee, Fogg L, Menon U. Knowledge and Beliefs Related to cervical cancer and screening among Korean American women. West J Nurs Res 2008; 30(8): 960–974.
17. Armstrong N, Eborall H. Resistance through risk: Women and cervical cancer screening. Health Risk Soc 2005; 7(2):161-176.
18. Hakama M, Pokhrel A, Malila N, Hakulinen T. Sensitivity, effect and overdiagnosis in screening for cancers with detectable pre-invasive phase. Int J Cancer 2015; 136: 928-935.

Sukob interesa: Nije prijavljen.
Primljen: 12.09.2021.
Revizija: 28.09.2021.
Prihvaćen: 02.10.2021.

Autor za korespondenciju: dr sci. med. Vesna M. Jovanović, Visoka zdravstvena škola, Akademija strukovnih studija Beograd, Cara Dušana 254, 11000 Beograd, Srbija; e-mail: vesna72.jovanovic@gmail.com

Literature

1. The European Network of Cancer Registries ENCR, 2016.
2. Oranratanaphan S, Amatyakul P, Iramaneerat K, Srithipayawan S. Knowledge, attitudes and practices about the Pap smear among medical workers in Naresuan University Hospital. Thailand. Asian Pac J Cancer Prev 2010; 11:1727-30.
3. Nacionalni program organizovanog skrininga za rak grlića materice. Republička stručna komisija za prevenciju i kontrolu raka grlića materice, 2013.
4. Matejić B, Vuković D, Pekmezović T, Kesić V, Marković M. Determinants of preventive health behavior in relation to cervical cancer screening among the female population of Belgrade. Health Educ Res 2011; 26(2):201-211,
5. Glanz K, Rimer BK, Lewis FM. Health behavior and health education: theory, research, and practice, (3rd ed). New Jersey: Jossey-Bass, 2002.
6. Hoque M, Ibekwe CM, Ntuli-Ngcobo B. Screening and perceived severity of cervical cancer among women attending Mahalapye District Hospital, Botswana, APJPH2009; 10(6):1095-1100.
7. Urrutia MT, Araya A, Rivera S, Viviani P, Villarroel L. A predictive model for the quality of sexual life in hysterectomized women. Rev Med Chile 2007; 135(3):317-325.
8. Leyva M, Byrd T, Tarwater P. Attitudes towards cervical cancer screening: A study of beliefs among women in Mexico. CJHP 2006; 4(2):13-24,
9. Jirojwong S, Maclennan R, Manderson L, Health beliefs and Pap smears among Thai women in Brisbane, Australia. APJPH 2001; 13(1):20-23.
10. Holroyd E, Taylor Piliae RE, Twinn SF. Investigating Hong Kong's Filipino domestic workers' healthcare behavior, knowledge, beliefs and attitudes towards cervical cancer and cervical screening, Women Health 2003; 38(1):69-82.
11. Moreland S, Engelman K, Greiner KA, Mayo MS. Papanicolaou testing among Native American and Hispanic populations, Ethn Dis 2006; 16(1):223-227.
12. Byrel TL, Peterson SK, Chavez R. Cervical cancer screening beliefs among young Hispanic women. Prev Med 2004; 38:192-197.
13. Seow A, Wong ML, Smith WCS, Lee HP. Beliefs and attitudes as determinants of cervical cancer screening: a community-based study in Singapore. Prev Med 1995; 24:134-41.
14. Fernandez Esquer ME, Cardenas Turanza M. Cervical cancer screening among Latinas recently immigrated to the United States. Prev Med 2004; 38:529-535.
15. Taylor VM, Jackson JC, Tu SP, Yasui Y, Schwartz SM, Kuniyuki A. Cervical cancer screening among Chinese Americans. Cancer Detec Prev 2002; 26(2):139-145.
16. Eunice E, Lee, Fogg L, Menon U. Knowledge and Beliefs Related to cervical cancer and screening among Korean American women. West J Nurs Res 2008; 30(8): 960–974.
17. Armstrong N, Eborall H. Resistance through risk: Women and cervical cancer screening. Health Risk Soc 2005; 7(2):161-176.
18. Hakama M, Pokhrel A, Malila N, Hakulinen T. Sensitivity, effect and overdiagnosis in screening for cancers with detectable pre-invasive phase. Int J Cancer 2015; 136: 928-935.

Conflict of interest: None declared.
Received: 09/12/2021
Revised: 09/28/2021
Accepted: 10/02/2021

Corresponding author: dr sci. med. Vesna M. Jovanović, Academy of Applied Studies, The College of Health Sciences, Cara Dušana 254, 11000 Belgrade, Serbia; e-mail: vesna72.jovanovic@gmail.com

ORIGINALNI RAD

UDK BROJEVI:
616.98:578.834(497.16)
DOI: https://doi.org/ 10.5937/zdravzast50-33907

ZNAČAJ NACIONALNE KOORDINACIJE ZA BRZI ODGOVOR NA COVID-19 U CRNOJ GORI TOKOM PRVOG TALASA PANDEMIJE

Aleksandar Obradović[1], Marija Raičević Todorović[1], Dragan Laušević[1]

[1]Institut za javno zdravlje Crne Gore, Podgorica, Crna Gora

SAŽETAK

Uvod/Cilj: Prvi detektovani slučaj SARS-CoV-2 infekcije u Crnoj Gori zabilježen je 17. marta 2020. godine, a već 20. marta počeo je sa radom nacionalni kol-centar za COVID-19, čija je osnovna svrha bila blagovremena reakcija usljed sumnje na COVID-19, kao i pružanje podrške lokalnim epidemiološkim službama uključivanjem velikog broja prethodno edukovanih volontera kao operatera kol-centra. Cilj ove deskriptivne studije bio je prikazivanje rezultata rada nacionalne koordinacije za brzi odgovor na COVID-19 u Crnoj Gori tokom prvog talasa pandemije.

Metode: Podaci o broju realizovanih i primljenih poziva preuzeti su iz kontakt aplikacije koja se koristila u svakodnevnom radu i koja je omogućavala detaljno izvještavanje o statusu poziva, dok su podaci o anketiranim licima preuzeti iz baze nastale popunjavanjem online upitnika tokom anketiranja osoba od strane operatera kol-centra.

Rezultati: U periodu od 20.03. do 18.05.2020. godine realizovano je 27.380 poziva, od čega 16.130 dolaznih, uz prisutne dnevne varijacije (u odnosu na dnevni broj novoregistrovanih slučajeva, kao i u odnosu na to da li je riječ o radnim danima ili danima vikenda i državnih praznika). Takođe, u istom periodu je, zbog sumnje na COVID-19, anketirano je 2.288 osoba, od čega neznatno više muškaraca (50,5%). Najveći broj anketiranih lica bio je iz Podgorice (59,8%), a najzastupljenija uzrasna grupa bila je 60 ili više godina (24,9%). Od ukupnog broja registrovanih slučajeva SARS-CoV-2 infekcija u Crnoj Gori, tokom perioda obuhvaćenog studijom, njih 40,4% bilo je neposredno ili posredno povezano sa kol-centrom.

Zaključak: Nacionalna linija u koordinisanju brzog odgovora na COVID-19 ispunila je prvenstveni cilj, da bude brana dok se zdravstveni sistem ne konsoliduje i omogući smanjivanje pritiska na ograničene ljudske kapacitete, a znatan procenat detektovanih slučajeva putem linije pripomogao je brzom otkrivanju kontakata inficiranih osoba i njihovom stavljanju u karantin, što je u značajnoj mjeri doprinijelo sprečavanju širenja infekcije, pa i njenom potpunom suzbijanju.

Ključne reči: kol-centar, telefonska trijaža, COVID-19, SARS-CoV-2

Uvod

Sami kraj 2019. godine označio je ujedno i početak kraja jedne ere koju smo poznavali. Naime, godinama su vodeći zdravstveni autoriteti upozoravali na neminovnost izbijanja pandemije zaraznih bolesti dalekosežnih posljedica, a 31.12.2019. godine, kada je iz Vuhana (Kina), izviješteno o klasteru slučajeva pneumonije nepoznate etiologije, postalo je jasno da se strepnje ostvaruju (1).

Nepunih mjesec dana kasnije, 24. januara 2020. godine, Francuska je prijavila 3 slučaja kod kojih je detektovan SARS-CoV-2, u tom momentu poznat kao *Novel Coronavirus* (2019-nCoV). Sva 3 lica bila su neposredno prije obolijevanja u Vuhanu i to su ujedno bili prvi zabilježeni slučajevi COVID-19 u Evropi (2). Dan za danom slučajevi su nastavili da se detektuju i u ostalim evropskim zemljama.

Najprije zbog male populacije, a potom i zbog manje frekventnosti putnog saobraćaja van ljetnje sezone, Crna Gora je na svoj prvi detektovani slučaj čekala do 17. marta 2020. godine (posljednja zemlja u Evropi sa registrovanim slučajem SARS-CoV-2). Uprkos tadašnjim definicijama SZO za suspektni slučaj, koje su u početku bile više specifične nego senzitivne, u Crnoj Gori, u namjeri da se što prije

ORIGINAL PAPER

UDK NUMBERS:
616.98:578.834(497.16)
DOI: https://doi.org/ 10.5937/zdravzast50-33907

THE IMPORTANCE OF NATIONAL COORDINATION FOR A QUICK RESPONSE TO COVID-19 IN MONTENEGRO DURING THE FIRST WAVE OF THE PANDEMIC

Aleksandar Obradovic[1], Marija Raicevic Todorovic[1], Dragan Laucevic[1]

[1] Institute for Public Health of Montenegro, Podgorica, Montenegro

SUMMARY

Introduction/Aim: The first detected case of SARS-CoV-2 infection in Montenegro was reported on March 17th, 2020. On March 20th, the National call center for COVID-19 started operating, with the main purpose to timely respond to the suspicion of COVID-19, as well as to provide support to local epidemiological services by involving a large number of previously trained volunteers as call center operators. The aim of this descriptive study was to present the results of the national coordination for a quick response to COVID-19 in Montenegro during the first wave of the pandemic.
Methods: The data about the number of calls were taken from the contact application which enables detailed reporting on the status of calls, while data of interviewed people were taken from the database created by filling out an online questionnaire during the interview.
Results: In period from March 20th to May 18th, 2020, 27,380 calls were realized, 16.130 of which were incoming calls, with daily variations (in relation to the daily number of reported cases, as well as in relation to whether it was a working day or weekend/state holiday). Also, in the same period, due to suspicion of COVID-19, 2,288 persons were interviewed and slightly more were men (50.5%). The largest number of respondents were from Podgorica (59.8%), and from the age group 60 or older (24.9%). During the study period, 40.4% of the total number of reported cases of SARS-CoV-2 infections in Montenegro were directly or indirectly related to the Call center.
Conclusion: The national phone-line in coordinating for a quick response to COVID-19 met the primary goal, to be a „dam" until the health system consolidates, and to reduce pressure on limited human capacities. A notable percentage of detected cases through the line contributed to quicker detection of contacts of infected persons and helped quarantine them, which significantly contributed to the control of infection spreading.

Keywords: call center, phone triage, COVID-19, SARS-CoV-2

Introduction

The very end of the year 2019 was also marked as the beginning of the end of an era we knew. For years, leading health authorities have been warning about the inevitability of infectious diseases pandemic with far-reaching consequences, and on December 31st 2019, when a cluster of pneumonia cases with unknown etiology was reported in Wuhan (China), it became clear that the predictions were accurate (1).

Less than a month later, on January 24th, 2020, France reported 3 cases of SARS-CoV-2, at that time known as Novel Coronavirus (2019-nCoV). All three were travelers who arrived from Wuhan just before the disease onset and these were the first reported cases of COVID-19 in Europe (2). Day after day, cases continued to be detected in other European countries as well.

Mainly due to the small population and lower international traffic frequency outside the summer season, Montenegro waited for its first detected case until March 17th, 2020 (the last country in Europe with a detected case of SARS-CoV-2). Despite the WHO definitions of a suspected case from that period of time, which were initially more specific than sensitive (included recent history of traveling to China), in Montenegro, in order to identify a possible imported case as soon as possible, every case of hospital pneumonia with

identifikuje mogući importovani slučaj, testirao se i svaki slučaj hospitalne pneumonije nerazjašnjene etiologije. Međutim, sve do 17. marta nije detektovan nijedan slučaj SARS-CoV-2 u Crnoj Gori (3).

Kako je postajalo jasno da je pitanje dana kada će se otkriti prvi slučaj COVID-19, Institut za javno zdravlje Crne Gore (IJZCG) je 12. marta 2020. godine, shodno instrukcijama tima koji je kreirao Komunikacionu strategiju COVID-19, započeo rad na uspostavljanju jedinstvene nacionalne linije za koordinaciju brzim odgovorom na COVID-19 (4). U narednim danima, napravljena je infrastruktura, instalirana oprema, kreirani trijažni upitnik i detaljni algoritam o postupanju u slučaju sumnje na COVID-19. Nakon izrade navedenog, sprovedena je edukacija o postupanju po kreiranom algoritmu, kojoj su prisustvovali predstavnici IJZCG, Kliničkog centra Crne Gore, lokalnih Higijensko-epidemioloških službi i timova izabranih doktora domova zdravlja, kao i Zavoda za hitnu medicinsku pomoć iz čitave Crne Gore.

Nacionalni kol-centar za COVID-19 (SOS linija 1616) počeo je sa radom 20.03.2020. godine (radno vrijeme od 08.00 do 23.00, svakog dana, uključujući dane vikenda i državne praznike). U centru su zajedno radili operateri i dežurni epidemiolozi/ljekari iz IJZCG.

Obuku za operaterski rad u kol-centru prošlo je četrdeset devet studenta Medicinskog fakulteta u Podgorici. Rad operatera bio je organizovan u 3 smjene po 5 sati, a rad dva epidemiologa i pet ljekara drugih javnozdravstvenih specijalnosti bio je organizovan u 2 smjene.

Ovako osmišljena SOS linija 1616 imala je više ciljeva. Jedan od prvih ciljeva bio je da se povratnici u zemlju, koji su stigli prije stupanja na snagu mjera obaveznog stavljanja pod zdravstveno-sanitarni nadzor, ukoliko dolaze iz zemalja gdje su registrovani slučajevi SARS-CoV-2, usmjere na jedinstveni broj gdje će dobiti potrebne instrukcije, a ukoliko razviju simptome koji bude sumnju na COVID-19 da se organizuje i testiranje. Osobe koje su pozivale iz nekog od sljedećih razloga: a) povratnici iz inostranstva unazad 2 do 4 sedmice i tom prilikom su posjetili neku od zemalja sa uspostavljenom lokalnom transmisijom (operateri su imali pred sobom listu zemalja ažuriranu u realnom vremenu), b) osobe koje sumnjaju ili znaju da su bile u kontaktu sa SARS-CoV-2 pozitivnom osobom, a od momenta uspostavljanja lokalne transmisije i c) sve osobe sa simptomima COVID-19, bivale su anketirane korišćenjem trijažnog upitnika sa setom skorovanih pitanja koji je omogućavao da se kreira lista prioriteta za povratne pozive od strane ljekara angažovanih na liniji.

Prilikom povratnog poziva ljekari su uzimali detaljnu anamnezu i spisak kontaka koje je osoba ostvarila 48 do 72 časa prije nastanka simptoma ili dobijanja pozitivnog nalaza kod asimptomatskih lica. Po zaključenom razgovoru ljekari bi, shodno situaciji, osobi saopštavali šta su sljedeći postupci po predviđenom algoritmu, a onda bi kontaktirali zdravstveno-sanitarnu inspekciju sa ciljem da je informišu da je licu neophodno izdati rješnje o izolaciji odnosno karantinu, ali i lokalnu epidemiološku službu sa ciljem da prenesu instrukcije o daljem postupanju sa licem (testiranju, neophodnosti zdravstvenog pregleda, transprorta u bolnicu i sl).

Osim centralne koordinacije na državnom nivou i blagovremene reakcije usljed sumnje na COVID-19, na ovaj način se postigla i velika ušteda kadra. Upotrebom trijažnog upitnika od strane studenata volontera značajno se smanjio pritisak na ljekarski kadar, što je imalo posebnu važnost jer su kolege iz lokalnih epidemioloških službi, tokom prva dva mjeseca, za svaki sumnjivi slučaj, u pratnji tehničara izlazili na teren.

Takođe, sistem je od samog početka, zbog svjesnosti da će od momenta detektovanja prvog slučaja do uspostavljanja lokalne transmisije proći najviše dvije sedmice, bio uspostavljen tako da ga je lako moguće transformisati davanjem prioriteta simptomima u odnosu na pozitivnu epidemiološku anamnezu (putovanje u inostranstvo ili kontakt sa licem koje se nedavno vratilo iz inostranstva), o čemu se vodilo računa prilikom kreiranja upitnika i algoritma o postupanju.

Cilj ove deskriptivne studije bio je prikazivanje rezultata nacionalne koordinacije za brzi odgovor na COVID-19 u Crnoj Gori tokom prvog talasa COVID-19 pandemije.

Metod

U ovoj deskriptivnoj studiji opisan je rad nacionalne telefonske linije 1616 za SARS-CoV-2 tokom perioda od 20.03. do 18.05.2020. godine, odnosno tokom prvog talasa novog korona virusa u Crnoj Gori.

Podaci o broju realizovanih i primljenih poziva preuzeti su iz komercijalne kontakt aplikacije koja se koristila u radu i koja je omogućavala detaljno izvještavanje o statusu poziva po danima i operateri-

unexplained etiology was tested. However, no SARS-CoV-2 cases were detected in Montenegro until March 17th (3).

As it became clear that it is a matter of days when the first case of COVID-19 will be discovered, on March 12th, 2020 the Institute for Public Health of Montenegro (IPH), in accordance with the instructions from COVID-19 Communication Strategy team, began working on the establishment of a unique national coordination line in a quick response to COVID-19 (4). In the following days, the infrastructure was built, the equipment was installed, a triage questionnaire was created and a detailed algorithm was developed on how to act in the event of suspicion of COVID-19. In addition, training on how to act according to the developed algorithm was conducted. That training was attended by representatives of IPH, Clinical Center of Montenegro, local Epidemiological Services and teams of general practitioners from primary health centers, as well as emergency medicine doctors from Montenegro.

The National Call Center for COVID-19 (CC 1616) started operating on March 20th, 2020 (working hours from 08:00 to 23:00, every day, including weekends and state holidays). Operators and on-duty epidemiologists/medical doctors from IPH worked together at the CC 1616.

Forty-nine students from the Medical Faculty in Podgorica were trained for operator work in the CC 1616. The operators were organized in 3 shifts per 5 hours, and two epidemiologists and five doctors of other public health specialties were organized in 2 shifts.

The CC 1616 work designed in this way had several goals. One of the main objectives was to direct returnees from countries with reported SARS-CoV-2, that arrived in Montenegro before health surveillance measures became mandatory, to a unique phone number where they could receive the necessary instructions, and, if they develop symptoms that are suspected of COVID-19 to organize testing. Persons who made calls for any of the following reasons: a) returnees from abroad in the past 2 to 4 weeks and who visited one of the countries with established local transmission (operators had a list of countries updated in real time), b) persons who suspect or know that they have been in contact with a SARS-CoV-2 positive person, and, since the moment of established local transmission c) all persons with COVID-19 symptoms have been interviewed using a triage questionnaire with a set of scoring questions that allowed to create a priority list for callbacks by physicians engaged on the line.

During the call back, the physicians took a detailed anamnesis and a list of contacts that the person had 48 to 72 hours before the onset of symptoms or before a positive test in asymptomatic individuals. After the conversation, the physicians would, according to the situation, tell the person what the next procedures are according to the algorithm, and then they would contact the Health and Sanitary Inspection in order to inform them that the person needs to be mandatory isolated or quarantined, but also they would contact the local epidemiological service to give instructions on further treatment of the person (testing, necessity of medical examination, transport to the hospital, etc.).

Apart from the central coordination at the state level and the prompt reaction in the event of COVID-19 suspicion, in this way of work organization great staff savings were achieved. The usage of the triage questionnaire by student volunteers significantly reduced the pressure on and need for the medical staff, which was especially important because colleagues from local epidemiological services, during the first two months of outbreak, went out to the field accompanied by technicians, in every case of COVID-19 suspicion.

Also, from the very beginning, due to the awareness that a maximum of two weeks will pass from the moment of detection of the first case to the established local transmission, the system was designed so it can be easily transformed by prioritizing symptoms over positive epidemiological history (travel abroad or contact with a person who has recently returned from abroad), which was taken into account when creating the questionnaire and the algorithm on the procedure.

The objective of this descriptive study was to present the results of national coordination in quick response to COVID-19 in Montenegro during the first wave of the COVID-19 pandemic.

Methods

This descriptive study describes the operation of the national phone line 1616 for SARS-CoV-2 in the period from March 20th to May 18th, 2020.

Numbers of realized and received calls were taken from the commercial contact application

ma (propušteni i realizovani dolazni i odlazni pozivi). Anketiranje osoba sprovodilo se tako što je operater tokom razgovora popunjavao online upitnik kreiran u javno dostupnom servisu *Google Forms*, a rezultati iz uptinika bili su vidljivi korišćenjem, takođe javno dostupnog servisa, *Google Sheets*.

Podaci o pojedinačnim novoregistrovanim slučajevima dostavljani su ljekarima angažovanim u kol-centru koji su svakog dana vršili provjere da li među njima ima onih koji su inicijalno kontaktirali liniju 1616 ili su navedeni kao kontakti od strane onih koji su se javili, pa su zbog toga bili naknadno kontaktirani. Takođe, prilikom povratnih razgovora ljekara sa osobama kod kojih je upitnik pokazao da postoji sumnja na COVID-19, popunjavan je zaseban upitnik (koji nije bio trijažnog karaktera već je više bio vodič kroz ranije osmišljeni algoritam) gdje su, između ostalih, unošeni i podaci o ostvarenim kontaktima u vremenu za koje se smatralo da su te osobe mogle biti rizične po okolinu.

Za izradu grafikona korišćen je *Excel* iz paketa *Microsoft Office* 2016, dok je za statističku obradu podataka, prije svega za izračunavanje koeficijenta korelacije, korišćen *EZR* (*Easy R*) *plugin* (verzija 1.42) na *R Commander*-u (verzija 2.6-2).

Rezultati

Od pokretanja SOS linije 1616, 20.3.2020. godine, zaključno sa 18.05.2020. godine (60 dana), ostvareno je 27.380 poziva (dolazni+odlazni).

Dnevno je bilo prosječno 456 poziva, uz prisutne varijacije, najmanje 91 (nedelja 17.05.2020. godine), a najviše 1.400 (srijeda 25.03.2020. godine). Broj poziva varirao je od dana do dana, tj. ako se nešto aktuelno dešavalo (npr. objavljivanje većeg broja osoba koje su pozitivne) rastao je i broj poziva i suprotno (Grafikon 1). Pokazalo se da je postojala jaka pozitivna korelacija između broja realizovanih poziva i broja novoregistrovanih slučajeva po danima (r_s= 0,73, p < 0,01) (Tabela 1).

Takođe, evidentan je i pad broja poziva tokom vikenda i državnih praznika, pa tako ako posmatramo dane vikenda i državne praznike, prosječan broj poziva je 345, dok je tokom radnih dana prosječan broj poziva 516.

U periodu od 20.03. do 18.05.2020. godine u Crnoj Gori registrovano je ukupno 311 slučajeva SARS-CoV-2 infekcije, od čega je njih 126 (40,4%) detektovano ili navedeno kao kontakt inficiranih lica inicijalno detektovanih preko linije 1616 (Grafikon 2).

U naznačenom periodu primljeno je 16.130 poziva, prosječno 269 dolaznih poziva dnevno, uz prisutne varijacije u broju primljenih poziva, najmanje 54 (nedelja 17.05.2020. godine), a najviše 774 (srijeda 25.3.2020. godine) (Grafikon 3).

Broj osoba za koje su operateri procijenili da je neophodno da ih provedu kroz upitnik, tj. broj anketiranih osoba bio je 2.288, odnosno prosječno 38 anketiranih osoba dnevno, uz prisutne varijacije, najmanje 6 (nedelja 10.05.2020. godine), a najviše 133 (petak 20.03.2020. godine) (Grafikon 3).

Procenat trijažiranih osoba bio je 14,2% (procenat ukupno anketiranih osoba u odnosu na ukupan broj primljenih poziva), najmanje 5,2% (subota 09.05.2020. godine), a najviše 27,6% (petak 20.03.2020. godine).

Pokazalo se da ne postoji koorelacija između procenta trijažiranih osoba i broja dolaznih poziva (p > 0,05) (Tabela 1).

Od momenta kada je uspostavljena lokalna transmisija u Crnoj Gori (negdje početkom druge sedmice rada nacionalne linije), sve osobe, nezavisno od težine simptoma, a koje su anketirane od strane operatera, bile su i pozvane od strane epidemiologa/ljekara iz kol-centra, i to unutar sat vremena od njihovog poziva SOS liniji 1616.

Daleko najveći procenat anketiranih osoba bio je iz Podgorice (59,8%), slijede Nikšić, Herceg Novi, Bar, Danilovgrad, Budva i Tuzi (Grafikon 4).

Od ukupnog broja anketiranih osoba, neznatno je više bilo muškaraca 1.156 (50,5%) (Grafikon 5).

Prosječan uzrast anketiranih osoba iznosio je 44 godine. Najveći procenat anketiranih osoba,

Tabela 1. Uporedni prikaz koeficijenata korelacije

Korelacije	r_s* (broj novoregistrovanih slučajeva)	r_s* (procenat trijažiranih osoba)
Realizovani pozivi po danu	0,73 (p<0,01)	0,23 (p>0,05)

*Spirmanov koeficijent korelacije sa p vrijednošću

which was used in the work and which enabled detailed reporting on call status by days and operators (missed and realized incoming and outgoing calls). Interviews of persons were conducted by the operator filling out an online questionnaire created in the publicly available Google Forms service, and the results from the questionnaires were visible in also publicly available service, Google Sheets.

Data on individual newly reported cases per day were provided to epidemiologists from CC 1616 who checked daily for those who had initially contacted the 1616 line or were listed as contacts by those who called and were subsequently contacted. Also, during the physician's callback to persons for whom the questionnaire showed a suspicion of COVID-19, a separate questionnaire was filled out (which did not have a triage character, but was more of a guide through a previously designed algorithm) where, among other information, data about contacts, made during the period of time when these persons could be considered as risky for the environment, were entered.

Excel from Microsoft Office 2016 was used to create charts, while the EZR (Easy R) plugin (version 1.42) on R Commander (version 2.6-2) was used for statistical data processing, primarily for calculating the correlation coefficient.

Results

Since the launch of the CC 1616, on March 20th, 2020, ending on May 18th, 2020 (60 days), 27.380 calls were made (incoming and outgoing).

In average, there were 456 calls per day, with variations present, a minimum of 91 (Sunday, May 17th, 2020) and a maximum of 1,400 (Wednesday, March 25th, 2020). The number of calls varied from day to day, i.e. if something currently happened (e.g. publishing a higher number of cases), the number of calls increased and vice versa (Figure 1). It was shown that there was a strong positive correlation between the number of realized calls and the number of reported cases per day (r_s = 0.73, p <0.01) (Table 1).

Also, there was an evident decrease in the number of calls during weekends and state holidays, so if we observe weekends and state holidays, the average number of calls is 345 per day, while during working days the average number of calls is 516.

During the period from March 20th to May 18th, 2020, a total of 311 cases of SARS-CoV-2 infection were reported in Montenegro, of which 126 (40,4%) were detected or listed as contacts of cases initially detected via phone-line 1616 (Figure 2).

In the indicated period, 16,130 calls were received, 269 incoming calls per day in average, with present variations in the number of received calls, minimum 54 (Sunday, May 17th, 2020), and maximum 774 (Wednesday, March 25th, 2020) (Figure 3).

The number of persons for whom operators estimated that it was necessary to lead them through the questionnaire, i.e. the number of interviewed persons was 2,288 or 38 persons per day in average, with variations present, minimum 6 (Sunday, May 10th, 2020) and maximum 133 (Friday, March 20th, 2020) (Figure 3).

The percentage of triaged persons was 14.2% (percentage of total number of interviewed persons in relation to the total number of received calls), minimum 5.2% (Saturday, May 9th, 2020), maximum 27.6% (Friday, March 20th, 2020).

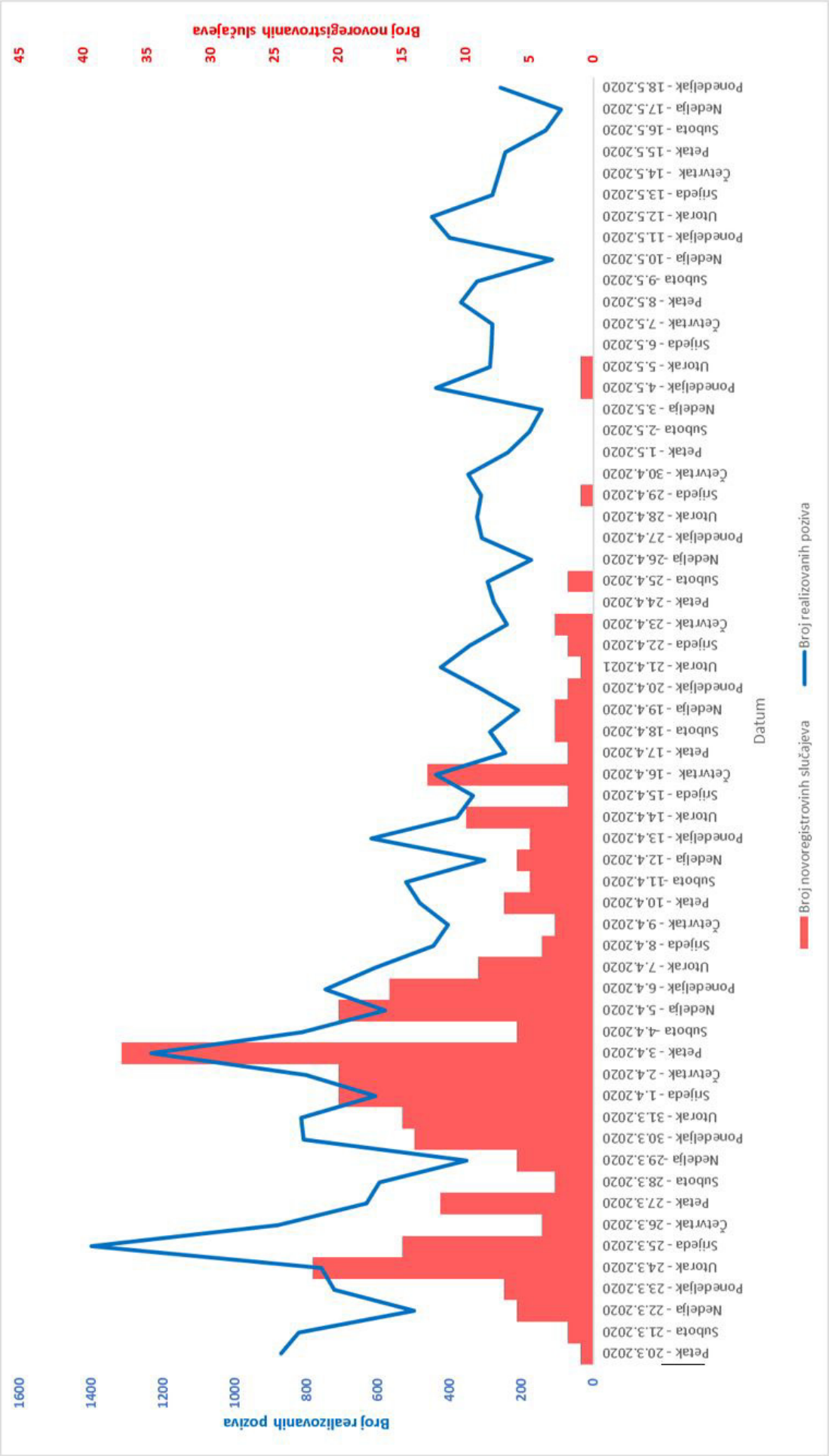
There was no correlation between the percentage of triaged persons and the number of incoming calls (p > 0.05) (Table 1).

From the moment when local transmission was established in Montenegro (somewhere in the beginning of the CC 1616 second week of work), all persons, regardless of the severity of symptoms, who were interviewed by the operator, were also called by an epidemiologist/medical doctor from CC 1616, within an hour.

Table 1. Comparative view of correlation coefficients

Correlation	r_s* (daily reported cases)	r_s* (percentage of triaged persons per day)
Realized calls per day	0.73 (p<0,01)	0.23 (p>0,05)

*Spearman correlation coefficient with p value



Grafikon 1. Uporedni prikaz broja realizovanih poziva (dolazni i odlazni) i broja novoregistrovanih slučajeva COVID-19 u Crnoj Gori, period 20.03-18.05.2020. godine

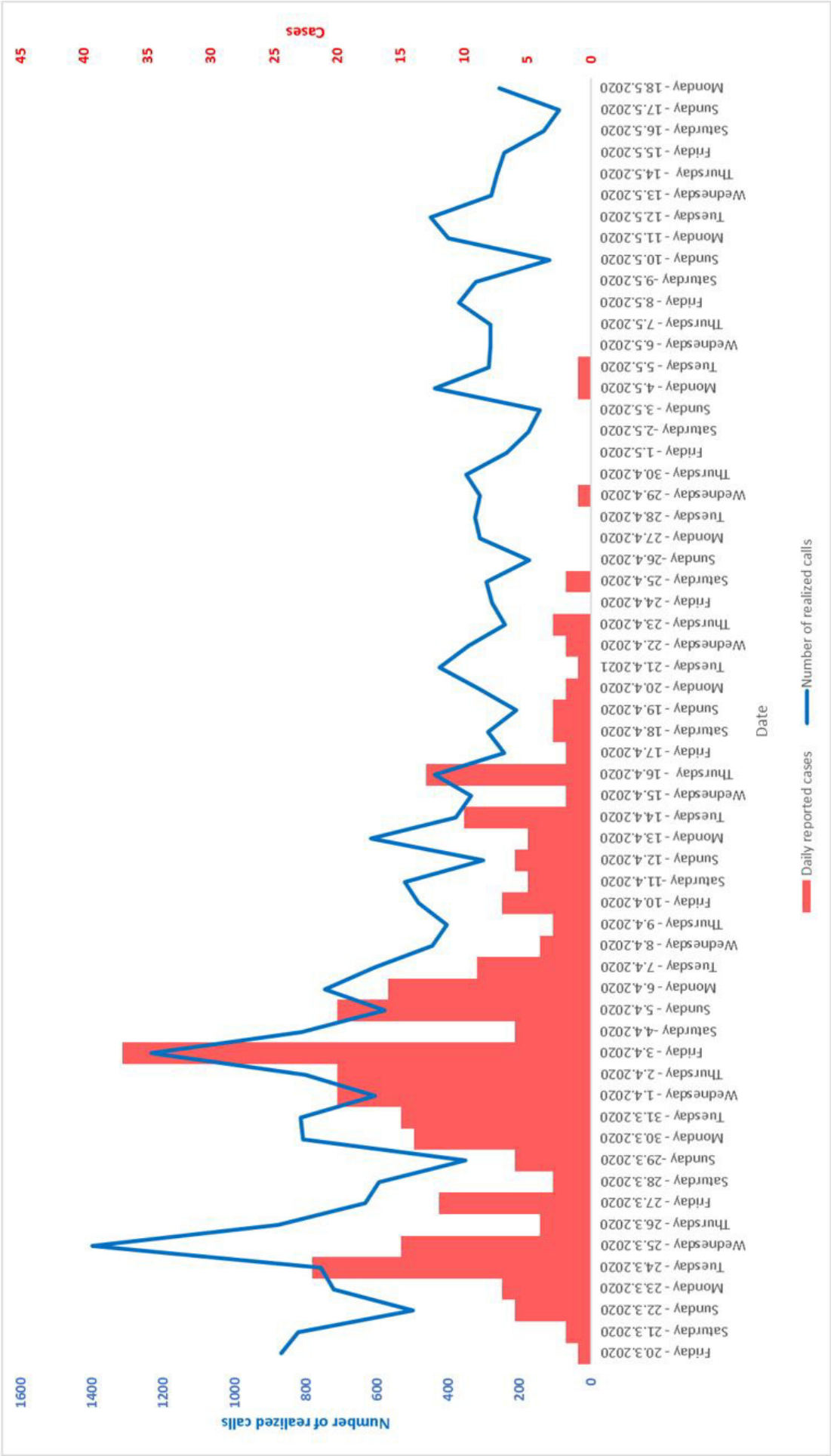
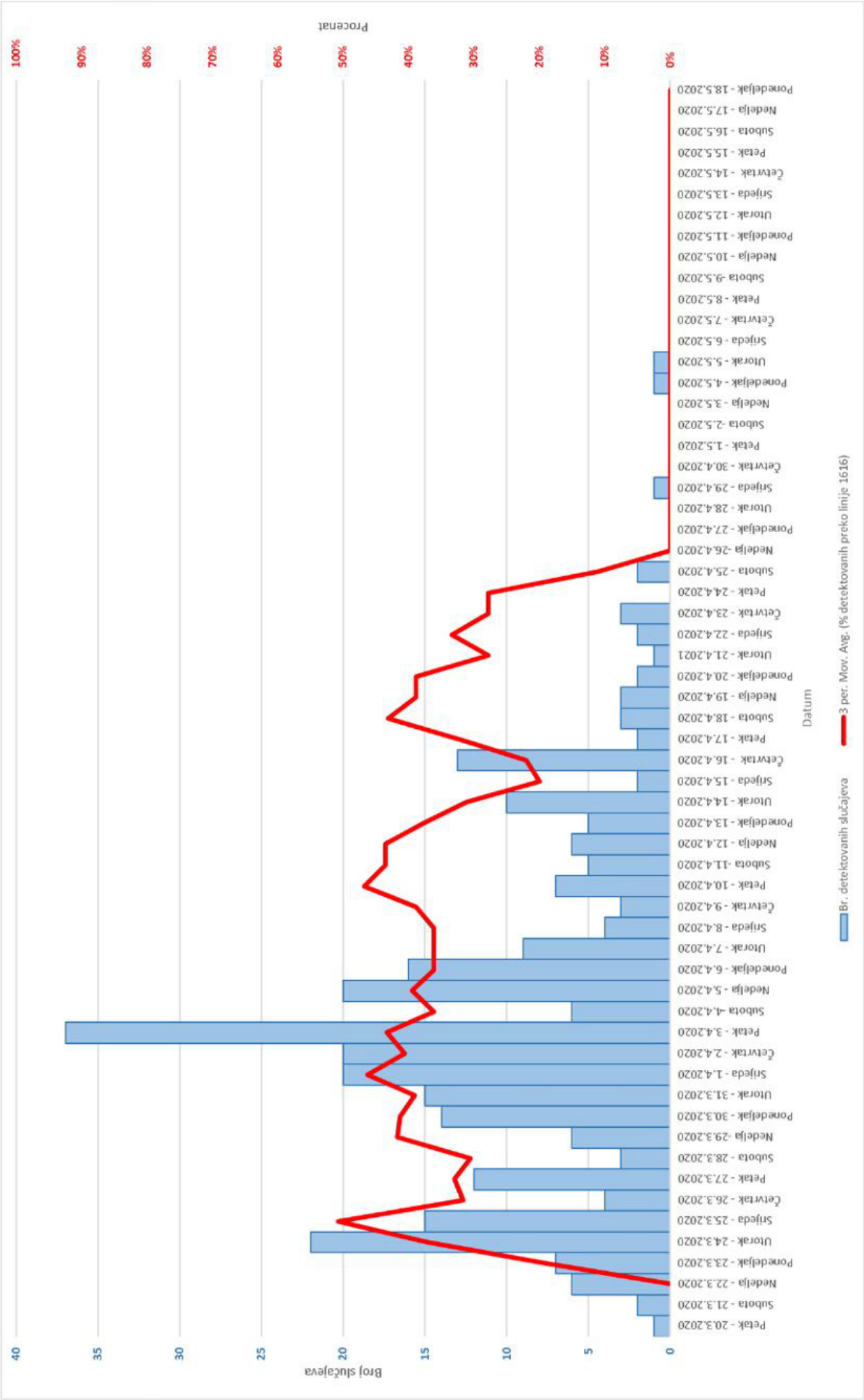


Figure 1. Comparative view of number of realized calls (incoming and outgoing) and number of daily reported cases of COVID-19 in Montenegro, 20 March-18 May 2020.



