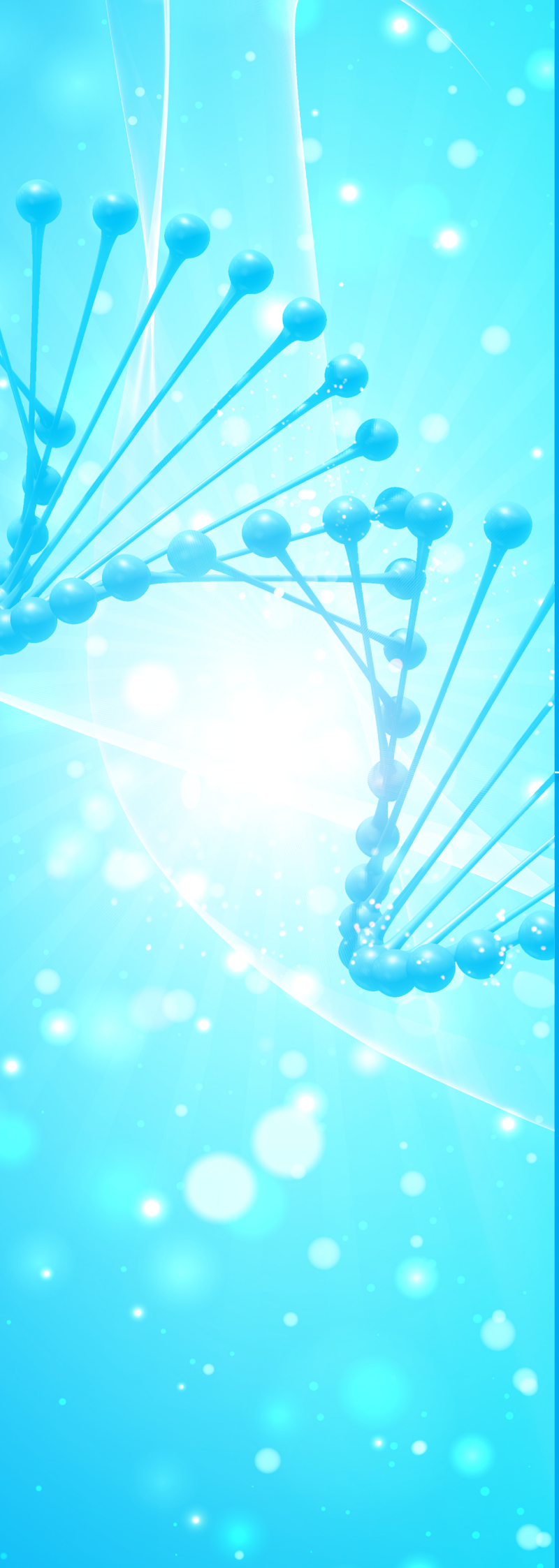

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KLINIČKE KARAKTERISTIKE OBOLELIH OD MAJMUNSKIH BOGINJA PRILIKOM PRVE POSETE DERMATOLOGU – SERIJE SLUČAJEVA

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SAŽETAK

Uvod/Cilj: Majmunske boginje (engl. *Monkeypox* –MPX) predstavljaju oboljenje izazvano istoimenim *Monkeypox* virusom (MPXV) koje je endemsko u zemljama Centralne i Zapadne Afrike. Od maja 2022. godine beleži se epidemija majmunskih boginja u različitim regijama sveta, uključujući i Republiku Srbiju. Cilj ovog rada je bio da prikažemo kliničke karakteristike obolelih od majmunskih boginja prilikom njihovog prvog javljanja dermatologu.
Serija slučajeva: U rad je uključeno 7 pacijenata koji su tokom juna i jula 2022. godine došli na pregled kod dermatologa zbog promena u genitalnoj i analnoj regiji kod kojih je infekcija majmunskim boginjama potvrđena PCR (engl. *Polymerase chain reaction*) testom. Prosečan uzrast obolelih bio je 35,8 godina, svi su bili muškog pola i homoseksualne orijentacije, 3 osobe su bile HIV-pozitivne, a infekciju su dobili putem seksualnih odnosa. Tri osobe su se inficirale u inostranstvu, a četiri u Beogradu. Kod većine obolelih inkubacija je trajala kraće od nedelju dana i prodromalni znaci su bili odsutni. Kožne promene su se najčešće javljale na penisu u vidu beličastih umbilikovanih papula, pustula i krusta. Serološki testovi na sifilis su pokazali da nijedan oboleli nije imao recentnu infekciju.
Zaključak: Dermatolozi bi trebalo da budu senzibilisani na prisustvo ovog oboljenja u našoj sredini, da rano posumnjaju, upute pacijenta na laboratorijsku dijagnostiku i evaluaciju kod infektologa, kao i da pacijentu savetuju izolaciju i seksualnu apstinenciju jer se bolest može preneti i seksualnim putem.