Grafikon 2. Uporedni prikaz novoregistrovanih slučajeva i procentualnog udjela onih koji su inicijalno kontaktirali SOS liniju 1616, period 20.03-18.05.2020. godine

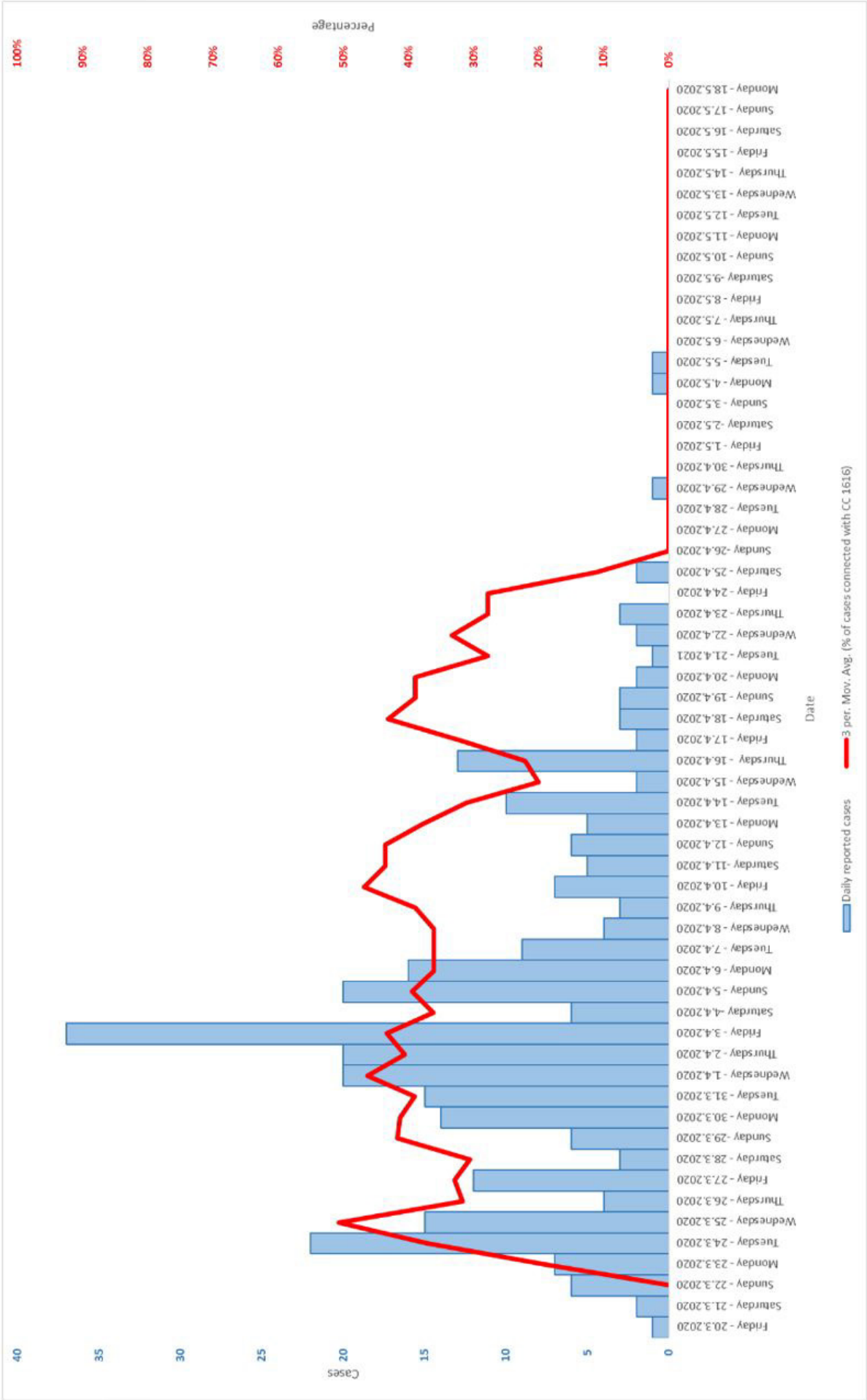
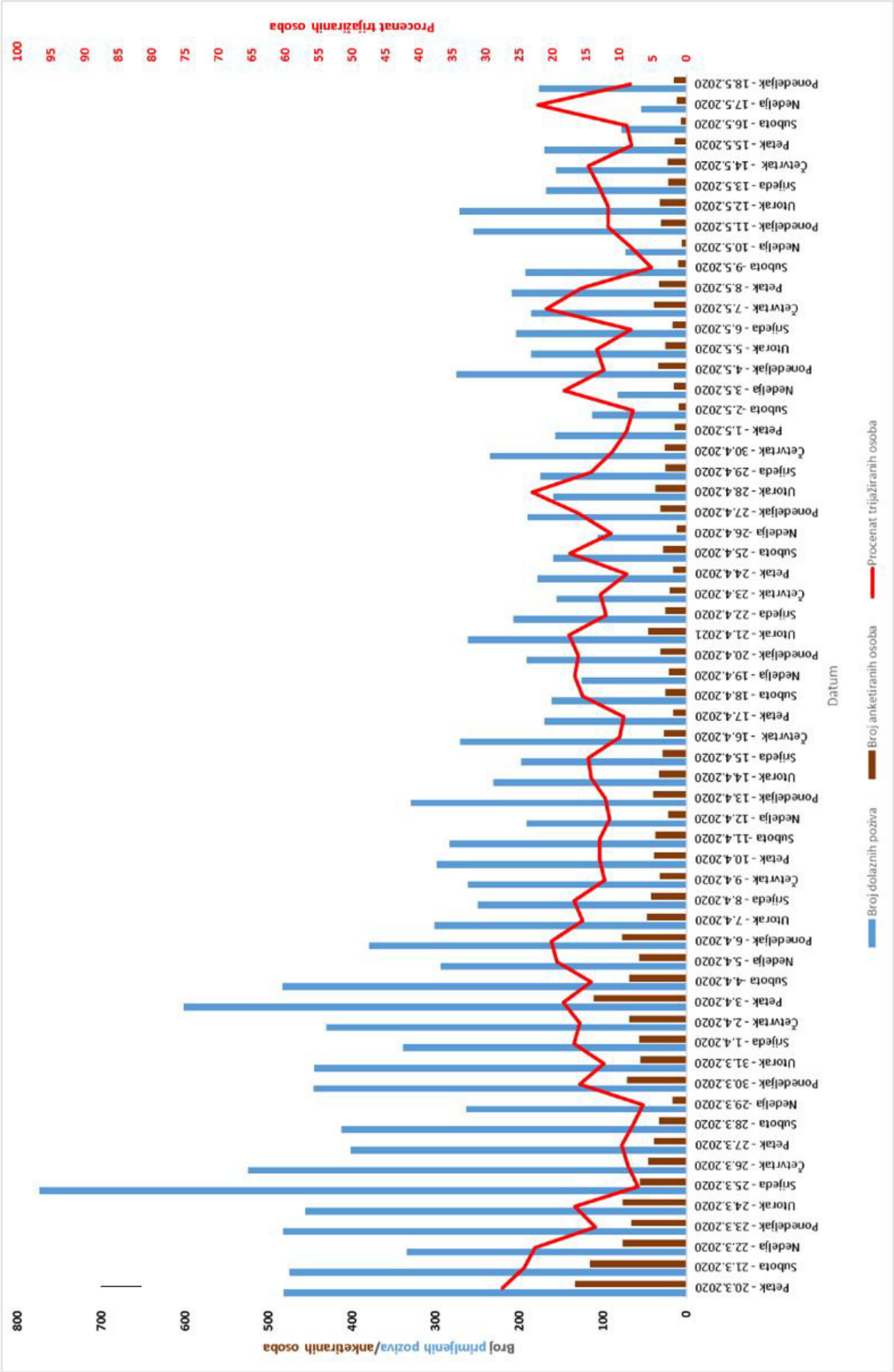


Figure 2. Comparative view of daily reported cases and percentage of cases connected with CC 1616, 20 March-18 May 2020.



Grafikon 3. Uporedni prikaz broja dolaznih poziva, anketiranih osoba i procenat trijažiranih osoba po danima (procenat anketiranih osoba u odnosu na broj primljenih poziva po danima), period 20.03-18.05.2020. god

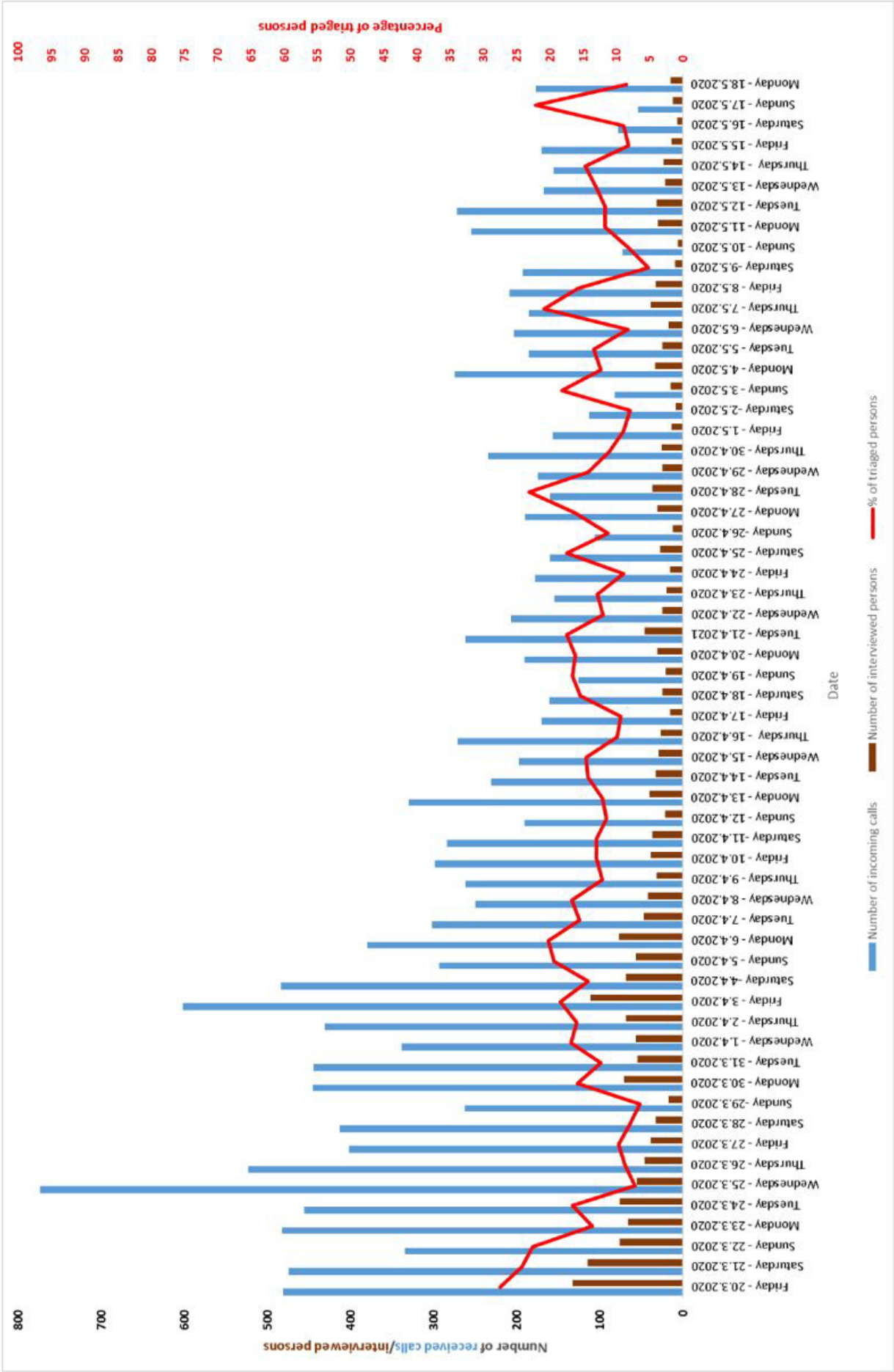
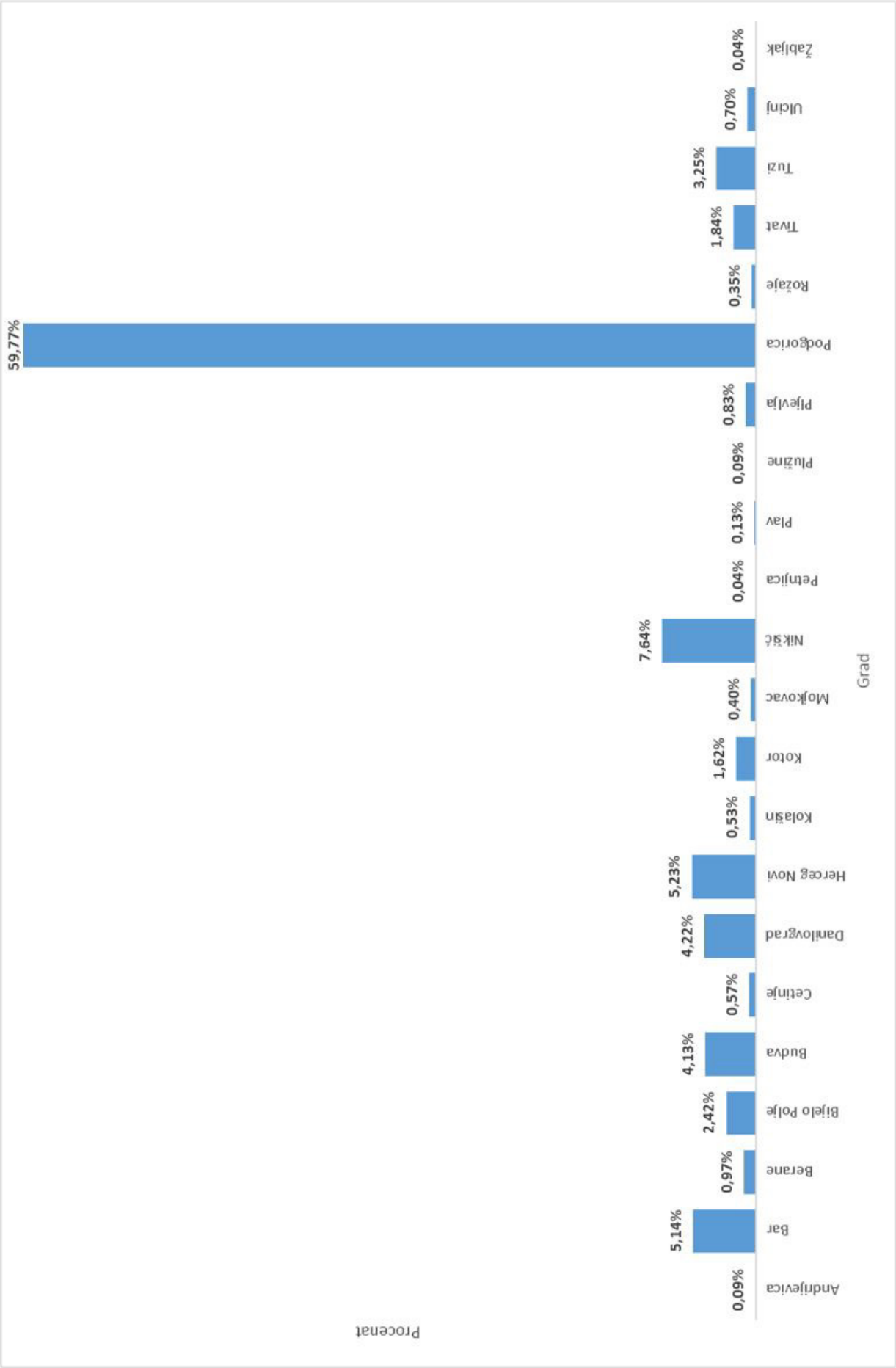


Figure 3. Comparative view of number of incoming calls, number of interviewed persons and percentage of triaged persons per day (percentage of number of interviewed persons per day in relation to the number of received calls per day), 20 March-18 May 2020.



Grafikon 4. Procentualna zastupljenost anketiranih osoba u odnosu na opštine iz kojih su pozivali, period 20.03-18.05.2020. godine

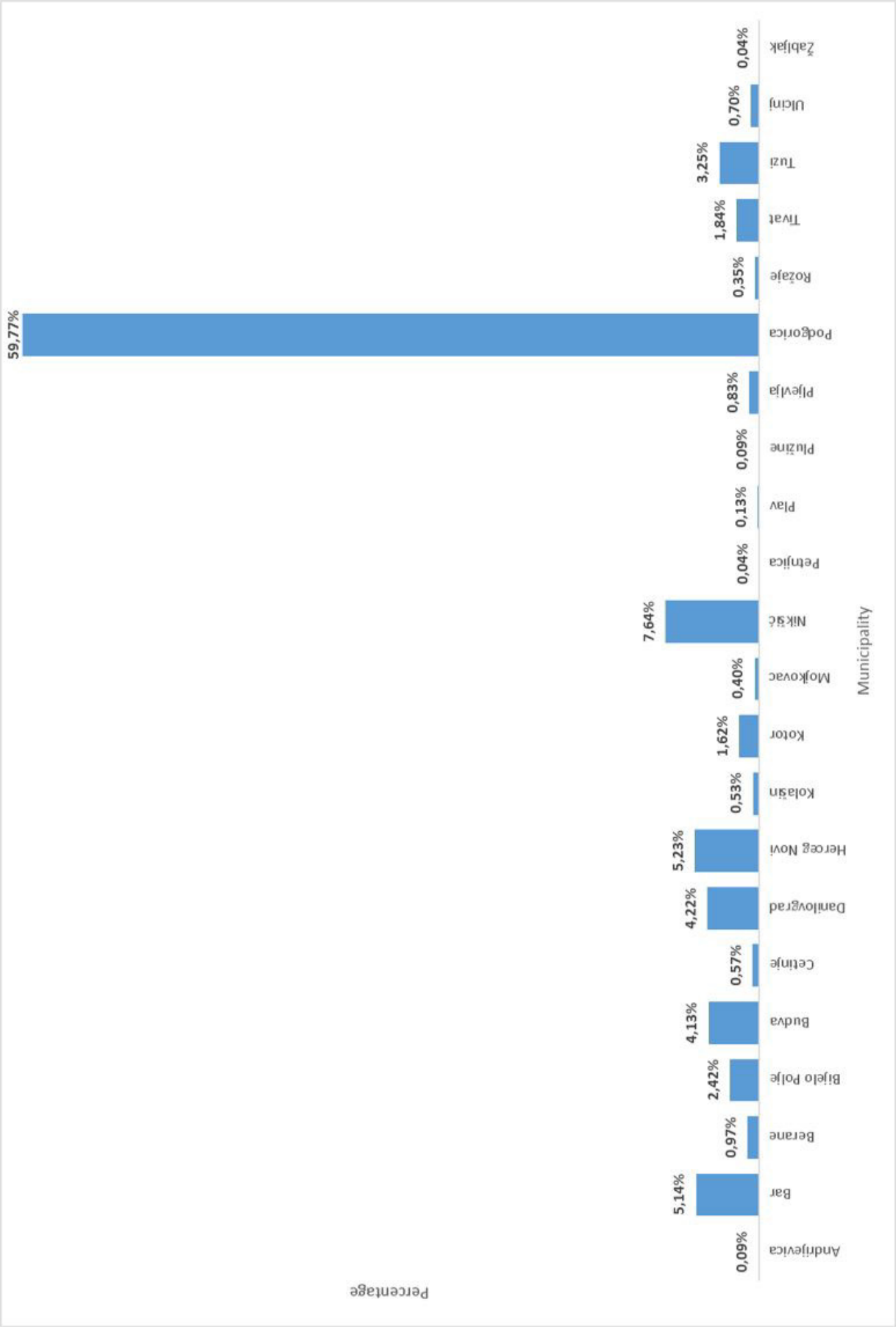
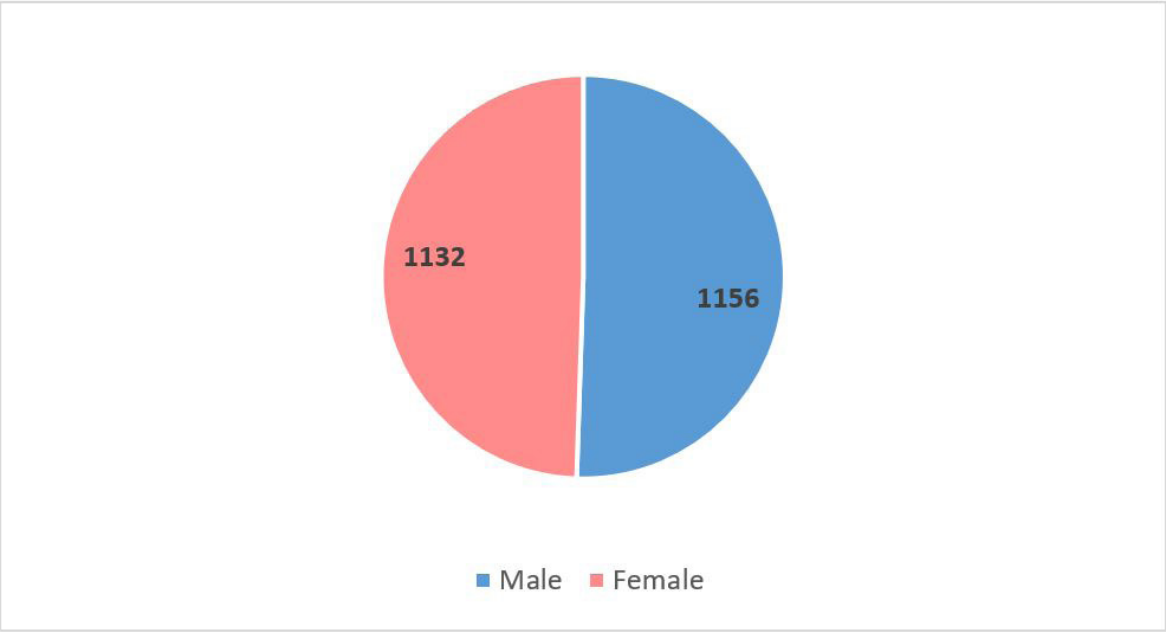
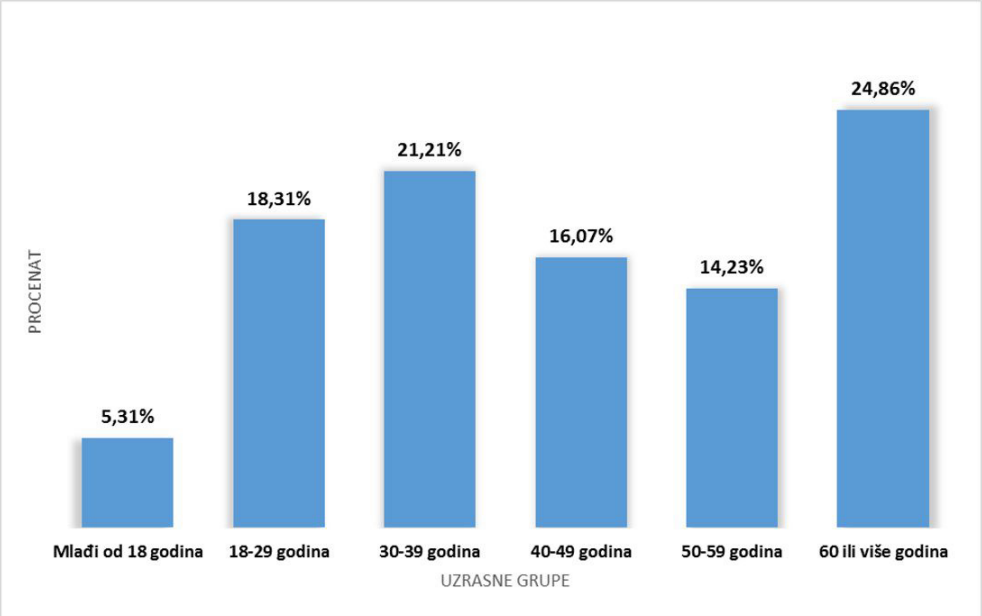


Figure 4. Distribution among interviewed people according to municipality, 20 March-18 May 2020.



Grafikon 5. Polna struktura anketiranih osoba, period 20.03-18.05.2020. godine



Grafikon 6. Uzrasna distribucija anketiranih osoba, period 20.03-18.05.2020. godine

24,9%, bio je iz uzrasne grupe 60 ili više godina starosti, a najmanji, 5,3%, iz uzrasne grupe mlađi od 18 godina (Grafikon 6).

Diskusija

Po izbijanju COVID-19 pandemije, prije ili kasnije, većina zemalja uspostavila je sisteme za samoprijavljivanje sumnje na COVID-19. Ti sistemi varirali su od online trijažnih upitnika, preko linija nalik opisanoj, ali autorima ovog članka nije poznato da je, u tom trenutku, postojao sistem sa sličnim povratnim mehanizmom reakcije, odnosno centralna, nacionalna, koordinacija lokalnih epidemioloških službi. Naravno, to je u Crnoj Gori bilo moguće i smisleno jer, u momentu započinjanja rada SOS linije 1616, nije bila uspostavljena lokalna transmisija, a po uspostavljanju iste, između ostalog i korišćenjem ove linije, spriječena je širokopojasna transmisija (5).

Navedena linija inicirala je opsežna i pravovremena istraživanja kontakata, gdje su se zbog malog broja detektovanih slučajeva, pod zdravstveno-sanitarni nadzor i u karantin stavljala i ona lica čiji je ostvareni konakt sa SARS-CoV-2 pozitivnim

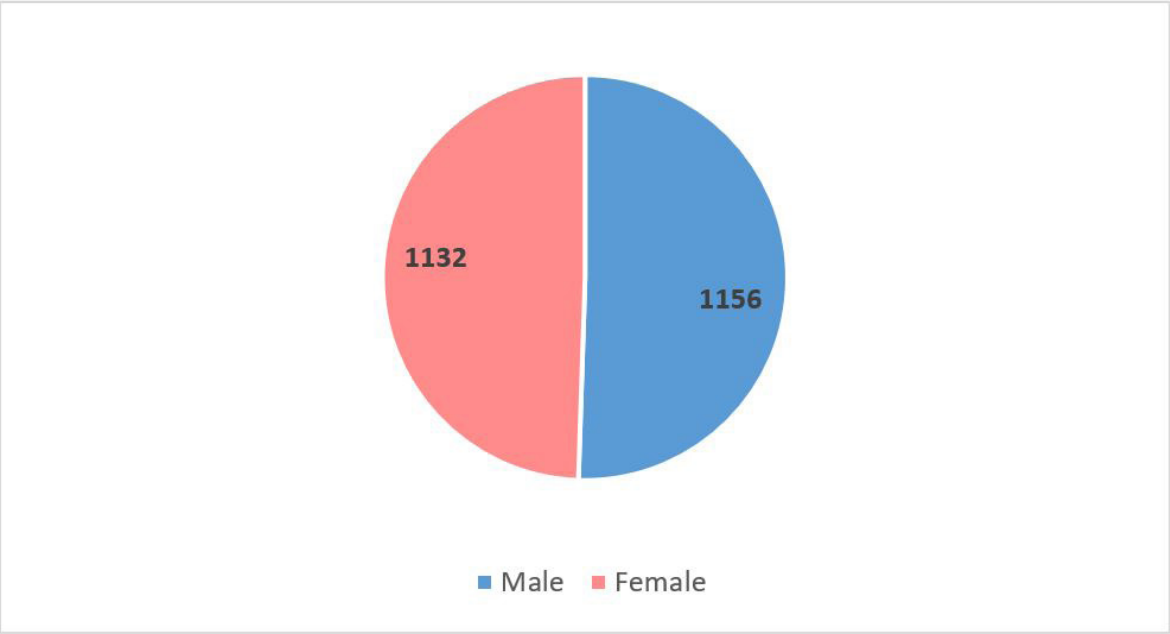


Figure 5. Sex distribution among interviewed persons, 20 March-18 May 2020.

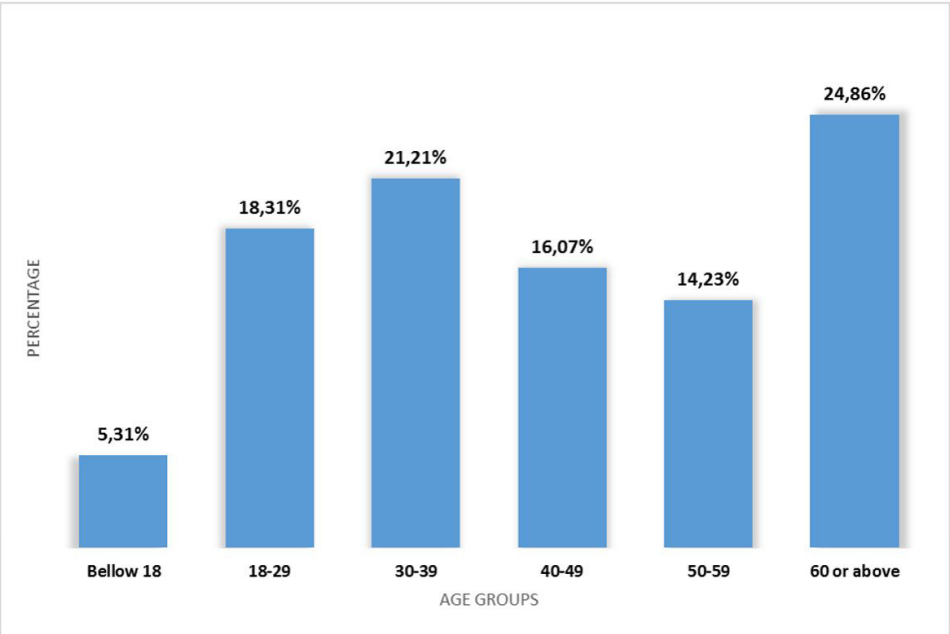


Figure 6. Age distribution among interviewed persons, 20 March-18 May 2020.

The largest percentage of interviewed persons were from Podgorica (59.8%), followed by Niksic, Herceg Novi, Bar, Danilovgrad, Budva and Tuzi (Figure 4).

From total number of interviewed persons, slightly more were men, 1,156 (50.5%) (Figure 5).

The average age of the interviewed persons was 44. The highest percentage of interviewed persons, 24.9%, was from the age group 60 or older, and the lowest, 5.3%, from the age group younger than 18 (Figure 6).

Discussion

Following the beginning of COVID-19 pandemic, sooner or later, most countries established systems for self-reporting suspicions of COVID-19. These systems ranged from online triage questionnaires to phone-lines similar to the one described in this article, but the authors of this article are not aware that, at the time covered by this study, there was a system with a similar feedback mechanism, i.e. central national coordination of local epidemiological services.

licima bio manjeg do umjerenog rizika (6).

Aktuelna epidemiološka situacija diktirala je i prirodu rada SOS linije 1616, pa se tako već u drugoj sedmici utvrdio „proboj" inicijalnog algoritma jer je pronađena osoba sa negativnom anamnezom putovanja u zemlje gdje je uspostavljena lokalna transmisija i koja je negirala kontakte sa licima koja su se nedavno vratila iz takvih zemalja. Ta osoba inicijalno je kontaktirala nacionalnu liniju zbog simptoma sumnjivih na COVID-19, ali je zbog negativne epidemiološke anamneze upućena na pregled umjesto na dalju epidemiološku obradu. U daljem toku bolesti ispostavilo se da je lice pozitivno na SARS-CoV-2, te je time dokazan prvi slučaj lokalne transmije nepoznatog porijekla. To je iniciralo momentalnu promjenu protokola i od tog dana su sva lica koja su kontaktirala SOS liniju 1616 i požalila se na simptome koji su budili sumnju na COVID-19 bila kontaktirana od strane ljekara sa linije, gdje se u opsežnom razgovoru procjenjivao rizik da li se radi o realnoj sumnji ili je veća vjerovatnoća da je riječ o nekom drugom uzročniku. Ne treba gubiti iz vida činjenicu da su u tom momentu laboratorijski kapaciteti za obradu PCR testova na SARS-CoV-2 u Crnoj Gori bili veličine više desetina obrađenih testova dnevno.

Naravno, bilo je jasno od samog početka da će, ako dođe do uspostavljanja širokopojasne transmisije, telefonska trijaža izgubiti svaki značaj i sistem morati u nastavku da se bazira na kliničkoj trijaži na nivou primarne zdravstvene zaštite, to jest u takozvanim COVID ambulantama. Međutim, prije svega zbog dobre komplijanse opšte populacije, ali i zbog izuzetno strogih mjera, nije došlo do razbuktavanja epidemije u Crnoj Gori i 04.05.2020. godine, 49 dana od prvog, detektovan je posljednji slučaj COVID-19 u prvom talasu (7).

Interesovanje za SOS liniju 1616 i njena uloga bili su značajniji na samom početku njenog funkcionisanja što se može vidjeti i po značajnom broju realizovanih poziva dok je kasnije, što zbog potencijalnog smirivanja situacije, što zbog dodatnog uključivanja lokalnih epidemioloških službi, došlo do pada interesovanja. Takođe, linija je uprkos svom inicijalnoj postavci imala i značajnu dozu informativno-edukativnog karaktera, jer je od starta bila zamišljena i kao dodatni komunikacioni kanal IJZCG (4).

Kapacitet linije od maksimalno 20 poziva u svakom trenutku i bilježenje propuštenih poziva, učinili su da se operateri odazovu na apsolutno svaki poziv upućen liniji.

Najveći izazov SOS linije 1616 ogledao se u manjem broju ljekara koji je svakodnevno radio iscrpljujući i odgovoran posao, obavljajući više desetina zahtjevnih telefonskih poziva, posebno imajući u vidu da je anksioznost osoba koje su sumnjale da su bile izložene uzročniku COVID-19 u prvim danima bila značajno pojačana usljed opšteg straha od nepoznatog. Nije bilo posebnih priprema ljekara za ovaj posao, tako da su se ovi izazovi savladavali u hodu (8,9).

Takozvani „efekat vikenda", tj, smanjenje, kako broja realizovanih poziva, tako i detektovanih slučajeva, primjetno je bio izražen uprkos tome što je linija svakog dana radila od 8-23 časa (10). Sa druge strane, postojanje jake pozitivne korelacije između broja ostvarenih poziva i novoregistrovanih slučajeva ne čudi jer je povećanjem broja slučajeva rastao i rizik po okruženje, a shodno tim i broj osoba koje su potencijalno bile u kontaktu sa SARS-CoV-2 pozitivnim licima.

Visok procenat osoba detektovanih preko SOS linije 1616 odraz je više stvari. Naime, usljed straha od nepoznatog, opšta populacija je u IJZCG prepoznala zdravstveni autoritet na koji se mogu osloniti, a sa druge strane zabrana putovanja i stroge opšte mjere koje su uslovile i manju mobilnost ljudi unutar zemlje, olakšale su ljekarima proces telefonske trijaže i procjenu rizika osoba da li imaju COVID-19 ili su mogle biti izložene SARS-CoV-2. Takođe, tim koji je kreirao komunikacionu strategiju IJZCG izvršio je i brendiranje SOS linije 1616 i učinio je vidljivom opštoj populaciji.

Uzrasna distribucija anketiranih osoba bila je u skladu sa očekivanom u odnosu na rizik od komplikacija usljed COVID-19, tj. najviše su se javljali ljudi uzrasta 60 ili više godina, a potom oni najmobilniji, lica uzrasta 18 do 39 godina (11-13). Takođe, i procentualna zastupljenost anketiranih lica u odnosu na opštine iz kojih se pozivali bila je u skladu sa distribucijom detektovanih slučajeva po opštinama i posljedičnom postojanju klastera u istima (14).

Zaključak

Korišćenje nacionalne telefonske linije u koordinisanju odgovorom na COVID-19 pokazalo se u potpunosti opravdanim jer je linija omogućila ono šta joj je bio prvenstveni cilj, da bude „brana" dok se zdravstveni sistem ne konsoliduje i omogući smanjivanje pritiska na ograničene ljudske

Of course, this was possible and meaningful in Montenegro because, at the time of introducing CC 1616, there was no local transmission established, and after its establishment, using this phone-line, among other things, widespread community transmission was prevented (5).

CC 1616 initiated extensive and timely contact tracing where, due to the small number of detected cases, those persons whose contact with SARS-CoV-2 cases was defined as low to moderate risk were also placed under health and sanitary supervision and quarantine. (6).

The current epidemiological situation dictated the nature of the CC 1616 work, so in the second week the "breakthrough" of the initial algorithm was registered because a person with a negative history of traveling to countries where local transmission was established and who denied contact with persons who had recently returned from abroad was found. That person initially contacted the CC 1616 because of symptoms suspected to be of COVID-19, but due to a negative epidemiological history was referred for clinical examination instead of further epidemiological processing. In the further course of the disease, it turned out that the person was positive for SARS-CoV-2, thus proving the first case of local transmission of unknown origin. This initiated an immediate change in protocol and from that day on, all persons who contacted the 1616 line and complained of symptoms that raised suspicion of COVID-19 were contacted by physicians from the line, who, through an extensive interview, assessed the risk of whether it was a real suspicion of COVID-19 or it was more probable that it is some other cause. It should be noted that, at that time, the laboratory capacities for processing PCR tests on SARS-CoV-2 in Montenegro were the size of dozens processed tests per day.

Of course, it was clear from the very beginning that, if wider community transmission is established, telephone triage will lose all significance and the system will have to be based on clinical triage at the primary health care level, i.e. in the so-called COVID ambulances. However, primarily due to the good compliance of the general population, but also due to extremely strict public health measures, there was no outbreak escalation, and in Montenegro on May 4th 2020, 49 days from the first, the last case of COVID-19 was detected in the first wave (7).

Interest in the CC 1616 and its role were more significant at the very beginning of its functioning, which can be seen in a high number of realized calls, while later, due to the potential calming of the situation and additional involvement of local epidemiological services, interest decreased. Also, the CC 1616, despite its initial set-up, had a significant dose of informative and educational character, because from the start it was conceived as an additional communication channel of the IPH (4).

The line capacity of a maximum of 20 calls at once and the recordings of missed calls, made the operators able to answer absolutely every call made to the line.

The main challenge for the CC 1616 was reflected in the small number of medical doctors engaged in exhausting and responsible work every day, making dozens of demanding phone calls, especially bearing in mind that the anxiety of people who suspected of being exposed to COVID-19 was significantly increased in the first days, due to a general fear of the unknown. There were no special preparations of medical doctors for this job, so these challenges were overcome in time (8,9).

The so-called "weekend effect", i.e. the reduction of both the number of realized calls per day and the number of daily reported cases, was noticeably pronounced despite the fact that the line worked from 8 am to 11 pm every day (10). On the other hand, the existence of a strong positive correlation between the number of realized calls and daily reported cases is not surprising because the higher number of cases increased the risk to the environment, and consequently there was a rise in number of people who were potentially in contact with SARS-CoV-2 cases.

High percentage of people detected by CC 1616 is a reflection of several things. Namely, due to the fear of the unknown, the general population have recognized the IPH as the health authority they can rely on, and on the other hand, travel bans and strict public health measures that caused less mobility within the country, facilitated the process of telephone triage and risk assessment of persons, in terms of whether they have COVID-19 or may have been exposed to SARS-CoV-2. Also, the team that created the communication strategy of the IPH branded the CC 1616 and made it visible to the general population.

Age distribution among interviewed persons was in accordance with expectations in relation

kapacitete, gdje se trijažnim upitnikom kroz koje su osobe provodili obučeni operateri, jasno definisalo dalje postupanje sa osobama. Zahvaljujući korišćenju adaptabilnog skorovanog upitnika, ljekari angažovani na SOS liniji 1616 su mogli promtno da reaguju na svaki slučaj sumnje jer su pred sobom, u realnom vremenu, imali hijerarhiju prioriteta.

Takođe, značajan procenat detektovanih slučajeva nakon što su ostvarili kontakt putem SOS linije 1616 pripomogao je brzom otkrivanju kontakata inficiranih osoba i njihovom stavljanju u karantin, što je u značajnoj mjeri doprinijelo sprečavanju širenja infekcije pa i njenom potpunom suzbijanju. Ovo ukazuje da opisani način funkcionisanja kol-centra treba uvijek imati na umu za slučaj ponovljanja situacija velikog epidemiološkog rizika usljed importacije određenih potencijalnih uzročnika epidemijskog javljanja zaraznih bolesti. Naravno, ne treba gubiti iz vida da je sve to bilo moguće jer su najsnažnije epidemiološke mjere bile na snazi (zatvorene granice, kafići, tržni centri itd) i da je u tom periodu postojala velika komplijansa od strane opšte populacije.

Zbog svega navedenog, treba u kontinuitetu komunicirati sa opštom populacijom kako jedini razlog za visoku komplijansu, u narednim izazovima, ne bi bio strah koji se u jednom momentu uvijek nadvlada, već visok stepen prosvijećenosti i povjerenja u krovne javnozdravstvene ustanove.

Literatura

1. WHO Timeline - COVID-19. Available from: https://www.who.int/news/item/27-04-2020-who-timeline---covid-19 [cited 2021 July 16]
2. 2019-nCoV outbreak: first cases confirmed in Europe. Available from: https://www.euro.who.int/en/health-topics/health-emergencies/coronavirus-covid-19/news/news/2020/01/2019-ncov-outbreak-first-cases-confirmed-in-europe [cited 2021 July 16]
3. Sekularac I. Montenegro reports first case of corona virus infection 2020. Available from: https://www.reuters.com/article/us-health-coronavirus-montenegro/montenegro-reports-first-case-of-corona-virus-infection-idUSKBN2143SY [cited 2021 July 16]
4. Cerović R, Redžepagić N, Strugar M, Boljević N. Komunikaciona strategija: COVID-19 faza II. Podgorica [Internet]. Available from: https://www.ijzcg.me/me/publikacije/komunikaciona-strategija-covid-19-faza-ii [cited 2021 July 16]
5. Digital solutions to fight COVID-19. Council of Europe [Internet]. Available from: https://rm.coe.int/prems-120820-gbr-2051-digital-solutions-to-fight-covid-19-text-a4-web-/16809fe49c [cited 2021 July 16]
6. Resource estimation for contact tracing, quarantine and monitoring activities for COVID-19 cases in the EU/EEA. Available from: https://www.ecdc.europa.eu/sites/default/files/documents/COVID-19-resources-for-contact-tracing-2-March-2020.pdf [cited 2021 July 16]
7. Daily new confirmed COVD-19 cases in Montenegro. COVID-19 Data Repository by the Center for Systems Science and Engineering (CSSE) at Johns Hopkins University. Available from: https://ourworldindata.org/explorers/coronavirus-data-explorer?zoomToSelection=true&time=2020-03-20..2020-05-18&pickerSort=desc&pickerMetric=new_cases_smoothed_per_
8. Roy D, Tripathy S, Kar SK, Sharma N, Verma SK, Kaushal V. Study of knowledge, attitude, anxiety & perceived mental healthcare need in Indian population during COVID-19 pandemic. Asian J Psychiatr. 2020
9. Harlianty RA, Widyastuti T, Mukhlis H, Susanti S. Study on Awareness of Covid-19, Anxiety and Compliance on Social Distancing in Indonesia During Coronavirus Disease 2019 (COVID-19) Pandemic. Research Square 2020, [Preprints]. DOI: 10.21203/rs.3.rs-44598/v1.
10. Becker IR, Tarrasch R, Blinder P, Ben-Eliyahu S. A seven-day cycle in COVID-19 infection, hospitalization, and mortality rates: Do weekend social interactions kill susceptible people? BMH Yale [Preprints], 2020.
11. Risk factors and risk groups. Available from: https://www.ecdc.europa.eu/en/covid-19/latest-evidence/risk-factors-risk-groups [cited 2021 July 16]
12. Yanez ND, Weiss NS, Romand JA, Treggiari MM. COVID-19 mortality risk for older men and women. BMC Public Health 2020; 20(1):1742.
13. Yechezkel M, Weiss A, Rejwan I, Shahmoon E, Ben-Gal S, Yamin D. Human mobility and poverty as key drivers of COVID-19 transmission and control. BMC Public Health 2021; 21(1):596.
14. Presjek situacije u Crnoj Gori u utorak, 05.05. u 08:45. Available from: https://www.ijzcg.me/me/novosti/presjek-situacije-u-crnoj-gori-u-utorak-0505-u-0845 [cited 2021 July 16]

to the risk of complications due to COVID-19, i.e. most calls were made from people aged 60 or more, followed by the most mobile people, aged 18 to 39 (11-13). Also, the distribution among interviewed persons by municipalities was in line with the distribution among reported cases by municipalities and consequent existence of clusters in those municipalities (14).

Conclusion

Using the national phone line in coordinating COVID-19 response proved to be fully justified because the line made possible what was its primary goal, to be a "dam" until the health system consolidates and to reduce the pressure on limited human capacities, where the triage questionnaire through which persons were led by trained operators, clearly defined their further treatment. Thanks to the use of an adaptive scoring questionnaire, CC 1616 medical doctors were able to respond promptly to any case of suspicion because they had a hierarchy of priorities in front of them, in real time.

Also, a notable percentage of cases detected after making contact through this phone line has helped to quickly detect contacts of infected people and put them in quarantine, which significantly contributed to controlling the spread of infection and to its complete suppression. This indicates that the described way of functioning of the CC 1616 should always be kept in mind in case of recurrence of high epidemiological risk situations due to the importation of certain potential causes of infectious diseases outbreaks. Of course, all of this was possible because the strongest epidemiological public measures were in force (sealed country borders, closed schools, restaurants, shopping malls, etc.) and because in that period there was great compliance by the general population.

Given such experience, it is important to have continuous communication with the general population so that the only reason for high compliance, in the next challenges, would not be fear that is always overcome at some point, but a high degree of enlightenment and trust in the main public health institutions.

Literature

1. WHO Timeline - COVID-19. Available from: https://www.who.int/news/item/27-04-2020-who-timeline---covid-19 [cited 2021 July 16]
2. 2019-nCoV outbreak: first cases confirmed in Europe. Available from: https://www.euro.who.int/en/health-topics/health-emergencies/coronavirus-covid-19/news/news/2020/01/2019-ncov-outbreak-first-cases-confirmed-in-europe [cited 2021 July 16]
3. Sekularac I. Montenegro reports first case of corona virus infection. 2020. Available from: https://www.reuters.com/article/us-health-coronavirus-montenegro/montenegro-reports-first-case-of-corona-virus-infection-idUSKBN2143SY [cited 2021 July 16]
4. Cerović R, Redžepagić N, Strugar M, Boljević N. Komunikaciona strategija: COVID-19 faza II. Podgorica [Internet]. Available from: https://www.ijzcg.me/me/publikacije/komunikaciona-strategija-covid-19-faza-ii [cited 2021 July 16]
5. Digital solutions to fight COVID-19. Council of Europe [Internet]. Available from: https://rm.coe.int/prems-120820-gbr-2051-digital-solutions-to-fight-covid-19-text-a4-web-/16809fe49c [cited 2021 July 16]
6. Resource estimation for contact tracing, quarantine and monitoring activities for COVID-19 cases in the EU/EEA. Available from: https://www.ecdc.europa.eu/sites/default/files/documents/COVID-19-resources-for-contact-tracing-2-March-2020.pdf [cited 2021 July 16]
7. Daily new confirmed COVD-19 cases in Montenegro. COVID-19 Data Repository by the Center for Systems Science and Engineering (CSSE) at Johns Hopkins University. Available from: https://ourworldindata.org/explorers/coronavirus-data-explorer?zoomToSelection=true&time=2020-03-20..2020-05-18&pickerSort=desc&pickerMetric=new_cases_smoothed_per_
8. Roy D, Tripathy S, Kar SK, Sharma N, Verma SK, Kaushal V. Study of knowledge, attitude, anxiety & perceived mental healthcare need in Indian population during COVID-19 pandemic. Asian J Psychiatr. 2020
9. Harlianty RA, Widyastuti T, Mukhlis H, Susanti S. Study on Awareness of Covid-19, Anxiety and Compliance on Social Distancing in Indonesia During Coronavirus Disease 2019 (COVID-19) Pandemic. Research Square 2020, [Preprints]. DOI: 10.21203/rs.3.rs-44598/v1.
10. Becker IR, Tarrasch R, Blinder P, Ben-Eliyahu S. A seven-day cycle in COVID-19 infection, hospitalization, and mortality rates: Do weekend social interactions kill susceptible people? BMH Yale [Preprints], 2020.
11. Risk factors and risk groups. Available from: https://www.ecdc.europa.eu/en/covid-19/latest-evidence/risk-factors-risk-groups [cited 2021 July 16]
12. Yanez ND, Weiss NS, Romand JA, Treggiari MM. COVID-19 mortality risk for older men and women. BMC Public Health 2020; 20(1):1742.
13. Yechezkel M, Weiss A, Rejwan I, Shahmoon E, Ben-Gal S, Yamin D. Human mobility and poverty as key drivers of

Sukob interesa: Nije prijavljen.
Primljen: 12.09.2021.
Revizija: 28.09.2021.
Prihvaćen: 02.10.2021.

Autor za korespondenciju: dr Marija Raičević Todorović, Institut za javno zdravlje Crne Gore, Džona Džeksona bb, 81000 Podgorica, Crna Gora; e-mail: marija.todorovic@ijzcg.me

COVID-19 transmission and control. BMC Public Health 2021; 21(1):596.

14. Cross-section of the situation in Montenegro on Tuesday, 05.05. at 08:45. Available from: https://www.ijzcg.me/me/novosti/presjek-situacije-u-crnoj-gori-u-utorak-0505-u-0845 [cited 2021 July 16]

Conflict of interest: None declared.
Received: 09/02/2021
Revised: 09/28/2021
Accepted: 10/02/2021

Corresponding author: Marija Raicevic Todorovic MD, Institute for Public Health of Montenegro, Dzona Dzeksona bb, 81000 Podgorica, Montenegro; e-mail: marija.todorovic@ijzcg.me

ORIGINALNI RAD

UDK BROJEVI:
616.43-008.6:618.17
616.43-008.6:616.64
616-056.7
DOI: https://doi.org/10.5937/zdravzast50-33283

POREMEĆAJI POLNE DIFERENCIJACIJE: ISKUSTVO TERCIJERNOG CENTRA

Snježana Mijatović[1], Tatjana Milenković[2], Katarina Sedlecki[2], Vladimir Kojović[1,2], Predrag Ilić[1,2], Jelena Martić[1,2], Katarina Mitrović[1,2], Slađana Todorović[2], Marko Marjanović[2], Jovana Tončev[2], Sanja Panić-Zarić[2], Rade Vuković[1,2*]

[1] Medicinski fakultet Univerziteta u Beogradu, Beograd, Republika Srbija
[2] Institut za zdravstvenu zaštitu majke i deteta Srbije „Dr Vukan Čupić", Beograd, Republika Srbija

SAŽETAK

Uvod/cilj: Poremećaji polne diferencijacije (PPD) obuhvataju heterogenu grupu urođenih stanja kod kojih postoji neusklađenost hromozomskog pola, gonadnog pola i izgleda spoljašnjih genitalija. Učestalost PPD-a iznosi 1 na 4.500-5.500 novorođene dece godišnje. Kongenitalna adrenalna hiperplazija (KAH) usled deficita enzima 21-hidroksilaze predstavlja jedan od najčešćih i najbolje poznatih uzroka PPD. Ostale forme KAH-a, kao i drugi uzroci PPD-a, se javljaju sa značajno manjom pojedinačnom učestalošću i samim time predstavljaju daleko veći dijagnostički i terapijski izazov. Cilj istraživanja bila je analiza etiologije i kliničkih karakteristika PPD-a, kao i promena u dijagnostičkom i terapijskom pristupu PPD-u, u tercijernom centru tokom prethodnih 13 godina.
Metode: Istraživanje je sprovedeno po tipu retrospektivne kohortne studije kojom su bili obuhvaćeni svi pacijenti ispitivani zbog PPD-a, a koji su dijagnostikovani u Službi za endokrinologiju Instituta za zdravstvenu zaštitu majke i deteta Srbije „Dr Vukan Čupić" u periodu od decembra 2007. godine do novembra 2020. godine. U istraživanje nisu uključena deca sa PPD-om kod kojih je utvrđena dijagnoza KAH usled deficita 21-hidroksilaze.
Rezultati: Studijom je obuhvaćeno 31 dete sa PPD-om i to 24 (77%) dece imalo je 46XY PPD, 3 (10%) 46XX PPD, a 4 (13%) hromozomski PPD. Definitivna dijagnoza je postavljena kod 25 dece (81%), a najčešća etiologija PPD-a je bila gonadna disgenezija (55%), zatim sindrom neosetljivosti na androgene (10%) i atipične forme KAH-a (7%). U periodu 2016-2020. godine (period 2) ispitivano je 18 dece sa PPD-om, a 13 dece u periodu 2007-2015. godine (period 1). Specifična dijagnoza etiologije PPD-a je utvrđena kod većeg broja dece (89%) u periodu 2 u odnosu na period 1 (69%). Takođe, tokom perioda 2 genitalna hirurgija je učinjena kod značajno manjeg broja dece (11%) i u starijem uzrastu (prosečan uzrast 6,8 godina) nego u periodu 1 (64%, $p < 0,05$; prosečan uzrast 4,8 godina).
Zaključak: Tokom kasnijeg perioda uočava se povećanje broja ispitivane dece, kao i procenat PPD-a sa utvrđenom etiologijom. Takođe, dijagnoza se sve češće postavlja na osnovu genetskih analiza, a genitoplastika se sprovodi kod sve manjeg broja dece i u kasnijem uzrastu. Deca sa PPD-om zahtevaju holistički i multidisciplinarni pristup radi što preciznije evaluacije pacijenata i pružanja adekvatnog i individualizovanog tretmana i nege.

Ključne reči: poremećaji polne diferencijacije, kongenitalna adrenalna hiperplazija, gonadna disgenezija, sindrom neosetljivosti na androgene

Uvod

Poremećaji polne diferencijacije (PPD), odnosno različitosti u polnoj diferencijaciji, obuhvataju klinički i patofiziološki heterogenu grupu stanja koja se najčešće manifestuju na rođenju u vidu ambivalentnog izgleda spoljašnjih genitalija ili u adolescentnom periodu u vidu izmenjenog razvoja sekundarnih polnih karakteristika. Poremećaji polne diferencijacije javljaju se sa učestalošću od 1:4.500-5.500 novorođene dece godišnje (1,2). Kod ovih pacijenata postoje odstupanja u fiziološkom razvoju hromozomskog, gonadnog ili fenotipskog pola (3,4).

Determinacija i diferencijacija pola su složeni procesi koji se odvijaju tokom prenatalnog perioda, a zatim se nastavljaju u postnatalnom periodu sve do sticanja polne zrelosti tokom puberteta i

ORIGINAL PAPER

UDK NUMBERS:
616.43-008.6:618.17
616.43-008.6:616.64
616-056.7
DOI: https://doi.org/10.5937/zdravzast50-33283

DISORDERS/DIFFERENCES OF SEX DEVELOPMENT: TERTIARY CENTRE EXPERIENCE

Snjezana Mijatovic[1], Tatjana Milenkovic[2], Katarina Sedlecki[2], Vladimir Kojovic[1,2], Predrag Ilic[1,2], Jelena Martic[1,2], Katarina Mitrovic[1,2], Sladjana Todorovic[2], Marko Marjanovic[2], Jovana Toncev[2], Sanja Panic-Zaric[2], Rade Vukovic[1,2*]

[1] School of Medicine, University of Belgrade, Belgrade, Republic of Serbia
[2] Mother and Child Health Care Institute of Serbia „Dr Vukan Cupic", Belgrade, Republic of Serbia

SUMMARY

Introduction/aim: Disorders of sex development (DSD) comprise a heterogeneous group of congenital conditions with a difference between chromosomal, gonadal sex and the appearance of the external genitalia. The frequency of DSD is 1: 4,500-5,500 newborns per year. Congenital adrenal hyperplasia (CAH) due to the deficiency of the 21-hydroxylase enzyme is one of the most common and best-known causes of DSD. Other forms of CAH, as well as other causes of DSD, occur with significantly lower individual frequencies and are thus more challenging to diagnose and treat. The aim of the study was to analyse the etiology and clinical characteristics of DSD, as well as changes in the diagnostic and therapeutic approach to DSD in the tertiary center during the previous 13 years.
Methods: The study was conducted in the form of a retrospective cohort study which included all patients investigated for DSD at the Department of Endocrinology of Mother and Child Health Care Institute of Serbia "Dr Vukan Cupic" during the period from December 2007 until November 2020. Children with DSD caused by CAH due to 21-hydroxylase deficiency were not included in this study.
Results: The study included a total of 31 children with DSD: 24 children (77%) had 46XY DSD, 3 (10%) had 46XX DSD, and 4 children (13%) had chromosomal DSD. A definitive diagnosis of specific etiology has been made in 25 children (81%), and the most common etiology of DSD was gonadal dysgenesis (55%), followed by the androgen insensitivity syndrome (10%) and atypical forms of CAH (7%). During the period 2 (2016-2020) more children with DSD (n = 18) were examined compared to the period 1 (2007-2015) and the specific etiological diagnosis was established in a larger number of children with DSD (89%) compared to the period 1 (69%). Also, during period 2 (2016-2020) genital surgery was performed in a significantly lesser number of children (11%) and at a later age (average age 6.8 years) than in period 1 (64%, average age 4.8 years; $p < 0.05$).
Conclusion: During the latter period (2016-2020), there has been an increase in the number of investigated children as well as the percentage of DSD with established specific etiology. Also, the diagnosis is increasingly being made on the basis of genetic analysis, and genitoplasty is performed in a decreasing number of children and at a later age. A holistic and multidisciplinary approach is required for the evaluation treatment of children with DSD.

Keywords: disorders of sex development, differences of sex development, congenital adrenal hyperplasia, gonadal dysgenesis, androgen insensitivity syndrome.

Introduction

Disorders of sex development (DSD), that is, differences of sex development encompass a heterogeneous group of conditions with diverse clinical features and pathophysiology that are manifested at birth as ambiguous external genitalia and in adolescence as changes in the development of secondary sexual characteristics. The frequency of disorders of sex development is 1: 4,500-5500 newborns per year (1,2). In these patients, deviations in the physiological development of chromosomal, gonadal and phenotypic sex occur (3,4).

Sex determination and differentiation are complex processes that evolve during the prenatal period, and then continue in the postnatal period until sexual maturation during puberty and adolescence. During fertilization, male and female

adolescencije. Prilikom oplođenja spajaju se muški i ženski gameti, i nastaje zigot, pri čemu dolazi do determinacije *hromozomskog (genetskog) pola*, odnosno do nastanka ploda koji je najčešće uobičajenog ženskog (46,XX) ili muškog (46,XY) kariotipa. Hromozomski pol ima glavnu ulogu u diferencijaciji primordijalne gonade u muške (testis) ili u ženske (ovarijumi) gonade, što označava *gonadni pol*. *Fenotipski pol* se poslednji diferencira i podrazumeva uspostavljanje razlika u izgledu i građi između muških i ženskih unutrašnjih i spoljašnjih genitalija. Sticanje muškog ili ženskog fenotipskog pola odvija se tokom prenatalnog i postnatalog perioda pod uticajem muških, odnosno ženskih polnih hormona koji se luče iz gonada. Odstupanje u nekom od ovih procesa vodi ka nastanku poremećaja u diferencijaciji pola, što se može manifestovati spektrom različitih kliničkih nalaza kroz različite faze rasta i razvoja deteta (1-4).

Postoje brojne podele poremećaja polne diferencijacije, a jedna od najčešće korišćenih u kliničkoj praksi je podela na osnovu nalaza kariotipa. Na ovaj način se svi poremećaji polne diferencijacije mogu podeliti na: 46,XY PPD, 46,XX PPD i hromozomske PPD (Tabela 1). Jedan od najznačajnijih uzroka PPD je kongenitalna adrenalna hiperplazija (KAH), kao najčešći uzrok PPD kod dece sa 46,XX kariotipom (1,3,5). Ostale forme KAH-a, kao i drugi uzroci PPD-a, se javljaju sa značajno manjom pojedinačnom učestalošću i samim time predstavljaju daleko veći dijagnostički i terapijski izazov. Zbog vrlo složenog dijagnostičkog i terapijskog pristupa, kod dece sa PPD-om neophodno je učešće multidisciplinarnog tima specijalista, koji sačinjavaju brojni specijalisti, najčešće neonatolog, pedijatrijski endokrinolog i urolog, ginekolog, radiolog i psihijatar (4,6).

Cilj ovog istraživanja bio je pregled etiologije i kliničkih karakteristika PPD-a, kao i promena u dijagnostičkom i terapijskom pristupu PPD-u, u tercijernom centru tokom prethodnih 13 godina.

Metode

Istraživanje je sprovedeno po tipu retrospektivne kohortne studije kojom su obuhvaćeni pacijenti ispitivani zbog PPD-a, a koji su se javili Službi za endokrinologiju Instituta za zdravstvenu zaštitu majke i deteta „Dr Vukan Čupić“ (IMD) u periodu od decembra 2007. godine do novembra 2020. godine. U istraživanje nisu uključena deca sa potvrđenom dijagnozom KAH-a usled deficita 21-hidroksilaze, a uključena su sva ostala deca sa PPD-om kod kojih je utvrđena bilo koja druga etiologija: gonadna disgenezija, sindrom rezistencije na androgene, atipični oblici KAH-a i drugi poremećaji steroidogeneze, kao i pacijenti kod kojih nije utvrđena etiologija PPD-a.

Iz medicinske dokumentacije Službe za endokrinologiju IMD-a prikupljeni su osnovni demografski podaci o pacijentima (pol dodeljen na rođenju, uzrast), podaci o razlogu javljanja lekaru i uzrastu u kojem su se pacijenti, odnosno roditelji, javili zbog neke od manifestacija PPD-a, podaci o grupi PPD-a, porodičnoj anamnezi, podaci o postavljenim specifičnim dijagnozama etiologije PPD-a na osnovu sprovedenih dijagnostičkih postupaka, uključujući analizu kariotipa i hormona, biopsiju gonada i vizualizacione dijagnostičke metode (ultrazvučni - UZ i preged magnetnom rezonancom - MR). Takođe su prikupljeni podaci o utvrđenoj dijagnozi, podaci o postojanju pridruženih anomalija, kao i podaci o sprovedenoj hormonskoj i hirurškoj terapiji pacijenata, kao i o datom savetu za podizanje deteta u određenom polu. Za sve pacijente su prikupljeni podaci o izgledu spoljašnjih genitalija koji su zatim kvantitativno analizirani korišćenjem skora izgleda spoljašnjih genitalija (engl. *External genitalia score* - EGS) (7).

U cilju analize promena u dijagnostičkom i terapijskom pristupu PPD-u tokom ukupnog perioda posmatranja od 13 godina, pacijenti su, u zavisnosti od godine u kojoj su zbog PPD-a ispitivani u IMD-u podeljeni u dve grupe: period 1 (pacijenti koji su se javili lekaru u periodu od 2007. godine do 2015. godine) i period 2 (pacijenti koji su se javili lekaru u periodu od 2016. godine do 2020. godine).

Statistička obrada prikupljenih podataka izvršena je u programu SPSS, metodama deskriptivne i analitčke statistike (*Hi-kvadrat test* i *Mann-Witney U test*). Prikupljeni podaci o pacijentima su upoređivani u odnosu na period ispitivajna u IMD-u, kao i u odnosu na grupu PPD-a (46XY PPD, 46XX PPD i hromozomski PPD). Rezultati su prikazani kao apsolutni brojevi (%), odnosno kao aritmetička sredina ± standardna devijacija, sa rasponom ekstremnih vrednosti u zagradi. Statistički značajnim rezultatima smatrani su rezultati kod kojih je p vrednost iznosila manje od 0,05.

gametes unite to form a zygote, resulting in chromosomal (genetic) sex determination, that is, the development of fetus with the most common karyotypes for females (46, XX) or males (46, XY). Chromosomal sex has a key role in the differentiation of the primordial gonad into male (testes) or female (ovaries) gonads, which is gonadal sex. Phenotypic sex differentiates last and it refers to differences in the appearance and structure of male and female internal and external genitalia. The development of male or female phenotypic sex occurs during the prenatal and postnatal period under the influence of male or female sexual hormones secreted by the gonads. Deviations of some of these processes lead to disorders of sex development, which may be manifested by a range of diverse clinical findings during different stages of children's growth and development (1-4).

There are numerous classifications of disorders of sex development, and one of the most frequently used in the clinical practice is the classification according to the karyotype findings. Thus, all disorders of sex development can be classified into: 46,XY DSD, 46,XX DSD and chromosomal DSD (Table 1). One of the most significant causes of DSD is congenital adrenal hyperplasia (CAH), as the most common DSD in children with 46,XX karyotype (1,3,5). Other forms of CAH, as well as other causes of DSD, occur with significantly lower individual frequency and are, therefore, more challenging to diagnose and treat. Due to a very complex diagnostic and therapeutic approach, in children with DSD a multidisciplinary team of specialists should necessarily be involved, including numerous specialists, most frequently neonatologists, pediatric endocrinologists and urologists, gynecologists, radiologists and psychiatrists (4,6).

The aim of this study was to analyze the etiology and clinical characteristics of DSD, as well as changes in the diagnostic and therapeutic approach to DSD in the tertiary center during the previous 13 years.

Methods

The study was conducted as a retrospective cohort study, which included all patients that were examined due to DSD at the Department of Endocrinology of Mother and Child Health Care Institute of Serbia "Dr Vukan Cupic" from December 2007 to November 2020. Children with the confirmed diagnosis of CAH due to the deficiency of 21-hydroxylase were not included in the study, while all the other children with DSD of different etiology were included in the study, including gonadal dysgenesis, androgen insensitivity syndrome, atypical forms of CAH and other disorders of steroidogenesis, as well as patients without established etiology of DSD.

Basic demographic data about patients (sex attributed at birth, age) were collected from the medical documentation of the Department of Endocrinology of Mother and Child Health Care Institute of Serbia "Dr Vukan Cupic", as well as data about reasons for visiting the doctor and age at which patients, that is, their parents visited the doctor due to some of the manifestations of DSD, data about DSD group, family anamnesis, data about specific etiology of DSD according to performed diagnostic procedures, including the analysis of karyotype and hormones, biopsy of gonads and visualization diagnostic methods (ultrasound and MR imaging). Also, data about the established diagnosis were collected, as well as data about the existence of comorbid anomalies, and data about the hormonal and surgical therapy, and the advice how to bring up a child of certain sex. Data about the appearance of external genitalia were collected for all patients, followed by quantitative analysis which was done with the help of external genitalia score (EGS) (7).

In order to analyze changes in the diagnostic and therapeutic approach to DSD during the whole period of observation that lasted 13 years, patients were divided into two groups depending on the year when they were examined due to DSD at Mother and Child Health Care Institute of Serbia "Dr Vukan Cupic": period 1 (patients who visited their doctor from 2007 to 2015) and period 2 (patients who visited their doctor during the period 2016-2020).

The statistical analysis of collected data was done with the help of SPSS program, using the methods of descriptive and analytical statistics (Chi-squared test and Mann-Whitney test). Collected data were compared in relation to the period of examination at the Institute, as well as in relation to the group of DSD (46 XY DSD, 46XX DSD and chromosomal DSD). The results were presented as absolute numbers (%), that is, as arithmetic mean ± standard deviation, with a range of extreme values in the brackets. The results were considered statistically significant if the p value was less than 0.05.

Rezultati

Tokom celokupnog perioda ovog istraživanja (2007-2020. godine) u našem centru je ispitano 31 dete sa PPD-om (uz isključivanje dece sa KAH-om usled deficita 21-hidroksilaze). Najveći broj (n=24, 77%) dece imao je 46XY PPD, troje (10%) 46XX PPD i 4 (13%) hromozomski PPD (Tabela 2). Uzrast u trenutku ispitivanja zbog PPD-a je najčešće bio neonatalni period (n=22, 71%), a najčešći razlog ispitivanja je bio ambivalentni izgled spoljašnjih genitalija (n=20, 64%) ili nepodudarnost nalaza prenatalnog/postnatalnog kariotipa sa izgledom spoljašnjih genitalija na rođenju (n=4, 13%), kao što je prikazano u Tabeli 2. Definitivna etiološka dijagnoza PPD-a je postavljena kod 25 dece (81%), a najčešća dijagnoza je bila gonadna disgenezija (n=17, 55%). Hormonska terapija je bila indikovana kod 10 dece (32%), genitalna hirurgija je učinjena kod 9 (31%), a gonadektomija kod 7 (23%). Klinički i laboratorijski podaci o ispitanicima, podaci dobijeni vizualizacionim dijagnostičkim metodama (UZ i MR) i podaci iz anamneze koji su od značaja u evaluaciji nastanka PPD-a prikazani su u Tabeli 2.

Od ukupnog broja dece sa PPD-om, 13 (41,9%) je ispitivano u IMD-u u periodu 1 (2007-2015), a 18 (58,1%) u periodu 2 (2016-2020). Karakteristike pacijenata u pogledu fizikalnog, laboratorijskog, UZ i MR nalaza, kao i sprovedenog lečenja, u zavisnosti od perioda javljanja i ispitivanja u IMD-u, prikazane su u Tabeli 3. Definitivna dijagnoza postavljena je u većem procentu kod dece koja su se javila zbog PPD-a u periodu 2 (n=16; 89%), u odnosu na decu koja su se javila u periodu 1 (n=9; 69%) (p=0,208). Veći broj dijagnoza potvrđen je hormonskim analizama kod pacijenata koji su se javili u periodu 2 (n=15; 83%), u odnosu na broj potvrđenih dijagnoza hormonskim analizama u periodu 1 (n=5; 38%) ($p < 0,05$, Tabela 3). Genitalna hirurgija se u periodu 2 sprovodila statistički značajno manje često (n=2; 11%) nego u periodu 1 (n=7; 64%) ($p<0,05$). Uzrast dece u trenutku sprovođenja prve genitalne hirurgije bio je viši u periodu 2 (6,8 godina) nego u periodu 1 (4,8 godina), kao što je prikazano u Tabeli 3.

Grupe PPD-a (46XY PPD, 46XX PPD i hromozomski PPD) razlikovale su se u odnosu na zastupljenost pojedinih tipova gonadne disgenezije (Tabela 4). Gonadna disgenezija bila je uzrok PPD-a kod 50% dece sa 46XY PPD-om, 33,3% dece sa 46XX PPD-om i kod sve dece sa hromozomskim PPD-om, odnosno mešovitom gonadnom disgenezijom (MGD). Kod 46XY grupe PPD-a najzastupljenija (58,3%) je bila parcijalna gonadna disgenezija (PGD), dok je u 46XX grupi PPD-a jedno dete sa gonadnom dis-

Tabela 1. Klasifikacija poremećaja polne diferencijacije (PPD) u zavisnosti od kariotipa (18)

46XY PPD	46XX PPD	Hromozomski PPD
Gonadna disgenezija (potpuna ili parcijalna)	Gonadna disgenezija	Mešovita gonadna disgenezija (45X/46XY i 46XX/46XY)
Ovotestikularni PPD	(Ovo)testikularni PPD	Varijante Tarnerovog sindroma
Poremećaji sinteze androgena (izolovani ili u sklopu atipičnih oblika KAH)	KAH, tipična forma (deficit 21-hidroksilaze)	Varijante Klinefelterovog sindroma
Sindrom potpune ili parcijalne neosetljivosti na androgene	KAH, atipične forme (deficit 11β-hidroksilaze, 17α-hidroksilaze, 3β-hidroksisteroid dehidrogenaze)	
Sindrom perzistentnih *Muller*-ovih kanala	Deficit placentalne aromataze	
Sindromske forme 46XY PPD (Smith-Lemli-Opitz, Denys-Drash, itd.)	Maternalni androgen-produkujući tumor	
	Uticaj egzogenih androgena (lekovi sa virilizujućim dejstvom)	
	Virilizujući luteom u trudnoći	
	Sindromske forme 46XX PPD	

PPD- poremećajima polne diferencijacije, KAH – kongenitalna adrenalna hiperplazija

Results

During the whole period of this study (2007-2020), 31 children with DSD were examined at the centre (with the exclusion of children with CAH caused by 21-hydroxylase deficiency). The largest number of children (n=24, 77%) had 46XY DSD, 3 children (10%) had 46XX DSD and 4 children (13%) had chromosomal DSD (Table 2). Age at the moment of examination due to DSD was most frequently the neonatal period (n=22, 71%), while the commonest reason for the examination was the ambiguous appearance of external genitalia at birth (n=20, 64%) or the incongruity between the analysis of prenatal/postnatal karyotype and the appearance of external genitalia at birth (n=4, 13%), as shown in Table 2. A definitive diagnosis of specific etiology was made in 25 children (81%), while the most common diagnosis was gonadal dysgenesis (n=17, 55%). Hormonal therapy was advised in 10 children (32%), while genital surgery was performed in 9 children (31%) and gonadectomy in 7 (23%). Clinical and laboratory data about examinees, data obtained with the help of visualization diagnostic methods (ultrasound and MR imaging) and data from anamnesis that were significant for the evaluation of DSD appearance are shown in Table 2.

Of the total number of children with DSD, 13 (41.9%) were examined at Mother and Child Health Care Institute during the first period (2007-2015), while 18 (58.1%) were examined in the second period (2016-2020). The characteristics of patients regarding the physical, laboratory, ultrasound and MR findings, as well as the performed treatment, depending on the period of visits and examination at the Institute, are shown in Table 3. A definitive diagnosis was established in a larger number of children who came to the doctor due to DSD during the second period (n=16, 89%), in comparison to children who visited the doctor during the first period (n=9, 69%) (p=0.208). Larger number of diagnoses was confirmed by hormonal analyses in patients during the second period (n=15, 83%) in comparison to the confirmed diagnoses using hormonal analyses in the first period (n=5, 38%) ($p<0.05$, Table 3). Genital surgery was performed less often during the second period and it was statistically significant (n=2, 11%) in comparison to the first period (n=7, 64%) ($p<0.05$). Also, during the second period, the first genital surgery was performed at a later age (6.8 years) than in the first period (4.8 years), as shown in Table 3.

Groups of DSD (46XY DSD, 46XX DSD and chromosomal DSD) were different depending on

Table 1. Disorders of sex development (DSD) depending on karyotype

46XY DSD	46XX DSD	Chromosomal DSD
Gonadal dysgenesis (complete or partial)	Gonadal dysgenesis	Mixed gonadal dysgenesis (45X/46XY i 46XX/46XY)
Ovotesticular DSD*	(Ovo)testicular DSD	Variants of Turner syndrome
Disorders of androgen synthesis (isolated or related to nonclassical types of CAH)	CAH, classic type (21-hydroxylase deficiency)	Variants of Klinefelter syndrome
Complete or partial androgen insensitivity syndrome	CAH. nonclassical types (11β-hydroxylase deficiency, 17α-hydroxylase deficiency, 3β hydroxysteroid dehydrogenase deficiency)	
Persistent Müllerian duct syndrome	Placental aromatase deficiency	
Syndrome forms of 46XY DSD (Smith-Lemli-Opitz, Denys-Drash, etc.)	Maternal androgen-producing tumor	
	Influence of exogenous androgens (drugs with virilizing effect)	
	Virilizing luteoma in pregnancy	
	Syndrome forms of 46XX DSD	

DSD-disorders of sex development, CAH-congenital adrenal hyperplasia

genezijom (GD) imalo *De la Chapell*-ov sindrom. Genitalna hirurgija je u većem procentu rađena kod dece sa hromozomskim PPD-om (kod 75,0% ove dece) i kod dece sa 46XX PPD (66,7%), dok je genitalnoj hirurgiji podrvgnuto znatno manje dece sa 46XY PPD (18,2%) ($p<0,05$). Karakteristike pacijenata u pogledu fizikalnog, laboratorijskog, UZ i MR nalaza, kao i sprovedenog lečenja, u zavisnosti od grupe PPD-a, prikazane su u Tabeli 4.

Diskusija

Poremećaji polne diferencijacije (PPD), odnosno različitosti u polnoj diferencijaciji, obuhvataju spektar kongenitalnih stanja kod kojih postoji nepodudarnost između izgleda spoljašnjih polnih organa (fenotipski pol) i hromozomskog ili gonadnog pola (1,8,9). U nastanku ovih stanja značajnu ulogu imaju genetski i hormonski faktori, kao i faktori sredine tokom prenatalnog i postnatalnog razvoja (8). Poremećaji polne diferencijacije mogu klinički da se manifestuju na rođenju, u vidu ambivalentnog izgleda spoljašnjih genitalija ili tokom detinjstva i adolescentnog perioda u vidu poremećaja u razvoju sekundarnih polnih karakteristika. Prema podacima iz literature učestalost javljanja poremećaja polne diferencijacije iznosi približno 1: 4.500-5.500 (1,2,4,8,10,11).

Učestalost KAH-a i MGD-a kao najčešćih uzroka PPD-a se procenjuje na približno 1:15.000, odnosno 1:10.000 rođene dece (4). Učestalost sindroma potpune neosetljivosti na androgene, kao uzroka PPD-a, varira između 1:60.000 i 1:99.000, dok je sindrom parcijalne neosetljivosti na androgene češći (1). Daleko ređi uzroci PPD-a su deficit 17β-hidroksisteroid dehidrogenaze (17β-HSD), koji se javlja u 1:150.000 dece sa PPD-om, kao i deficit 5α-reduktaze (5α-RD), koji se javlja još ređe (1). U našem istraživanju učestalost GD-a iznosila je 54,8%, pri čemu je 23,5% ove dece imalo mešovitu formu GD, 41,2% imalo je parcijalnu formu, dok je preostalih 35,3% ove dece imalo druge forme GD. Sindrom neosetljivosti na androgene bio je zastupljen kod 9,7% dece sa PPD-om. Atipične forme KAH-a bile su uzrok PPD-a kod 6,5% dece, a kod 19,3% dece etiologija nije utvrđena.

Studija sprovedena 2006. godine u Nemačkoj ukazuje na pozitivnu porodičnu anamnezu u 6,3% ispitivanih pacijenata sa PPD-om (1). Rezultati našeg istraživanja, koji pokazuju pozitivnu porodičnu anamnezu kod 6,5% dece sa PPD-om, u skladu su sa ovakvim podacima.

U zavisnosti od kariotipa pacijenata sa poremećajem polne diferencijacije, ovi poremećaji se mogu svrstati u tri grupe: 46XY PPD, 46XX PPD i hromozomski PPD (12). Hromozomski PPD obuhvataju različite poremećaje kod kojih postoje strukturne ili numeričke anomalije polnih hromozoma, kao što su varijante Tarnerovog (45X monozomija ili 45X/46XY mozaicizam) i Klinefelterovog sindroma (47XXY). 46XY grupa PPD-a najčešće se manifestuje ambivalentnim izgledom spoljašnjih genitalija ili spoljašnjim genitalijama potpuno ženskog tipa usled smanjene ili odsutne maskulinizacije in utero. Uzrok ovog tipa PPD-a može da bude: 1) poremećen razvoj gonada što rezultuje parcijalnom ili kompletnom gonadnom disgenezijom, 2) defekt u biosintezi testosterona ili dihidrotestosterona, 3) odsustvo efekata androgena usled neosetljivosti ciljnih tkiva na androgene (sindrom potpune/parcijalne neosetljivosti na androgene – CAIS/PAIS) (11). 46XX gupa PPD-a najčešće se karakteriše ambivalentnim izgledom spoljašnjih genitalija usled ekspozicije fetusa prekomernom nivou androgena, uz normalan razvoj Milerovih struktura i ovarijuma. Prekomerna ekspozicija androgenima najčešće nastaje usled: 1) poremećaja u sintezi hormona nadbubrežne žlezde sa prekomernom produkcijom androgena (KAH), 2) deficita placentalne aromataze, 3) dejstva maternalnih androgenih hormona (ovarijalni ili adrenalni tumor, hormonska terapija) (12).

Rezultati istraživanja sprovedenog u Kolumbiji, kao i istraživanja sprovedenog od strane EU COST (engl. *European Cooperation in Science and Technology*), ukazuju na najveću učestalost kariotipa 46,XY među decom sa PPD-om (75%), a zatim kariotipa 46,XX (10-15%) i hromozomskog PPD-a (12,5%) (1,7,13,14). Ovakvi podaci u skladu su sa rezultatima našeg istraživanja, gde je među ispitivanom decom sa PPD-om kariotip 46,XY bio najzastupljeniji (77,42%), a potom hromozomski PPD (12,9%) i kariotip 46,XX (9,7%).

Prema istraživanju sprovedenom 2006. godine u Nemačkoj, oko polovina dece sa PPD-om nema postavljenu definitivnu dijagnozu do 6. meseca života (1,15). Prema rezultatima istog istraživanja, definitivna dijagnoza postavljena putem genetskih analiza utvrđena je kod 20% pacijenata sa PPD-om (15). U okviru našeg istraživanja utvrđeno je da je definitivna dijagnoza bez obzira na uzrast postavljena kod 25 pacijenata (81%). Dijagnoza je definitivno postavljena na osnovu: 1) hormonskih anali-

the presence of certain forms of gonadal dysgenesis (Table 4). Gonadal dysgenesis was the cause of DSD in 50% of children with 46XY DSD, 33% of children with 46XX DSD and in all children with chromosomal DSD, that is, with mixed gonadal dysgenesis (MGD). In 46XY group of DSD the most frequent (58.3%) was partial gonadal dysgenesis (PGD), whereas in 46XX group of DSD one child with gonadal dysgenesis (GD) had De la Chapelle syndrome. Genital surgery was performed in greater number of children with chromosomal DSD (in 75% of these children) and in children with 46XX DSD (66.7%), while fewer children with 46XY DSD were subjected to genital surgery (18.2%) ($p<0.05$). The characteristics of patients relating to physical, laboratory, ultrasound and MR findings, as well as their treatment, depending on the group of DSD, are shown in Table 4.

Discussion

Disorders of sex development (DSD), that is, differences of sex development encompass a range of congenital conditions with a difference between the appearance of the external genitalia (phenotypic sex) and chromosomal or gonadal sex (1,8,9). Genetic and hormonal factors have a significant role in the onset of these conditions, as well as environmental factors during the prenatal and postnatal development (8). Disorders of sex development may be clinically manifested at birth as ambiguous external genitalia or during childhood and adolescence as disorders of secondary sexual development. According to the literature data, the frequency of disorders of sex development is approximately 1: 4,500-5,500 (1,2,4,8,10,11).

The frequency of CAH and MGD as the most frequent causes of DSD is estimated to be approximately 1:15,000, that is, 1:10,000 of newborns (4). The frequency of complete androgen insensitivity syndrome, as a cause of DSD, varies between 1:60,000 and 1:99,000, while of partial androgen insensitivity syndrome is more frequent (1). Far less frequent cause of DSD is the deficiency of 17ß-hydroxysteroid dehydrogenase (17ß-HSD), which occurs in 1:150,000 of children with DSD, as well as the deficiency of 5α-reductase (5α-RD) that occurs even less frequently (1). In our study, the frequency of GD amounted to 54.8%, while 23.5% of these children had a mixed form of GD, 41.2% had a partial form, and the remaining 35.3% of these children had other forms of GD. Androgen insensitivity syndrome was present in 9.7% of children with DSD. Atypical forms of CAH were causes of DSD in 6.5% of children, while in 19.3% of children the etiology was not established.

A study conducted in Germany in 2006 pointed to the positive family anamnesis in 6.3% of examined patients with DSD (1). The results of our study, which pointed to the positive family anamnesis in 6.5% of children with DSD, were in accordance with such data.

Depending on the karyotype of patients with disorders of sex development, these disorders may be classified into three groups: 46XY DSD, 46XX DSD and chromosomal DSD (12). Chromosomal DSD encompass different disorders with structural or numerical anomalies of sexual chromosomes, such as the variants of Turner (45X monosomia or 45X/46XY mosaicism) and Klinefelter syndrome (47XXY). 46XY group of DSD is most commonly manifested as ambiguous external genitalia or external genitalia that are completely female due to the decreased or absent masculinization in utero. A cause of this type of DSD may be: 1) disrupted development of gonads resulting in partial or complete gonadal dysgenesis, 2) defect in the biosynthesis of testosterone or dihydrotestosterone, 3) the absence of androgen effects due to insensitivity of target tissues to androgens (complete/partial androgen insensitivity syndrome – CAIS/PAIS) (11). 46XX group of DSD is most frequently manifested as ambiguous appearance of external genitalia due to the exposure of fetus to the excess level of androgen, with the normal development of Müllerian structures and ovaries. Exposure to excess androgens is most frequently caused by: 1) disorders of the synthesis of hormones of adrenal glands with the excessive production of androgens (CAH), 2) placental aromatase deficiency, 3) the activity of maternal androgen hormones (ovarian and adrenal tumor, hormonal therapy) (12).