Ključne reči: majmunske boginje, klinička prezentacija, epidemija, dermatolog

Uvod

Majmunske boginje (engl. *Monkeypox* – MPX) predstavljaju oboljenje izazvano istoimenim Monkeypox DNK virusom (MPXV) koji pripada familiji *Poxviridae* i rodu *Orthopoxvirus*. Virus je izolovan među laboratorijskim majmunima u Kopenhagenu 1958. godine (1), a oboljenje je prvi put opisano kod ljudi 1970. godine u Zairu i od tada se kao endemsko javlja u zemljama Centralne i Zapadne Afrike (2). Importovani slučajevi *monkeypox* infekcije opisani su kod osoba koje su putovale u endemske krajeve ili imale kontakt sa životinjama uvezenim iz Afrike (3,4). Početkom 2022. godine beleži se epidemija majmunskih boginja u različitim regijama sveta kod osoba koje nisu putovale u endemske krajeve. Od početka maja 2022. godine pa do 26. jula 2022. godine u zemljama Evropske regije registrovano je 13.043 slučaja osoba obolelih od *monkeypox* infekcije, predominantno muškog pola, a od toga 10 registrovanih pacijenata bili su oboleli iz Republike Srbije (5).

Nakon inkubacije od jedne do dve nedelje, oboljenje najčešće počinje prodromalnom fazom u vidu povišene telesne temperature, glavobolje, bolova u mišićima i limfadenopatije, koju prati osip centrifugalnog rasporeda na licu i ekstremitetima uključujući dlanove i tabane, a kožne promene koje su kontagiozne i polimorfne (papule, vezikule, pustule i kruste) prolaze za nekoliko nedelja. Oboljenje se može preneti kapljičnim putem, bliskim fizičkim kontaktom, uključujući i seksualni odnos, kao i indirektno preko kontaminiranih predmeta (6,7).

Ono što karakteriše aktuelnu pandemiju su blagi prodromalni simptomi i pojava atipične kliničke prezentacije, sa primarnim lezijama u

CASE SERIES

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CLINICAL CHARACTERISTICS OF PATIENTS WITH MONKEYPOX INFECTION AT THEIR FIRST VISIT TO DERMATOLOGIST – A CASE SERIES

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SUMMARY

Introduction/Aim: Monkeypox disease (MPX) is caused by Monkeypox virus (MPXV) which is endemic in countries of Central and West Africa. Since May 2022 an outbreak of Monkeypox has been recorded in various regions of the world, including the Republic of Serbia. The aim of this study is to present the clinical characteristics of monkeypox cases during their first visit to a dermatologist.

Case series: The study includes seven patients who consulted a dermatologist during the months of June and July 2022 due to ano-genital rash and in whom Monkeypox infection was confirmed via PCR (Polymerase chain reaction) tests. The average age of patients was 35.8 years, all were men who have sex with men, three patients were HIV-positive and the infection was transmitted through sexual intercourse. Three patients were infected abroad and four in Belgrade. In most cases incubation lasted less than a week and prodromal signs were absent. Skin lesions most frequently appeared on the penis in the form of white umbilicated papules, pustules and crusts. Serological tests for syphilis showed that none of the patients had a recent infection.

Conclusion: Dermatologists should be sensitized to the presence of this disease in our country, they should suspect it early on, refer the patient to laboratory diagnostics and evaluation by an infectious disease specialist, and advise patients to isolate and abstain from sex because the disease can be transmitted during sexual intercourse.