The results of one study conducted in Columbia, as well as the study conducted by EU COST (European Cooperation in Science and Technology), pointed to the highest frequency of karyotype 46XY among children with DSD (75%), and then karyotype 46XX (10-15%) and chromosomal DSD (12.5%) (1,7,13,14). These data are in accordance with the results of our study, where karyotype 46XY was the commonest among the examined-

Tabela 2. Anamnestički i dijagnostički podaci o pacijentima sa poremećajima polne diferencijacije (PPD)

Karakteristike	N=31 Broj (%) / $\bar{x}$ ± SD
Uzrast u trenutku javljanja lekaru zbog PPD	
Neonatalni uzrast	22 (71,0)
Detinjstvo	5 (16,1)
Adolescencija	4 (12,9)
Razlog prvog javljanja lekaru	
Ambivalentan izgled spoljašnjih genitalija	20 (64,5)
Nepodudarnost između nalaza prenatalnog/ postnatalnog kariotipa i izgleda polnih organa na rođenju	4 (12,9)
Virilizacija kod devojčica	1 (3,2)
Primarna amenoreja	1 (3,2)
Udruženo primarna amenoreja i virilizacija	2 (6,4)
Nizak rast	1 (3,2)
Ostalo	2 (6,4)
Grupa PPD	
46,XX PPD	3 (9,7)
46,XY PPD	24 (77,4)
Hromozomski PPD	4 (12,9)
Pozitivna porodična anamneza	2 (6,4)
Postavljena definitivna dijagnoza	25 (80,6)
Dijagnoza	
Gonadna disgenezija	17 (54,8)
Sindrom rezistencije na androgene	3 (9,7)
KAH (atipične forme)	2 (6,4)
Ostalo	3 (9,7)
Nije utvrđena etiologija	6 (19,4)
Tip gonadne disgenezije (GD) (% od uk. broja GD)	
Mešovita GD	4 (23,5)
Parcijalna GD	7 (41,2)
Ostale forme GD	6 (35,3)
Dijagnoza definitivno postavljena na osnovu	
Hormonskih nalaza	12 (48,0)
Genetskih analiza	8 (32,0)
Biopsije	2 (8,0)
Kliničkog ili radiološkog nalaza	3 (12,0)
Dijagnoza potvrđena hormonskim analizama	20 (64,5)
Prisustvo pridruženih malformacija	16 (51,6)
Pridružene malformacije (% od uk. broja malformacija)	
Urogenitalne	5 (31,2)
Malformacije CNS-a	1 (6,3)
Malformacije ekstremiteta	1 (6,3)
Nizak rast	4 (25,0)
Ostale malformacije	2 (12,5)
Udružene urogenitalne malformacije, malformacije CNS-a, ekstremiteta, srca, i nizak rast	1 (6,3)
Udružene malformacije CNS-a, srca i ostale malformacije	1 (6,3)
Udružene malformacije ekstremiteta, srca i ostale malormacije	1 (6,3)
Smrtni ishod pacijenta u prvih 12 meseci	2 (6,5)
Savetovano podizanje detata	
Muški pol	9 (29)
Ženski pol	5 (16,1)
Nije data preporuka	17 (54,8)
Izgled spoljašnjih genitalija – EGS	5,35±3,22
Gonade viđene na MR	5 (16,1)
Gonade viđene na UZ	19 (61,3)
Uterus viđen na MR	3 (50,0)
Uterus viđen na UZ	12 (40,0)

Table 2. Anamnestic and diagnostic data about patients with disorders of sex development (DSD) (1/2)

Characteristics	N=31 No (%) / $\bar{x}$ ± SD
Age at the time of first visit to a doctor due to DSD	
Neonatal period	22 (71.0)
Childhood	5 (16.1)
Adolescence	4 (12.9)
Reason for the first visit to a doctor	
Ambiguous external genitalia	20 (64.5)
Mismatch between prenatal/postnatal karyotype findings and genital appearance at the birth	4 (12.9)
Virilization of females	1 (3.2)
Primary amenorrhea	1 (3.2)
Primary amenorrhea and virilization	2 (6.4)
Low growth	1 (3.2)
Other reasons	2 (6.4)
DSD categories	
46,XX DSD	3 (9.7)
46,XY DSD	24 (77.4)
Chromosomal DSD	4 (12.9)
Positive family anamnesis	2 (6.4)
Definitive diagnosis	25 (80.6)
Diagnosis	
Gonadal dysgenesis	17 (54.8)
Androgen insensitivity syndrome	3 (9.7)
CAH (nonclassical types)	2 (6.4)
Other	3 (9.7)
Unknown etiology	6 (19.4)
Type of gonadal dysgenesis (GD) (% of total no. of GD)	
Mixed GD	4 (23.5)
Partial GD	7 (41.2)
Other forms GD	6 (35.3)
A definitive diagnosis was made by	
Hormonal analysis	12 (48.0)
Genetic analysis	8 (32.0)
Biopsy	2 (8.0)
Clinical and radiology findings	3 (12.0)
Diagnosis confirmed by hormonal analysis	20 (64.5)
Malformations associated with DSD	16 (51.6)
Types of malformations (% of total no. of malformations)	
Urogenital	5 (31.2)
CNS malformations	1 (6.3)
Extremities malformations	1 (6.3)
Low growth	4 (25.0)
Other malformations	2 (12.5)
United urogenital malformations. malformations of CNS. extremities. heart and low growth	1 (6.3)
United malformations of CNS. heart and other malformations	1 (6.3)
United malformations of extremities. heart and other malformations	1 (6.3)
Death of patients with DSD in first 12 months	2 (6.5)
Recommended gender of the child with DSD	9 (29.0)
Male	5 (16.1)
Female	17 (54.8)
No recommendation	
External genitalia score (EGS) ($\bar{x}$ ± SD)	5.35±3.22
Gonads visualized by MR	5 (16.1)
Gonads visualized by ultrasound	19 (61.3)
Uterus visualized by MR	3 (50.0)
Uterus visualized by ultrasound	12 (40.0)

Tabela 2. Anamnestički i dijagnostički podaci o pacijentima sa poremećajima polne diferencijacije (PPD) - nastavak (2/2)

Karakteristike	N=31 Broj (%) / $\bar{x}$ ± SD
Sindrom gubitka soli	0 (0,0)
Akutna adrenalna insuficijencija	0 (0,0)
Medikamentna endokrinološka terapija	10 (32,3)
Hidrokortizon	3 (9,7)
Fludrokortizon	1 (3,2)
Testosteron	0 (0,0)
E2	5 (16,1)
GnRH analog	3 (9,7)
Dete podizano u:	
muškom polu	18 (60,0)
ženskom polu	12 (40,0)
Genitalna hirurgija	9 (31,0)
Uzrast u trenutku prve genitalne hirurgije (god) ($\bar{x}$ ± SD)	5,29±3,99
Gonadektomija	7 (23,3)
Uzrast u trenutku gonadektomije (god) ($\bar{x}$ ± SD)	7,84 ±5,30
Biopsija gonada (podatak dostupan za 29 pacijenata)	9 (31,0)
Uzrast u trenutku poslednje kontrole (god) ($\bar{x}$ ± SD)	6,93 ± 6,59

$\bar{x}$ - aritmetička sredina, SD - standardna devijacija, PPD – poremećaji polne diferencijacije, KAH– kongenitalna adrenalna hiperplazija, GD – gonadalna disgenezija, UZ – ultrazvuk, MR – magnetna rezonanca, E2 – estradiol, GnRH – gonadotropin oslobađajući hormon, CNS–centralni nervni sistem, EGS–*External genitalia score*

za kod 48% dece, 2) genetskih analiza (uključujući nalaz kariotipa) kod 32% dece, 3) biopsije kod 8% dece, 4) kliničkih i radioloških nalaza kod 3% dece sa PPD-om. Defintivna dijagnoza kod većeg broja dece (89%) postavljena je u periodu 2 (2016-2020) u odnosu 69% u periodu 1 (2007-2015). U periodu 2 takođe je veći broj dijagnoza postavljen putem genetskih analiza (37,5%), nego u periodu 1 (22,2%).

Prema ranijim preporukama Akademije američkih pedijatara (engl. American Academy of Pediatrics) optimalnim uzrastom za izvođenje genitalne hirurgije (genitoplastike) kod dece sa PPD-om smatrao se uzrast između 2. i 6. meseca života (4,16). Međutim, prema novijim preporukama hirurški tretman pacijenata sa PPD preporučuje se u značajno kasnijem uzrastu deteta, idealno u cilju omogućavanja učestvovanja u donošenju odluke o hirurškoj intervenciji (4).

Rezultati našeg istraživanja su u skladu sa ovom izmenom smernica, gde je pokazana statistički značajna razlika ($p<0,05$) između perioda 1 (2007-2015) i perioda 2 (2016-2020) u pogledu genitalne hirurgije. Genitoplastika je znatno češće vršena kod dece u periodu 1 (64%) nego kod dece u periodu 2 (11%). Takođe, genitalna hirurgija tokom perioda 2 je bila u proseku u starijem uzrastu dece (6,8 ± 6,5) u odnosu na decu podvrgnutu genitalnoj hirurgiji u periodu 1 (4,8 ± 3,6).

U našem istraživanju kod 51,6% dece sa PPD-om utvrđeno je prisustvo pridruženih nalaza, najčešće se radilo o urogenitalnim malforacijama (31,2%), niskom rastu (25,0%), malformacijama centralnog nervnog sistema (6,3%) i ekstremiteta (6,3%). S obzirom na složenost dijagnostičkog i terapijskog pristupa poremećajima polne diferencijacije, u ispitivanju i lečenju ove dece neophodan je multidisciplinarni pristup tima stručnjaka koji sačinjavaju brojni specijalisti, a najčešće podrazumeva: pedijatra endokrinologa, neonatologa, dečjeg urologa, ginekologa, radiologa, psihijatra ili psihologa i socijalnog radnika (4,6,17).

Zaključak

Poremećaji polne diferencijacije obuhvataju etiološki heterogenu grupu stanja, koja zahteva holistički pristup radi što preciznijeg sagledavanja pacijenata i pružanja adekvatnog i individualizovanog tretmana i nege. U našem istraživanju dece sa PPD-om (uz isključivanje dece sa KAH-om usled deficita 21-hidroksilaze) najčešći uzroci PPD-a bili su: gonadna disgenezija, sindrom rezistencije na

Table 2. Anamnestic and diagnostic data about patients with disorders of sex development (DSD) (2/2)

Characteristics	N=31 No (%) / $\bar{x}$ ± SD
Salt wasting syndrome	0 (0.0)
Acute adrenal insufficiency	0 (0.0)
Hormonal therapy	10 (32.3)
Hydrocortisone	3 (9.7)
Fludrocortisone	1 (3.2)
Testosterone	0 (0.0)
E2	5 (16.1)
GnRH analogues	3 (9.7)
Child raised as	
Male	18 (60.0)
Female	12 (40.0)
Genital surgery	9 (31.0)
Age at the moment of first genital surgery ($\bar{x}$ ± SD)	5.29±3.99
Gonadectomy	7 (23.3)
Age at the moment of gonadectomy ($\bar{x}$ ± SD)	7.84 ±5.30
Gonadal biopsy (data available for 29 patients)	9 (31.0)
Age at the moment of last control ($\bar{x}$ ± SD)	6.93 ± 6.59

$\bar{x}$ – Mean, SD –Standard deviation, DSD – Disorders of sex development, GD – Gonadal dysgenesis, CAH- Congenital adrenal hyperplasia, MR – Magnetic resonance, E2 – estradiol, GnRH – Gonadotropin-releasing hormone, CNS – Central nervnous system, EGS-external genitalia score

children with DSD (77.42%), and then chromosomal DSD (12.9%) and karyotype 46XX (9.7%).

According to a study conducted in Germany in 2006, around one half of children with DSD did not have a definitive diagnosis until the sixth month of age (1,15). According to the results of the same study, a definitive diagnosis was made with the help of genetic analyses in 20% of patients with DSD (15). Within the scope of our study, it was found out that a definitive diagnosis was made in 25 patients regardless of patients' age (81%). The diagnosis was definitely made with the help of: 1) hormonal analyses in 48% of children, 2) genetic analyses (including the karyotype analysis) in 32% of children, 3) biopsy in 8% of children, 4) clinical and radiological findings in 3% of children with DSD. A definitive diagnosis was established in a larger number of children (89%) in the second period (2016-2020) in comparison to 69% in the first period (2007-2015). In the second period, more diagnoses were established with the help of genetic analyses (37.5%) than in the first period (22.2%).

According to the previous recommendations of the American Academy of Pediatrics, age between 2 and 6 months of life was deemed to be the optimal age for the introduction of genital surgery (genitoplasty) in children with DSD. However, according to recent recommendations, surgical treatment of patients with DSD is recommended at a significantly later age, aimed at enabling patients to make decisions about surgical interventions (4).

The results of our study are in accordance with these changes of guidelines, where statistically significant difference ($p<0.05$) between the period 1 (2007-2015) and period 2 (2016-2020) regarding genital surgery was shown. Genitoplasty was significantly more frequently performed in children in the first period (64%) than in the second period (11%). Also, during the second period genital surgery was performed at a later age (6.8 + 6.5) in comparison to children subjected to genital surgery in the first period (4.8 + 3.6).

In our study, in 51.6% of children with DSD, comorbid conditions were found, most commonly urogenital malformations (31.2%), short stature (25.0%), malformations of the central nervous system (6.3%) and extremities (6.3%). Considering the complexity of diagnostic and therapeutic approach to disorders of sex development, the examination and treatment of these children should necessarily involve a multidisciplinary approach of the team of specialists, including numerous specialists, most frequently pediatric endocrinologists, neonatologists, pediatric urologists, gynecologists, radiologists, psychiatrists or psychologists and social workers (4,6,17).

Tabela 3. Karakteristike ispitanika u zavisnosti od perioda ispitivanja u Institutu za zdravstvenu zaštitu majke i deteta Srbije - (1/2)

Karakteristike	Period 1 N= 13 Broj (%)	Period 2 N=18 Broj (%)	p vrednost
Uzrast u trenutku javljanja lekaru zbogPPD			
Neonatalni	9 (69,2)	13 (72,2)	> 0,05
Detinjstvo	3 (23,1)	2 (11,1)	
Adolescencija	1 (7,7)	3 (16,7)	
Razlog prvog javljanja lekaru			> 0,05
Ambivalentan izgled spoljašnjih genitalija	10 (76,9)	10 (55,6)	
Nepodudarnost između nalaza prenatalnog/postnatalnog kariotipa izgleda polnih organa na rođenju	1 (7,7)	3 (16,7)	
Virilizacija kod devojčica	0 (0,0)	3 (16,7)	
Primarna amenoreja	1 (7,7)	0 (0,0)	
Udruženo primarna amenoreja i virilizacija	0 (0,0)	2 (11,1)	
Nizak rast	1 (7,7)	0 (0,0)	
Ostalo	0 (0,0)	2 (11,1)	
Grupa PPD			
46,XX DSD	3 (23,1)	0 (0,0)	> 0,05
46,XY DSD	8 (61,5)	16 (88,9)	
Hromozomski DSD	2 (15,4)	2 (11,1)	
Pozitivna porodična anamneza	0 (0,0)	2 (11,1)	> 0,05
Postavljena definitivna dijagnoza	9 (69,2)	16 (88,9)	> 0,05
Dijagnoza			
Gonadna disgenezija	7(53,8)	10 (55,6)	> 0,05
Sindrom rezistencije na androgene	2 (15,4)	1 (5,6)	
KAH (atipične forme)	0 (0,0)	2 (11,1)	
Ostalo	0 (0,0)	3 (16,7)	
Nije utvrđena etiologija	4 (30,8)	2 (11,1)	
Izgled spoljašnjih genitalija-EGS ($\bar{x}$ ± SD)	4,7 ± 3,1	5,8 ± 3,3	> 0,05
Prisustvo pridruženih malformacija	5 (38,5)	11 (61,1)	> 0,05
Pridružene malformacije (% od uk. broja malformacija)			
Urogenitalne	1 (20,0)	4 (36,4)	> 0,05
Malformacije CNS-a	0 (0,0)	1 (9,1)	
Malformacije ekstremiteta	0 (0,0)	1 (9,1)	
Nizak rast	3 (60,0)	1 (9,1)	
Ostale malformacije	0 (0,0)	2 (18,2)	
Udružene urogenitalne malformacije, malformacije CNS-a, srca i ostale malformacije	0 (0,0)	1 (9,1)	
Udružene malformacije CNS-a, srca i ostale malformacije	1 (20,0)	0 (0,0)	
Udružene malformacije ekstremiteta, srca i ostale malformacije	1 (9,1)	0 (0,0)	
Smrtni ishod pacijenta u prvih 12 meseci	0 (0,0)	2 (11,1)	> 0,05
Gonadalna disgenezija tip (% od uk. broja GD)			
MGD	2 (28,6)	2 (20,0)	> 0,05
PGD	3 (42,9)	4 (40,0)	
Ostale forme GD	2 (28,6)	4 (40,0)	
Dijagnoza postavljena definitivno na osnovu			
Hormonskih nalaza	4 (44,4)	8 (50,0)	> 0,05
Genetskih analiza	2 (22,2)	6 (37,5)	
Biopsije	1 (11,1)	1 (6,3)	
Kliničkog i radiološkog nalaza	2 (22,2)	1 (6,3)	
Dijagnoza potvrđena hormonskim analizama	5 (38,5)	15 (83,3)	0,021
Dijagnoza potvrđena genetskim analizama	3 (23,1)	6 (33,3)	> 0,05
Gonade viđene na UZ	8 (61,5)	11 (61,1)	> 0,05
Gonade viđene na MR	1 (7,7)	4 (22,2)	> 0,05
Uterus viđen na UZ	6 (46,2)	6 (35,3)	> 0,05
Uterus viđen na MR	1 (50,0)	2 (50,0)	> 0,05
Sindrom gubitka soli	0 (0,0)	0 (0,0)	

Table 3. Characteristics of patients depending on the period of examination in the Mother and Child Health Care Institute of Serbia „Dr Vukan Cupic", Belgrade, Serbia (1/2)

Characteristics	Period 1 N= 13 No (%)	Period 2 N=18 No (%)	p value
Age at the time of first visit to a doctor due to DSD			
Neonatal period	9 (69.2)	13 (72.2)	> 0,05
Childhood	3 (23.1)	2 (11.1)	
Adolescence	1 (7.7)	3 (16.7)	
Reason for the first visit to a doctor			
Ambiguous external genitalia	10 (76.9)	10 (55.6)	> 0,05
Mismatch between prenatal/postnatal karyotype findings and genital appearance at the birth	1 (7.7)	3 (16.7)	
Virilization of females	0 (0.0)	3 (16.7)	
Primary amenorrhea	1 (7.7)	0 (0.0)	
Primary amenorrhea and virilization	0 (0.0)	2 (11.1)	
Low growth	1 (7.7)	0 (0.0)	
Other reasons	0 (0.0)	2 (11.1)	
DSD categories			
46.XX DSD	3 (23.1)	0 (0.0)	> 0,05
46.XY DSD	8 (61.5)	16 (88.9)	
Chromosomal DSD	2 (15.4)	2 (11.1)	
Positive family anamnesis	0 (0.0)	2 (11.1)	> 0.05
Definitive diagnosis	9 (69.2)	16 (88.9)	> 0.05
Diagnosis			
Gonadal dysgenesis	7(53.8)	10 (55.6)	> 0.05
Androgen insensitivity syndrome	2 (15.4)	1 (5.6)	
CAH (nonclassical types)	0 (0.0)	2 (11.1)	
Other	0 (0.0)	3 (16.7)	
Unknown etiology	4 (30.8)	2 (11.1)	
External genitalia score (EGS) $\bar{x} \pm SD$	4.7 ± 3.1	5.8 ± 3.3	> 0.05
Malformations associated with DSD	5 (38.5)	11 (61.1)	> 0.05
Types of malformations (% of total no. of malformations)			
Urogenital	1 (20.0)	4 (36.4)	> 0.05
CNS malformations	0 (0.0)	1 (9.1)	
Extremities malformations	0 (0.0)	1 (9.1)	
Low growth	3 (60.0)	1 (9.1)	
Other malformations	0 (0.0)	2 (18.2)	
United urogenital malformations. malformations of CNS. extremities. heart and low growth	0 (0.0)	1 (9.1)	
United malformations of CNS. heart and other malformations	1 (20.0)	0 (0.0)	
United malformations of extremities. heart and other malformations	1 (9.1)	0 (0.0)	
Death of patients with DSD in first 12 months	0 (0.0)	2 (11.1)	> 0.05
Type of gonadal dysgenesis (GD) (% of total no. of GD)			
Mixed GD	2 (28.6)	2 (20.0)	> 0.05
Partial GD	3 (42.9)	4 (40.0)	
Other forms GD	2 (28.6)	4 (40.0)	
A definitive diagnosis was made by			
Hormonal analysis	4 (44.4)	8 (50.0)	> 0.05
Genetic analysis	2 (22.2)	6 (37.5)	
Biopsy	1 (11.1)	1 (6.3)	
Clinical and radiology findings	2 (22.2)	1 (6.3)	
Diagnosis confirmed by hormonal analysis	5 (38.5)	15 (83.3)	0.021
Diagnosis confirmed by genetic analysis	3 (23.1)	6 (33.3)	> 0.05
Gonads visualized by MR	8 (61.5)	11 (61.1)	> 0.05
Gonads visualized by ultrasound	1 (7.7)	4 (22.2)	> 0.05
Uterus visualized by MR	6 (46.2)	6 (35.3)	> 0.05
Uterus visualized by ultrasound	1 (50.0)	2 (50.0)	> 0.05

Tabela 3. Karakteristike ispitanika u zavisnosti od perioda ispitivanja u Institutu za zdravstvenu zaštitu majke i deteta Srbije - nastavak (2/2)

Karakteristike	Period 1 N= 13 Broj (%)	Period 2 N=18 Broj (%)	p vrednost
Akutna adrenalna insuficijencija	13 (100,0)	18 (100,0)	
Medikamentna endokrinološka terapija	4 (30,8)	6 (33,3)	> 0,05
Hidrokortizon	0 (0,0)	3 (16,7)	> 0,05
Fludrokortizon	0 (0,0)	1 (5,6)	> 0,05
Testosteron	0 (0,0)	0 (0,0)	
E2	3 (23,1)	2 (11,1)	> 0,05
GnRH analog	0 (0,0)	3 (16,7)	> 0,05
Genitalna hirurgija	7 (63,6)	2 (11,1)	0,010*
Uzrast u trenutku prve genitalne hirurgije	4,8±3,6	6,83±6,46	> 0,05
Biopsija gonada	5 (45,5)	4 (22,2)	> 0,05
Gonandektomija	5 (41,7)	2 (11,1)	> 0,05
Uzrast u trenutku gonadektomije	7,2±6,3	9,3±2,6	> 0,05
Uzrast u trenutku poslednje kontrole	10,2±6,3	4,7±6,00	0,019*
Savetovano podizanje deteta			
Muški pol	5 (38,5)	4 (22,2)	> 0,05
Ženski pol	3 (23,1)	2 (11,1)	
Nije data preporuka	5 (38,5)	12 (66,7)	
Dete podizano u			
Muškom polu	6 (50,0)	12 (66,7)	> 0,05
Ženskom polu	6 (50,0)	6 (33,3)	

$\bar{x}$ – aritmetička sredina, SD– standardna devijacija, PPD – poremećaji polne diferencijacije, KAH–kongenitalna adrenalna hiperplazija, GD – gonadalna disgenezija, UZ – ultrazvuk, MR – magnetna rezonanca, E2 – estradiol, GnRH – gonadotropin oslobađajući hormon, CNS–centralni nervni sistem, EGS–*External genitalia score*, *p vrednost dobijena primenom Studentovog t testa

Table 3. Characteristics of patients depending on the period of examination in the Mother and Child Health Care Institute of Serbia „Dr Vukan Cupic", Belgrade, Serbia - continued (2/2)

Characteristics	Period 1 N= 13 No (%)	Period 2 N=18 No (%)	p value
Salt wasting syndrome	0 (0.0)	0 (0.0)	
Acute adrenal insufficiency	13 (100.0)	18 (100.0)	
Hormonal therapy	4 (30.8)	6 (33.3)	> 0.05
Hydrocortisone	0 (0.0)	3 (16.7)	> 0.05
Fludrocortisone	0 (0.0)	1 (5.6)	> 0.05
Testosterone	0 (0.0)	0 (0.0)	
E2	3 (23.1)	2 (11.1)	> 0.05
GnRH analogues	0 (0.0)	3 (16.7)	> 0.05
Genital surgery	7 (63.6)	2 (11.1)	0.010*
Age at the moment of first genital surgery	4.8±3.6	6.83±6.46	> 0.05
Gonadal biopsy	5 (45.5)	4 (22.2)	> 0.05
Gonadectomy	5 (41.7)	2 (11.1)	> 0.05
Age at the moment of gonadectomy	7.2±6.3	9.3±2.6	> 0.05
Age at the moment of last control	10.2±6.3	4.7±6.00	0.019*
Recommended gender of the child with DSD			
Male	5 (38.5)	4 (22.2)	> 0.05
Female	3 (23.1)	2 (11.1)	
No recommendation	5 (38.5)	12 (66.7)	
Child raised as			
Male	6 (50.0)	12 (66.7)	> 0.05
Female	6 (50.0)	6 (33.3)	

$\bar{x}$ – Mean, SD –Standard deviation, DSD – Disorders of sex development, GD – Gonadal dysgenesis, CAH- Congenital adrenal hyperplasia, MR – Magnetic resonance, E2 – estradiol, GnRH – Gonadotropin-releasing hormone, CNS – Central nervnous system, EGS-external genitalia score *p value obtained using Student's t test

Tabela 4. Karakteristike ispitanika u zavisnosti od grupe PPD - (1/2)

Karakteristike	46XX PPD N=3 Broj (%)	46XY PPD N=24 Broj (%)	Hromozomski PPD N=4 Broj (%)
Uzrast u trenutku prvog obraćanja lekaru zbog PPD			
Neonatalni/ odojački	2 (66,7)	18 (75,0)	2 (50,0)
Detinjstvo	1 (33,3)	3 (12,5)	1 (25,0)
Adolescencija	0 (0,0)	3 (12,5)	1 (25,0)
Razlog prvog javljanja lekaru zbog PPD			
Ambivalentan izgled spoljašnjih genitalija	2 (66,7)	15 (62,5)	3 (75,0)
Nepodudarnost između nalaza prenatalnog/ postanatalnog kariotipa i izgleda polnih organa na rođenju	1 (33,3)	3 (12,5)	0 (0,0)
Virilizacija kod devojčica	0 (0,0)	1 (4,2)	3 (12,5)
Primarnaamenoreja	0 (0,0)	2 (8,3)	0 (0,0)
Udruženo primarna amenoreja i virilizacija	0 (0,0)	1 (4,2)	0 (0,0)
Nizak rast	0 (0,0)	2 (8,3)	0 (0,0)
Ostalo	0 (0,0)	0 (0,0)	0 (0,0)
Pozitivna porodična anamneza	0 (0,0)	2 (8,3)	0 (0,0)
Postavljena definitivna dijagnoza	1 (33,3)	20 (83,3)	4 (100,0)
Dijagnoza			
Gonadna disgenezija	1 (33,3)	12 (50,0)	4 (100,0)
Sindrom rezistencije na androgene	0 (0,0)	3 (12,5)	0 (0,0)
KAH (atipične forme)	0 (0,0)	2 (8,0)	0 (0,0)
Ostalo	0 (0,0)	3 (12,5)	0 (0,0)
Nije utvrđena etiologija	2 (66,7)	4 (16,7)	0 (0,0)
Gonadalna disgenezija tip (% od uk. broja GD)			
MGD	0 (0,0)	0 (0,0)	4 (100,0)
PGD	0 (0,0)	7 (58,3)	0 (0,0)
Ostale forme	1 (100,0)	5 (41,7)	0 (0,0)
Dijagnoza postavljena defintivno na osnovu			
Hormonski nalazi	1 (100,0)	11 (55,0)	0 (0,0)
Genetske analize	0 (0,0)	4 (20,0)	0 (0,0)
Biopsija	0 (0,0)	2 (10,0)	0 (0,0)
Klinički i radiološki nalazi	0 (0,0)	3 (15,0)	0 (0,0)
Izgled spoljašnjih genitalija (EGS) ($\bar{x}$ ± SD)	6,5 ± 2,6	5,2 ± 3,5	5,5 ± 2,4
Dijagnoza potvrđena hormonskim analizama	0 (0,0)	17 (70,8)	3 (75,0)
Dijagnoza potvrđena genetskim analizama	1 (33,3)	4 (16,7)	4 (100,0)
Gonade viđene na UZ	2 (66,7)	14 (58,3)	3 (75,0)
Gonade viđene na MR	1 (100,0)	2 (100,0)	2 (100,0)
Uterus viđen na UZ	1 (33,3)	9 (39,1)	2 (50,0)
Uterus viđen na MR	1 (100,0)	0 (0,0)	0 (0,0)
Sindrom gubitka soli	0 (0,0)	0 (0,0)	0 (0,0)
Akutna adrenalna insuficijencija	0 (0,0)	0 (0,0)	0 (0,0)
Medikamentna endokrinološka terapija	0 (0,0)	7 (29,2)	3 (75,0)
Hidrokortizon	0 (0,0)	3 (12,5)	0 (0,0)
Fludrokortizon	0 (0,0)	1 (4,2)	0 (0,0)
Testosteron	0 (0,0)	0 (0,0)	0 (0,0)
E2	0 (0,0)	3 (12,5)	2 (50,0)
GnRH	0 (0,0)	2 (8,3)	1 (25,0)
Prisustvo pridruženih malformacija	1 (33,3)	12 (50,0)	3 (75,0)

Table 4. Characteristics of patients depending on DSD groups (1/2)

Characteristics	46XX DSD N=3 Number (%)	46XY DSD N=24 Number (%)	Chromosomal PPD N=4 Number (%)
Age at the time of first visit to a doctor due to DSD			
Neonatal period	2 (66.7)	18 (75.0)	2 (50.0)
Childhood	1 (33.3)	3 (12.5)	1 (25.0)
Adolescence	0 (0.0)	3 (12.5)	1 (25.0)
Reason for the first visit to a doctor			
Ambiguous external genitalia	2 (66.7)	15 (62.5)	3 (75.0)
Mismatch between prenatal/postnatal karyotype findings and genital appearance at the birth	1 (33.3)	3 (12.5)	0 (0.0)
Virilization of females	0 (0.0)	1 (4.2)	3 (12.5)
Primary amenorrhea	0 (0.0)	2 (8.3)	0 (0.0)
Primary amenorrhea and virilization	0 (0.0)	1 (4.2)	0 (0.0)
Low growth	0 (0.0)	2 (8.3)	0 (0.0)
Other reasons	0 (0.0)	0 (0.0)	0 (0.0)
Positive family anamnesis	0 (0.0)	2 (8.3)	0 (0.0)
Definitive diagnosis	1 (33.3)	20 (83.3)	4 (100.0)
Diagnosis			
Gonadal dysgenesis	1 (33.3)	12 (50.0)	4 (100.0)
Androgen insensitivity syndrome	0 (0.0)	3 (12.5)	0 (0.0)
CAH (nonclassical types)	0 (0.0)	2 (8.0)	0 (0.0)
Other	0 (0.0)	3 (12.5)	0 (0.0)
Unknown etiology	2 (66.7)	4 (16.7)	0 (0.0)
Type of gonadal dysgenesis (GD) (% of total no. of GD)			
Mixed GD	0 (0.0)	0 (0.0)	4 (100.0)
Partial GD	0 (0.0)	7 (58.3)	0 (0.0)
Other forms GD	1 (100.0)	5 (41.7)	0 (0.0)
Diagnosis confirmed by			
Hormonal analysis	1 (100.0)	11 (55.0)	0 (0.0)
Genetic analysis	0 (0.0)	4 (20.0)	0 (0.0)
Biopsy	0 (0.0)	2 (10.0)	0 (0.0)
Clinical and radiology findings	0 (0.0)	3 (15.0)	0 (0.0)
External genitalia score (EGS) ($\bar{x}$ ± SD)	6.5 ± 2.6	5.2 ± 3.5	5.5 ± 2.4
Diagnosis confirmed by hormonal analysis	0 (0.0)	17 (70.8)	3 (75.0)
Diagnosis confirmed by genetic analysis	1 (33.3)	4 (16.7)	4 (100.0)
Gonads visualized by MR	2 (66.7)	14 (58.3)	3 (75.0)
Gonads visualized by ultrasound	1 (100.0)	2 (100.0)	2 (100.0)
Uterus visualized by MR	1 (33.3)	9 (39.1)	2 (50.0)
Uterus visualized by ultrasound	1 (100.0)	0 (0.0)	0 (0.0)
Salt wasting syndrome	0 (0.0)	0 (0.0)	0 (0.0)
Acute adrenal insufficiency	0 (0.0)	0 (0.0)	0 (0.0)
Hormonaltherapy	0 (0.0)	7 (29.2)	3 (75.0)
Hydrocortisone	0 (0.0)	3 (12.5)	0 (0.0)
Fludrocortisone	0 (0.0)	1 (4.2)	0 (0.0)
Testosterone	0 (0.0)	0 (0.0)	0 (0.0)
E2	0 (0.0)	3 (12.5)	2 (50.0)
GnRH analogues	0 (0.0)	2 (8.3)	1 (25.0)
Malformations associated with DSD	1 (33.3)	12 (50.0)	3 (75.0)
Child raised as			
Male	1 (33.3)	15 (65.2)	2 (50.0)
Female	2 (66.7)	8 (34.8)	2 (50.0)
Genital surgery	2 (66.7)	4 (18.2)	3 (75.0)

Tabela 4. Karakteristike ispitanika u zavisnosti od grupe PPD nastavak - nastavak (2/2)

Karakteristike	46XX PPD N=3 Broj (%)	46XY PPD N=24 Broj (%)	Hromozomski PPD N=4 Broj (%)
Pridružene malformacije (% od uk. broja malformacija)			
Urogenitalne	1 (100,0)	4 (33,2)	0 (0,0)
Malformacije CNS-a	0 (0,0)	1 (8,3)	0 (0,0)
Malformacije ekstremiteta	0 (0,0)	1 (8,3)	0 (0,0)
Nizak rast	0 (0,0)	1 (8,3)	3 (100,0)
Ostale malformacije	0 (0,0)	2 (16,6)	0 (0,0)
Udružene urogenitalne malformacije, malformacije CNS-a, srca i ostale malformacije	0 (0,0)	1 (8,3)	0 (0,0)
Udružene malformacije CNS-a, srca i ostale malformacije	0 (0,0)	1 (8,3)	0 (0,0)
Udružene malformacije ekstremiteta, srca i ostale malormacije	0 (0,0)	1 (8,3)	0 (0,0)
Smrtni ishod pacijenta u prvih 12 meseci	0 (0,0)	2 (8,3)	0 (0,0)
Savetovano podizanje deteta			
Muški pol	1 (33,3)	7 (29,2)	1 (25,0)
Ženski pol	1 (33,3)	3 (12,5)	1 (25,0)
Nije data preporuka	1 (33,3)	14 (58,3)	2 (50,0)
Dete podizano u			
Muškom polu	1 (33,3)	15 (65,2)	2 (50,0)
Ženskom polu	2 (66,7)	8 (34,8)	2 (50,0)
Genitalna hirurgija	2 (66,7)	4 (18,2)	3 (75,0)
Uzrast u trenutku prve genitalne hirurgije (godine) ($\bar{x}$ ± SD)	7.90±5.35	2.48±1.16	7.28±4.33
Gonadektomija	0 (0,0)	4 (17,4)	3 (75,0)
Uzrast u trenutku gonadektomije (godine) ($\bar{x}$ ± SD)		8.33±5.48	7.17± 6.17
Biopsija gonada	0 (0,0)	6 (27,3)	3 (75,0)
Uzrast u trenutku poslednje kontrole ($\bar{x}$ ± SD)	8.95±10.50	6.20±6.36	9.65±5.64

$\bar{x}$ - aritmetička sredina, SD-standardna devijacija , PPD – poremećaji polne diferencijacije, GD – gonadalna disgenezija, UZ – ultrazvuk, MR – magnetna rezonanca, E2 – estradiol, GnRH – gonadotropin oslobađajući hormon, CNS-centralni nervni sistem, EGS– *External genitalia score*.

androgene i atipične forme KAH. Utvrđeno je da je u drugom periodu (2016-2020) zbog PPD-a ispitivan veći broj dece sa PPD-om u IMD-u, kao i da se povećao procenat PPD-a sa utvrđenom etiologijom u odnosu na prvi period (2007-2015). Takođe, naši podaci ukazuju da se dijagnoza sve češće postavlja na osnovu genetskih analiza, a da se genitoplastika sprovodi kod sve manjeg broja dece i u kasnijem uzrastu.

Literatura

1. Thyen U, Lanz K, Paul-Martin Holterhus PM, Hiort O. Epidemiology and Initial Management of Ambiguous Genitalia at Birth in Germany. Horm Res 2006; 66(4):195-203.
2. Lee PA, Nordenström A, P Houk CP, S Ahmed SF, Auchus R, Baratz A, et al. Global Disorders of Sex Development Update since 2006: Perceptions, Approachand Care. Horm Res Paediatr 2016; 85(3):158-80.
3. Kumar J, Kumar V, Bhatia V, Preeti Dabadghao P, Chaturvedi S, Kapoor R et al. Managing disorder of sexual development surgically: A single center experience. Indian J Urol 2012; 28(3):286-91.
4. Kim KS, Kim J. Disorder of sex development. Korean J Urol 2012; 53(1):1-8.
5. Man E, GOSH DSD MDT, Achermann J. A single centre experience of differences/disorders in sex development over 20 years. Endocrine Abstracts 2016; 45 OC6.9.
6. Ahmed SF, Achermann JC, Arlt W, Balen A, Gerry Conway G, Edwards Z et al. Society for Endocrinology UK guidance on the initial evaluation of an infant or an adolescent with a suspected disorder of sex development (Revised 2015). Clinical Endocrinol (Oxf) 2016; 84(5):771-88.
7. van der Straaten S, Springer A, Zecic A, Hebenstreit D, Tonnhofer U, Gawlik A et al. The External Genitalia Score (EGS): A European Multicenter Validation Study. J Clin Endocrinol Metab 2020; 105(3): dgz142.
8. García-Acero M, Moreno-Niño O, Suárez-Obando F, Molina M, Manotas MC, Prieto JC et al. Disorders of sex development: Genetic characterization of a patient cohort. Mol Med Rep 2020; 21(1):97-106.
9. Hiort O, Birnbaum W, Marshall L, Wünsch L, Werner R, Schröder T, et al. Management of disorders of sex

Table 4. Characteristics of patients depending on DSD groups - continued (2/2)

Characteristics	46XX DSD N=3 Number (%)	46XY DSD N=24 Number (%)	Chromosomal PPD N=4 Number (%)
Types of malformations (% of total no. of malformations)			
Urogenital	1 (100.0)	4 (33.2)	0 (0.0)
CNS malformations	0 (0.0)	1 (8.3)	0 (0.0)
Extremities malformations	0 (0.0)	1 (8.3)	0 (0.0)
Low growth	0 (0.0)	1 (8.3)	3 (100.0)
Other malformations	0 (0.0)	2 (16.6)	0 (0.0)
United urogenital malformations. malformations of CNS. extremities. heart and low growth	0 (0.0)	1 (8.3)	0 (0.0)
United malformations of CNS. heart and other malformations	0 (0.0)	1 (8.3)	
United malformations of extremities. heart and other malformations	0 (0.0)	1 (8.3)	0 (0.0) 0 (0.0)
Death of patients with DSD in first 12 months	0 (0.0)	2 (8.3)	0 (0.0)
Recommended gender of the child with DSD			
Male	1 (33.3)	7 (29.2)	1 (25.0)
Female	1 (33.3)	3 (12.5)	1 (25.0)
No recommendation	1 (33.3)	14 (58.3)	2 (50.0)
Age at the moment of first genital surgery ($\bar{x}$ ± SD)	7.90±5.35	2.48±1.16	7.28±4.33
Gonadectomy	0 (0.0)	4 (17.4)	3 (75.0)
Age at the moment of gonadectomy		8.33±5.48	7.17± 6.17
Gonadal biopsy	0 (0.0)	6 (27.3)	3 (75.0)
Age at the moment of last control ($\bar{x}$ ± SD)	8.95±10.50	6.20±6.36	9.65±5.64

$\bar{x}$ – Mean, SD –Standard deviation, DSD – Disorders of sex development, GD – Gonadal dysgenesis, MR – Magnetic resonance, E2 – estradiol, CAH- Congenital adrenal hyperplasia, GnRH – Gonadotropin-releasing hormone, CNS – Central nervnous system, EGS-external genitalia score.

Conclusion

Disorders of sex development encompass a heterogeneous group of conditions with different etiology, which demand a holistic approach in order to evaluate patients more precisely and provide appropriate and individualized treatment and care. In our study that included children with DSD (with the exclusion of children with CAH due to 21-hydroxylase), the most frequent reasons for DSD were the following: gonadal dysgenesis, androgen insensitivity syndrome and atypical forms of CAH. It was found out that in the second period (2016-2020) a larger number of children with DSD were examined at Mother and Child Health Care Institute, as well as that the percentage of DSD with the established etiology increased in comparison to the first period (2007-2015). Also, our data pointed to the fact that the diagnosis was more frequently established with the help of genetic methods, and that genitoplasty was performed in fewer children and at a later age.

Literature

1. Thyen U, Lanz K, Paul-Martin Holterhus PM, Hiort O. Epidemiology and Initial Management of Ambiguous Genitalia at Birth in Germany. Horm Res 2006; 66(4):195-203.
2. Lee PA, Nordenström A, P Houk CP, S Ahmed SF, Auchus R, Baratz A, et al. Global Disorders of Sex Development Update since 2006: Perceptions, Approachand Care. Horm Res Paediatr 2016; 85(3):158-80.
3. Kumar J, Kumar V, Bhatia V, Preeti Dabadghao P, Chaturvedi S, Kapoor R et al. Managing disorder of sexual development surgically: A single center experience. Indian J Urol 2012; 28(3):286-91.
4. Kim KS, Kim J. Disorder of sex development. Korean J Urol 2012; 53(1):1-8.
5. Man E, GOSH DSD MDT, Achermann J. A single centre experience of differences/disorders in sex development over 20 years. Endocrine Abstracts 2016; 45 OC6.9.
6. Ahmed SF, Achermann JC, Arlt W, Balen A, Gerry Conway G, Edwards Z et al. Society for Endocrinology UK guidance on the initial evaluation of an infant or an adolescent with a suspected disorder of sex development (Revised 2015). Clinical Endocrinol (Oxf) 2016; 84(5):771-88.
7. van der Straaten S, Springer A, Zecic A, Hebenstreit D, Tonnhofer U, Gawlik A et al. The External Genitalia Score (EGS): A European Multicenter Validation Study. J Clin Endocrinol Metab 2020; 105(3): dgz142.

development. Nature reviews: endocrinology 2014; 10(9):520-9.

10. Selma Feldman Witchel. Disorders of Sex Development. Best practice and researcs. Clinical obstetrics and gynaecology 2018; 48:90-102.
11. Michelle M Ernst , Lih-Mei Liao, Arlene B. Baratz and David E. Sandberg. Disorders of Sex Development/ Intersex: Gaps in Psychosocial Care for Children. Pediatrics 2018;142(2): e20174045.
12. Lisboa Gomes N, Tarini Chetty, Anne Jorgensen, Rod T Mitchell. Disorders of Sex Development-Novel Regulators, Impacts on Fertility, and Options for Fertility Preservation. Int J Mol Sci 2020; 21(7):2282.
13. Poyrazoglu S, Darendeliler F, Faisal Ahmed S, Hughes I, Bryce J, Jiang J et al. Birth weight in different etiologies of disorders of sex development. J Clin Endocrinol Metab 2017; 102(3):1044-1050.
14. Audi L, Ahmed SF, Krone N, Cools M, McElreavey K, Holterhus PM et al. GENETICS IN ENDOCRINOLOGY: Approaches to molecular genetic diagnosis in the management of differences/disorders of sex development (DSD): position paper of EU COST Action BM 1303 'DSDnet'. Eur J Endocrinol 2018; 179(4): R197-R206.
15. Anu Bashamboo A, Ken Mc Elreavey K. Mechanism of sex determination in humans: Insights from disorders of sex development. Sex Dev 2016; 10(5-6):313-325.
16. American Academy of Pediatrics. Timing of elective surgery on the genitalia of male children with particular reference to the risks, benefits, and psychological effects of surgery and anesthesia. Pediatrics 1996; 97:590-4.
17. Cools M, Nordenström A, Robeva R, Hall J , Westerveld P , Flück C et al. Caring for individuals with a difference of sex development (DSD): a Consensus Statement. Nat Rev Endocrinol 2018;14(7):415-429.
18. Acién P, Acién M. Disorders of Sex Development: Classification, Review, and Impact on Fertility. J Clin Med 2020; 9(11):3555.

Sukob interesa: Nije prijavljen.
Primljen: 27.07.2021.
Revizija: 29.09.2021.
Prihvaćen: 03.10.2021.