Key words: Monkeypox disease, clinical presentation, outbreak, dermatologist

Introduction

Monkeypox disease (MPX) is caused by Monkeypox virus (MPXV) that belongs to the family Poxviridae and the Orthopoxvirus genus. The virus was isolated among laboratory monkeys in Copenhagen in 1958 (1), while the disease was for the first time described in people in Zaire in 1970, and since then it has been endemic in countries of Central and West Africa (2). The imported cases of monkeypox infection were described in persons who traveled to endemic regions or who had contact with animals imported from Africa (3,4). Since the beginning of 2022, an outbreak of monkeypox has been recorded in different regions of the world in persons who did not travel to endemic regions. From the beginning of May 2022 to July 26th, 2022, 13,043 cases of monkeypox infection were recorded in the countries belonging to the European region, and they were predominantly males, while 10 registered patients were from the Republic of Serbia (5).

After the incubation period, the disease most frequently begins with the prodromal period in the form of fever, headache, muscle ache and lymphadenopathy, which is followed by rash on the face and extremities, including palms and soles with a central distribution, while skin lesions that are contagious and polymorphic (papules, vesicles, pustules and crusts) clear up in a few weeks. The disease may be transmitted by respiratory droplets, close physical contact, including the sexual intercourse, and through contaminated materials (6, 7).

Mild prodromal symptoms are characteristic of the current pandemic, as well as the appearance

anogenitalnoj regiji koje upućuju na bliski fizički kontakt tokom seksualnih aktivnosti, zabeleženi naročito u populaciji muškaraca koji imaju seksualne odnose sa muškarcima (5). Stoga, oboleli se neretko prvo javljaju u klinike za polne bolesti, dermatolozima koji nisu imali priliku da se susretnu sa *monkeypox* infekcijom u svojoj kliničkoj praksi. Cilj ovog rada je bio da prikažemo kliničke manifestacije *monkeypox* infekcije prisutne prilikom prvog javljanja obolelog dermatologu, kao i karakteristike inficiranih osoba.

Serija slučajeva

U rad je uključeno 7 pacijenata koji su tokom juna i jula 2022. godine došli u ambulantu Službe za polno prenosive infekcije Gradskog zavoda za kožne i venerične bolesti u Beogradu sa simptomima koji su upućivali na monkeypox infekciju i koji su potom upućivani na Infektivnu kliniku Kliničkog centra Srbije gde je sa briseva kožnih promena i farinksa lančanom reakcijom polimerazom (engl. *Polymerase chain reaction* - PCR) dijagnostikovan *monkeypox* virus. Svi pacijenti su popunili anonimnu anketu vezanu za seksualno ponašanje u poslednjih mesec dana pre pojave simptoma (vrsta seksualnog odnosa, seksualna orijentacija, seksualni odnosi u inostranstvu), aktuelne simptome i HIV status. Svim pacijentima su urađeni serološki testovi na sifilis (VDRL – *Venereal Disease Research Laboratory*; laboratorijski test za istraživanje venoričnih bolesti; TPHA – *Treponema Pallidum Haemagglutination Assay*; *Treponema pallidum* hemaglutacioni test). U ovom radu su prikazane samo inicijalne kožne manifestacije obolelih koji su nakon potvrđene dijagnoze lečeni prema preporukama infektologa i upućivani na kućnu/bolničku izolaciju.

Karakteristike obolelih i kliničke manifestacije *monkeypox* infekcije prikazane su u Tabeli 1. Prosečan uzrast obolelih bio je 35,8 godina (najmlađi pacijent imao je 26 a najstariji 44 godine) i svi su bili muškog pola i homoseksualne orijentacije, 3 osobe su bile HIV pozitivne, a infekciju su dobili putem oralnih i/ili analnih seksualnih odnosa. Tri osobe su se inficirale u inostranstvu (jedna u Nemačkoj, druga u Austriji, a treća u Grčkoj), a ostale u Beogradu. Kod većine obolelih inkubacija je trajala kraće od nedelju dana i prodromalni znaci su bili odsutni. Kod onih sa prisutnim prodromalnim znacima dominirala je povišena telesna temperatura i blaga malaksalost. Kožne promene su se najčešće javljale na penisu u vidu beličastih umbilikovanih papula (slika 1), diseminovanih pustula (slika 2) i krustoznih lezija (slika 3). Promene u perigenitalnoj regiji su bile u vidu brojnih papulopustula okruženih eritematoznim haloom (slika 4) dok su kod pacijenta sa glutealnim lezijama dominirale pustule (slika 5). Kod dvojice pacijenata promene na koži su bile praćene osećajem bola i pečenja. Serološki testovi na sifilis su pokazali da nijedan oboleli nije imao recentnu infekciju, ali da je troje imalo pozitivan TPHA test usled ranije lečenog sifilisa.

Diskusija

Podaci iz Evrope o epidemiji majmunskih boginja ukazuju da je najveći broj registrovanih slučajeva u Španiji, Velikoj Britaniji, Nemačkoj i Francuskoj, da je većina obolelih muškog pola (99,4%),

Tabela 1. Izabrane karakteristike obolelih i kliničke manifestacije *monkeypox* infekcije

Karakteristike:	Broj (N)=7
Uzrast (prosečan):	35,8 godina
Seksualna orijentacija:	
Homoseksualna	7
Heteroseksualna	0
HIV status:	
Negativan	4
Pozitivan	3
Serološki test na sifilis:	
Negativan	4
Pozitivan*	3
Vrsta seksualnog odnosa:	
Oralni seks	2
Analni seks	3
Oralni i analni seks	2
Period inkubacije	
≤ 7 dana	4
8 - 14 dana	3
Prodromalni znaci:	
Prisutni	3
Odsutni	4
Lokalizacija lezija:	
Samo na penisu	5
Perigenitalna regija	1
Perianalna regija	1
Dominantna kožna lezija:	
Papula	3
Pustula	3
Krusta	1

* Pozitivan test usled ranije lečenog sifilisa.

of atypical clinical presentation, with primary lesions in ano-genital region which indicate a close physical contact during sexual practices, and which have been particularly recorded in the population of men who have sex with men (5). Therefore, patients often first visit clinics for venereal diseases, dermatologists who have not had the chance to see the monkeypox infection in their clinical practice. The aim of this study is to present the clinical manifestations of monkeypox infection during the first visit to a dermatologist, as well as the characteristics of infected persons.

Case series

The study includes seven patients with the symptoms of the monkeypox infection who came to the STD outpatient clinic of the City Institute for Skin and Venereal Diseases in Belgrade and who were referred to the Clinic for Infectious Diseases of the Clinical Center of Serbia, where the monkeypox virus was diagnosed by PCR (Polymerase chain reaction) tests on swabs taken from skin lesions and pharynx. All patients filled out the anonymous questionnaire relating to their sexual behavior during the last month before the symptoms appeared (type of sexual relation, sexual orientation, sexual relations abroad), current symptoms and HIV status. Serological tests for syphilis were done for all patients (VDRL – Venereal Disease Research Laboratory; TPHA – Treponema Pallidum Haemagglutination Assay). Only initial skin manifestations of patients, who were treated according to the recommendations of infectious disease specialist after the diagnosis was confirmed and who were referred to home/hospital isolation, were presented in this paper.

The characteristics of patients and clinical manifestations of monkeypox infection are presented in Table 1. The average age of patients was 35.8 years (the youngest patient was 26, while the oldest was 44) and they were all men who have sex with men, three patients were HIV positive and the infection was transmitted via oral and/or anal sexual intercourse. Three persons were infected abroad (one in Germany, second in Austria and third in Greece), while four were infected in Belgrade. In most cases incubation lasted less than a week, and prodromal signs were absent. Fever and mild weakness were dominant in patients with prodromal signs. Skin lesions most frequently appeared on the penis in the form of white umbilicated papules (Figure 1), disseminated pustules (Figure 2) and crusted lesions (Figure 3). Lesions in the perigenital region were in the form of numerous papulopustular lesions surrounded by an erythematous halo (Figure 4), while pustules were dominant in patients with gluteal lesions (Figure 5). In two patients, skin lesions were accompanied by painful and itchy sensation. Serological tests for syphilis showed that none of the patients had a recent infection, but that three of them had a positive TPHA test due to the previously treated syphilis.