Autor za korespondenciju: klinički asistent, dr Rade Vuković, Medicinski fakultet Univerziteta u Beogradu, Institut za zdravstvenu zaštitu majke i deteta Srbije „Dr Vukan Cupic", Radoja Dakića 8, 11070 Beograd, Srbija; e-mail: radevukovic9@gmail.com

8. García-Acero M, Moreno-Niño O, Suárez-Obando F, Molina M, Manotas MC, Prieto JC et al. Disorders of sex development: Genetic characterization of a patient cohort. Mol Med Rep 2020; 21(1):97-106.
9. Hiort O, Birnbaum W, Marshall L, Wünsch L, Werner R, Schröder T, et al. Management of disorders of sex development. Nature reviews: endocrinology 2014; 10(9):520-9.
10. Selma Feldman Witchel. Disorders of Sex Development. Best practice and researcs. Clinical obstetrics and gynaecology 2018; 48:90-102.
11. Michelle M Ernst, Lih-Mei Liao, Arlene B. Baratz and David E. Sandberg. Disorders of Sex Development/ Intersex: Gaps in Psychosocial Care for Children. Pediatrics 2018;142(2): e20174045.
12. Lisboa Gomes N, Tarini Chetty, Anne Jorgensen, Rod T Mitchell. Disorders of Sex Development-Novel Regulators, Impacts on Fertility, and Options for Fertility Preservation. Int J Mol Sci 2020; 21(7):2282.
13. Poyrazoglu S, Darendeliler F, Faisal Ahmed S, Hughes I, Bryce J, Jiang J et al. Birth weight in different etiologies of disorders of sex development. J Clin Endocrinol Metab 2017; 102(3):1044-1050.
14. Audi L, Ahmed SF, Krone N, Cools M, McElreavey K, Holterhus PM et al. GENETICS IN ENDOCRINOLOGY: Approaches to molecular genetic diagnosis in the management of differences/disorders of sex development (DSD): position paper of EU COST Action BM 1303 'DSDnet'. Eur J Endocrinol 2018; 179(4): R197-R206.
15. Anu Bashamboo A, Ken Mc Elreavey K. Mechanism of sex determination in humans: Insights from disorders of sex development. Sex Dev 2016; 10(5-6):313-325.
16. American Academy of Pediatrics. Timing of elective surgery on the genitalia of male children with particular reference to the risks, benefits, and psychological effects of surgery and anesthesia. Pediatrics 1996; 97:590-4.
17. Cools M, Nordenström A, Robeva R , Hall J , Westerveld P , Flück C et al. Caring for individuals with a difference of sex development (DSD): a Consensus Statement. Nat Rev Endocrinol 2018;14(7):415-429.
18. Acién P, Acién M. Disorders of Sex Development: Classification, Review, and Impact on Fertility. J Clin Med 2020; 9(11):3555.

Conflict of interest: None declared.
Received: 07/24/2021
Revised: 09/29/2021
Accepted: 10/03/2021

Corresponding author: clinical assistant, Rade Vukovic, MD, School of Medicine, University of Belgrade, Mother and Child Health Care Institute of Serbia „Dr Vukan Cupic", Radoja Dakica 8, 11070 Belgrade, Serbia; e-mail: radevukovic9@gmail.com

PRIKAZ SLUČAJA

UDK BROJEVI:
616-001-085:615.279
DOI: https://doi.org/ 10.5937/zdravzast50-33583

ZNAČAJ ALFALIPOINSKE KISELINE ZA LEČENJE HRONIČNIH RANA NA NIVOU PRIMARNE ZDRAVSTVENE ZAŠTITE: PRIKAZI VIŠE POJEDINAČNIH SLUČAJEVA

Dragan Marinković[1]

[1] Služba za zaštitu zdravlja odraslog stanovništva, Zdravstveni centar Negotin, Republika Srbija

SAŽETAK

Iako se sam pojam „rane“ i njihovo lečenje u najvećoj meri vezuje za hirurške discipline i više nivoe zdravstvene zaštite, hronične rane su deo patoloških stanja sa kojima se lekari primarne zdravstvene zaštite u svojoj svakodnevnoj praksi susreću i koje zahvaljujući savremenim oblogama i sredstvima za lečenje hroničnih rana mogu biti uspešno lečene i na primarnom nivou zdravstvene zaštite ukoliko lokalizacija i stepen zahvaćenog tkiva to dozvoljavaju (najbolje je ako je zahvaćena površna tkiva). Uvođenje alfalipoinske kiseline, pored primene savremenih sredstava i obloga za lečenje hroničnih rana, čak i kod osoba sa dijabetesom, podspešuje i ubrzava proces zarastanja. Alfalipoinska kiselina ima jako antioksidativno i antiinflamatorno dejstvo.

Ključne reči: primarna zdravstvena zaštita, savremene obloge, alfalipoinska kiselina, dijabetes, hronične rane

Uvod

Rana koja i uz primenjeno lečenje standardnim procedurama ne pokazuje tendenciju zarastanja, te ostaje otvorena duže od mesec dana, smatra se hroničnom (1,2). Kompleksno zarastanje hronične rane nameće i sveobuhvatni, holistički pristup pacijentu i lečenje osnovne bolesti, s obzirom da i jedan broj rana nastaje kao komplikacija osnovne bolesti, kao što su hronična venska insuficijencija sa varikoznim sindromom, venskim refluksom, trombozom i posledičnim venskim ulkusima. Osim toga ,i kod šećerne bolesti progresivno oštećenje perifernih nerava predstavlja uvod za nastanak, najpre ulceracija na stopalu, a potom i gangrene, kao najteže komplikacije, koja neretko zahteva amputaciju obolelog ekstremiteta kao neophodnu terapijsku opciju.

Lečenje hroničnih rana za svaki zdravstveni sistem i samo društvo predstavlja značajno finansijsko opterećenje uzimajući u obzir troškove lečenja, te posledičnu invalidnost, pogotovu kod pacijenata sa dijabetesom. Epidemiološki podaci govore da 70-80% svih hroničnih ulkusa čini venski ulkus, te predstavlja najčešću hroničnu ulceraciju, čiji su recidivi jako česti i lečenje dugotrajno. Učestalost je veća kod ženskog pola, u 95% slučajeva lokalizovani su na unutrašnjoj strani potkolenice, u neposrednoj blizini medijalnog maleolusa (1-5). Takođe se procenjuje da 15% dijabetičara ima hroničnu uleraciju (6). Savremene obloge za lečenje hroničnih rana, zahvaljujući svojoj strukturi, omogućavaju autolitički debridman rane. U kontaktu sa ranom ostvaruju održavanje relativno konstantne temperature i vlažnosti rane, odnosno koncept vlažnog zarastanja rane, čime se proces zarastanja značajno ubrzava. U odnosu na široko primenjivanu klasičnu pamučnu gazu, savremene obloge imaju niz prednosti, počevši od jednostavne upotrebe, do omogućavanja nephodne razmene gasova između rane i spoljašnje sredine, sprečavanja penetracije mikroorganizama u ranu, sve do one najbitnije, sprečavanja isušivanja rane, a samim tim i produženog inflamatornog odgovora, čime se omogućava adekvatna sinteza i migracija ćelija neophodnih za epitelizaciju i zarastanje rane (3,4,6). Alfalipoinska kiselina predstavlja deo terapijske palete u lečenju pacijenata sa dijabetesom, ne samo u lečenju dijabetesne polineuropatije, već zbog svog izraženog antiiflamatornog dejstva pospešuje i ubrzava zarastanje hroničnih ulkusa (7,8).

CASE REPORT

UDK NUMBERS:
616-001-085:615.279
DOI: https://doi.org/ 10.5937/zdravzast50-33583

THE IMPORTANCE OF ALPHA-LIPOIC ACID FOR THE TREATMENT OF CHRONIC WOUNDS AT THE LEVEL OF PRIMARY HEALTH CARE: A CASE REPORT

Dragan Marinkovic[1]

[1] Healthcare service for adults, Health Care Center Negotin,Republic of Serbia

SUMMARY

Although the term "wounds" and their treatment is mostly associated with surgical disciplines and higher levels of health care, chronic wounds are part of the pathological conditions that primary care physicians encounter in their daily practice and which, thanks to modern dressings and means for the treatment of chronic wounds, can be successfully treated at the primary level of health care if the localization and the degree of affected tissues allow (the best case is when only surface tissues are damaged). In addition to the use of modern means and dressings for the treatment of chronic wounds, the introduction of alpha-lipoic acid, in addition to the use of modern means and dressings for the treatment of chronic wounds, even in people with diabetes, accelerates and accelerates the healing process. Alpha-lipoic acid has a strong antioxidant and anti-inflammatory effect.

Key words: primary health care, modern wound dressings, alpha-lipoic acid, diabetes, chronic wounds

Introduction

Wounds that fail to heal, although the treatment with standard procedures was applied, and stay open longer than a month are deemed to be chronic (1,2). The complex healing of a chronic wound demands a comprehensive, holistic approach to patient care and the treatment of the primary disease, considering the fact that some wounds occur as complications of primary diseases, such as chronic venous insufficiency with varicose veins, venous reflux, thrombosis, and the consequent venous ulcers, as well as diabetes, whose progressive damage of peripheral nerves represents the introduction to the appearance of foot ulcers at first, and then gangrene as the most severe complication which often demands the amputation of affected limbs as a necessary treatment option.

The treatment of chronic wounds represents a significant financial burden for the healthcare system and society, as well, considering the costs of that treatment and the consequent disability, especially in patients with diabetes. Epidemiological data indicate that venous ulcer is the most frequent chronic ulceration accounting for 70-80% of all chronic ulcers, with the frequent recurrence and long-lasting treatment. The incidence is higher in women, and in 95% of cases they are localized on the inner lower leg above the medial malleolus (1,2,4,5). Also, it has been estimated that 15 % of all people with diabetes have chronic ulcerations (6). Modern wound dressings used for the treatment of chronic wounds, thanks to their structure, enable autolytic wound debridement. In contact with the wound, they provide and maintain relatively constant temperatures and moist environment to the wound, that is, the concept of moist wound healing, thus accelerating the healing process. In comparison to the widely applied classic cotton gauze, modern wound dressings have several advantages. First, they are easy to apply, they allow gas exchange between the wound and environment, they prevent the penetration of microorganisms into the wound and most importantly, they prevent dehydration and therefore, prolonged inflammatory response, thus enabling the appropriate synthesis and migration of cells necessary for the epithelialization and wound healing (3,4,6). Alpha-lipoic acid is one part of

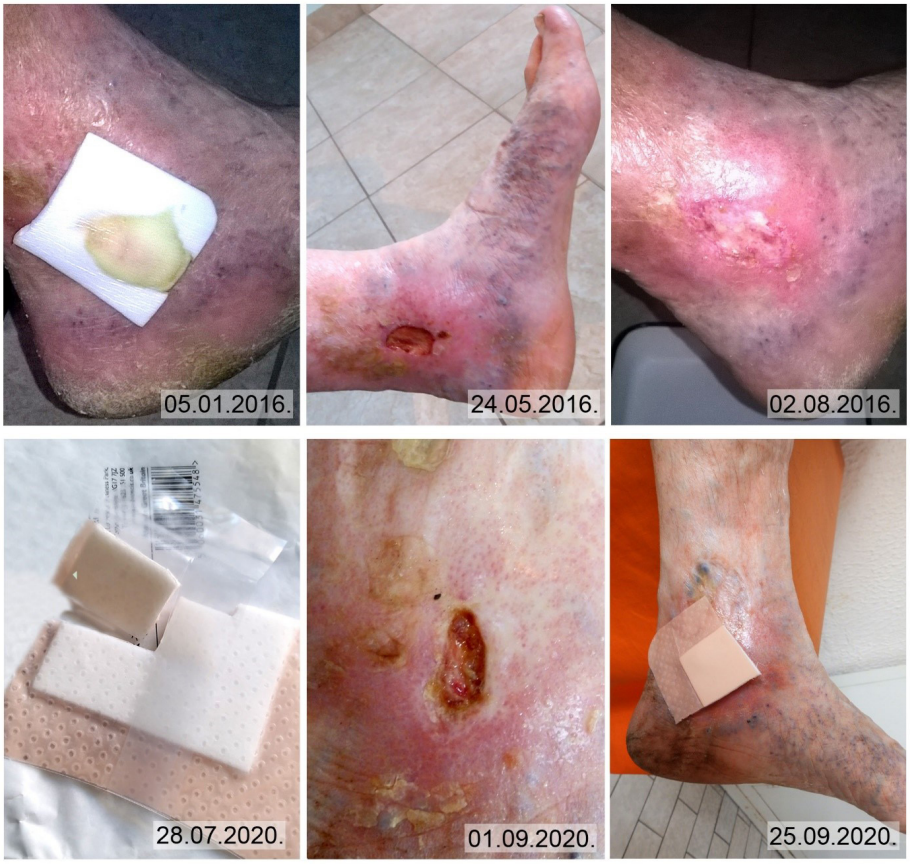
Cilj prikaza sva tri pacijenta je da se ukaže da se na primarnom nivou zdravstvene zaštite može koristiti, pored savremenih obloga i sredstava za lečenje hroničnih rana, kada su procesom zahvaćena površna tkiva, i alfalipoinska kiselina.

Prikazi slučajeva

Prikaz 1

Pacijentkinja starosne dobi 67 godina, zbog dijabetesa je na peroralnoj terapiji od 2004. godine i ima sledeće dijagnoze: *insuff. vv. perforantes, sy. varicosum cruris billat, ulcus venosum cruris lat. sin.*, zatražila je pomoć od lekara primarne zdravstvene zaštite usled recidiva venskog ulkusa na medijalnoj strani leve potkolenice. Ulkus je lokalizovan perimaleolarno, nepravilnog je oblika i ivica, sa nekrotičnim tkivom na dnu ulkusa.

U decembru 2015. godine prvi put se pristupa lečenju hronične venske ulceracije primenom dvoslojne semiokluzivne poliuretanske obloge u cilju ostvarivanja autolitičkog debridmana rane sa već prethodno uvedenim preparatom alfalipoinske kiseline u jutarnjoj dozi od 600 mg, pored svoje redovne terapije derivatima sulfonilureje i metforminom za lečenje šećerne bolesti, te antihipertenzivne terapije ACE inhibitorima, kao i acetilsalicilne kiseline. Takođe, u terapiju se uvode i antibiotici širokog spektra parenteralnim i peroralnim putem u trajanju od dve nedelje. Debridman hronične rane se od maja meseca 2016. godine, usled već oskudnije eksudacije, nastavlja upotrebom hidrokoloidne obloge, kao i aplikovanjem gela za epitelizaciju rana, deproteinizovanog hemodijalizata teleće krvi, sve do zarastanja ulkusa.



Slika 1. Venski ulkus leve potkolenice
05.01.2016. lečenje ulkusa dvoslojnom semiokluzivnom poliuretanskom oblogom se počinje još u decembru 2015. radi postizanja autolitičkog debridmana hronične rane;
24.05.2016. i 02.08.2016, debridman hronične rane usled već oskudnijeg eksudata se nastavlja hidrokoloidnom oblogom i aplikovanjem gela za epitelizaciju rana, deproteinizovanog hemodijalizata teleće krvi sve do zarastanja ulkusa;
28.07.2020, 01.09.2020. i 25.09.2020. recidiv hroničnog venskog ulkusa, lečenje se sprovodi hidrofilnom poliuretanskom oblogom i topikalnom primenom fusidinske kiseline.

the therapeutic palette used for the treatment of patients with diabetes. It is used not only for the treatment of diabetic polyneuropathy, but due to its pronounced anti-inflammatory effect, it improves and accelerates chronic ulcer healing (7,8).

The aim of case reports of three patients was to point to the fact that in addition to modern dressings and means for the treatment of chronic wounds, alpha-lipoic acid can be used at the level of primary healthcare when surface tissues are affected by the process.

Case reports

The first case report

A 67-year-old female patient has taken medications for diabetes orally and her diagnoses are the following: *Insuff. vv. perforantes, Sy. varicosum crusris billat, Ulcus venosum cruris lat. sin*. The woman has sought help from the general practitioner due to the recurrence of venous ulcer on the medial side of the left inner lower leg. It was a perimalleolar ulcer with an irregular shape and edges, and with the necrotic tissue at the base of the ulcer. In December 2015, chronic venous ulceration was treated for the first time by the two-layered polyurethane semi-occlusive dressing, aimed at achieving autolytic wound debridement with the previously introduced preparation of alpha-lipoic acid of 600 mg in the morning, in addition to the regular therapy including sulfonylurea derivatives and metformin used for the treatment of diabetes, antihypertensive therapy with ACE inhibitors, and acetylsalicylic acid. Also, parenteral

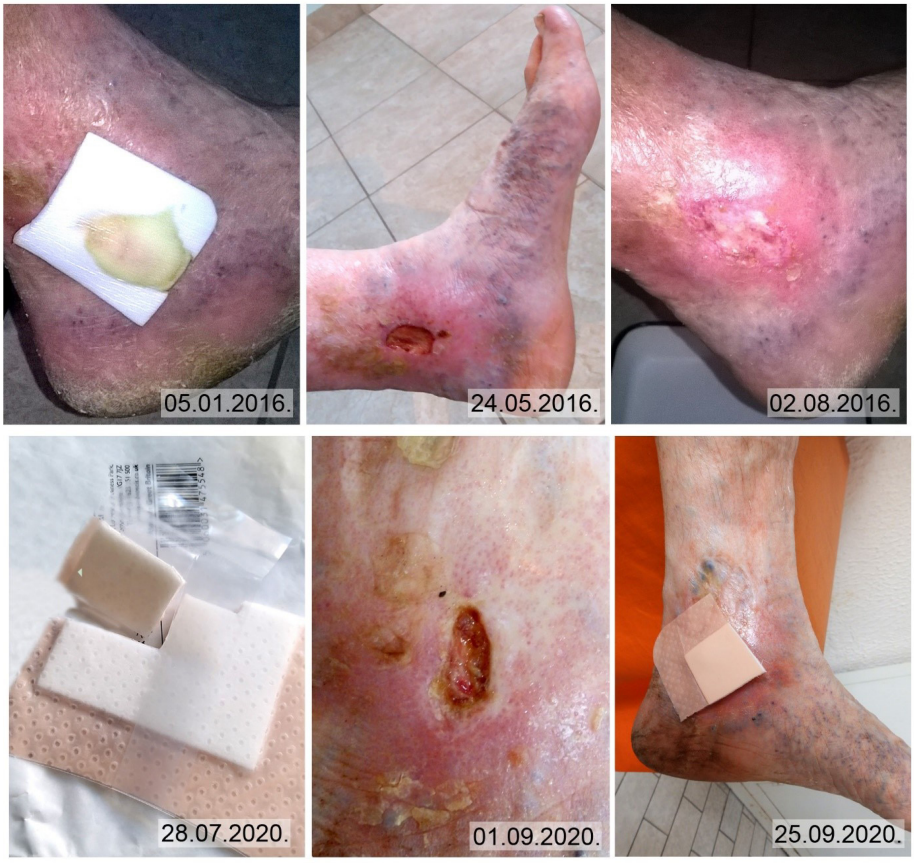


Figure 1. Venous ulcerations on the left shin

January 5th, 2016 – the treatment of the ulcer with the two-layered polyurethane semi-occlusive dressing that started in December, 2015 in order to achieve the autolytic wound debridement.

May 24th, 2016 and August 2nd, 2016 – due to the decreased exudation, chronic wound debridement was continued with the hydrocolloid dressing and application of gel for wound epithelialization, deproteinized calf blood hemoderivative until the ulcer healed.

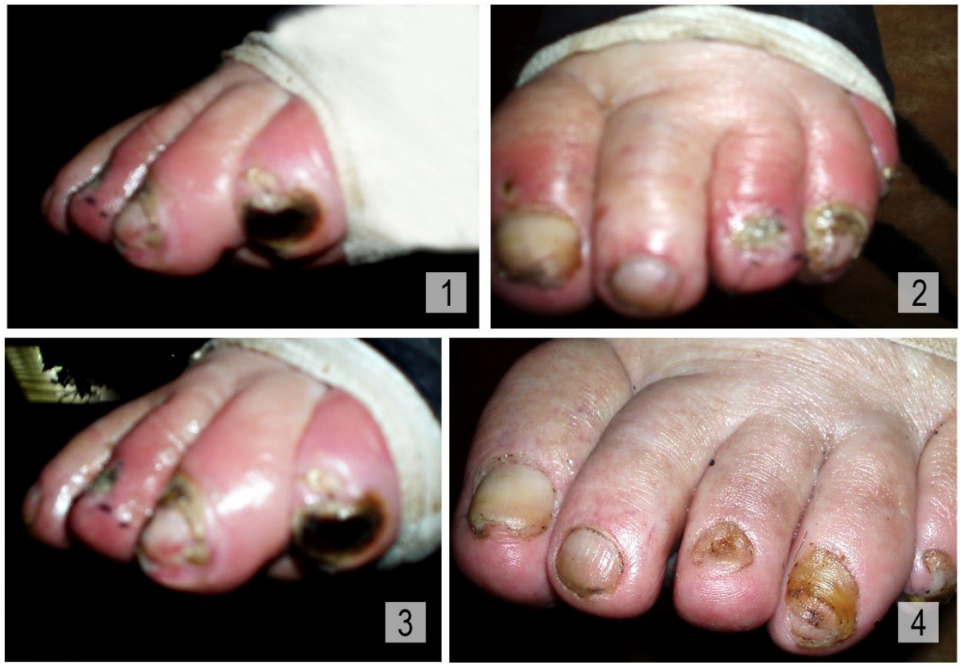
July 28th, September 1st, September 25th, 2020 – the relapse of chronic venous ulcer; it was treated with the hydrophilic polyurethane dressing and topical application of fusidic acid.

Primenjenom terapijom u trajanju od osam meseci, od decembra 2015. do početka avgusta 2016. godine, ostvaruje se zarastanje i epitelizacija hroničnog venskog ulkusa.

Već atrofirana koža leve potkolenice i konstantno izlaganje fizičkom naporu uslovljava pojavu novog recidiva nakon nepune četiri godine nakon zarastanja (slika 1). U 2020. debridman recidivantnog hroničnog venskog ulkusa se, takođe, sprovodi primenom savremene obloge za lečenje hroničnih rana uz topikalnu primenu fusidinske kiseline zbog znakova lokalne infekcije ulkusa. Aplikovanju hidrofilne poliuretanske obloge i fusidinske kiseline prethodi obavezno čišćenje rane. Zarastanje i epitelizacija recidivantnog hroničnog venskog ulkusa postiže se krajem 2020. godine.

Prikaz 2

Pacijentkinja starosne dobi 75 godina je na insulinskoj terapiji poslednjih dvadeset godina i ima varikozni sindromom. Lekaru primarne zdravstvene zaštite pacijentkinja se obraća mesec dana nakon povređivanja koje je inicijalno pokušala sama da leči. Motiv za obraćanje lekaru su bili lokalni znaci pogoršanja same povrede, edem stopala i početna gangrena distalnog dela petog prsta levog stopala. U postelji zauzima pasivan stav, telesna težina pacijentkinje je 120 kg, a indeks telesne težine ukazuje na patološku gojaznost. Odaje utisak teškog bolesnika. U terapiju se uvodi klindamicin što dovodi do delimičnog poboljšanja i regresije edema levog stopala. Međutim, davanje klindamicina moralo je biti obustavljeno usled alergijske reakcije koja se manifestovala nakon trećeg dana od započete antibiotske terapije (slika 2). Intenzitet bola je posle trećeg dana od antibiotske terapije bio manji. Zbog alergijske reakcije na klindamicin u terapiju se uvodi ceftriakson, intramuskularno dva puta dnevno po 1 gr u trajanju od sedam dana, i alfalipoinska kiselina 600 mg u infuzionim rastvorima, jedanput dnevno u toku sedam dana. Pacijentkinja je više godina unazad imala u terapiji 600 mg dnevno alfalipoinske kiseline peroralnim putem, što može da objasni odsusustvo simptoma i znakova dijabetesne polineuropatije, kao i povlačenje edema i početne gangrene u relativno kratkom vremenskom periodu. Primenom navedene medikamentne terapije antibioticima i alfalipoinskom kiselinom peroralnom i parenteralnom administracijom postiže se povlačenje edema i eritema, kao i početne gangrene lokalizovane na distalnom delu petog prsta levog stopala.



Slika 2. Početna gangrena petog prsta levog stopala

Slike 2.1, 2.2, i 2.3 prikazuju edematozno stopalo sa početnom gangrenom distalnog dela petog prsta levog stopala;

Slika 2.4 prikazuje povlačenje edema i početne gangrene na primenjenu antibiotsku terapiju i alfalipoinsku kiselinu.

and peroral broad-spectrum antibiotics have been administered for two weeks. Since 2016, due to scarce exudation, chronic wound debridement had continued using the hydrocolloid dressing, as well as the gel for the wound epithelialization, that is, deproteinized calf blood hemoderivative until the ulcer healed.

The epithelialization and healing of the chronic venous ulcer was achieved by the applied therapy that lasted eight months, from December 2015 to August 2016.

The atrophic skin of the left inner lower leg and constant exposure to physical strain caused the appearance of the new relapse after less than four years after healing (Picture 1). In 2020, the debridement of the recurrent chronic venous ulcer was also done with the help of a modern chronic wound dressing with the topical application of fusidic acid due to the signs of local infection. The wound should necessarily be cleansed before the application of hydrophilic polyurethane dressing and fusidic acid. The epithelialization and healing of the recurrent chronic venous ulcer was achieved at the end of 2020.

The second case report

A 75-year-old female patient has been on insulin therapy for twenty years and she has had the varicose syndrome. The patient came to the doctor at the primary health care center one month after she had fallen and tried to treat the wound on her own. The motives for the visit to the doctor were local signs of the exacerbation of that injury, foot edema and the initial gangrene of the distal part of the fifth toe on the left foot. Her posture was passive in bed, her body weight was 120 kg, and the body max index indicated pathological obesity. She gave the impression of a serious patient. Clindamycin was introduced into the therapy, which led to the partial improvement and regression of the edema of the left foot, but this therapy had to be stopped due to the allergic reaction that occurred three days after the beginning of antibiotic therapy (Picture 2). The intensity of pain decreased three days after the antibiotic therapy. Due to allergic reaction to Clindamycin, Ceftriaxone was introduced intramuscularly in a dose of 1 gr two times a day within 7 days and alpha-lipoic acid of 600 mg in infusion solutions once a day within seven days.

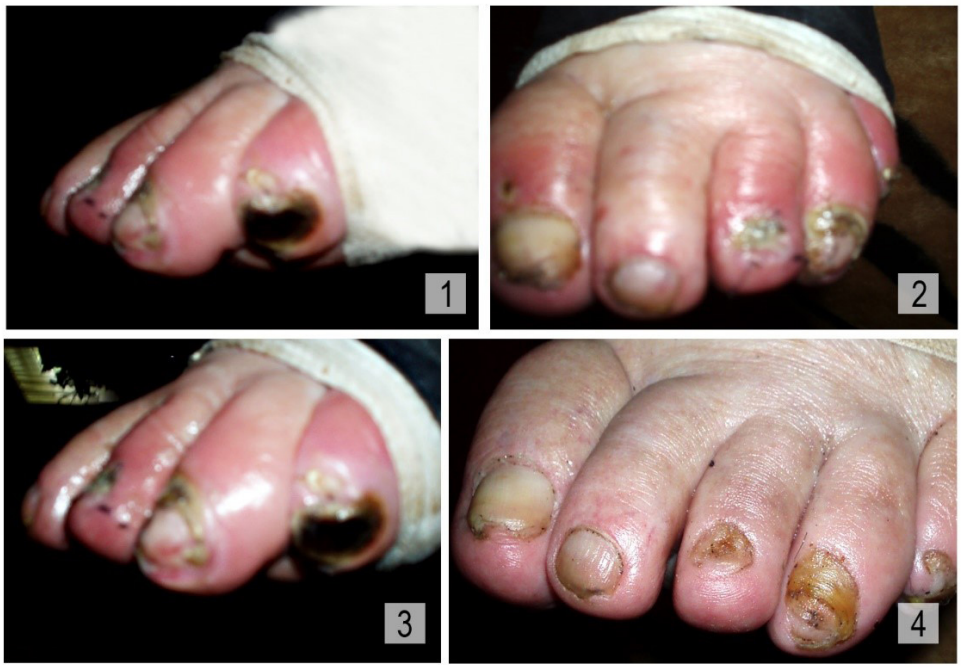


Figure 2. Initial gangrene on the distal part of the left fifth toe
The edematous foot with the initial gangrene on the distal part of the left fifth toe is shown in pictures 2.1, 2.2 and 2.3.
Picture 2.4 shows the regression of edema and the initial gangrene after the administration of antibiotic therapy and alpha-lipoic acid.

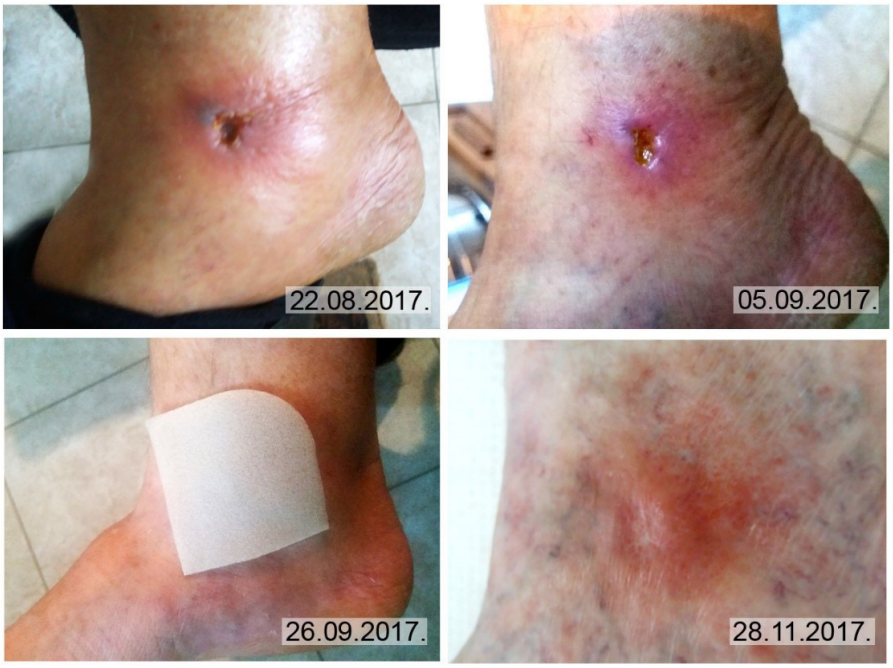
Prikaz 3

Pacijentkinja, stara 66 godina, je hronični srčani bolesnik i 2016. godine je imala infarkt miokarda. Od 2017. godine je na terapiji hidroksikarbamidom usled hroničnog mijeloproliferativnog oboljenja, kao i na antikoagulantnoj terapiji usled poremećaja srčanog ritma po tipu atrijalne fibrilacije. Javlja se izabranom lekaru dva meseca nakon nastale ubodne rane distalnog dela medijalne strane desne potkolenice i to zbog bolova, kao i zbog razvoja periulkusnog edema i eritema neposredno nakon povređivanja. Ordinira se klindamicin 600 mg peroralno, nakon čega se pacijentinja subjektivno oseća bolje, uz povlačenje lokalnog eritema i edema na ivicama rane. Mikrobiološkim brisom ulkusa izolovan je *Proteus mirabilis* te se ulkus leči topikalnom primenom antibiotika po antibiogramu uz primenu hidrokoloidne obloge, koja se oblikuje u odnosu na dimenzije rane. Pacijentkinja poreklom iz ruralne oblasti je bila edukovana da sama primenjuje oblogu u daljem toku lečenja. Nakon tromesečnog debridmana hronične rane hidrokoloidnom oblogom uz toaletu rane i lokalnim aplikovanjem antibiotika po rezultatima antibiograma postiže se zarastanje hroničnog ulkusa.

Diskusija

Superficijalni venski ulkus leve potkolenice u pacijentkinje sa dijabetesom na peroralnoj terapiji (prikaz 1), predstavlja hroničnu ranu koja najčešće recidivira i čije lečenje dugo traje, često više godina (6,9). Ujedno predstavlja i primer neophodnosti sveobuhvatnog lečenja pacijenata sa hroničnim ranama, a kod pacijenata sa dijabetesom adekvatna glikoregulacija je preduslov uspešnog lečenja. Početna gangrena distalnog dela petog prsta levog stopala (prikaz 2), kao i ubodna rana distalnog dela medijalne strane desne potkolenice (prikaz 3), nakon lečenja nisu pokazivale znake recidiva.

Svi prikazani pacijenti su poreklom iz ruralnih oblasti što često uslovljava potrebu za kućnim i lečenjem u terenskim ambulantama, ukoliko priroda i lokalizacija rane to dozvoljavaju. Jedan od kriterijuma za odabir adekvatne savremene obloge je i količina eksudata u rani. Tako su za lečenje hroničnog venskog ulkusa zbog umerene eksudacije korišćene poliuretanske i potom hidrokoloidne obloge, dok su za ubodnu ranu zbog oskudnog eksudata upotrebljene hidrokoloidne obloge. Preparati alfalipoinske kiseline u dozi od 600 mg dnevno su zbog svog dokazanog protektivnog i antiiflamatornog dejstva pogotovu u pacijenata sa dijabetesom ordinirani peroralnim i parenteralnim putem, čija je upotreba



Slika 3. Ubodna rana desne potkolenice
22.08.2017, 05.09.2017 i 26.09.2017. ubodna rana desne potkolenice i hidrokoloidna obloga; 28.11.2017. zarastanje ulkusa topikalnom primenom levofloksacina i hidrokoloidnom oblogom nakon tromesečnog lečenja.

The patient had used 600 mg of alpha-lipoic acid perorally for years, which could explain the absence of symptoms and signs of diabetic polyneuropathy, as well as the regression of edema and initial gangrene in a relatively short period of time. The regression of edema and erythema, as well as the initial gangrene localized on the distal part of the left fifth toe was achieved by the peroral and parenteral administration of the above mentioned medical therapy.

The edematous foot with the initial gangrene on the distal part of the left fifth toe is shown in pictures 1, 2 and 3. Picture 4 shows the regression of edema and the initial gangrene after the administration of antibiotic therapy and alpha-lipoic acid.

The third case report

The 66 year old patient has a chronic heart disease and she had myocardial infarction in 2016. Since 2017, she has used hydroxycarbamide due to chronic myeloproliferative disease, as well as anticoagulant therapy due to cardiac arrhythmias by type of atrial fibrillation. She came to her doctor two months after she had had stab wounds to the distal part of the medial side of the right lower leg, due to pain as well as due to development periulcus edema and erythema immediately after injuries. Clindamycine was administered in a dose of 600 mg perorally, and after that she felt better with the regression of local erythema and edema on the edges of the wound. *Proteus mirabilis* was isolated from the ulcer with the microbiological swab, and the ulcer was treated with the topical application of antibiotics according to the antibiogram, as well as with the application of hydrocolloid dressing whose shape depends on the dimension of the wound. The patient was from a rural area and therefore, she was educated to apply the dressing on her own during the further course of treatment. After the wound debridement with the hydrocolloid dressing that lasted three months, as well as cleansing and local application of antibiotics according to antibiogram results, ulcer healing was achieved.

Discussion

Superficial venous ulcer of the left lower leg in the patient with diabetes who received the therapy perorally (Case report 1) represents a chronic wound that usually recurs and whose treatment lasts a long time, often several years. The presence of diabetes is an aggravating circumstance for the healing process (6,9). It also confirms the necessity for the comprehensive treatment of patients with

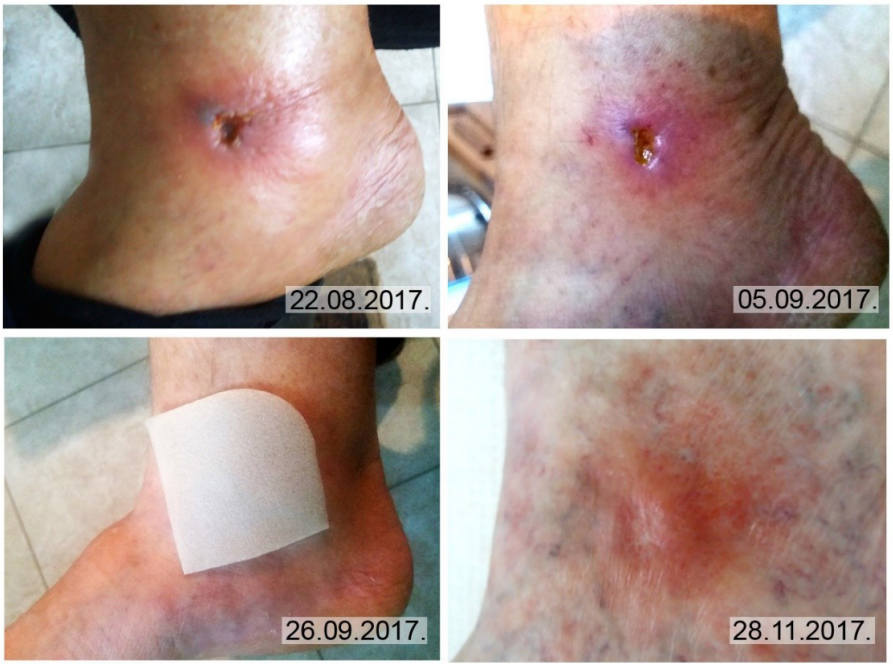


Figure 3. Stab wound on the distal part of the right shin medial side August 22th, September 5th, and September 26th, 2017 – a swab wound of the right lower leg and hydrocolloid dressing. November 28th, 2017 – healing of the ulcer with the help of topical levofloxacine and hydrocolloid dressing after the treatment that lasted three months.

zajedno sa savremenim oblogama uz antibiotsku terapiju značajno ubrzala lečenje.

Zaključak

Lečenje hroničnih rana je težak zadatak za zdravstveni sistem i samo društvo, uzimajući u obzir da može trajati više meseci, ponekad i godinama. Primarna zdravstvena zaštita sa svim svojim segmentima predstavlja nezaobilazan deo zdravstvenog sistema u lečenju hroničnih rana. Masovnija upotreba savremenih sredstava za lečenje hroničnih rana, kao i uvođenje u terapiju alfalipoinske kiseline, za osobe sa dijabetesom bi u značajnoj meri olakšala i ubrzala lečenje.

Literatura

1. Sen CK. Human Wounds and Its Burden: An Updated Compendium of Estimates. Adv Wound Care (New Rochelle) 2019; 8(2):39-48.
2. Zhao R, Liang H, Clarke E, Jackson C, Xue M. Inflammation in Chronic Wounds. Int J Mol Sci 2016; 17(12):2085.
3. Shi C, Wang C, Liu H, et al. Selection of Appropriate Wound Dressing for Various Wounds. Front Bioeng Biotechnol 2020; 8:182.
4. Delić J. Vodič za lečenje hronične venske ulceracije, 2020. Dostupno na: https://www.udruzenjezalecenjerana.org/wp-content/uploads/2018/09/Vodic-za-lecenje-hronicnih-rana.pdf
5. Matić M. Hronične rane vaskularne etiologije. U: Lečenje hroničnih rana, Borović S, Vučetić Č, Matić M, urednici. Beograd: Zavod za udžbenike, 2014; pp 83-132.
6. Han G, Ceilley R. Chronic Wound Healing: A Review of Current Management and Treatments. Adv Ther 2017; 34(3):599-610.
7. Alleva R, Nasole E, Di Donato F, Borghi B, Neuzil J, Tomasetti M. Alpha-Lipoic acid supplementation inhibits oxidative damage, accelerating chronic wound healing in patients undergoing hyperbaric oxygen therapy. Biochem Biophys Res Commun. 2005; 333(2):404-410.
8. Gomes MB., Negrato CA. Alpha-lipoic acid as a pleiotropic compound with potential therapeutic use in diabetes and other chronic diseases. Diabetol Metab Syndr 2014; 6:80.
9. Tan MKH, Luo R, Onida S, Maccatrozzo S, Davies AH. Venous Leg Ulcer Clinical Practice Guidelines: What is AGREEd? Eur J Vasc Endovasc Surg 2019; 57(1):121-129.

Sukob interesa: Nije prijavljen.
Primljen: 17.08.2021.
Revizija: 28.09.2021.
Prihvaćen: 03.10.2021.

Autor za korespondenciju: Spec. opšte medicine dr Dragan Marinković, Služba za zaštitu zdravlja odraslog stanovništva Zdravstveni centar Negotin, Negotin, Republika Srbija; e-mail: drdragan303899@gmail.com

chronic wounds, while adequate glicoregulation is a precondition for the successful treatment in patients with diabetes. The initial gangrene of the distal part of the left fifth toe (Case report 2), as well as a swab wound of the distal part of the medial side of left lower leg (Case report 3) did not show signs of relapse after the treatment.

All presented patients were from rural areas, which often conditioned the need for the treatment at home or in field ambulances if the nature and localization of the wound allowed that. One of the criteria for the choice of appropriate modern dressing was the quantity of exudation in the wound. Therefore, polyurethane and hydrocolloid dressings were used for the treatment of chronic venous ulcers due to the moderate exudation, while hydrocolloid dressings were used for the swab wound due to the decreased exudation. The preparations of alpha-lipoic acid in a dose of 600 mg a day were administered perorally and parenterally in patients with diabetes due to their protective and anti-inflammatory effects, and their application together with modern dressings and antibiotic therapy accelerated healing significantly.

Conclusion

The treatment of chronic wounds is a difficult task for the healthcare system and society, as well, considering the fact that it can last several months, sometimes even years. The primary healthcare with all its segments presents an unavoidable part of the healthcare system in the treatment of chronic wounds. A wider usage of modern means for the treatment of chronic wounds, as well as alpha-lipoic acid, for people with diabetes would alleviate and accelerate the treatment significantly.

Literature

1. Sen CK. Human Wounds and Its Burden: An Updated Compendium of Estimates. Adv Wound Care (New Rochelle) 2019; 8(2):39-48.
2. Zhao R, Liang H, Clarke E, Jackson C, Xue M. Inflammation in Chronic Wounds. Int J Mol Sci 2016; 17(12):2085.
3. Shi C, Wang C, Liu H, et al. Selection of Appropriate Wound Dressing for Various Wounds. Front BioengBiotechnol 2020;8:182.
4. Delic J. Guidelines for the treatment of chronic venous ulceration, 2020. Available at: https://www.udruzenjezalecenjerana.org/wp-content/uploads/2018/09/Vodic-za-lecenje-hronicnih-rana.pdf
5. Matic M. Chronic wounds of vascular etiology. In: The Treatment of Chronic wounds, Borovic S, Vucetic C, Matic M, editors. Belgrade: Publishing House for Textbooks, 2014; pp83-132.
6. Han G, Ceilley R. Chronic Wound Healing: A Review of Current Management and Treatments. Adv Ther 2017; 34(3):599-610.
7. Alleva R, Nasole E, Di Donato F, Borghi B, Neuzil J, Tomasetti M. Alpha-Lipoic acid supplementation inhibits oxidative damage, accelerating chronic wound healing in patients undergoing hyperbaric oxygen therapy. BiochemBiophys Res Commun. 2005; 333(2):404-410.
8. Gomes MB., Negrato CA. Alpha-lipoic acid as a pleiotropic compound with potential therapeutic use in diabetes and other chronic diseases. DiabetolMetabSyndr 2014; 6:80.
9. Tan MKH, Luo R, Onida S, Maccatrozzo S, Davies AH. Venous Leg Ulcer Clinical Practice Guidelines: What is AGREEd? Eur J VascEndovasc Surg 2019; 57(1):121-129.