Discussion

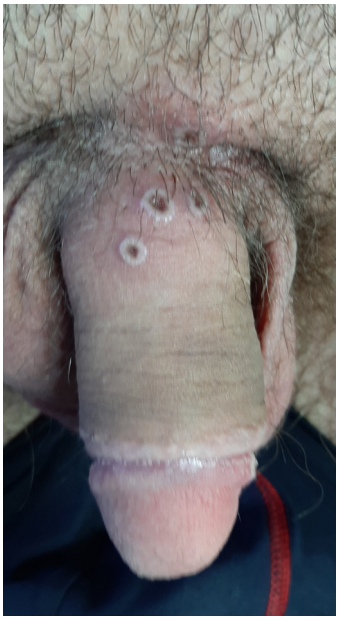
Data from Europe on the outbreak of MPX show that the largest number of registered cases have been reported in Spain, Great Britain,

Table 1. Some selected characteristics of cases and clinical manifestations of monkeypox infection

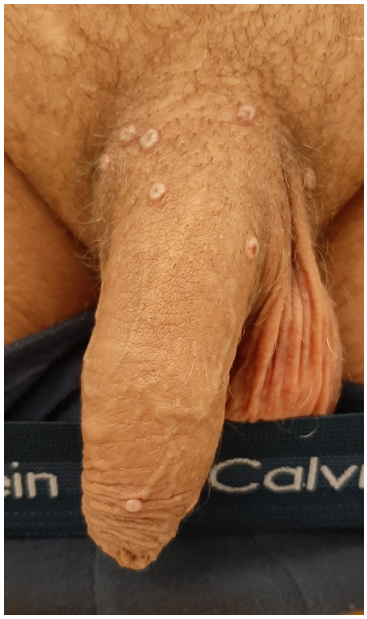
Characteristics:	Number (N)=7
Age (average):	35.8 years
Sexual orientation:	
Homosexual	7
Heterosexual	0
HIV status:	
Negative	4
Positive	3
Serological test for syphilis:	
Negative	4
Positive*	3
Type of sexual practice	
Oral sex	2
Anal sex	3
Oral and anal sex	2
Incubation period:	
≤ 7 days	4
8 - 14 days	3
Prodromal signs:	
Present	3
Absent	4
Localization of lesions:	
Only on penis	5
Perigenital region	1
Perianal region	1
Dominant skin lesion:	
Papule	3
Pustule	3
Crust	1

* Positive test due to the previously treated syphilis

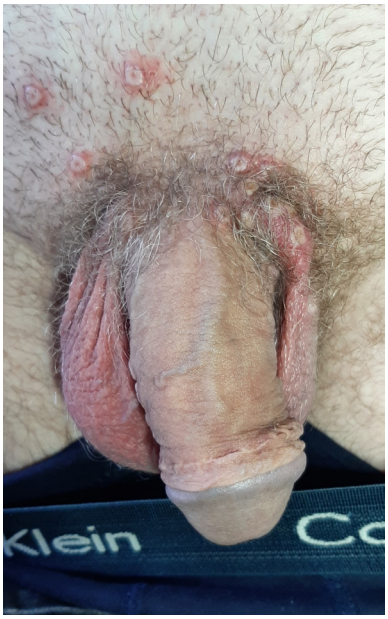
Slika 1. Beličaste papule sa centralnom umbilikacijom na glansu i telu penisa



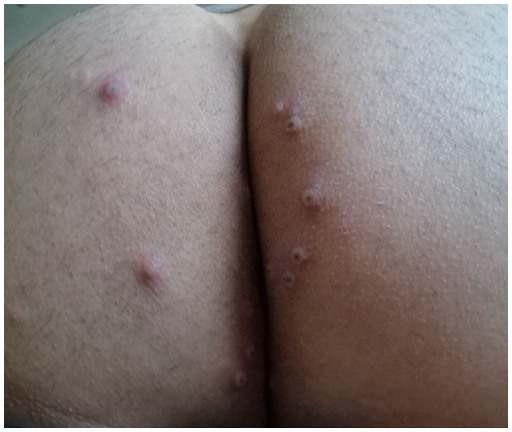
Slika 3. Krustozne lezije na telu penisa



Slika 2. Brojne jasno ograničene pustulozne lezije na telu penisa



Slika 4. Brojne papulopustulozne lezije u perigenitalnoj regiji okružene eritematoznim haloom



Slika 5. Pustulozna ospa u predelu gluteusa

Figure 1. White papules with central umbilication on the glans and the shaft of the penis

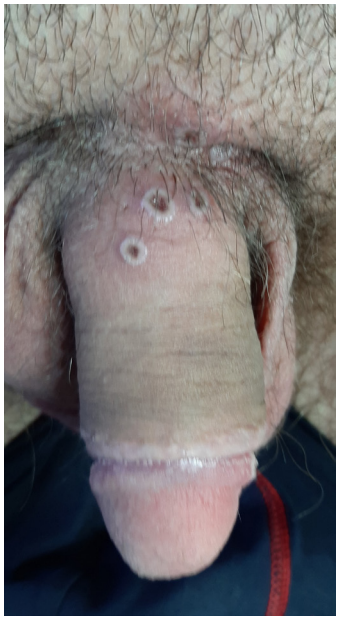


Figure 3. Crusted lesions on the shaft of the penis

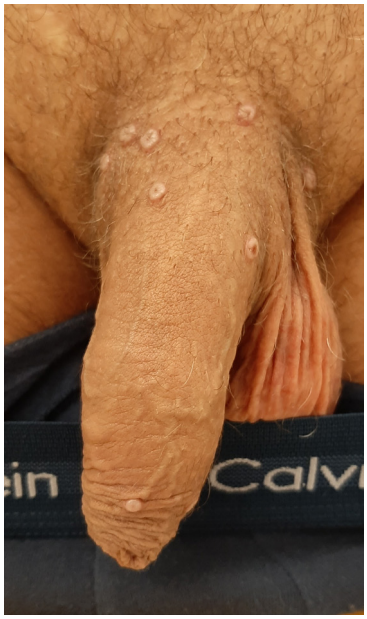


Figure 2. Multiple well circumscribed pustular rash on the shaft of the penis

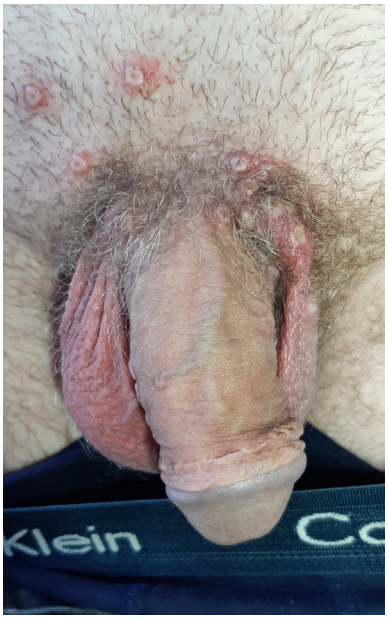


Figure 4. Multiple papulopustular lesions on the perigenital regionsurrounded by an erythematous halo

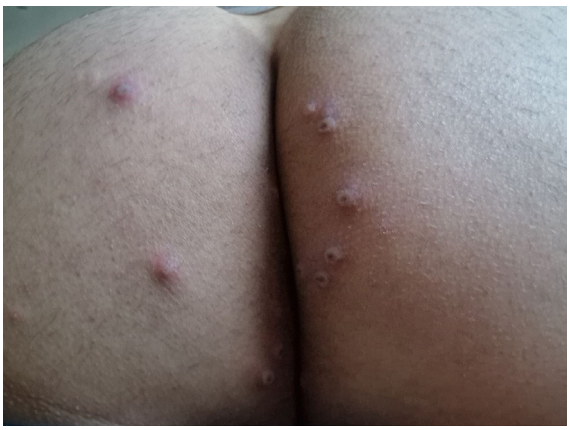


Figure 5. Pustular rash on the gluteal region

uzrasta od 31 do 40 godina, da 37% obolelih ima HIV-koinfekciju, da je kod 94,7% obolelih prisutan osip na koži, a da su opšti simptomi poput povišene temperature, slabosti, bolova u mišićima ili glavobolje prisutni kod 67% slučajeva (5). I u našem radu svi oboleli su bili muškarci, prosečnog uzrasta 35,8 godina, a skoro polovina je imala HIV-koinfekciju. S obzirom na to da da opisujemo samo kliničku sliku prilikom prvog obraćanja obolelih zdravstvenoj službi (u ovom slučaju dermatologu), svi naši pacijenti su imali kožni osip.