Conflict of interest: None declared.
Received: 08/17/2021
Revised: 09/28/2021
Accepted: 10/03/2021

Corresponding author: specialist of general practice dr Dragan Marinkovic, Healthcare service for adults ,Health Care Center Negotin, Negotin, Republic of Serbia; E-mail:drdragan303899@gmail.com

PREGLEDNI RAD

UDK BROJEVI:
616-036.1
615.279:547.56
DOI: https://doi.org/10.5937/zdravzast50-33638

ZNAČAJ POLIFENOLA U PREVENCIJI HRONIČNIH NEZARAZNIH BOLESTI

Marijana Jandrić-Kočić[1]

[1] JZU Dom zdravlja Krupa na Uni, Republika Srpska, Bosna i Hercegovina

SAŽETAK

Polifenoli su spojevi koji u svojoj strukturi sadrže jednu ili više hidroksilnih skupina vezanih izravno na jedan ili više aromatskih ugljovodonika. Predstavljaju jednu od najbrojnijih i najrasprostranjenijih skupina sekundarnih biljnih metabolita sa više od 8000 polifenolnih struktura. Polifenoli su antioksidansi koji smanjuju disfunkciju endotela i krvni pritisak, unapređuju imunološku odbranu, ublažavaju upalni odgovor, blokiraju agregaciju trombocita i oksidaciju lipoproteina male gustine. Neke studije ukazuju na postojanje indirektne veze između unosa flavonoida i obolevanja od infarkta miokarda i umiranja od koronarne bolesti. Crna čokolada, orašasti plovodi, grožđe, crveno vino i mediteranska dijeta (bazirana na voću i povrću, ribi i maslinovom ulju) su bogati polifenolima i ključni su u prevenciji kardiovaskularnih bolesti. Polifenoli sprečavaju neurodegenerativne promjene povezane s cerebralnom ishemijom. Antocijanini iz borovnica imaju antiaterogena i protivupalna svojstva tako da djeluju neuroprotektivno. Ekstrakt crnog vina, bogat antocijaninom, smanjuje ozljede izazvane cerebralnom ishemijom. Unošenje polifenola hranom može redukovati hipertenziju, protivupalnim i antioksidativnim efektima, kao i povećanom proizvodnjom oksida azota. Posebno se ukazuje na značaj crnog i zelenog čaja u snižavanju vrednosti krvnog pritiska. Neophodna su dalja istraživanja polifenola u cilju donošenja što jasnijih preporuka za njihovu opštu preventivnu primenu.

Ključne riječi: polifenoli, hronične nezarazne bolesti, prevencija, ishrana

Uvod

Prema hemijskim obilježjima, polifenoli su spojevi koji u svojoj strukturi sadrže jednu ili više hidroksilnih skupina vezanih izravno na jedan ili više aromatskih ugljikovodika (1,2). Predstavljaju jednu od najbrojnijih i najrasprostranjenijih skupina sekundarnih biljnih metabolita sa više od 8000 polifenolnih struktura (2,3). Cijela skupina ima naziv po osnovnom predstavniku, fenolu (1,2). Polifenoli nastaju od zajedničkog intermedijera, fenilalanina, odnosno bliskog prekursora, šikiminske kiseline (3). Klasifikacija polifenola se temelji na strukturi, biološkoj aktivnosti i biosintetskom putu (2). Generalno, razlikujemo flavonoide i neflavonoide (4). Flavonoidi pripadaju klasi nisko-molekularnih bioaktivnih fenolnih jedinjenja (5,6). Imaju zajedničku osnovnu strukturu s dva aromatična prstena i tri atoma ugljika povezana u oksigenirani heterocikl (4). Na temelju varijacije u tipu heterocikla podijeljeni u šest glavnih potklasa: flavonoli, flavanoni, flavanoli, flavoni, antocijani i izoflavoni (4). Ostale grupe flavonoida uključuju manje prisutne halkone, dihidrohalkone, dihidroflavonole, flavan-3,4-diole, kumarine i aurone (2). Unutar svake grupe postoje pojedinačne razlike u broju i rasporedu hidroksilnih grupa i njihovog stepena alkilacije i/ili glikozilacije (4). Flavonoidi se pojavljuju i kao hidroksilirani i metoksirani derivati, glikozilirani s monosaharidima ili oligosaharidima, esterificirani s organskim kiselinama (2).

Prehrambeni izvori polifenola

Voće, povrće i pića, poput čaja i crnog vina, predstavljaju glavne izvore polifenola (tabela 1) (7-9). Određeni polifenoli, poput kvercetina, nalaze se u svim biljnim proizvodima (voće, povrće, žitarice, mahunarke, voćni sokovi, čaj, vino), dok su drugi specifični za određenu hranu (flavanoni u citrusnom voću, izoflavoni u soji) (7). Flavanoni su prisutni u začinskim biljkama (kapari, šafran, sušeni meksički origano) i povrću (luk, špinat), a izoflavoni

REVIEW ARTICLE

UDK NUMBERS:
616-036.1
615.279:547.56
DOI: https://doi.org/10.5937/zdravzast50-33638

THE IMPORTANCE OF POLYPHENOLS IN THE PREVENTION OF CHRONIC NON-COMMUNICABLE DISEASES

Mariana Jandric-Kocic[1]

[1] JZU Health center Krupa na Uni, Republic of Srpska, Bosnia and Herzegovina

SUMMARY

Polyphenols are compounds that contain in their structure one or more hydroxyl groups attached directly to one or more aromatic hydrocarbons. They represent one of the most numerous and widespread groups of secondary plant metabolites with more than 8000 polyphenolic structures. Polyphenols are antioxidants that reduce endothelial dysfunction and blood pressure, improve the immune defense, alleviate the inflammatory response, block platelet aggregation and oxidation of low-density lipoproteins. Some studies suggest that there is an indirect link between flavonoid intake and myocardial infarction and coronary heart disease. Dark chocolate, nuts, grapes, red wine and the Mediterranean diet (based on fruits and vegetables, fish and olive oil) are rich in polyphenols and are key in preventing cardiovascular disease. Polyphenols prevent neurodegenerative changes associated with cerebral ischemia. Blueberry anthocyanins have antiatherogenic and anti-inflammatory properties so they have a neuroprotective effect. Red wine extract, rich in anthocyanin, reduces injuries caused by cerebral ischemia. Food intake of polyphenols can reduce hypertension, anti-inflammatory and antioxidant effects, as well as increased production of nitric oxide. The importance of black and green tea in lowering blood pressure is especially pointed out. Further research on polyphenols is needed in order to make as clear recommendations as possible for their general preventive use.

Key words: polyphenols, chronic noncommunicable diseases, prevention, diet

Introduction

According to their chemical characteristics, polyphenols are compounds that contain in their structure one or more hydroxyl groups attached directly to one or more aromatic hydrocarbons (1,2). They represent one of the most numerous and widespread groups of secondary plant metabolites with more than 8000 polyphenolic structures (2,3). The whole group was named after its main representative, phenol (1,2). Polyphenols arise from a common intermediate, phenylalanine, that is, a close precursor, shikimic acid (3). The classification of polyphenols is based on their structure, biological function and biosynthetic pathway (2). Generally, they are classified into flavonoids and non-flavonoids (4). Flavonoids belong to a class of low-molecular bioactive phenolic compounds (5,6). They have a common basic structure consisting of two aromatic rings that are bound together by three carbon atoms that form an oxygenated heterocycle (4). They are classified into six main sub-groups according to the variations of the type of heterocycle: flavonols, flavanones, flavanols, flavones, anthocyanins and isoflavones (4). The other groups of flavonoids include the less present chalcones, dihydrochalcones, dihydroflavonols, flavan-3,4-diols, coumarin and aurone (2). Within each group, there are individual differences regarding the number and location of hydrohyl groups and their degree of alkylation and/or glycosylation (4). Flavonoids appear as hydroxylated and methoxylated derivatives, glycosylated with monosaccharides or oligosaccharides, esterified with organic acids (2).

Food sources of polyphenols

Fruit, vegetables and beverages, such as tea and red wine, represent the main sources of polyphenols (Table 1) (7-9). Certain polyphenols, such as quercetin, are present in all plant products

Tabela 1. Prehrambeni izvori polifenola (7)

Polifenoli u hrani	Izvor polifenola	Sadržaj polifenola mg/kg (mg/l)
Hydroxybenzoic acids	Kupina	80–270
Protokatekuinska kiselina	Malina	60–100
Galna kiselina	Crna ribizla	40–130
p -hidroksibenzojeva kiselina	Jagoda	20–90
Hidroksicinamske kiseline	Borovnica	2000–2200
Kofeinska kiselina	Kivi	600–1000
Klorogenska kiselina	Trešnja	180–1150
Kumarna kiselina	Šljiva	140–1150
Ferulinska kiselina	Patlidžan	600–660
Sinapinska kiselina	Jabuka	50–600
	Kruška	15–600
	Cikorija	200–500
	Artičoka	450
	Krompir	100–190
	Kukuruzno brašno	310
	Brašno	70–90
	Jabukovača	10–500
	Kafa	350–1750
Antocijani	Patlidžan	7500
Cianidin	Kupina	1000–4000
Pelargonidin	Crna ribizla	1300–4000
Peonidin	Borovnica	250–5000
Definidin	Crno grožđe	300–750
Malvidin	Trešnja	350–4500
	Rabarbara	2000
	Jagoda	150–750
	Crno vino	200–350
	Šljiva	20–250
	Crveni kupus	250
Flavonoli	Žuti luk	350–1200
Kvercetin	Uvijeni kelj	300–600
Kaempferol	Praziluk	30–225
Miricetin	Šeri paradajz	15–200
	Brokoli	40–100
	Crna ribizla	30-70
	Borovnica	30–160
	Kajsija	25–50
	Jabuka	20–40
	Pasulj, zeleni ili beli	10–50
	Crno grožđe	15–40
	Paradajz	2–15
	Zeleni čaj	20–35
	Crni čaj	30–45
	Crno vino	2–30
Flavoni	Peršun	240–1850
Apigenin	Celer	20–140
Luteolin	Paprika	5–10
Flavanoni	Sok od naranče	215–685
Hesperetin	Sok od grejpfruta	100–650
Naringenin	Sok od limuna	50–300
Eriodiktiol		
Izoflavoni	Sojino brašno	800–1800
Daidzein	Soja, kuvana	200–900
Genistein	Tofu	80–700
Glicitein	Miso	250-900
	Sojino mlijeko	30–175

Table 1. Food sources of polyphenols (7)

Polyphenols in foods	Source	Polyphenol content mg/kg (mg/l)
Hydroxybenzoic acids	Blackberry	80–270
Protocatechuic acid	Raspberry	60–100
Gallic acid	Black currant	40–130
p-Hydroxybenzoic acid	Strawberry	20–90
Hydroxycinnamic acids	Blueberry	2000–2200
Caffeic acid	Kiwi	600–1000
Chlorogenic acid	Cherry	180–1150
Coumaric acid	Plum	140–1150
Ferulic acid	Aubergine	600–660
Sinapic acid	Apple	50–600
	Pear	15–600
	Chicory	200–500
	Artichoke	450
	Potato	100–190
	Corn flour	310
	Flour: wheat, rice, oat	70–90
	Jabukovača	10–500
	Coffee	350–1750
Anthocyanins	Aubergine	7500
Cyanidin	Blackberry	1000–4000
Pelargonidin	Black currant	1300–4000
Peonidin	Blueberry	250–5000
Delphinidin	Black grape	300–750
Malvidin	Cherry	350–4500
	Rhubarb	2000
	Strawberry	150–750
	Red wine	200–350
	Plum	20–250
	Red cabbage	250
Flavonols	Yellow onion	350–1200
Quercetin	Curly kale	300–600
Kaempferol	Leek	30–225
Myricetin	Cherry tomato	15–200
	Broccoli	40–100
	Black currant	30-70
	Blueberry	30–160
	Apricot	25–50
	Apple	20–40
	Beans, green or white	10–50
	Black grape	15–40
	Tomato	2–15
	Green tea infusion	20–35
	Black tea infusion	30-45
	Red wine	2–30
Flavones	Parsley	240–1850
Apigenin	Celery	20–140
Luteolin	Capsicum pepper	5–10
Flavanones	Orange juice	215–685
Hesperetin	Grapefruit juice	100–650
Naringenin	Lemon juice	50–300
Eriodictyol		
Isoflavones	Soy flour	800–1800
Daidzein	Soybeans, boiled	200–900
Genistein	Miso	250-900
Glycitein	Tofu	80–700
	Soy milk	30–175

Tabela 1. Prehrambeni izvori polifenola (7) - nastavak

Polifenoli u hrani	Izvor polifenola	Sadržaj polifenola mg/kg (mg/l)
Monomerni flavanoli	Čokolada	460–610
Katehin	Pasulj	350–550
Epikatehin	Kajsija	100–250
	Trešnja	50–220
	Grožđe	30–175
	Breskva	50–140
	Kupina	130
	Jabuka	20–120
	Zeleni čaj	100–800
	Crni čaj	60–500
	Crno vino	80–300
	Jabukovača	40

u mahunarkama i njihovim prerađenim proizvodima (značajna količina daidzeina i genisteina) (8). Flavoni su široko rasprostranjeni u sušenom bilju i agrumima, dok su antocijanini prisutni u obojenom voću i povrću (bobičasto voće, šljive, trešnje, crveni kupus, patlidžan, crveni luk i crvena rotkva) (8). Flavanoli se nalaze u voću (jagoda, jabuka i breskva), proizvodima od kakaa, crnom i zelenom čaju (8). Voće (jagoda, malina, brusnica, nar) i orašasti plodovi (kesten) su bogati fenolnim kiselinama, sjemenke (predominantno lan) i žitarice lignanima, grožđe i crno vino stilbenima (resveratrol) (8).

Bioraspoloživost polifenola

Bioraspoloživost polifenola određuju pedoklimatski i agronomski uslovi, kao i stepen zrelosti (7). Sa sa zrelošću plodova masline smanjuje se koncentracija fenolnih spojeva (10). Zrenje povećava koncentraciju antocijanina (10). Homogenizacija, liofilizacija, kuhanje i termička obrada različito utiču na sadržaj i količinu apsorbiranih polifenolnih spojeva (10). Termička obrada uzrokuje značajno smanjenje ukupnog sadržaja fenola u grahu i mahunarkama (10,11). Polifenoli u mrkvi se u potpunosti gube s ključanjem (10). Kuhanje na pari i prženje ima manje negativan učinak (10). S druge strane, kuhanje na pari brokuli povećava sadržaj polifenola (10). Homogenizacije povrća povećava bioraspoloživost polifenola (10). Skladištenje u mraku u trajanju od sedam mjeseci rezultuje smanjenjem sadržaja antocijana za 88% (10). Bioraspoloživost određuju izravne interakcije između polifenola i nekih sastojaka hrane, poput proteina, ugljikohidrata, vlakana, masti i alkohola (14). Prisustvo masti u ishrani pojačava apsorpciju flavonoida (10). S druge strane, dijetalna vlakna usporavaju resorpciju polifenola (10). Fitohemijske osobine polifenola (strukture molekula, lipofilnost, konstanta disocijacije i topljivost) imaju značajnu ulogu u apsorpciji istih (7). Molekule veće molekulske mase se sporije resorbuju (7). Stepen glikolizacije i tip šećerne jedinice znatno utječu na apsorpciju flavonoida u tankom crijevu (izuzev flavan-3ola koji nisu prisutni u obliku glikozida) (7). Katehini esterificirani s galnom kiselinom pokazuju značajno manju bioraspoloživost od pripadajućih slobodnih oblika (7). Faktori domaćina (crijevni i sistemski) su značajni u apsorpciji polifenola (10). Ograničena apsorpcija u probavnom sustavu predstavlja uzrok relativno niske bioraspoloživosti polifenola (2).

Metabolizam polifenola

Polifenoli u tanko crijevo uglavnom dospijevaju u nepromijenjenom obliku (slika 1) (7). U istom polifenoli podliježu hidroksilaciji djelovanje laktaze floridzin hidrolaze i citosolne β-glukozidaze (10,12,17). Laktaza floridzin hidrolaza ima dva katalitička mjesta, jedno za hidrolizu laktoze, a drugo za deglikozilaciju hidrofobnih supstrata (10,11). Nastali aglikoni pasivnom difuzijom ulaze u epitelnu ćeliju (povećana lipofilnost) (10). Citosolna β-glukozidaza učestvuje u transportu polarnih glukozida (prenos aktivnim, natrijum zavisnim, transporterom glukoze) (10). Polifenoli koji se ne apsorbuju u tankom crijevu (95%) u debelom crijevu prolaze kroz značajne strukturne modifikacije (10). Mikroflora debelog crijeva hidrolizira glikozide do aglikona i jednostavnih fenolnih kiselina (10). Prije ulaska u krvotok, jednostavni aglikoni

Table 1. Food sources of polyphenols (7) - continued

Polyphenols in foods	Source	Polyphenol content mg/kg (mg/l)
Monomeric flavanols	Chocolate	460–610
Catechin	Beans	350–550
Epicatechin	Apricot	100–250
	Cherry	50–220
	Grape	30–175
	Peach	50–140
	Blackberry	130
	Apple	20–120
	Green tea	100–800
	Black tea	60–500
	Red wine	80–300
	Cider	40

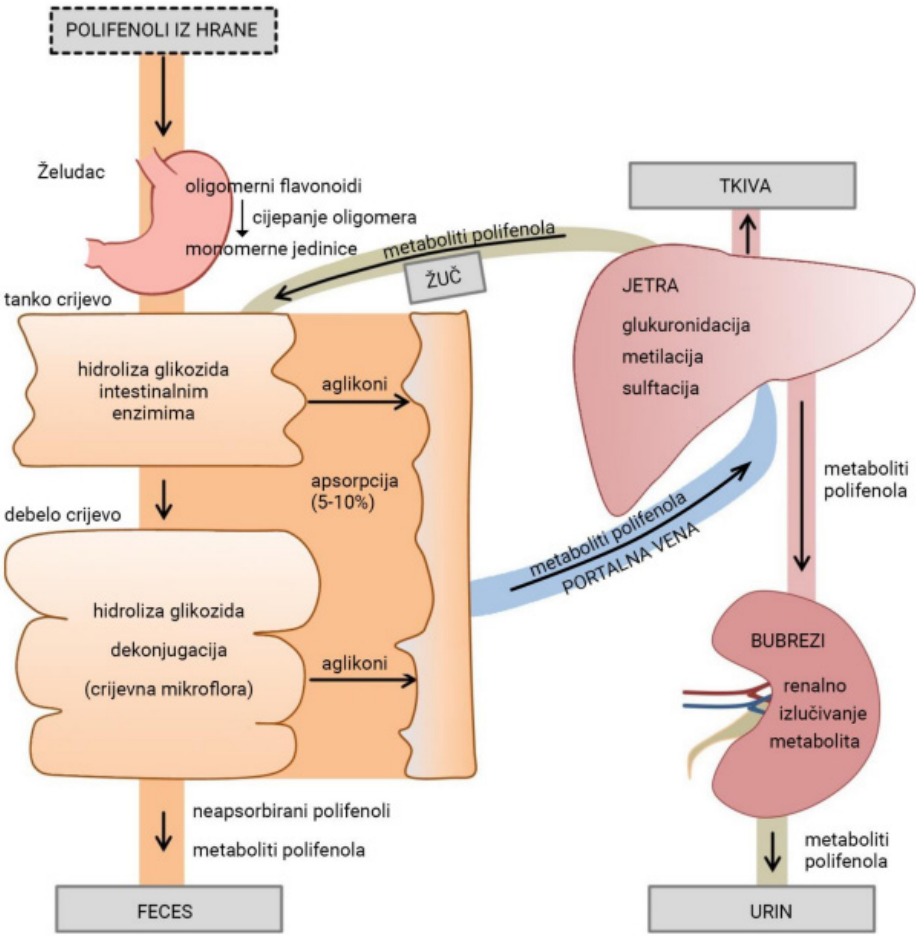
(fruit, vegetables, cereals, leguminous plants, fruit juices, tea, wine), while other products are specific to particular food (flavanones in citrus fruit, isoflavones in soybean) (7). Flavanones are present in spices (cappar, saffron, dried Mexican oregano) and vegetables (onions, spinach), while isoflavones are present in legumes and their processed products (significant amount of daidzein and genistein) (8). Flavones are widely present in dried herbs and citrus fruit, while anthocyanins are present in colored fruit and vegetables (berries, plums, cherries, red cabbage, aubergine, red onion and radish) (8). Flavanols are present in fruit (strawberry, apple and peach), cacao products, black and green tea (8). Fruit (strawberry, raspberry, cranberry and pomegranate) and nuts (chestnut) are rich in phenol acids, seeds (predominantly flax) and cereals are rich in lignans, while grapes and red wine are rich in stilbenes (resveratrol) (8).

Bioavailability of polyphenols

The bioavailability of polyphenols is determined by pedoclimatic and agronomic conditions, as well as by the degree of ripeness (7). The concentration of phenol compounds decreases with the ripeness of olives (10). During ripening, anthocyanin concentrations increase (10). Homogenization, lyophilisation, cooking and thermal processing influence the content and the quantity of absorbed polyphenolic compounds in different ways (10). Thermal processing causes a significant decrease in the total amount of phenols in peas and leguminous plants (10,11). Polyphenols in carrots are completely lost during boiling (10). Steaming and frying have a less negative effect (10). On the other hand, the amount of polyphenols increases when steaming broccoli (10). Homogenization of vegetables increases the bioavailability of polyphenols 910). Storage over seven months in the dark caused a decrease in anthocyanins for 88% (10). Bioavailability is determined by direct interaction between polyphenols and some ingredients, such as proteins, carbohydrates, fibers, fat and alcohol (14). The presence of fat in diet increases the absorption of flavonoids (10). On the other hand, dietary fibers slow down the absorption of polyphenols (10). Phytochemical properties of polyphenols (molecular structure, lipophilic properties, dissociation constant and melting point) have a significant role in their absorption (7). Molecules of higher molecular mass are absorbed more slowly (7). The degree of glycosylation and the type of glucose unit significantly influence the absorption of flavonoids in small intestines (except flavan-3-ols which are not present in the form of glycosides) (7). Catechins which are esterified with gallic acid show significantly reduced bioavailability than the affiliated free forms (7). Factors belonging to the host (intestinal and systemic) are also important for the absorption of polyphenols (10). Limited absorption in the digestive system is a cause of relatively low bioavailability of polyphenols (2).

Metabolism of polyphenols

Polyphenols reach the small intestine in their unchanged form (Picture 1). There polyphenols are subject to hydroxylation under the influence of lactase phlorizin hydrolase and cytosolic ß-glucosidase (10,12,17). Lactase phlorizin hydrolase has two catalytic sites, one



Slika 1. Apsorpscija i metabolizam polifenola (2)

se podvrgavaju procesu konjugacije koji se odvija u tankom crijevu i jetri (predominantno) (10). Konjugacija (metilacija, sulfacija i glukuronidacija) predstavlja metabolički proces detoksikacije zajednički mnogim ksenobiotičkim spojevima koji ograničava potencijalne toksične efekte i olakšava uklanjanje istih iz žuči i urina (10). Katehol-O-metiltransferaza katalizira prijenos metilne grupe iz adenozil-metionina u polifenole s difenolnom sekvencom, poput kvercetina, katehina, kafene kiseline i cijanidina (10). Enzim je prisutan u širokom spektru tkiva, s najvećom aktivnošću u jetri i bubrezima (10). Sulfotransferaze kataliziraju transfer sulfatnog dijela iz fosfoadenozin-fosfosulfata u hidroksilnu grupu polifenola (10,13). Sulfacija se predominantno odvija u jetri (10,13). Uridin 5 difosfat glukuroniziltransferaze unutar endoplazmatskog retikuluma kataliziraju transfer glukuronske kiseline iz uridin difosfat glukuronske kiseline u polifenole (10). Glukuronidacija polifenola u enterocitima prethodi konjugaciji u jetri (10). Proces konjugacije s jedne strane stvara aktivne metabolite dijetalnih polifenola, s druge strane smanjuje ukupnu količinu polifenola u krvotoku, povećavajući njihovo izlučivanje (10). Ekskrecija polifenola se odvija putem bubrega i manjim dijelom žuči (5).

Polifenoli i kardiovaskularne bolesti

Polifenoli smanjuju kardiovaskularni rizik (14). Redukuju biosintezu lipida, povećavaju fekalnu ekskreciju holesterola, inhibiraju lipoprotein male gustine (engl. *Low-density lipoprotein* - LDL), povećavaju koncentraciju lipoproteina visoke gustine (engl. *High-density lipoprotein* - HDL), smanjuju agregaciju lipoproteina male gustine, posljedičnu fagocitozu makrofaga i stvaranje pjenastih stanica, održavaju elastičnost stijenke krvnih žila i inhibiraju trombocitnu aktivnost (5).

Brojna istraživanja ukazuju na protektivnu ulogu polifenola koji se unose ishranom. Konzumiranje tamne čokolade s visokim udjelom flavonoida povećava koncentraciju epikatehina u plazmi kod zdravih odraslih osoba uzrokujući endotel zavisnu vazodilataciju (15). Konzumiranja grožđa i crvenog vina rezultuje porastom cikličnog guanozin monofosfata (aktivaciju endotelne dušik oksid

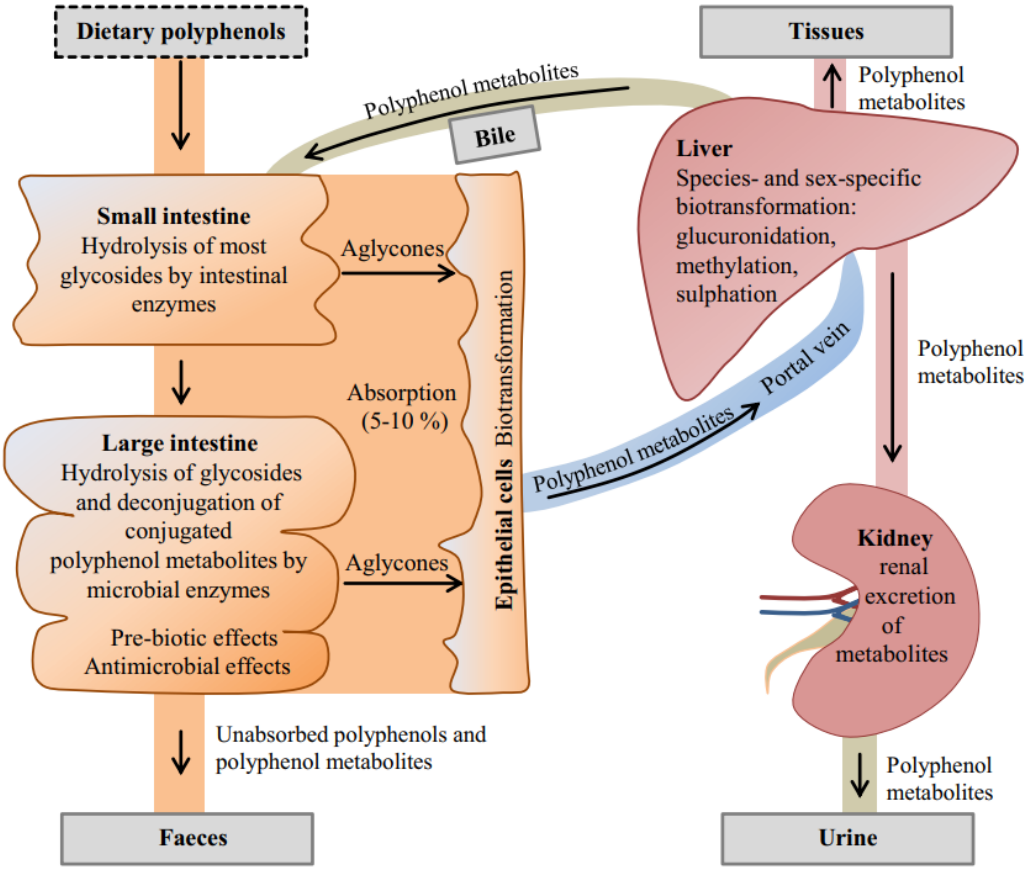


Figure 1. Absorption and metabolism of polyphenols (2)

for the hydrolysis of lactose, and the other for the deglycosylation of hydrophobic substrates (10,11). Aglycones that are caused by passive diffusion reach the epithelial cell (lipophility increases) (10). Cytosolic ß-glucosidase participates in the transport of polar glucosides (transport with the active, sodium-dependent glucose transporter) (10). Polyphenols that are not absorbed in the small intestine (95) undergo significant structural modifications in the large intestine (10). The microflora of the large intestine hydrolyzes glycosides to aglycones and simple phenolic acids (10). Prior to passage into the blood stream, simple aglycones undergo the process of conjugation in the small intestine and liver (predominantly) (10). The conjugation (methylation, sulfation and glucuronidation) is a metabolic process of detoxication that is common in many xenobiotic compounds and that limits the potential toxic effects and alleviates the excretion from the gall bladder and urine (10). Catechol-O-methyltransferase catalyzes the transfer of methyl group from adenosyl-methionine into polyphenols with diphenolic sequence, such as quercetin, catechin, caffeic acid, cyanidin (10). The enzyme is present in many tissues, with the greatest activity in the liver and kidneys (10). Sulfotransferase catalyze the transfer of sulfate part from phosphoadenosine-phosphosulfate into the hydroxyl group of polyphenols (10,13). Sulfation is predominant in the liver (10,13). Uridine-5-diphosphate glucuroniziltransferase within the endoplasmic reticulum catalyze the transfer of glucuronic acid from uridine diphosphate glucuronic acid into polyphenols (10). Glucuronidation of polyphenols in enterocytes is prior to the conjugation in the liver (10). The process of conjugation creates active metabolites of dietary polyphenols on the one hand, while on the other hand, it reduces the total amount of polyphenols in the blood stream, by increasing their excretion (10). Polyphenols are excreted via kidneys and to a lesser extent, via gallbladder (5).

Polyphenols and cardiovascular diseases

Polyphenols reduce the cardiovascular risk (14). They reduce the biosynthesis of lipids, increase the fecal excretion of cholesterol,

sintaze) i opuštanjem vaskularnih glatkih mišića (16). Oksidacija LDL-a ima značajnu ulogu za nastanak ateroskleroze (16). Fenoli u crvenom vinu inhibiraju oksidaciju LDL-a kataliziranu bakrom (16).

Upotreba čokolade bogate procijanidinom rezultuje povećanjem plazmatskog antioksidativnog kapaciteta i odlaganjem LDL oksidacije (17). Katehini zelenog čaja se ugrađuju u čestice LDL-a i redukuju oksidaciju istih (18). Listovi hibiskusa sabdarife su bogati flavonoidima, posjeduju antioksidativno i antiaterosklerotsko djelovanje (19).

Proliferacija i migracija vaskularnih glatkih mišićnih ćelija indukuje progresivno zadebljanje intime i razvoj skleroze arterijskog zida (16). Polifenoli crvenog vina i borovnice inhibiraju receptor aktiviran proliferatorom peroksizoma γ koja redukuje aktivnost signalnog puta proliferacije (16).

Makrofagi igraju ključnu ulogu u aterogenezi svojim proinflamatornim djelovanjem (20). Polifenoli mogu smanjiti proupalne medijatore hronične upale (22). Resveratrol, izorhamnetin, kurkumin i vanilinska kiselina mogu smanjiti oslobađanje proupalnog citokina iz T limfocita (16).

Aterosklerozu karakteriše pojačana aktivacija trombocita (16). Resveratrol smanjuje aktivaciju trombocita izazvanu kolagenom, adenozin difosfatom i trombinom i agregaciju istih. Polifenoli u naru smanjuju agregaciju trombocita (inhibiraju proizvodnju tromboksana A2, kolagena i arahidonske kiseline) (16).

Kohortna studija sprovedena u Finskoj među 2748 muškaraca i 2385 žena uzrasta od 30 do 69 godina (bez poznate koronarne bolesti), u trajanju od 26 godina, identifikovala je značajan inverzni gradijent unosa flavonoida u ishrani i ukupnog i koronarnog mortaliteta (21). Istraživanje sprovedeno u Grčkoj unutar 30 muškaraca starosti preko 70 godina, s nedavnim (< 2 mjeseca) akutnim koronarnim sindromom ili bajpasom koronarne arterije i fibrilacijom atrija pokazuje da ekstrakt polifenola crvenog grožđa uzrokuje vazodilataciju posredovanu protokom (vrhunac za 60 minuta) (22). Rezultati petogodišnje studije u Holandiji ukazuju na obrnutu povezanost unosa flavonoida i obolevanja od infarkta miokarda i umiranja od koronarne bolesti (23). Dvostruko slijepo, placebo kontrolisano ispitivanje 40 pacijenata nakon infarkta, sprovedeno u Kavkazu uočilo je da resveratrol u osoba s bolestima koronarnih arterija poboljšava dijastoličku funkciju lijeve komore, funkciju endotela, smanjuje nivo LDL-holesterola i štiti od nepovoljnih hemodinamskih promjena (24). Kohortna studija sprovedena u Ujedinjenom Kraljevstvu među 30.458 žena (starosne dobi od 35 do 69 godina) u trajanju od 16,7 godina identifikovala je direktnu povezanost ukupnog unosa voća i smanjene smrtnosti od koronarne bolesti srca i kardiovaskularnih bolesti (smanjenje rizika za 6-7% za svaki konzumirani obrok od 80 g/dan) (25). Multicentrično, randomizirano, ispitivanje primarne prevencije (PREDIMEDIRANA studija) pružilo je snažne dokaze da orašasti plovodi i mediteranska dijeta na bazi povrća bogatog polifenolima predstaljaju idealan i održiv model prevencije kardiovaskularnih bolesti (26).

Polifenoli i moždani udar

Moždani udar je drugi uzrok smrtnosti u svijetu i glavni uzrok invaliditeta odraslih u zemljama sa srednjim (12,8%) i visokim prihodima (8,7%) (27). Oksidativni stres predstavlja ključni događaj u patogenezi cerebralne ishemije (27). Prekomjerna proizvodnja reaktivnih vrsta kiseonika u toku ishemije prouzrokuje neravnotežu oksidativnih i antioksidativnih procesa koja oštećuje lipide, proteine i nukleinske kiseline, indukujući apoptozu odnosno nekrozu (28). Polifenoli sprečavaju neurodegenerativne promjene povezane s cerebralnom ishemijom (28). Stimulišu redoks enzime, poput endotelne dušik oksid sintaze, katalaze, superoksid dizmutaze 1 i superoksid dizmutaze 2, te moduliraju imunološki odgovor inhibiranjem protivupalnih biomarkera (28).

Polifenoli zelenog čaja i epigalokatehin-3-galat mogu inhibirati apoptozu neurona regulacijom nivoa neurotrofina (16,27). Resveratrol i polifenoli zelenog čaja smanjuju reaktivne vrste kiseonika u mitohondrijima i edem endotelnih ćelija mozga (smanjenjem jonizovanog kalcijuma) (27). Resveratrol značajno smanjuje apoptozu, peroksidaciju mitohondrijskih lipida, zapreminu moždanog infarkta i edem (27). Mangiferin i morin, dva prirodna antioksidansa iz kore manga predstavljaju perspektivne neuroprotektore cerebrovaskularnog insulta, epilepsije, traume mozga i oštećenja kičmene moždine (antioksidativna i antiapoptotička aktivnost) (27). Neuroprotektor kvercetin smanjuje nivo matrične metalopeptidaze, pokazuje antilipidno, peroksidativno, antioksidativno i protivupalno djelovanje (27). Polifenoli iz grožđa

inhibit low-density lipoprotein, increase high-density lipoprotein, decrease the aggregation of low-density lipoprotein, the consequential phagocytosis of macrophages and formation of foam cells, maintain blood vessel elasticity and inhibit thrombin activity (5).

Numerous studies point out the protective role of polyphenols that we get through certain foods. The consumption of dark chocolate with high percentage of flavonoids increases the concentration of epicatechin in plasma of healthy adults, thus inducing endothelium-dependent vasodilation (15). The consumption of grapes and red wine results in the increase of cyclic guanosine monophosphate (activation of endothelial nitric oxide synthase) and the relaxation of vascular smooth muscles (16). Oxidation of LDL has an important role in the occurrence of atherosclerosis (16). Phenols in red wine inhibit copper-catalyzed oxidation of LDL (16).

The use of chocolate rich in procyanidin increases plasma antioxidant capacity and postpones LDL oxidation (17). Catechins in green tea are incorporated into LDL particles and reduce their oxidation (18). Hibiscus sabdariffa leaves are rich in flavonoids, and they have antioxidant and antiatherosclerotic effects (19).

The proliferation and migration of vascular smooth muscle cells induce progressive intimal thickening and development of sclerosis of arterial walls (16). Polyphenols from red wine and blueberries inhibit the peroxisome proliferator-activated reactor that reduces the activity of signaling pathway of proliferation (16).

Macrophages have an important role in atherogenesis with their proinflammatory activity (20). Polyphenols can reduce proinflammatory mediators of chronic inflammation (22). Resveratrol, isorhamnetin, curcumin and vanillic acid can decrease the liberation of proinflammatory cytocine from T-lymphocytes (16).

Atherosclerosis is characterized by the increased thrombocyte activation (16). Resveratrol reduces thrombocyte activation caused by collagen, adenosine diphosphate and thrombin and their aggregation. Polyphenols in pomegranate reduce thrombocyte aggregation (they inhibit the production of thromboxane A2, collagen and arachidonic acid) (16).

A cohort study conducted in Finland, which included 2748 men and 2385 women aged 30-69 years (without known coronary disease) and which lasted 26 years, identified a significant inverse gradient between the flavonoid intake and total coronary mortality (21). A study conducted in Greece, which included 30 men older than 70 with a recent (<2 months) coronary syndrome or bypass of coronary artery and atrial fibrillation, showed that red grape polyphenol extract caused flow-mediated vasodilation (peaking at 60 minutes) (22). The results of five-year study conducted in the Netherlands showed the inverse relationship between the flavonoid intake and myocardial incidence and mortality associated with coronary disease (23). A double-blind, placebo-controlled trial of 40 patients after myocardial infarction, which was conducted in the Caucasus, found that resveratrol in patients with diseases of coronary arteries, improved the diastolic function of left ventricle, endothelial function and decreased the level of LDL cholesterol and protected from unfavorable chemodynamic changes (24). A cohort study conducted in the United Kingdom, which included 30458 women (aged 35 to 69 years) and lasted 16.7 years, identified a direct relationship between the total fruit intake and decreased mortality of coronary disease and cardiovascular disease (reduction of risk for 6-7% for each intake of 80g/daily) (25). A multicenter, randomized study of primary prevention (the PREDIMED study) proved that nuts and Mediterranean diet based on vegetables rich in polyphenols present an ideal and sustainable model of cardiovascular disease prevention (26).

Polyphenols and stroke

Stroke is the second leading cause of death worldwide and major cause of disability of adults in middle-income countries (12.8%) and high-income countries (8.7%) (27). Oxidative stress is a key event in the pathogenesis of cerebral ischemia (27). The excessive production of reactive oxygen species during ischemia causes the imbalance between the oxidative and antioxidative process that damages lipids, proteins and nucleic acids, thus inducing apoptosis, that is, necrosis (28). Polyphenols prevent neurodegenerative changes associated with cerebral ischemia (28). They stimulate redox enzymes, such as endothelial nitric oxide synthase, catalase, superoxide dismutase and 1 superoxide dismutase 2, and therefore, they modulate the immune response by inhibiting anti-inflammatory biomarkers (28).

u prahu, koji se daju kao dodatak prehrani, štite mozak od ishemijskih oštećenja (27). Antocijanini iz borovnica ima antiaterogena i protivupalna svojstva te djeluje neuroprotektivno (27). Ekstrakt crnog vina, bogat antocijaninom, smanjuje ozljede izazvane cerebralnom ishemijom, štiti od ekscitotoksičnosti izazvane ishemijom i energetskim zatajenjem i oksidativnim stresom (27,29).

Meta-analiza 11 prospektivnih studija utvrdila je da umjerana konzumacija kafe smanjuje rizik od nastanka moždanog udara (30). Kohortna studija sprovedena u Sjedinjenim Američkim Državama među 229.119 muškaraca i 173.141 žena uzrasta od 50 do 71 godine (bez prisustva karcinoma, srčanih bolesti i moždanog udara) identifikovala je postojanje obrnute veze između konzumiranja kafe i razvoja moždanog udara (31). Slični rezultati prikazani su u jednoj kohortnoj studiji koju je činilo 83.076 žena u Sjedinjenim Američkim Državama, a koje su bile praćene 24 godine (41). Meta-analiza osam studija, sa 5228 osoba sa moždanim udarom među 280.174 učesnika identifikovala je povezanost značajnog unosa flavonola i smanjenja rizika za nastanak moždanog udara (32). Unos flavonola veći od 20 mg/dan smanjuje rizik razvoja moždanog udara za 14% (32).

Polifenoli i hipertenzija

Arterijska hipertenzija predstavlja glavni faktor rizika za nastanak kardiovaskularnih bolesti (33). Polifenoli koji su unose ishranom mogu smanjiti ublažiti hipertenziju protivupalnim i antioksidativnim efektima, kao i povećanom proizvodnjom oksida azota (33). Protivupalni učinak nastaje kao rezultat smanjene ekspresije redoks-osjetljivog nuklearnog faktora-kB, dok je antioksidativni učinak povezan s poboljšanim enzimskim aktivnostima superoksid dismutaze, katalaze i glutation peroksidaze (33). Osim toga, polifenoli sudjeluju u aktivaciji redoks-osjetljivog puta fosfoinozitid 3- kinaze, što rezultuje povećanim stvaranjem oksida azota (33). Izoflavoni stimulišu endotelnu sintezu dušik oksida, što dovodi do povećanja endogene proizvodnje oksida azota (34). Antocijanini smanjuju ekspresiju NF-κB signalnog puta, aktivatora protein 1 (AP-1) i signalni put mitogen-aktivirane protein kinaze (34). Cijanidin 3-O-glukozid iz kupina aktivira endotelnu dušik oksid sintazu sprečava disfunkciju endotela i vaskularno zatajenje uklanjanjem peroksinitrata (jakog oksidansa odgovornog za oštećenje dezoksiribonukleinske kiseline i proteina) (34). Delfhinidin, antocijanin prisutan u crnom vinu, inhibira apoptozu ćelija endotela putem stvaranja dušikovog oksida i regulacije homeostaze kalcijuma (34).