Tipična slika majmunskih boginja je praćena pojavom febrilnog prodroma sa limfadenopatijom koji se javlja u proseku od 5 do 13 dana nakon infekcije, a njega prati kožni osip u vidu jasno ograničenih često umbilikovanih papula, vezikula, pustula i krusti sa centrifugalnom distribucijom, a nekad može biti diseminovan po čitavoj koži (8). Podaci iz Sjedinjenih Američkih Država tokom epidemije malignih boginja ukazuju na sve češću pojavu atipičnog osipa u ano-genitalnoj regiji, bez prethodnog prodromalnog stadijuma i opštih simptoma što može izazvati sumnju da se radi o *varicella-zoster* infekciji ili nekoj polno prenosivoj bolesti (9). U našoj studiji prodromalna faza je bila prisutna u manje od polovine obolelih, a sve inicijalne lezije su bile lokalizovane u genitalnoj ili perianalnoj regiji i bile su polimorfnog karaktera (umbilikovane papule, pustule ili kruste), ali kod svakog pacijenta su zabeležene promene u istoj evolutivnoj fazi (identične papularne ili pustulozne ili krustozne lezije) što je karakteristično za majmunske boginje (9). Lokacija ospe kod naših obolelih upućuje na direktan seksualni put prenošenja infekcije jer su kožne promene u svim fazama majmunskih boginja kontagiozne. Svi naši pacijenti su pripadali populaciji muškaraca koji imaju seksualne odnose sa muškarcima kod kojih su polne bolesti poput sifilisa i HIV infekcije u našoj zemlji češće (10), te ne čudi što je kod tri pacijenta bio pozitivan seroloski test na sifilis koji ukazuje da su oni već bili lečeni od ove infekcije. Prva tri pacijenta su se inficirala tokom seksualnih odnosa u inostranstvu, a ostali u Beogradu što upućuje na to da je virus sve više prisutan i u našoj sredini, te dolazi do lokalne transmisije naročito u populaciji pod rizikom za polno prenosive infekcije. Iako je veći broj inficiranih u inicijalnoj fazi pandemije zabeležen među muškarcima koji imaji seksualne odnose sa muškarcima (5,9), ova infekcija nije ograničena ni na jednu populacionu grupu jer se bolest prenosi bliskim fizičkim kontaktom sa inficiranim, te se svako može zaraziti.

U diferencijalnoj dijagnozi majmunskih boginja kod kojih su inicijalne promene u anogenitalnoj regiji u obzir dolaze polno prenosive infekcije poput ranog sifilisa (primarni i sekundarni stadijum), genitalnog herpesa, šankroida i *molluscum contagiosum* infekcije, ili, pak, infekcije izazvane *varicella-zoster* virusom (ovčije boginje i herpes zoster) stoga dermatolozi predstavljaju važnu kariku u ranoj dijagnostici ove bolesti.

Zaključak

Dermatolozi bi trebalo da budu posebno senzibilisani na prisustvo ovog oboljenja i u našoj sredini, da rano posumnjaju, upute pacijenta na laboratorijsku dijagnostiku i evaluaciju infektologu, a da pacijentu savetuju izolaciju zbog kontagioznosti oboljenja i seksualnu apstinenciju jer iako ne pripada u klasične polno prenosive infekcije, može se preneti i seksualnim putem.

Konflikt interesa

Autor je izjavio da nema konflikta interesa.

Literatura

1. von Magnus P, Andersen EA, Petersen KB, Birch-Andersen A. A pox-like disease in cynomolgus monkeys. Acta Path Microbiol Scand 1959; 46(2):156-176. doi: 10.2217/fvl.12.130
2. Breman JG, Kalisa R, Steniowski MV, Zanotto E, Gromyko AI, Arita I. Human monkeypox, 1970–79. Bull World Health Organ 1980; 58(2):165–182.
3. Centers for Disease Control and Prevention (CDC) Update: multistate outbreak of monkeypox--Illinois, Indiana, Kansas, Missouri, Ohio, and Wisconsin, 2003. MMWR Morb Mortal Wkly Rep 2003; 52(27):642-6.
4. Vaughan A, Aarons E, Astbury J, Balasegaram S, Beadsworth M, Beck CR et al. Two cases of monkeypox imported to the United Kingdom, Septembar 2018. Euro Surveill 2018; 23(38):1800509. doi: 10.2807/1560-7917.ES.2018.23.38.1800509
5. European Centre for Disease Prevention and Control/WHO Regional Office for Europe. Monkeypox, Joint Epidemiological overview, 27 July 2022.
6. Centres for Disease Control and Prevention. Monkeypox. Available from: https://www.cdc.gov/poxvirus/monkeypox/index.html [Accessed on Avgust 3, 2022]
7. Petersen E, Kantele A, Koopmans M, Asogyn D, Yinke-Ogunleye A, Ihekweazu C, et al. Human monkeypox: epidemiologic and clinical characteristics, diagnosis and prevention. Infect Dis Clin North Am 2019; 33(4):1027-43. doi: 10.1016/j.idc.2019.03.001