Randomizirano, dvostruko slijepo, kontrolirano, unakrsno istraživanje sprovedeno u Australiji među 61 ispitanikom uzrasta od 24 do 72 godine utrdilo je hipotenzivni kapacitet ekstrakta lišća masline (35). Istraživanje koje je obuhvatilo 550 odraslih i starijih osoba u Sao Paulu identifikovalo je inverznu povezanost unosa lignana, stilbena, tirosola, alkilfenola i drugih polifenola i hipertenzije (36). Istraživanje u Poljskoj unutar prospektivne kohortne studije o zdravlju, alkoholu i psihosocijalnim faktorima u istočnoj Evropi (HAPIEE) sa 8.821 učesnika uzrasta od 45 do 69 godina u trajanju od pet godina utvrdio je zaštitni učinak fenolne kiseline na razvoj hipertenzije (37). Studija o mediteranskoj zdravoj ishrani, starenju i životnom stilu (MEAL) sprovedena među 2.044 muškarca i žena starijim od 18 godina u Italiji pokazuje linearnu inverznu povezanost unosa fenolne kiseline i hipertenzije u skupini odraslih osoba koje žive na mediteranskom ostrvu (38). Dijetalni unos fenolnih kiselina (hidroksibenzojeva i hidroksifeniloctena kiselina) u najvišim kvartilima (približno > 400 mg/dan) je bila obrnuto povezana sa hipertenzijom (38). Istraživanje sprovedeno u Koreji među 2.204 muškaraca i 3305 žena starijih od 40 godina koji nisu imali metabolički sindrom ukazuje na postojanje inverzne povezanosti konzumiranja izoflavona i hipertenzije kod žena (39). Metaanaliza osam kontrolisanih ispitivanja s ukupno 843 sudionika utvrdila je da konzumacija susama (bogatog fitosterolima i lignaninima) smanjuje krvni pritisak (40). Rezultati istraživanja sprovedenog u Kini koje je obuhvatilo 1.476 ispitanika uočilo je da konzumiranje crnog i zelenog čaja snižava krvni pritisak (41). Konzumacija zelenog čaja (u prosjeku 400 mL) rezultuje smanjenjem sistolnog krvnog pritiska za 2,1 mmHg i padom dijastolnog krvni pritisak za 1,7 mmHg (41). Konzumacija crnog čaja (u prosjeku 400 ml) uzrokuje pad sistolnog krvnog pritiska za 1,4 mmHg i dijastolnog krvnog pritiska za 1,1 mmHg (41).

Zaključak

Polifenoli smanjuju disfunkciju endotela, redukuju krvni pritisak, unapređuju antioksidativnu

Polyphenols in green tea and epigallocatechin-3-gallate may inhibit apoptosis of neurons by regulating the level of neurotrophin (16,27). Resveratrol and polyphenols in green tea reduce the reactive oxygen species in mitochondria and edema of endothelial brain cells (by decreasing the ionized calcium) (27). Resveratrol significantly reduces apoptosis, peroxidation of mitochondrial lipids, volume of stroke and edema (27). Mangiferin and morin, two natural antioxidants from mango peel, are promising neuroprotectors of cerebrovascular insult, epilepsy, brain trauma and damage of spinal cord (antioxidative and antiapoptotic activity) (27). Neuroprotector quercetin decreases the level of matrix metallopeptidase, shows antilipid, peroxidative, antioxidative and anti-inflammatory activity (27). Polyphenols from grapes in powder, which are given as food supplements, protect the brain from ischemic damage (27). Anthocyanins from blueberries have antiatherogenic and anti-inflammatory properties and neuroprotective effect (27). Red wine extract, rich in antocyanin, decreases damage caused by cerebral ischemia, protects from excitotoxicity caused by ischemia and reduced energy and oxidative stress (27,29).

A meta-analysis of 11 prospective studies found that moderate coffee consumption reduced the risk of stroke (30). A cohort study, which was conducted in the United States of America among 229,119 men and 173,141 women aged 50 to 71 (with no cancer, heart diseases and stroke) identified the inverse relationship between coffee consumption and development of stroke (31). Similar results were shown in a cohort study which included 83.076 women in the United States of America, who had been observed for 24 years (41). A meta-analysis of eight studies, which included 5228 persons with stroke among 280174 respondents, identified the relationship between the significant intake of flavonols and decrease of the risk for the appearance of stroke (32). The intake of flavonols higher than 20mg/daily reduces the risk of stroke development for 14% (32).

Polyphenols and hypertension

Arterial hypertension is the main risk factor for the appearance of cardiovascular diseases (33). Polyphenols that we get through food can alleviate hypertension with their anti-inflammatory and antioxidative effects, as well as the increased production of nitric oxide (33). Anti-inflammatory effect is caused by the decreased expression of redox-sensitive nuclear factor-kB, while the antioxidative effect is associated with the improved enzyme activities of superoxide dismutase, catalase and glutation peroxidase (33). In addition, polyphenols take part in the activation of redox-sensitive pathway phosphoinositide kinase, which results in the increased production of nitric oxide (33). Isoflavones stimulate the endothelial nitric oxide synthase, resulting in the increased endogen production of nitric oxide (34). Anthocyanins reduce the expression of NF-kB signaling pathway, activator of protein 1 (AP-1) and signaling pathway of mitogen-activated protein kinase (34). Cyanidin 3-O-glucoside from blackberries activates endothelial nitric oxide sintase, prevents endothelial dysfunction and vascular failure by removing peroxynitrite (strong oxidant responsible for the damage of deoxyribonucleic acid and proteins) (34). Delphinidin, anthocyanin present in red wine, inhibits endothelial cells apoptosis by creating nitric oxide and regulation of calcium homeostasis (34).

A randomized, double-blind, controlled, cross-sectional study, which was conducted in Australia and included 61 respondents aged 24 to 72 years, found the hypotensive capacity of extract of olive leaves (35). A study, which included 550 elderly adults in Sao Paolo, indentified the inverse relationship between the intake of lignans, stilbenes, tyrosol, alkifenol and other polyphenols and hypertension (36). A study from Poland, which was a prospective, cohort study about health, alcohol and psychosocial factors in Eastern Europe (HAPIEE) with 8821 respondents aged 45 to 69 years that lasted five years, found the protective effect of phenolic acid on the development of hypertension (37). A study about the Mediterranean healthy eating, ageing and lifestyle (MEAL) was conducted among 2044 men and women older than 18 years in Italy and it showed a linear inverse relationship between the intake of phenolic acid and hypertension among adults who lived in the Mediterranean (38). Dietary intake of phenolic acids (hydroxybenzoic and hydroxyphenyllactic acid) in highest quartiles (approximately > 400 mg/daily) was inversely related with hypertension (38). A study conducted in Korea among 2204 men and 3305 women older than 40 years, who did not have metabolic syndrome, pointed out

odbranu, ublažavaju upalni odgovor, blokiraju agregaciju trombocita i oksidaciju lipoproteina male gustine. Snižena intrinzička aktivnost polifenola zbog otežane apsorpcije, visokog stepena biotransformacije i brze eliminacije iz organizma značajno limitira njihovo dejstvo. Brojne studije ukazuju na njihov ogroman značaj u prevenciji hroničnih nezaraznih bolesti, ali su neophodna dalja istraživanja koja bi precizirala dozvoljene protektivne doze koje se unose hranom i/ili suplementima.

Literatura

1. Tsao R. Chemistry and biochemistry of dietary polyphenols. Nutrients 2010; 2(12):1231-46.
2. Balta V. Bioraspoloživost, metabolizam i antioksidacijska sposobnost polifenola u organima miša C57BL/6 [doktorska disertacija]. Zagreb (Republika Hrvatska): Prirodoslovno-matematički fakultet, Biološki odsjek, Sveučilišta u Zagrebu, 2018.
3. Truzzi F, Tibaldi C, Zhang Y, Dinelli G, D'Amen E. An overview on dietary polyphenols and their biopharmaceutical classification system (BCS). Int J Mol Sci 2021; 22(11):5514.
4. Pandey KB, Rizvi SI. Plant polyphenols as dietary antioxidants in human health and disease. Oxid Med Cell Longev 2009; 2(5):270-8.
5. Kamenjašević M, Oršolić N, Matković A, Matković BR. Učinkovitost polifenolne prehrane na zdravlje i funkcionalnu sposobnost sportaša i rekreativaca. Hrvatski športskomedicinski vjesnik 2017; 32(1/2):5-21.
6. Panche AN, Diwan AD, Chandra SR. Flavonoids: an overview. J Nutr Sci 2016; 5:e47.
7. Manach C, Scalbert A, Morand C, Rémésy C, Jiménez L. Polyphenols: food sources and bioavailability. Am J Clin Nutr 2004; 79(5):727-47.
8. Mrduljaš N, Krešić G, Bilušić T. Polyphenols - food sources and health benefits. In: Chavarri Hueda M, editor. Functional food - improve health through adequate food. Rijeka (Croatia): InTechOpen, 2017. p. 23-41.
9. Silva RFM, Pogačnik L. Polyphenols from food and natural products: neuroprotection and safety. Antioxidants (Basel) 2020; 9(1):61.
10. D'Archivio M, Filesi C, Varì R, Scazzocchio B, Masella R. Bioavailability of the polyphenols: status and controversies. Int J Mol Sci 2010; 11(4):1321-42.
11. Xu B, Chang SKC. Total phenolic, phenolic acid, anthocyanin, flavan-3-ol, and flavonol profiles and antioxidant properties of pinto and black beans (Phaseolus vulgaris L.) as affected by thermal processing. J Agric Food Chem 2009; 57(11):4754-64.
12. Day AJ, Cañada FJ, Díaz JC, Kroon PA, Mclauchlan R, Faulds CB, et al. Dietary flavonoid and isoflavone glycosides are hydrolysed by the lactase site of lactase phlorizin hydrolase. FEBS Lett 2000; 468(2-3):166-70.
13. Falany CN. Enzymology of human cytosolic sulfotransferases. FASEB J. 1997; 11(4):206-16.
14. Fraga CG, Croft KD, Kennedy DO, Tomás-Barberán FA. The effects of polyphenols and other bioactives on human health. Food Funct 2019; 10(2):514-28.
15. 15. Storniolo CE, Roselló-Catafau J, Pintó X, Mitjavila MT, Moreno JJ. Polyphenol fraction of extra virgin olive oil protects against endothelial dysfunction induced by high glucose and free fatty acids through modulation of nitric oxide and endothelin-1. Redox Biol 2014; 2:971-7.
16. Cheng YC, Sheen JM, Hu WL, Hung YC. Polyphenols and oxidative stress in atherosclerosis-related ischemic heart disease and stroke. Oxid Med Cell Longev 2017; 2017:8526438.
17. Mathur S, Devaraj S, Grundy SM, Jialal I. Cocoa products decrease low density lipoprotein oxidative susceptibility but do not affect biomarkers of inflammation in humans. J Nutr 2002; 132(12):3663-7.
18. Suzuki-Sugihara N, Kishimoto Y, Saita E, Taguchi C, Kobayashi M, Ichitani M, et al. Green tea catechins prevent low-density lipoprotein oxidation via their accumulation in low-density lipoprotein particles in humans. Nutr Res 2016; 36(1):16-23.
19. Chen JH, Lee MS, Wang CP, Hsu CC, Lin HH. Autophagic effects of Hibiscus sabdariffa leaf polyphenols and epicatechin gallate (ECG) against oxidized LDL-induced injury of human endothelial cells. Eur J Nutr 2017; 56(5):1963-81.
20. Rodella LF, Favero G. Atherosclerosis and current antioxidant strategies for atheroprotection. In: Rezzani R, editor. Current trends in atherogenesis. Rijeka (Croatia): InTechOpen, 2013. p. 1-26.
21. Knekt P, Jarvinen R, Reunanen A, Maatela J. Flavonoid intake and coronary mortality in Finland: a cohort study. BMJ 1996; 312(7029):478-81.
22. Lekakis J, Rallidis LS, Andreadou I, Vamvakou G, Kazantzoglou G, Magiatis P, et al. Polyphenols compounds from red grapes acutely improve endothelial function in patients with coronary heart disease. Eur J Cardiovasc Prev Rehabil 2005; 12(6):596-600.
23. Hertog MG, Feskens EJ, Hollman PC, Katan MB, Kromhout D. Dietary antioxidant flavonoids and risk of coronary heart disease: the Zutphen Elderly Study. Lancet 1993; 342(8878):1007-11.
24. Magyar K, Halmosi R, Palfi A, Feher G, Czopf L, Fulop A, et al. Cardioprotection by resveratrol: a human clinical trial in patients with stable coronary artery disease. Clin Hemorheol Microcirc 2012; 50(3):179-87.
25. Lai HTM, Threapleton DE, Day AJ, Williamson G, Cade JE, Burley VJ. Fruit intake and cardiovascular disease mortality in the UK Women's Cohort Study. Eur J Epidemiol 2015; 30(9):1035-48.
26. Martínez-González MA, Salas-Salvadó J, Estruch R, Corella D, Fitó M, Ros E. Benefits of the Mediterranean diet: insights from the PREDIMED study. Prog Cardiovasc Dis 2015; 58(1):50-60.

the existence of inverse relationship between the consumption of isoflavones and hypertension in women (39). A meta-analysis of eight controlled studies with 843 respondents found that sesame consumption (rich in phytosterols and lignans) decreases the blood pressure (40). The results of study conducted in China, which included 1476 respondents, found that the consumption of black and green tea lowers the blood pressure (41). The consumption of green tea (on average 400 ml) resulted in the decrease of systolic blood pressure for 2.1 mmHg and lowering of diastolic blood pressure for 1.7 mmHg (41). The consumption of black tea (on average 400 ml) causes lowering of systolic blood pressure for 1.4 mmHg and diastolic blood pressure for 1.1 mmHg (41).

Conclusion

Polyphenols decrease endothelial dysfunction and blood pressure, improve the antioxidative defense, alleviate the inflammatory response, block platelet aggregation and oxidation of low-density lipoproteins. Reduced intrinsic activity of polyphenols due to malabsorption, high level of biotransformation and fast elimination from the body, significantly limit their activity. Numerous studies point to their huge significance in the prevention of chronic non-contagious diseases. However, further research is needed that would state precisely the allowed preventive doses that are taken in with food and/or supplements.

Literature

1. Tsao R. Chemistry and biochemistry of dietary polyphenols. Nutrients 2010; 2(12):1231-46.
2. Balta V. Bioraspoloživost, metabolizam i antioksidacijska sposobnost polifenola u organima miša C57BL/6 [doktorska disertacija]. Zagreb (Republika Hrvatska): Prirodoslovno-matematički fakultet, Biološki odsjek, Sveučilišta u Zagrebu, 2018.
3. Truzzi F, Tibaldi C, Zhang Y, Dinelli G, D'Amen E. An overview on dietary polyphenols and their biopharmaceutical classification system (BCS). Int J Mol Sci 2021; 22(11):5514.
4. Pandey KB, Rizvi SI. Plant polyphenols as dietary antioxidants in human health and disease. Oxid Med Cell Longev 2009; 2(5):270-8.
5. Kamenjašević M, Oršolić N, Matković A, Matković BR. Učinkovitost polifenolne prehrane na zdravlje i funkcionalnu sposobnost sportaša i rekreativaca. Hrvatski športskomedicinski vjesnik 2017; 32(1/2):5-21.
6. Panche AN, Diwan AD, Chandra SR. Flavonoids: an overview. J Nutr Sci 2016; 5:e47.
7. Manach C, Scalbert A, Morand C, Rémésy C, Jiménez L. Polyphenols: food sources and bioavailability. Am J Clin Nutr 2004; 79(5):727-47.
8. Mrduljaš N, Krešić G, Bilušić T. Polyphenols - food sources and health benefits. In: Chavarri Hueda M, editor. Functional food - improve health through adequate food. Rijeka (Croatia): InTechOpen, 2017. p. 23-41.
9. Silva RFM, Pogačnik L. Polyphenols from food and natural products: neuroprotection and safety. Antioxidants (Basel) 2020; 9(1):61.
10. D'Archivio M, Filesi C, Varì R, Scazzocchio B, Masella R. Bioavailability of the polyphenols: status and controversies. Int J Mol Sci 2010; 11(4):1321-42.
11. Xu B, Chang SKC. Total phenolic, phenolic acid, anthocyanin, flavan-3-ol, and flavonol profiles and antioxidant properties of pinto and black beans (Phaseolus vulgaris L.) as affected by thermal processing. J Agric Food Chem 2009; 57(11):4754-64.
12. Day AJ, Cañada FJ, Díaz JC, Kroon PA, Mclauchlan R, Faulds CB, et al. Dietary flavonoid and isoflavone glycosides are hydrolysed by the lactase site of lactase phlorizin hydrolase. FEBS Lett 2000; 468(2-3):166-70.
13. Falany CN. Enzymology of human cytosolic sulfotransferases. FASEB J. 1997; 11(4):206-16.
14. Fraga CG, Croft KD, Kennedy DO, Tomás-Barberán FA. The effects of polyphenols and other bioactives on human health. Food Funct 2019; 10(2):514-28.
15. 15. Storniolo CE, Roselló-Catafau J, Pintó X, Mitjavila MT, Moreno JJ. Polyphenol fraction of extra virgin olive oil protects against endothelial dysfunction induced by high glucose and free fatty acids through modulation of nitric oxide and endothelin-1. Redox Biol 2014; 2:971-7.
16. Cheng YC, Sheen JM, Hu WL, Hung YC. Polyphenols and oxidative stress in atherosclerosis-related ischemic heart disease and stroke. Oxid Med Cell Longev 2017; 2017:8526438.
17. Mathur S, Devaraj S, Grundy SM, Jialal I. Cocoa products decrease low density lipoprotein oxidative susceptibility but do not affect biomarkers of inflammation in humans. J Nutr 2002; 132(12):3663-7.
18. Suzuki-Sugihara N, Kishimoto Y, Saita E, Taguchi C, Kobayashi M, Ichitani M, et al. Green tea catechins prevent low-density lipoprotein oxidation via their accumulation in low-density lipoprotein particles in humans. Nutr Res 2016; 36(1):16-23.
19. Chen JH, Lee MS, Wang CP, Hsu CC, Lin HH. Autophagic effects of Hibiscus sabdariffa leaf polyphenols and epicatechin gallate (ECG) against oxidized LDL-induced injury of human endothelial cells. Eur J Nutr 2017; 56(5):1963-81.
20. Rodella LF, Favero G. Atherosclerosis and current anti-oxidant strategies for atheroprotection. In: Rezzani R, editor. Current trends in atherogenesis. Rijeka (Croatia): InTechOpen, 2013. p. 1-26.

27. Tressera-Rimbau A, Arranz S, Eder M, Vallverdú-Queralt A. Dietary polyphenols in the prevention of stroke. Oxid Med Cell Longev 2017; 2017:7467962.
28. Simonyi A, Wang Q, Miller RL, Yusof M, Shelat PB, Sun AY, et al. Polyphenols in cerebral ischemia: novel targets for neuroprotection. Mol Neurobiol 2005; 31(1-3):135-47.
29. Ayuso MI, Montaner J. Advanced neuroprotection for brain ischemia: an alternative approach to minimize stroke damage. Expert Opinion on Investigational Drugs 2015; 24(9):1137-1142.
30. Larsson SC, Orsini N. Coffee consumption and risk of stroke: a dose-response meta-analysis of prospective studies. Am J Epidemiol 2011; 174(9):993-1001.
31. Freedman ND, Park Y, Abnet CC, Hollenbeck AR, Sinha R. Association of coffee drinking with total and cause-specific mortality. N Engl J Med 2012; 366(20):1891-904.
32. Wang ZM, Zhao D, Nie ZL, Zhao H, Zhou B, Gao W, et al. Flavonol intake and stroke risk: a meta-analysis of cohort studies. Nutrition 2014; 30(5):518-23.
33. de Brito-Alves JL, de Sousa VP, Cavalcanti-Neto MP, Magnani M, de Andrade-Braga V, da Costa-Silva JH, et al. New insights on the use of dietary polyphenols or probiotics for the management of arterial hypertension. Front Physiol 2016; 7:448.
34. Godos J, Vitale M, Micek A, Ray S, Martini D, Del Rio D, et al. Dietary polyphenol intake, blood pressure, and hypertension: a systematic review and meta-analysis of observational studies. Antioxidants (Basel) 2019; 8(6):152.
35. Lockyer S, Rowland I, Spencer JPE, Yaqoob P, Stonehouse W. Impact of phenolic-rich olive leaf extract on blood pressure, plasma lipids and inflammatory markers: a randomised controlled trial. Eur J Nutr 2017; 56(4):1421-32.
36. Miranda AM, Steluti J, Fisberg RM, Marchioni DM. Association between polyphenol intake and hypertension in adults and older adults: a population-based study in Brazil. PLoS One 2016; 11(10):e0165791.
37. Grosso G, Stepaniak U, Micek A, Stefler D, Bobak M, Pająk A. Dietary polyphenols are inversely associated with metabolic syndrome in Polish adults of the HAPIEE study. Eur J Nutr 2017; 56(4):1409-20.
38. Godos J, Sinatra D, Blanco I, Mulè S, La Verde M, Marranzano M. Association between dietary phenolic acids and hypertension in a Mediterranean cohort. Nutrients 2017; 9(10):1069.
39. Woo HW, Kim MK, Lee YH, Shin DH, Shin MH, Choi BY. Habitual consumption of soy protein and isoflavones and risk of metabolic syndrome in adults ≥ 40 years old: a prospective analysis of the Korean Multi-Rural Communities Cohort Study (MRCohort). Eur J Nutr 2019; 58(7):2835-50.
40. Khosravi-Boroujeni H, Nikbakht E, Natanelov E, Khalesi S. Can sesame consumption improve blood pressure? A systematic review and meta-analysis of controlled trials. J Sci Food Agric 2017; 97(10):3087-94.
41. Liu G, Mi XN, Zheng XX, Xu YL, Lu J, Huang XH. Effects of tea intake on blood pressure: a meta-analysis of randomised controlled trials. Br J Nutr 2014; 112(7):1043-54.

Sukob interesa: Nije prijavljen.
Primljen: 30.08.2021.
Revizija: 30.09.2021.
Prihvaćen: 03.10.2021.

Autor za korespondenciju: Marijana Jandrić-Kočić, JZU Dom zdravlja Krupa na Uni, Stevana Jakovljevića 23, 78000 Banjaluka; e-mail: marijanajandrickocic@gmail.com

21. Knekt P, Jarvinen R, Reunanen A, Maatela J. Flavonoid intake and coronary mortality in Finland: a cohort study. BMJ 1996; 312(7029):478-81.
22. Lekakis J, Rallidis LS, Andreadou I, Vamvakou G, Kazantzoglou G, Magiatis P, et al. Polyphenols compounds from red grapes acutely improve endothelial function in patients with coronary heart disease. Eur J Cardiovasc Prev Rehabil 2005; 12(6):596-600.
23. Hertog MG, Feskens EJ, Hollman PC, Katan MB, Kromhout D. Dietary antioxidant flavonoids and risk of coronary heart disease: the Zutphen Elderly Study. Lancet 1993; 342(8878):1007-11.
24. Magyar K, Halmosi R, Palfi A, Feher G, Czopf L, Fulop A, et al. Cardioprotection by resveratrol: a human clinical trial in patients with stable coronary artery disease. Clin Hemorheol Microcirc 2012; 50(3):179-87.
25. Lai HTM, Threapleton DE, Day AJ, Williamson G, Cade JE, Burley VJ. Fruit intake and cardiovascular disease mortality in the UK Women's Cohort Study. Eur J Epidemiol 2015; 30(9):1035-48.
26. Martínez-González MA, Salas-Salvadó J, Estruch R, Corella D, Fitó M, Ros E. Benefits of the Mediterranean diet: insights from the PREDIMED study. Prog Cardiovasc Dis 2015; 58(1):50-60.
27. Tressera-Rimbau A, Arranz S, Eder M, Vallverdú-Queralt A. Dietary polyphenols in the prevention of stroke. Oxid Med Cell Longev 2017; 2017:7467962.
28. Simonyi A, Wang Q, Miller RL, Yusof M, Shelat PB, Sun AY, et al. Polyphenols in cerebral ischemia: novel targets for neuroprotection. Mol Neurobiol 2005; 31(1-3):135-47.
29. Ayuso MI, Montaner J. Advanced neuroprotection for brain ischemia: an alternative approach to minimize stroke damage. Expert Opinion on Investigational Drugs 2015; 24(9):1137-1142.
30. Larsson SC, Orsini N. Coffee consumption and risk of stroke: a dose-response meta-analysis of prospective studies. Am J Epidemiol 2011; 174(9):993-1001.
31. Freedman ND, Park Y, Abnet CC, Hollenbeck AR, Sinha R. Association of coffee drinking with total and cause-specific mortality. N Engl J Med 2012; 366(20):1891-904.
32. Wang ZM, Zhao D, Nie ZL, Zhao H, Zhou B, Gao W, et al. Flavonol intake and stroke risk: a meta-analysis of cohort studies. Nutrition 2014; 30(5):518-23.
33. de Brito-Alves JL, de Sousa VP, Cavalcanti-Neto MP, Magnani M, de Andrade-Braga V, da Costa-Silva JH, et al. New insights on the use of dietary polyphenols or probiotics for the management of arterial hypertension. Front Physiol 2016; 7:448.
34. Godos J, Vitale M, Micek A, Ray S, Martini D, Del Rio D, et al. Dietary polyphenol intake, blood pressure, and hypertension: a systematic review and meta-analysis of observational studies. Antioxidants (Basel) 2019; 8(6):152.
35. Lockyer S, Rowland I, Spencer JPE, Yaqoob P, Stonehouse W. Impact of phenolic-rich olive leaf extract on blood pressure, plasma lipids and inflammatory markers: a randomised controlled trial. Eur J Nutr 2017; 56(4):1421-32.
36. Miranda AM, Steluti J, Fisberg RM, Marchioni DM. Association between polyphenol intake and hypertension in adults and older adults: a population-based study in Brazil. PLoS One 2016; 11(10):e0165791.
37. Grosso G, Stepaniak U, Micek A, Stefler D, Bobak M, Pająk A. Dietary polyphenols are inversely associated with metabolic syndrome in Polish adults of the HAPIEE study. Eur J Nutr 2017; 56(4):1409-20.
38. Godos J, Sinatra D, Blanco I, Mulè S, La Verde M, Marranzano M. Association between dietary phenolic acids and hypertension in a Mediterranean cohort. Nutrients 2017; 9(10):1069.
39. Woo HW, Kim MK, Lee YH, Shin DH, Shin MH, Choi BY. Habitual consumption of soy protein and isoflavones and risk of metabolic syndrome in adults ≥ 40 years old: a prospective analysis of the Korean Multi-Rural Communities Cohort Study (MRCohort). Eur J Nutr 2019; 58(7):2835-50.
40. Khosravi-Boroujeni H, Nikbakht E, Natanelov E, Khalesi S. Can sesame consumption improve blood pressure? A systematic review and meta-analysis of controlled trials. J Sci Food Agric 2017; 97(10):3087-94.
41. Liu G, Mi XN, Zheng XX, Xu YL, Lu J, Huang XH. Effects of tea intake on blood pressure: a meta-analysis of randomised controlled trials. Br J Nutr 2014; 112(7):1043-54.

Conflict of interest: None declared.
Received: 08/30/2021
Revised: 09/30/2021
Accepted: 10/03/2021

Corresponding author: Mariana Jandric-Kocic, PHI Health Center Krupa na Uni, Stevana Jakovljevica 23, 78000 Banja Luka, Republic of Srpska, Bosnia and Herzegovina; e-mail: marijanajandrickocic@gmail.com

PREGLEDNI RAD

UDK BROJEVI:
502.3:504.5:66
616:504.5:661.715.7
DOI: https://doi.org/10.5937/zdravzast50-32819

POLICIKLIČNI AROMATIČNI UGLJOVODONICI, NJIHOVI URINARNI METABOLITI I ZDRAVLJE

Vladan Radosavljević[1]

[1] Institut za epidemiologiju Vojnomedicinske akademije, Beograd, Republika Srbija

SAŽETAK

Svetska zdravstvena organizacija je kao jedan od zaključaka navela da je zagađenje vazduha vodeći ekološki rizik po zdravlje ljudi. Policiklični aromatični ugljovodonici (PAU) su dobro poznati kancerogeni agensi (preko pet stotina jedinjenja) koji uzrokuju rak pluća i kože, naročito kod profesionalno izloženih radnika. Značajnim smanjenjem emisije iz savremenih industrijskih postrojenja za sagorevanje, redukcijom emisije izduvnih gasova u saobraćaju i strogim zabranama pušenja na javnim mestama, može se smanjiti ekspozicija PAU. Izloženost PAU bi trebalo bolje istražiti, posebno u oblasti masovnog biomonitoringa koncentracija njihovih glavnih metabolita u urinu. Takav biomonitoring bi trebalo da integriše ekspoziciju hemijskim kancerogenima iz različitih izvora (vazduha, vode, hrane, potrošačkih proizvoda, profesionalnih postupaka, itd.), kao i izloženost hemijskim noksama preko respiratornog trakta (udisanjem), digestivnog trakta (gutanjem) ili preko kože. Analiza koncentracije glavnih metabolita PAU u urinu visoko sofisticiranom opremom je potrebna za dobijanje validne baze podataka. Tako dobijeni podaci neophodni su za procenu rizika i kreiranje zdravstvene politike, a sve u cilju smanjivanja izloženosti hemijskim karcinogenima.

Ključne reči: poličiklični aromatični ugljovodonici, hemijske nokse, zagađenje vazduha

Uvod

Prema podacima Svetske zdravstvene organizacije (SZO) zagađenje vazduha je među najvećim javno zdravstvenim problemima (1), što potvrđuje činjenica da preko 90% svetskog stanovništva živi u područjima gde je kvalitet vazduha ispod procenjenih normi SZO. Zagađenje vazduha ne potiče samo od čestica, nego i od toksičnih hemikalija (1). Upravo iz navednog razloga jako je važan biomonitoring, odnosno procena izloženosti ljudi hemikalijama (2). Od svih hemijskih zagađivača vazduha jedan od najvažnijih su policiklični aromatični ugljovodonici (engl. *Polycyclic Aromatic Hydrocarbons* - PAU) (Tabela 1), koji su imunotoksični, mogu da izazovu karcinome i aterosklerozu, a imaju i teratogena dejstva (1,3). Brojna istraživanja pokazuju da se PAU najviše dovode u vezu sa nastankom kancera pluća i kože (3,4). Procene su da će do 2025. godine zagađenje vazduha biti vodeći uzrok prevremenog umiranja (3,5). Voda se najčešće zagađuje izlivanjem industrijskih i komunalnih voda, koje takođe zagađuju zemljište. Zemljište se dodatno zagađuje čvrstim komunalnim otpadom, šumskim požarima, aktivnostima vulkana, odlaganjem industrijskog otpada itd. Cilj ovog rada je da analizira rasprostranjenost PAU u životnoj sredini, metabolizam nakon prodiranja u ljudski organizam i mogućnosti redukcije zagađenja ovim jedinjenjima i prevencije oboljevanja ljudi.

Rasprostranjenost policikličnih armatičnih ugljovodonika

PAU su perzistentna organska jedinjenja koja se sastoje od dva ili više kondenzovanih aromatičnih prstenova. Prisutni su u vodi, vazduhu i zemljištu. Nastaju nepotpunim sagorevanjem organske materije i postaju glavni izvor zagađenja atmosfere (6). PAU mogu nastati prirodnim ili antropogenim putem. Prirodnim putem, PAU u atmosferu dospevaju vulkanskim erupcijama i požarima. Međutim, antropogene aktivnosti koje dovode do nastanka PAU su najčešće gust saobraćaj, industrija, građevinski radovi, sagorevanja otpada na otvore-

REVIEW ARTICLE

UDK NUMBERS:
502.3:504.5:66
616:504.5:661.715.7
DOI: https://doi.org/10.5937/zdravzast50-32819

POLYCYCLIC AROMATIC HYDROCARBONS, THEIR URINARY METABOLITES AND HEALTH

Vladan Radosavljević[1]

[1]Institute of Epidemiology, Military Medical Academy, Belgrade, Republic of Serbia

SUMMARY

The World Health Organization (WHO) stated as one of its conclusions that air pollution is a leading environmental health risk. Polycyclic aromatic hydrocarbons (PAHs) are well-known carcinogens (above five hundred compounds) that cause lung and skin cancer, especially in occupationally exposed workers. By significantly reducing emissions from modern combustion plants, traffic control, and strict smoking bans in public places, PAHs exposure can be reduced. PAH exposure ought to be better investigated, especially in the field of mass biomonitoring of the urinary concentrations of their major metabolites. Such biomonitoring ought to integrate exposures to chemical carcinogens from different sources (air, water, food, consumer products, professional procedures, etc.), as well as exposure to chemical noxae through the respiratory tract by (inhalation), digestive tract (ingestion), or through the skin. Analysis of the concentration of main PAHs metabolites in urine must be done with highly sophisticated equipment for a valid database to be obtained. The data thus obtained are necessary for risk assessment and health policymaking in order to reduce exposure to chemical carcinogens.

Key words: polycyclic aromatic hydrocarbons, chemical noxae, pollution

Introduction

According to the World Health Organization (WHO), air pollution is one of the greatest public health problems (1), which is confirmed by the fact that more than 90% of the world's population lives in regions where air quality is below the norms estimated by the WHO. Air pollution is caused by particles, as well as by toxic chemicals (1). Due to the above-mentioned reason, biomonitoring is of great importance, that is, the assessment of human exposure to chemicals (2). One of the most important chemical air pollutants are polycyclic aromatic hydrocarbons (PAHs) (Table 1), they are immunotoxic, can cause cancers and atherosclerosis, and have teratogenic effects (1,3). Numerous studies have shown that PAHs are mostly associated with the appearance of skin and lung cancer (3,4). It is estimated that by 2025 air pollution will be the main cause of early deaths (3,5). Water is most frequently polluted by industrial and communal waters that also pollute the soil. The soil is additionally contaminated by solid communal waste, forest fires, volcanic activity, industrial waste disposal, etc. This review article aims to analyze the ubiquity of PAHs in the environment, metabolism after penetration into the human body, and the possibility of reducing pollution with these compounds and preventing human disease.

The ubiquity of polycyclic aromatic hydrocarbons

PAHs are persistent organic compounds that consist of two or more condensed aromatic rings. They are present in water, air, and soil. They originate from the incomplete combustion of organic matter and become the main source of atmospheric pollution (6). PAHs may originate from natural or anthropogenic activities. The natural ways for PAHs to reach the atmosphere are volcanic eruptions and forest fires. However, the most common anthropogenic activities that lead to the appearance of PAHs are dense traffic, industry,

nom, grejanje domaćinstava fosilnim gorivima, spaljivanje bolničkog medicinskog otpada, duvanski dim, itd. (7,8).

U duvanskom dimu identifikovano je preko 500 različitih PAU, a najčešće su prisutni naftalen, fluoren i fenantren (9). Interesantno je da korišćenje dizel generatora za snabdevanje električnom energijom u Bejrutu, Bagdadu i Nju Delhiju doprinose da je kod nepušača srednja vrednosti 1-hidroksipirena (engl. *1-hydroxypyrene*) dva puta veća nego kod pušača u Sjedinjenim Američkim Državama, a 1,5 puta veća nego kod pušača u Italiji (10). Inače, 1-hidroksipiren je glavni urinarni metabolit PAU i najvažniji za njihov biomonitoring. Kina i Indija imaju najveću godišnju emisiju PAU usled sagorevanja fosilnih goriva, saobraćaja i sagorevanja duvana koja iznosi od 67.000 tona do 106.000 tona. Mnogo manja emisija je zabeležena u Sjedinjenim Američkim Državama, negde oko 8.500 tona PAU (11).

Takođe se smatra da je hrana potencijalno značajan izvor PAU, jer može da bude kontaminirana jedinjenjima PAU iz vode, vazduha i zemljišta (12,13). Dimljenje, grilovanje, pečenje i kuvanje hrane može još više da poveća sadržaj PAU (13). Kuvari koji više vremena provode u prženju hrane, u odnosu na one koji više kuvaju hranu, imaju veće vrednosti hidroksi-policikličnih aromatičnih ugljovodonika, ali ove razlike nisu bile statistički značajne (13). Takođe, isparenja tokom kuvanja ulja povećavaju koncentraciju 8-hidroksideoksiguanozina (engl. *8-hydroxydeoxyguanosine*) u urinu ljudi što ukazuje na oksidativno oštećenje DNK i lipidnu peroksidaciju koja favorizuje kancerogenezu (14). Mnoge osobe su svakodnevno izložene niskim vrednostima PAU, i zato kod njih postoji veliki rizik za nastanak malignoma (15). Istraživanja pokazuju da gojazne osobe imaju veći nivo PAU u odnosu na normalno uhranjene i pothranjene (8).

Metabolizam policikličnih aromatičnih ugljovodonika

U organizmu ljudi metabolizmom PAU nastaje 1-hidroksipiren (Tabela 1), koji reakcijama prelazi u1-hidroksipiren-glukuronid i 1-hidroksipiren-sulfat i kao takva jedinjenja se izlučuje urinom (3). Jedinjenje 1-hidroksipiren predstavlja najznačajniji metabolit pirena u ljudskom urinu (3,16,17). Vreme poluraspada metabolita PAU u urinu ljudi kreće se od 5 sati do 17 dana (3). Njihove vrednosti u urinu ljudi mogu biti veće usled prisustva zagađivača u vazduhu i vodi, načina ishrane i genetskih polimorfizama metaboličkih enzima (1).

Putem urina izlučuju se PAU manje molekulske mase (npr. fenantren, fluoren, antracen i naftalen), a fecesom veće molekulske mase (benzo[a]piren, koranulen, ovalen, koronen) (18,19). Urinarni metaboliti fenantrena i naftalena ukazuju na izloženost PAU iz vazduha (20-22), a 1-hidroksipiren na izloženost PAU ne samo iz vazduha, nego i na njihovo unošenje putem kože (20-22).

Usled izloženosti populacije velikom broju hemikalija, kao i sagledavanjem značaja PAU za nastanak brojnih oboljenja, započeto je sa inicijativom da se koordinira i unapredi humani biomonitoring u Evropi (23-25). Benzo[a]piren-diol-epoksid-N2deoksiguanozin je najviše proučavan produkt nastao iz PAU, konkretno iz benzo[a]pirena (26-29). Postoje interakcije između izloženosti kadmijuma, hroma i nikla i izloženosti PAU, koje utiču na povećanje oksidativnih markera u urinu (30).

Nakon prodiranja u organizam PAU se neravnomerno raspoređuju. U krvi, PAU imaju vreme poluraspada od 5 časova, u nemasnom tkivu od 22 časa, a u masnom tkivu od 400 časova (3). Vrednost 1-hidroksipirena u urinu može se posmatrati kao biomarker za trenutnu izloženost PAU i oslobađanje PAU iz masnog i nemasnog tkiva, kao i iz drugih delova tela.

Tabela 1. Policiklični aromatični ugljovodonici (PAU), njihovi intermedijarni i urinarni metaboliti

Glavni policiklični aromatični ugljovodonik (PAU)	Intermedijarni metabolit PAU	Urinarni metabolii PAU
Benzopiren	Epoksidi →Fenoli→Kinoni Benzopiren-7,8-Epoksid → → Benzopiren-7,8-diol → →Benzopiren-7,8-diol-9,10-epoksid	1-Hidroksipirenglukuronid i 1-Hidroksipirensulfat Benzopiren-7,8-diol-9,10-epoksid deoksiguanozin

construction sites, open waste burning, domestic heating with fossil fuels, hospital incineration, tobacco smoke, etc. (7,8).

More than 500 different PAHs have been identified in tobacco smoke, while the most common ones are naphthalene, fluorene, and phenanthrene (9). It is interesting that diesel generators that are used for electric supply systems in Beirut, Baghdad, and New Delhi contribute to the fact that the median levels of 1-hydroxypyrene in non-smokers are two times higher in comparison to smokers in the United States of America, and 1.5 higher in comparison to smokers in Italy (10). In addition, 1-hydroxypyrene is the main urinary metabolite of PAHs and the most important for their biomonitoring. China and India have the highest annual emission of PAHs due to the combustion of fossil fuels, traffic, and tobacco smoke, and it ranges from 67.000 tons to 106.000 tons. Lower emissions were recorded in the United States of America, around 8.500 tons of PAHs (11).

Also, food is thought to be a significant source of PAHs because it can be contaminated with PAHs from the air, soil, and water (12,13). Smoking, grilling, roasting, and boiling may increase the contents of PAHs (13). Cooks, who spent more time frying food, had higher levels of hydroxyl-polycyclic aromatic hydrocarbons than those who boiled food, but this difference was not statistically significant (13). Also, oil fumes increase the concentration of 8-hydroxydeoxyguanosine in human urine, indicating oxidative DNA damage and lipid peroxidation that favors carcinogenesis (14). Many people are exposed to low levels of PAHs every day, and therefore, they have a high risk of malignant disease occurrence (15). Research has shown that obese persons have higher PAHs levels than those who have normal weight or are underweight (8).

The metabolism of polycyclic aromatic hydrocarbons

In the human organism, PAHs metabolism produces 1-hydroxypyrene (Table 1), which becomes 1-hydroxypyrene-glucuronide and 1-hydroxypyrene-sulfate through chemical reactions and is excreted through urine in those forms (3). 1-hydroxypyrene conjugate is the most important pyrene metabolite in human urine (3,16,17). Half-lives of PAHs metabolites in urine range from 5 hours to 17 days (3). Their levels in human urine may be higher due to the higher presence of air and water pollutants, diet, and genetic polymorphisms in metabolizing enzymes (1).

PAHs of lower molecular weight are excreted via urine (e.g., phenanthrene, fluorene, anthracene, and naphthalene), while PAHs of higher molecular weight are excreted via feces (benzo[a]pyrene, corannulene, ovalene, coronene) (18,19). Urinary metabolites of phenanthrene and naphthalene indicate exposure to PAHs from the air (20-22), while 1-hydroxypyrene indicates exposure to PAHs not only through inhalation but also through dermal intake (20-22).

Due to the exposure of the population to a wide range of chemicals and the realization that PAHs are significant for the occurrence of numerous diseases, an initiative was started to coordinate and advance human biomonitoring in Europe (23-25). Benzo[a]pyrene diol epoxide N2 deoxyguanosine is the most studied PAH-derived adduct formed by Benzo[a]pyrene (26-29). There are interactions between Cd, Cr, Ni exposure, and PAHs exposure that have an effect on the urinary increase of oxidative markers (30).

After penetrating the body, PAHs are unevenly distributed. In the blood, PAHs have half-lives of 5 hours, 22 hours in the lean tissue, and 400 hours in the adipose tissue (3). The value of 1-hydroxypyrene

Table 1. Polycyclic aromatic hydrocarbons (PAHs), their intermediate metabolites and their final urinary metabolites

The main Polycyclic aromatic hydrocarbon (PAH)	Intermediate metabolites of the PAHs	Final urinary metabolites of the PAHs
Benzo[a]pyrene	Epoxides → Phenols → Quinones	1-Hydroxypyrene Glucuronides and Sulfates
	Benzo[a]pyrene-7,8-Epoxide → → Benzo[a]pyrene-7,8-diol) → → Benzo[a]pyrene-7,8- diol-9,10-epoxide)	Benzo[a]pyrene-7,8- diol-9,10-epoxide deoxyguanosine

Rezultati populacionih studija urinarnih metabolita PAU

U Nemačkoj, između 2014. i 2017. godine, sprovedeno je istraživanje na 2.294 dece i adolescenata uzrasta 3-17 godina. Rezultati istraživanja su pokazali da su ispitanici koji su koristili fosilno gorivo za grejanje ili gas za kuvanje, imali veće koncentracije urinarnih metabolita PAU (31).

U studiji sprovedenoj u Kanadi uočeno je da su koncentracije metabolita PAU u urinu u vezi sa promenama vrednosti kreatinina (32). Zapaženo je da su pušenje, godine starosti i pol značajno povezani sa koncentracijama 1-hidroksipirena u urinu.

Takođe, ishrana predstavlja najverovatniji uzrok neobjašnjene varijacije u vrednostima urinarnih metabolita PAU (33-35).

Studija sprovedana u Flandriji obuhvatila je tinejdžere uzrasta 14-15 godina sa područja gde je bila razvijena industrija i to fabrika čelika (n=197; od januara 2010. do novembra 2010.) i fabrika sekača i drobilica (n=199; od maja 2010. do februara 2011.) (15). Urinarni biomarkeri za PAU su u značajnoj meri bili povezani sa višim koncentracijama 8-hidroksideoksiguanozina (8-OhdG) u urinu. To znači da eksponiranost višim koncentracijama PAU dovodi i do oštećenja DNK, što može da se potvrdi prisustvom 8-OhdG u urinu.

Na Tajvanu je 61 vojni kuvar (koji su prosečno dnevno provodili 6,3 sati kuvajući) i 37 muškaraca kontrolne grupe (koji nisu bili kuvari i nisu bili izloženi isparenjima tokom kuvanja ulja) dali uzorke urina prvog (pre smene) i petog radnog dana (posle smene) (14). Uočeno je da su srednje vrednosti 1-hidroksipirena pre smene i posle smene bile statistički značajno veće kod vojnih kuvara. Brojne studije su ukazale da izlaganje isparenjima tokom kuvanja ulja vodi oksidativnom oštećenju DNK (36,37).

U studiji sprovedenoj u Južnoj Koreji u periodu od 2009. do 2017. analizirano je 15.125 uzoraka urina (3). Visoke vrednosti PAU otkrivene su po prvi put kod građevinaca (putara, radnika koji su pokrivali krovove), rudara i dostavljača (uličnih prodavaca, prevoznika, radnika u ribarnicama). Ukupna prosečna vrednost 1-hidroksipirena u urinu je bila manja od 1 µg/L za analiziranih 15.125 uzoraka (3). Kod neizloženih urinarna koncentracija 1-hidroksipirena bila je 0,5 µg/L.

Zaključak

Zabrana pušenja na javnim mestima i smanjenje emisije iz industrijskih postrojenja za sagorevanje fosilnih goriva i ostalih organskih materija mogu doprineti smanjenoj emisiji a samim tim i izloženosti PAU. Izloženost PAU bi trebalo i dalje istraživati, posebno u oblasti biomonitoringa koncentracije 1-hidroksipirena u urinu. Podaci biomonitoringa reflektuju aktuelni unos i/ili metabolizam praćenih hemikalija. Trenutno se koriste slučajni uzorci urina (eng. spot urine sample) da bi se odredile koncentracije PAU hidroksi metabolita. Buduće studije bi trebalo da sadrže podatke o personalnoj izloženosti PAU iz različitih izvora, čiji je unos omogućen na različite načine (udisanjem, preko kože ili ingestijom). Visoko sofisticirana oprema i instrumenti kao i neinvazivno uzorkovanje (recimo uzorkovanje urina) su neophodni za formiranje baze podataka radi procene izloženosti PAU. Biomonitoring PAU na velikom broju uzoraka u cilju validne procene eksponiranosti PAU bi omogućio bolji uvid u štetan uticaj ovih hemijskih agenasa i preduzimanje preventivnih mera za smanjenje ili eliminaciju izloženosti njima.

Literatura

1. Luo K, Stepanov I, Hecht S. Chemical biomarkers of exposure and early damage from potentially carcinogenic airborne pollutants. Ann Cancer Epidemiol 2019; 3(5):1-31.
2. Vorkamp K, Castano A, Antignac J-P, Boada L, Cequier E, Covaci A, et al. Biomarkers, matrices and analytical methods targeting human exposure to chemicals selected for a European human biomonitoring initiative. EnvironInt 2021; 146:106082.
3. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans: Some Non-heterocyclic Polycyclic Aromatic Hydrocarbons and Some Related Exposures, 92; Lion, France: 2010.
4. Koh D-H, Park J-H, Lee S-G, Kim H-C, Choi S, Jung H, et al. Comparison of Polycyclic Aromatic Hydrocarbons Exposure Across Occupations Using Urinary Metabolite 1-Hydroxypyrene. Annals Work Expo Health 2020; 64(4):445–454.
5. GBD 2015 Risk Factors Collaborators: Forouzanfar MH, Afshin A, Alexander LT, Anderson HR, Bhutta ZA, Biryukov S, et al. Global, regional, and national comparative risk assessment of 79 behavioural, environmentaland occupational, and metabolic risks or clusters of risks, 1990-2015: a systematic analysis for the Global Burden of Disease Study 2015. Lancet 2016; 388(10053):1659-1724.
6. OECD. Health and Environment Chapter of the OECD Environmental Outlook to 2050: The Consequences of

in urine may be observed as a biomarker for the current exposure to PAHs and release of PAHs from the adipose and lean tissue, as well as from other body parts.

The results of studies on polycyclic aromatic hydrocarbons

In Germany, a study was conducted between 2014 and 2017, and it included 2294 children and adolescents aged 3 to 17. The results of the study showed that examinees, who used domestic fuel for heating or gas for cooking, had higher concentrations of urinary metabolites of PAHs (31).

In a study conducted in Canada, it was noticed that concentrations of PAHs metabolites in urine were connected with the changed creatinine levels (32). It was reported that tobacco smoking, age, and sex were significantly connected with the urinary concentrations of 1-hydroxypyrene.

Also, diet was the most likely cause of the unexplained variation of levels of urinary PAHs metabolites (33-35).

A study that was conducted in Flanders included teenagers aged 14-15 from the area with developed industry, that is, a stainless steel factory (n=199; from January 2010 to November 2010), and a shredder factory (n=199; from May 2010 to February 2011) (15). Urinary biomarkers for PAHs were significantly correlated with higher concentrations of 8-hydoxydeoxyguanozine (8-OhdG) in urine. This means that exposure to higher concentrations of PAHs leads to DNA damage, which can be confirmed by the presence of 8-OhdG in urine.

In Taiwan, sixty-one military cooks (daily average time spent cooking was 6.3 hours) and 37 men from the control group (who were not cooks and were not exposed to cooking oil fumes) gave urine samples on the first weekday (pre-shift) and the fifth workday (post-shift) (14). It was found that the mean value of 1-hydroxypyrene before the shift and after the shift was significantly higher in military cooks. Numerous studies have shown that exposure to cooking oil fumes leads to oxidative DNA damage (36,37).

In one study conducted in South Korea, 15125 urine samples were analyzed from 2009 to 2017 (3). High values of PAHs were found for the first time among construction workers (road pavers, roofers), miners, and deliverers (street vendors, transporters, fishery workers). The overall average value of 1-hydroxypyrene in urine was lower than 1 µg/L for the 15125 analyzed samples (3). In workers who were not exposed, the urinary concentration of 1-hydroxypyree was 0.5 µg/L.

Conclusion

The prohibition of smoking in public places and the reduction of emissions from modern plants for the combustion of fossil fuels and other organic material may contribute to the decreased emission of and exposure to PAHs. Exposure to PAHs should be investigated further, especially monitoring the observed increase in concentrations of 1-hydroxypyrene in urine. Biomonitoring data reflect the actual intake and/or metabolism of the monitored chemicals. Currently, single spot urine samples are used to determine the concentrations of PAHs hydroxyl metabolites. Future studies should provide data on the personal PAH exposure from different sources whose intake was enabled via different routes (inhalation, skin contact, and ingestion). Highly sophisticated equipment and instruments and non-invasive sampling (for example, urine sampling) are necessary for forming a database to estimate PAH exposure. Large-scale biomonitoring of PAHs for the purpose of valid assessment of PAH exposure would provide better insight into the harmful effects of these chemical agents and taking preventive measures to reduce or eliminate exposure to them.

Literature

1. Luo K, Stepanov I, Hecht S. Chemical biomarkers of exposure and early damage from potentially carcinogenic airborne pollutants. Ann Cancer Epidemiol 2019; 3(5):1-31.
2. Vorkamp K, Castano A, Antignac J-P, Boada L, Cequier E, Covaci A, et al. Biomarkers, matrices and analytical methods targeting human exposure to chemicals selected for a European human biomonitoring initiative. EnvironInt 2021; 146:106082.
3. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans: Some Non-heterocyclic Polycyclic Aromatic Hydrocarbons and Some Related Exposures, 92; Lion, France: 2010.
4. Koh D-H, Park J-H, Lee S-G, Kim H-C, Choi S, Jung H, et al. Comparison of Polycyclic Aromatic Hydrocarbons Exposure Across Occupations Using Urinary Metabolite 1-Hydroxypyrene. Annals Work Expo Health 2020; 64(4):445–454.
5. GBD 2015 Risk Factors Collaborators: Forouzanfar MH, Afshin A, Alexander LT, Anderson HR, Bhutta ZA, Biryukov

Inaction. March 2012. [cited 2021 March 19]; [about 5 p.]. Available from: https://read.oecd-ilibrary.org/environment/oecd-environmental-outlook-to-2050/summary/english_env_outlook-2012-sum-en#page1.

7. Abdel-Shafy HI, Mansour MSM,. A review on polycyclic aromatic hydrocarbons: source, environmental impact, effect on human health and remediation. Egypt J Petrol 2016; 25(1):107–123.
8. Murawski A, Roth A, Schwedler G, Schmied-Tobies M, Rucic E, Pluym N, et al. Polycyclic aromatic hydrocarbons (PAH) in urine of children and adolescents in Germany – human biomonitoring results of the German Environmental Survey 2014–2017 (GerES V) Int J Hyg Environ Health 2020; 226:113491.
9. Rodgman A, Perfetti T. The Chemical Components of Tobacco and Tobacco Smoke. 2nd ed. Boca Raton: CRC Press; 2013.
10. Salwa H, Aarajl Al, Zgheib N, BadrK, Ismaeel H, Abchee A, et al. The association of urinary metabolites of polycyclic aromatic hydrocarbons with obstructive coronary artery disease: A red alert for action. Environmental Pollution 2021; 272:115967.
11. Shen H, Huang Y, Wang R, Zhu D, Li W, Shen G, et al. Global atmospheric emissions of polycyclic aromatic hydrocarbons from 1960 to 2008 and future predictions. Environ Sci Technol 2013; 47(12):6415-6424.
12. Alomirah H, Al-Zenki S, Al-Hooti S, Zaghloul S, Sawaya W, Ahmed N, et al. Concentrations and dietary exposure to polycyclic aromatic hydrocarbons (pahs) from grilled and smoked foods. Food Control 2011; 22(12):2028-2035.
13. Cao L, Wang D, Wen Y, He H, Chen A, Hu D, et al. Effects of environmental and lifestyle exposures on urinary levels of polycyclic aromatic hydro- carbon metabolites: A cross-sectional study of urban adults in China. Chemosphere 2020; 240:124898.
14. Lai CH, Jaakkola J, Chuang C-Y, Liou S-H, Lung S-C, Loh C-H, et al. Exposure to co- oking oil fumes and oxidative damages: a longitudinal study in Chinese military cooks. J Expo Sci Environ Epidemiol 2013; 23.
15. Franken C, Koppen G, Lambrechts N, Govarts E, Bruckers L, Den Hond E, et al. Environmental exposure to human carcinogens in teenagers and the association with DNA damage. Environ Res 2017; 152:165–174.
16. Strickland PT, Kang D, Bowman ED, Fitzwilliam A, Downing TE, Rothman N, et al. Identification of 1-hydroxypyrene glucuronide as a major pyrene metabolite in human urine by synchronous fluorescence spectroscopy and gas-chromatography mass-spectrometry. Carcinogenesis 1994;15(3):483-487.
17. U.S. Department of Health and Human Services CDC. Fourth Report on Human Exposure to Environmental Chemicals, Updated Tables. 2019; 1.
18. Guo Y, Senthilkumar K, Alomirah H, Moon H-B, Minh TB, Mohd MA, et al. Concentra- tions and profiles of urinary polycyclic aromatic hydrocarbon metabolites (OH-PAHs) in several Asian countries. Environ Sci Technol. 2013;47(6):2932–2938.
19. Ruby MV, Lowney YW, Bunge AL, Roberts SM, Gomez-Eyles JL, Ghosh U, et al. Oral Bioavailability, Bioaccessibility, and Dermal Absorption of PAHs from Soil State of the Science. Environ Sci Technol 2016; 50(5):2151–2164.
20. Jansen E, Schenk E. Use of biomarkers in exposure assessment of polycyclic aromatic hydrocarbons. Clin Chem 1995; 41(12):1905–1906.
21. Kim H, Cho S, Kang J, Kim Y, Nan H, Lee C-H, et al. Urinary 1-hydroxypyrene and 2-naphthol concentrations in male Koreans. Int Arch Occup Environ Health 2000; 74:59–62.
22. Serdar B, Waidyanatha S, Zheng Y, Rappaport SM. Simultaneous determination of urinary 1-and 2-naphthols, 3-and 9-phenanthrols, and 1-pyrenol in coke oven workers. Biomarkers 2003; 8(2):93–109.
23. Oberg T, Iqbal MS. The chemical and environmental property space of REACH chemicals. Chemosphere 2012; 87(8):975–981.
24. Ganzleben C, Antignac J-P, Barouki R, Castano A, Fiddicke U, Klanova J, et al. Human biomonitoring as a tool to support chemicals regulation in the European Union. Int. J. Hyg Environ Health 2017; 220(2 Pt A): 94–97.
25. Yuan JM, Butler LM, Gao YT, Murphy S, Carmella S, Wang R, et al. Urinary metabolites of a polycyclic aromatic hydrocarbon and volatile organic compounds in relation to lung cancer development in lifelong never smokers in the Shanghai Cohort Study. Carcinogenesis 2014;35(2):339-345.
26. Pavanello S, Favretto D, Brugnone F, Mastrangelo G, Dal Pra G, Clonfero E. HPLC/ fluorescence determination of anti-BPDE-DNA adducts in mononuclear white blood cells from PAH-exposed humans. Carcinogenesis 1999; 20(3):431-435.
27. Jin Y, Xu P, Liu X, Zhang C, Tan C, Chen C, et al. Cigarette smoking, BPDE-DNA adducts, and aberrant promoter methylations of tumor suppressor genes (TSGs) in NSCLC from Chinese population. Cancer Invest 2016;34(4):173-180.
28. Naidoo RN, Makwela MH, Chuturgoon A, Tiloke C, Ramkaran P, Phulukdaree A. Petrol exposure and DNA integrity of peripheral lymphocytes. Int Arch Occup Environ Health 2016;89(5):785-792.
29. Rundle A, Richards C, Neslund-Dudas C, Tang D, Rybicki B. Neighborhood socioeconomic status modifies the association between individual smoking status and PAH-DNA adduct levels in prostate tissue. Environ Mol Mutagen 2012;53(5):384-391.
30. Wang T, Feng W, Kuang D, Deng Q, Zhang W, Wang S, et al. The effects of heavy metals and their interactions with polycyclic aromatic hydrocarbons on the oxidative stress among coke-oven workers. Environ Res 2015; 140:405-413.
31. Dobraca D, Lum R, Sjodin A, Calafat AM, Laurent CA, Kushi LH, et al. Urinary biomarkers of polycyclic aromatic hydrocarbons in pre- and peripubertal girls in Northern California: predictors of exposure and temporal variability. Environ Res 2018; 165: 46–54.

S, et al. Global, regional, and national comparative risk assessment of 79 behavioural, environmentaland occupational, and metabolic risks or clusters of risks, 1990-2015: a systematic analysis for the Global Burden of Disease Study 2015. Lancet 2016; 388(10053):1659-1724.

6. OECD. Health and Environment Chapter of the OECD Environmental Outlook to 2050: The Consequences of Inaction. March 2012. [cited 2021 March 19]; [about 5 p.]. Available from: https://read.oecd-ilibrary.org/environment/oecd-environmental-outlook-to-2050/summary/english_env_outlook-2012-sum-en#page1.
7. Abdel-Shafy HI, Mansour MSM,. A review on polycyclic aromatic hydrocarbons: source, environmental impact, effect on human health and remediation. Egypt J Petrol 2016; 25(1):107–123.
8. Murawski A, Roth A, Schwedler G, Schmied-Tobies M, Rucic E, Pluym N, et al. Polycyclic aromatic hydrocarbons (PAH) in urine of children and adolescents in Germany – human biomonitoring results of the German Environmental Survey 2014–2017 (GerES V) Int J Hyg Environ Health 2020; 226:113491.
9. Rodgman A, Perfetti T. The Chemical Components of Tobacco and Tobacco Smoke. 2nd ed. Boca Raton: CRC Press; 2013.
10. Salwa H, Aarajl Al, Zgheib N, BadrK, Ismaeel H, Abchee A, et al. The association of urinary metabolites of polycyclic aromatic hydrocarbons with obstructive coronary artery disease: A red alert for action. Environmental Pollution 2021; 272:115967.
11. Shen H, Huang Y, Wang R, Zhu D, Li W, Shen G, et al. Global atmospheric emissions of polycyclic aromatic hydrocarbons from 1960 to 2008 and future predictions. Environ Sci Technol 2013; 47(12):6415-6424.
12. Alomirah H, Al-Zenki S, Al-Hooti S, Zaghloul S, Sawaya W, Ahmed N, et al. Concentrations and dietary exposure to polycyclic aromatic hydrocarbons (pahs) from grilled and smoked foods. Food Control 2011; 22(12):2028-2035.
13. Cao L, Wang D, Wen Y, He H, Chen A, Hu D, et al. Effects of environmental and lifestyle exposures on urinary levels of polycyclic aromatic hydro- carbon metabolites: A cross-sectional study of urban adults in China. Chemosphere 2020; 240:124898.
14. Lai CH, Jaakkola J, Chuang C-Y, Liou S-H, Lung S-C, Loh C-H, et al. Exposure to co- oking oil fumes and oxidative damages: a longitudinal study in Chinese military cooks. J Expo Sci Environ Epidemiol 2013; 23.
15. Franken C, Koppen G, Lambrechts N, Govarts E, Bruckers L, Den Hond E, et al. Environmental exposure to human carcinogens in teenagers and the association with DNA damage. Environ Res 2017; 152:165–174.
16. Strickland PT, Kang D, Bowman ED, Fitzwilliam A, Downing TE, Rothman N, et al. Identification of 1-hydroxypyrene glucuronide as a major pyrene metabolite in human urine by synchronous fluorescence spectroscopy and gas-chromatography mass-spectrometry. Carcinogenesis 1994;15(3):483-487.
17. U.S. Department of Health and Human Services CDC. Fourth Report on Human Exposure to Environmental Chemicals, Updated Tables. 2019; 1.
18. Guo Y, Senthilkumar K, Alomirah H, Moon H-B, Minh TB, Mohd MA, et al. Concentra- tions and profiles of urinary polycyclic aromatic hydrocarbon metabolites (OH-PAHs) in several Asian countries. Environ Sci Technol. 2013; 47(6):2932–2938.
19. Ruby MV, Lowney YW, Bunge AL, Roberts SM, Gomez-Eyles JL, Ghosh U, et al. Oral Bioavailability, Bioaccessibility, and Dermal Absorption of PAHs from Soil State of the Science. Environ Sci Technol 2016; 50(5):2151–2164.
20. Jansen E, Schenk E. Use of biomarkers in exposure assessment of polycyclic aromatic hydrocarbons. Clin Chem 1995; 41(12):1905–1906.
21. Kim H, Cho S, Kang J, Kim Y, Nan H, Lee C-H, et al. Urinary 1-hydroxypyrene and 2-naphthol concentrations in male Koreans. Int Arch Occup Environ Health 2000; 74:59–62.
22. Serdar B, Waidyanatha S, Zheng Y, Rappaport SM. Simultaneous determination of urinary 1-and 2-naphthols, 3-and 9-phenanthrols, and 1-pyrenol in coke oven workers. Biomarkers 2003; 8(2):93–109.
23. Oberg T, Iqbal MS. The chemical and environmental property space of REACH chemicals. Chemosphere 2012; 87(8):975–981.
24. Ganzleben C, Antignac J-P, Barouki R, Castano A, Fiddicke U, Klanova J, et al. Human biomonitoring as a tool to support chemicals regulation in the European Union. Int J Hyg Environ Health 2017; 220(2 Pt A): 94–97.
25. Yuan JM, Butler LM, Gao YT, Murphy S, Carmella S, Wang R, et al. Urinary metabolites of a polycyclic aromatic hydrocarbon and volatile organic compounds in relation to lung cancer development in lifelong never smokers in the Shanghai Cohort Study. Carcinogenesis 2014; 35(2):339-345.
26. Pavanello S, Favretto D, Brugnone F, Mastrangelo G, Dal Pra G, Clonfero E. HPLC/ fluorescence determination of anti-BPDE-DNA adducts in mononuclear white blood cells from PAH-exposed humans. Carcinogenesis 1999; 20(3):431-435.
27. Jin Y, Xu P, Liu X, Zhang C, Tan C, Chen C, et al. Cigarette smoking, BPDE-DNA adducts, and aberrant promoter methylations of tumor suppressor genes (TSGs) in NSCLC from Chinese population. Cancer Invest 2016; 34(4):173-180.
28. Naidoo RN, Makwela MH, Chuturgoon A, Tiloke C, Ramkaran P, Phulukdaree A. Petrol exposure and DNA integrity of peripheral lymphocytes. Int Arch Occup Environ Health 2016; 89(5):785-792.
29. Rundle A, Richards C, Neslund-Dudas C, Tang D, Rybicki B. Neighborhood socioeconomic status modifies the association between individual smoking status and PAH-DNA adduct levels in prostate tissue. Environ Mol Mutagen 2012;53(5):384-391.
30. Wang T, Feng W, Kuang D, Deng Q, Zhang W, Wang S, et al. The effects of heavy metals and their interactions

32. Jennifer LA, Keir, Sabit Cakmak, Jules M Blais, Paul A. White. The influence of demographic and lifestyle factors on urinary levels of PAH metabolites—empirical analyses of Cycle 2 (2009–2011) CHMS data. J Expo Sci Environ Epidemiol 2021; 31(2): 386–397.
33. Goldman R, Enewold L, Pellizzari E, Beach JB, Bowman ED, Krishnan SS, et al. Smoking increases carcinogenic polycyclic aromatic hydrocarbons in human lung tissue. Cancer Res 2001; 61(17):6367–6371.
34. St Helen G, Goniewicz ML, Dempsey D, Wilson M, III PJ, Benowitz NL. Exposure and kinetics of polycyclic aromatic hydrocarbons (PAHs) in cigarette smokers. Chem Res Toxicol. 2012; 25(4):952–964.
35. Ramesh A, Walker SA, Hood DB, Guillen MD, Schneider K, Weyand EH. Bioavaila- bility and risk assessment of orally ingested polycyclic aromatic hydrocarbons. Int J Toxicol. 2004; 23(5):301–333.
36. Pan CH, Chan CC, Wu KY. Effects on Chinese restaurant workers of exposure to cooking oil fumes: a cautionary note on urinary 8-hydroxy-20-deoxyguanosine. Cancer Epidemiol Biomarkers Prev 2008; 17(12):3351–3357.
37. Wu SC, Yen GC, Sheu F. Mutagenicity and identification of mutagenic compounds of fumes obtained from heating peanut oil. J Food Prot 2001; 64(2):240–245.

Sukob interesa: Nije prijavljen.
Primljen: 19.06.2021.
Revizija: 01.07.2021.
Prihvaćen: 02.07.2021.

Autor za korespondenciju: Dr Vladan Radosavljević, Institut za epidemiologiju Vojnomedicinske akademije, Crnotravska 17, 11000 Beograd, Republika Srbija; e-mail: vladanr4@gmail.com

with polycyclic aromatic hydrocarbons on the oxidative stress among coke-oven workers. Environ Res 2015; 140:405-413.

31. Dobraca D, Lum R, Sjodin A, Calafat AM, Laurent CA, Kushi LH, et al. Urinary biomarkers of polycyclic aromatic hydrocarbons in pre- and peripubertal girls in Northern California: predictors of exposure and temporal variability. Environ Res 2018; 165: 46–54.
32. Jennifer LA, Keir, Sabit Cakmak, Jules M Blais, Paul A. White. The influence of demographic and lifestyle factors on urinary levels of PAH metabolites—empirical analyses of Cycle 2 (2009–2011) CHMS data. J Expo Sci Environ Epidemiol 2021; 31(2): 386–397.
33. Goldman R, Enewold L, Pellizzari E, Beach JB, Bowman ED, Krishnan SS, et al. Smoking increases carcinogenic polycyclic aromatic hydrocarbons in human lung tissue. Cancer Res 2001; 61(17):6367–6371.
34. St Helen G, Goniewicz ML, Dempsey D, Wilson M, III PJ, Benowitz NL. Exposure and kinetics of polycyclic aromatic hydrocarbons (PAHs) in cigarette smokers. Chem Res Toxicol. 2012; 25(4):952–964.
35. Ramesh A, Walker SA, Hood DB, Guillen MD, Schneider K, Weyand EH. Bioavaila- bility and risk assessment of orally ingested polycyclic aromatic hydrocarbons. Int J Toxicol. 2004; 23(5):301–333.
36. Pan CH, Chan CC, Wu KY. Effects on Chinese restaurant workers of exposure to cooking oil fumes: a cautionary note on urinary 8-hydroxy-20-deoxyguanosine. Cancer Epidemiol Biomarkers Prev 2008; 17(12):3351–3357.
37. Wu SC, Yen GC, Sheu F. Mutagenicity and identification of mutagenic compounds of fumes obtained from heating peanut oil. J Food Prot 2001; 64(2):240–245.

Conflict of interest: None declared.
Received: 06/19/2021
Revised: 07/01/2021
Accepted: 07/02/2021

Corresponding author: Dr Vladan Radosavljević, Institute of Epidemiology Military Medical Academy, Crnotravska 17, 11000 Belgrade, Republic of Serbia; e-mail: vladanr4@gmail.com

UPUTSTVO AUTORIMA

U časopisu **Zdravstvena zaštita** objavljuju se originalni naučni radovi, prethodna saopštenja, pregledi i stručni radovi, kratka saopštenja, uvodnici, pisma uredniku, meta-analize, prikazi bolesnika, aktuelne teme, prikazi stručnih knjiga i skupova, i drugo, iz svih oblasti medicine, farmacije, biohemije, stomatologije i menadžmenta u zdravstvu.

Uz rukopis za objavljivanje u časopisu **Zdravstvena zaštita** treba dostaviti propratno pismo (izjavu) potpisanu od svih autora: 1) da rad nije prethodno objavljivan i da nije istovremeno podnet za objavljivanje u nekom drugom časopisu; 2) da su rukopis pročitali i odobrili svi autori koji ispunjavaju kriterijume autorstva; 3) da su u radu kontakt podaci za sve autore tačni; 4) da autor za korespondenciju, u ime drugih autora, potpisuje Ugovor o autorskim pravima, kojim svi autori rada prenose svoja autorska prava na izdavača časopisa - Komoru zdravstvenih ustanova Srbije.

Časopis je u režimu otvorenog pristupa (engl. *Open Access*) od septembra 2019. godine i objavljuje se četiri puta godišnje. Svi autori da bi poslali rukopis za objavljivanje u časopisu elektronski treba da budu registrovani e-mail adresom na internet stranici časopisa: **https://aseestant.ceon.rs/index.php/zdravzast**. Ukoliko postoji bilo koji problem u procesu elektronskog slanja rukopisa, rukopis se može proslediti na e-mail: **urednik@komorazus.org.rs.**

Priprema rukopisa za objavljivanje u časopisu

Rukopis dostaviti na engleskom ili spskom jeziku (latinicom), sa rezimeima na srpskom i engleskom. Tekst rada piše se sa proredom 1,5 na stranicama A4 formata. Kuca se u programu za obradu teksta Word, fontom *Times New Roman* i veličinom slova 12 i marginama od 25 mm. Izbegavati deljenje reči (hifenacije), kao i **bold** i *italic* slova. Originalni članci, opšti pregledi (uslov da autori navedu 5 autocitata) i meta-analize ne smeju prelaziti 16 strana (bez priloga), stručni rad i aktuelne teme 10 strana, članci iz istorije medicine, kazuistika (prikaz jednog ili serije slučajeva) i prethodna saopštenja – 8 strana, a komentari, pisma uredniku, izveštaji sa skupova i prikazi knjiga 3 strane. Za izradu grafičkih priloga koristiti program *Windows* iz programskog paketa *Microsoft Office* (*Excel, Word Graph*).

Delovi rada su: naslovna strana, sažetak na srpskom i engleskom jeziku sa ključnim rečima na srpskom i engleskom jeziku, tekst rada (Uvod, Metode, Rezultati, Diskusija, Zaključak, Literatura, Zahvalnica) i prilozi.
Tekst rada pisati kratko i jasno, a skraćenice koristiti samo za veoma dugačke nazive i za nazive koji su poznati kao skraćenice (npr. sida, HIV, itd).

Naslovna strana

Navesti naziv rada (velikim slovima), puna imena i prezimena autora, njihove stručne titule i nazive ustanova i mesta u kojima rade. Imena autora povezati sa nazivima ustanova indeksiranim arapskim brojevima. Takođe navesti ime i prezime autora za korespondenciju, njegovu ustanovu, adresu ustanove, broj telefona i e-mail adresu.

Sažetak i ključne reči

Druga strana treba da sadrži: naslov rada, strukturisani sažetak do 250 reči i ključne reči na srpskom jeziku. Sažetak se sastoji iz četiri dela: Uvod/Cilj, Metode, Rezultati i Zaključak. Ispod sažetka navodi se 3-8 ključnih reči. Kod prikaza jednog ili serije slučajeva sažetak ima tri dela: Uvod/cilj, Prikaz bolesnika i Zaključak, a kod preglednih radova sažetak je deskriptivan (bez podcelina). Treća strana je identična drugoj, ali je na engleskom jeziku.

INSTRUCTIONS FOR AUTHORS

The Journal of **Health Care** publishes original scientific papers, short communications, reviews and professional papers, short press releases, editorials, letters to the editor, meta-analyses, case reports, actual topics, reviews of expert books and conferences, in all fields of medicine, pharmacy, biochemistry, dentistry and healthcare management.

The manuscript for publication in the Journal of **Health Care** should be accompanied by a cover letter (statement) signed by all authors: 1) that the manuscript has not been previously published and that it has not been simultaneously submitted for publication in another journal; 2) that the manuscript has been read and approved by all authors who meet the authorship criteria; 3) that the contact information for all authors is correct in the manuscript; 4) that the corresponding author, on behalf of other authors, signs the Copyright Agreement, by which all authors of the work transfer their copyright to the publisher of the journal - the Chamber of Health Institutions of Serbia.

The journal has been in Open Access mode since September 2019 and it is published four times a year. All authors must be registered electronically by e-mail on the journal website: **https://aseestant.ceon.rs/index.php/zdravzast** in order to submit the manuscript for publication in the journal. If there is any problem in the process of sending the manuscript electronically, the manuscript can be forwarded to e-mail: **urednik@komorazus.org.rs.**

Preparation of a manuscript for publication in the Journal

The manuscript should be submitted in English or Serbian (latin alphabet), with summaries in Serbian and English. The text of the manuscript should be written with a 1.5 line spacing on A4 pages and 25 mm margins. The text should be typed in Word, Times New Roman font and font size 12. Avoid hyphenation as well as **bold** and *italic* letters. Original articles, reviews (condition for authors to cite 5 self-citations) and meta-analyses must not exceed 16 pages (without attachments), professional articles and actual topics 10 pages, articles in medical history, case reports and case series (presentation of one or a series of cases) and previous reports - 8 pages, and comments, letters to the editor, conference reports and book reviews 3 pages. To create graphical attachments, use Windows from Microsoft Office (Excel, Word Graph).

Parts of the manuscript are: title page, summary in Serbian and English with keywords in Serbian and English, text of the manuscript (Introduction, Methods, Results, Discussion, Conclusion, Literature, Acknowledgment) and appendices.

The manuscript should be written briefly and clearly and abbreviations used only for very long names and for names known as abbreviations (eg AIDS, HIV, etc.).

Title page

Give the name of the manuscript (in capital letters), full names of the authors, their affiliation. Associate author names with institution names indexed by Arabic numerals. Also provide the first and last name for the corresponding author, their institution, institution address, telephone number and e-mail address.

Summary and keywords

The second page should include: the title of the manuscript, a structured summary up to 250 words and keywords in Serbian. The summary consists of four parts: Introduction/Aim, Methods, Results and Conclusion. Below the summary 3-8 keywords, should be listed. When presenting one or a series of

Uvod/cilj

Uvod treba da bude jasan i direktno povezan sa predmetom istraživanja. Treba da pruži najvažnije informacije o problematici kojom se bavi rad, kao i to šta je do sada o tom problemu istraživano tj. poznato, a šta je nepoznato, malo poznato, ili postoje kontroverzni podaci. Posle uvodnih napomena potrebno je navesti cilj rada.

Metode

U ovom delu autori opisuju kako je studija izvedena, obrazlažu izbor metoda i dizajn istraživanja.Podceline metoda rada mogu biti: dizajn studije (npr. kvantititavno ili kvalitativno istraživanje, deskriptivna ili analitička ili eksperimetalna studija, itd.), izbor ispitanika (kriterijumi za uključivanje i isključivanje iz studije), etički aspekti (broj pod kojim je studija odobrena od etičkog komiteta), instrumenti istraživanja (način prikupljanja podataka, specifičnosti korišćenih instrumenata) i statistička analiza podataka (vrste testova). Važno je navesti podatke iz literature za poznate metode, uključujući i statističke.

Rezultati

Tekstualno opisati rezultate istraživanja prezentovane logičkim redosledom kroz tabele, grafikone i ilustracije (prilozi se navode iza Literature).

Diskusija

Rezultate istraživanja uporedite sa rezultatima drugih već publikovanih relevantnih istraživanja (ako je to moguće ne starijim od pet godina).

Literatura

Rukopisi se pripremaju u skladu sa Vankuverskim dogovorom. Literaturni podaci označavaju se arapskim brojevima, npr. (6), redosledom kojim se pojavljuju u tekstu. Informacije o citiranju mogu se naći na internet stranici https://www.nlm.nih.gov/bsd/uniform_requirements.html. Pri citiranju literature, navode se svi autori, ali ako broj autora prelazi 6, navodi se prvih šest autora i dodaje et al. Broj radova u spisku literature ne treba da prelazi 30. Podaci sa Interneta citiraju se uz navođenje datuma pristupa tim podacima. Članke koji su prihvaćeni za publikovanje, ali nisu objavljeni, treba označiti sa u štampi (*in press*). Uz svaku referencu treba navesti DOI broj članka.

Zahvalnica

Potrebno je uputiti zahvalnicu svim saradnicima koji su doprineli realizaciji rada, ali koji ne ispunjavaju kriterijume za autorstvo, kao i svima koji su finansijski i materijalno pomogli realizaciji istraživanja.

Prilozi

Priloge čine tabele, slike (fotografije, crteži, sheme, grafikoni) i video-prilozi. Svi prilozi moraju biti na srpskom i engleskom jeziku. Za sve priloge mora postojati naslov koji se navodi iznad priloga. Svi prilozi se označavaju arapskim brojevima prema redosledu navođenja u tekstu. Korišćenje skraćenica u naslovima ili bilo kom delu priloga obavezno objasniti ispod datog priloga.

cases, the abstract should consist of three parts: Introduction/Aim, Case report and Conclusion, and in review papers, the summary is descriptive (without subsections). The third page is identical to the other, but is in English.

Introduction/Aim

The introduction should be clear and directly related to the subject of the research. It should provide the most important information about the problem that is being dealt with, as well as what has been investigated so far about the problem, what is known and what is unknown, or little known, or if there is controversial information. After the introductory notes, the aim of the paper should be stated.

Methods

In this section, the authors describe how the study was conducted, explain the choice of methods and design of the research. The sub-sections of the methods may be: study design (eg quantitative or qualitative research, descriptive or analytical or experimental study, etc.), choice of respondents (inclusion and exclusion criteria from the study), ethical aspects (the number under which the study was approved by the ethics committee), research instruments (method of data collection, specificity of instruments used), and statistical analysis of the data (types of tests). It is important to provide literature data for known methods, including statistical methods.

The results

Describe the results of the research presented in a logical order through tables, charts and illustrations (appendices are cited after the Literature).

Discussion

Compare the results of your research with the results of other relevant research already published (if possible not older than five years).

Literature

Manuscripts are prepared in accordance with the Vancouver Arrangement. Literature data are indicated by Arabic numerals, e.g. (6), in the order in which they appear in the text. Citation information can be found at https://www.nlm.nih.gov/bsd/uniform_requirements.html. When citing the literature, all authors should be cited, but if the number of authors exceeds 6, the first six authors are cited and added by et al. The number of references in the literature should not exceed 30. Data from the Internet are cited indicating the date of access to that data. Articles accepted for publication but not published should be marked in press. Each reference should include a DOI article number.

Acknowledgment

Acknowledgments should be given to all contributors who have contributed to the realization of the work but who haven't met the criteria for authorship, as well as to all those who have financially and materially assisted in the realization of the research.

Appendices

Appendices include tables, pictures (photos, drawings, diagrams, charts) and video attachments. All appendices must be in Serbian and English. There must be a title above all appendices for each appendix. All appendices are indicated by Arabic numerals in the order in which they appear in the text. The use of abbreviations in the headings or any part of the appendix must be explained below.

POZIV ZA REKLAMIRANJE

Poštovani,
U okviru časopisa **Zdravstvena zaštita** imate mogućnost oglašavanja i reklamiranja vaših proizvoda i usluga, kao i svih vidova kontinuirane edukacije i publikacija (monografija, knjiga itd.) svim našim korisnicima.

Ovaj naučni časopis je za sve lekare, farmaceute i stomatologe. U njemu se objavljuju neobjavljeni originalni naučni radovi, pregledni i stručni članci, kratka saopštenja, uvodnici, pisma uredniku, meta-analize, prikazi bolesnika, aktuelne teme, prikazi stručnih knjiga i skupova, i drugo, iz javnog zdravlja, zdravstvenog osiguranja i ekonomike, menadžmenta u zdravstvu i svih drugih oblasti medicine, farmacije i stomatologije, čime se doprinosi promociji i razvoju nauke, struke i naučno-istraživačkog rada. Štampa se na srpskom ili engleskom jeziku sa rezimeima na srpskom i engleskom.

Cene reklama i oglasa u časopisu su:

1. Oglas u crno-beloj tehnici A4 formata za jedan broj 10.000,00 dinara, a za celu godinu (četiri broja) 30.000,00 dinara.
2. Oglas u boji A4 formata za jedan broj 20.000,00 dinara, a za celu godinu (četiri broja) 60.000,00 dinara.
3. Oglas u crno-beloj tehnici na koricama A4 formata za jedan broj 20.000,00 dinara, a za celu godinu (četiri broja) 60.000,00 dinara.
4. Oglas u boji na koricama A4 formata za jedan broj 40.000,00 dinara, a za celu godinu (četiri broja) 120.000,00 dinara.

Za sva obaveštenja, uputstva i ponude obratite se Uredniku časopisa: **urednik@komorazus.org.rs**
Sredstva se uplaćuju Komori zdravstvenih ustanova Srbije na žiro račun broj 205-4707-32 preko Komercijalne banke.

INVITATION TO ADVERTISE

To whom it may concern:

The Journal of **Health Care**, issued by the Chamber of Health Institutions offers the possibility of advertisement and promotion of all forms of continued education and publications (books, monographs, etc.), as well as your products and services, to all our users.

This journal is a scientific publication for all doctors, pharmacologists, biochemists, dentists and managers in health industry. Previously unpublished scientific papers are published in the journal, as well as reviews and short articles, announcements, introductions, letters to the editor, meta – analysis, case reports and case series, actual topics, depictions of expert books and conferences. In this way, the journal contributes to the promotion and development of science, as well as expertise and scientific and research work.

Pricelist for the commercials and ads in the journal are:

1. Advertisment in black - and - white technique in A4 format is 10.000,00 RSD for one issue, and 30.000,00 RSD for the entire year (four issues).
2. Advertisment in color in A4 format for one issue is 20.000,00 RSD, and 60.000,00 RSD for the entire year (four issues).
3. Advertisment in black – and - white technique on the covers of A4 format is 20.000,00 RSD for one issue, and 60.000,00 RSD for the entire year (four issues).
4. Advertisment in color on the covers of A4 format is 40.000,00 RSD, and 120.000,00 RSD for the entire year (four issues).

Feel free to contact Editorial board for all additional information, questions or inquiries: **urednik@komorazus.org.rs**

CIP - Каталогизација у публикацији
Народна библиотека Србије, Београд
613/614
ZDRAVSTVENA zaštita = Health care : zvanični časopis Komore zdravstvenih ustanova Srbije za medicinu, farmaciju, biohemiju, stomatologiju i menadžment u zdravstvu / glavni i odgovorni urednik Sandra Grujičić. - God. 1, br. 1 (1972)- . - Beograd : Komora zdravstvenih ustanova Srbije, 1972- (Beograd : Cakum Pakum). - 26 cm
Tromesečno. - Tekst na srp i engl. jeziku. - Drugo izdanje na drugom medijumu: Здравствена заштита (Online) = ISSN 2683-4286
ISSN 0350-3208 = Zdravstvena zaštita
COBISS.SR-ID 3033858