Germany and France, and that the majority of them are males (99.4%), aged 31-40 years, while 37% of patients have HIV-coinfection, and that 94.7% of patients have skin rash, while general symptoms such as fever, feebleness, muscle ache or headache are present in 67% of cases (5). Also, in our study all patients were men, aged 35.8 years on average, and almost half of them had HIV-coinfection. Considering the fact that we have described only the clinical presentation during the first visit to the healthcare service (in this case a dermatologist), all our patients had skin rash.

A typical presentation of MPX is accompanied by febrile prodrome with lymphadenopathy, which appears on average 5 to 13 days after exposure, and it is followed by the skin rash in the form of clearly circumscribed papules that often umbilicate, vesicles, pustules and crusts with a centrifugal distribution, while sometimes it can be disseminated across the entire skin (8). Data from the United States of America during the outbreak of MPX have pointed to the atypical rash in the ano-genital region, without the previous prodromal period and general symptoms and therefore, *varicella zoster* infection or other sexually-transmitted diseases may be suspected (9). In our study, the prodromal period was present in less than half of patients, while initial lesions were localized in the genital or perianal region and they were polymorphic (umbilicated papules, pustules and crusts), but in each patient lesions were registered in the same evolution stage (identical papular or pustular or crusted lesions), which is characteristic of MPX (9). The location of rash in our patients indicates a direct sexual way of transmission because skin lesions are contagious in all stages of MPX. All our patients belong to the population of men who have sex with men, in whom venereal diseases such as syphilis and HIV are more frequent in our country (10), and therefore, it is not surprising that in three patients, serological test for syphilis was positive which indicates that they were previously treated due to this infection. Three patients were infected abroad during sexual intercourse, whereas other patients were infected in Belgrade, which points to the fact that the virus is increasingly present in our country, so there comes to the local transmission, especially in the population at risk of sexually-transmitted infections. Although a large number of cases in the initial phase of the pandemic was reported among men who have sex with men (5, 9), this infection has not been limited to particular population groups because it is transmitted by close physical contact with the infected person, so everybody can get exposed.

In a differential diagnosis of MPX when the initial changes are in the ano-genital region, sexually-transmitted diseases may be taken into consideration, such as early syphilis (primary and secondary stage), genital herpes, chancroid and *molluscum contagiosum* infections, or infections caused by the *varicella zoster* virus (varicella and herpes zoster). Therefore, dermatologists are an important link in the chain of early diagnostics of this disease.

Conclusion

Dermatologists should be particularly sensitized to the presence of this disease in our country, they should suspect it early on, refer the patient to laboratory diagnostics and evaluation by an infectious disease specialist, and advise patients to isolate due to the contagiousness of this disease and abstain from sex because this disease can be transmitted during sexual intercourse although it does not belong to sexually-transmitted diseases in the classic sense.

Competing interests

The author declares no competing interests.

Literature

1. von Magnus P, Andersen EA, Petersen KB, Birch-Andersen A. A pox-like disease in cynomolgus monkeys. Acta Path Microbiol Scand 1959; 46(2):156-176. doi: 10.2217/fvl.12.130
2. Breman JG, Kalisa R, Steniowski MV, Zanotto E, Gromyko AI, Arita I. Human monkeypox, 1970–79. Bull World Health Organ 1980; 58(2):165–182.
3. Centers for Disease Control and Prevention (CDC) Update: multistate outbreak of monkeypox--Illinois, Indiana, Kansas, Missouri, Ohio, and Wisconsin, 2003. MMWR Morb Mortal Wkly Rep 2003; 52(27):642-6.
4. Vaughan A, Aarons E, Astbury J, Balasegaram S, Beadsworth M, Beck CR et al. Two cases of monkeypox imported to the United Kingdom, Septembar 2018. Euro Surveill 2018; 23(38):1800509. doi: 10.2807/1560-7917.ES.2018.23.38.1800509
5. European Centre for Disease Prevention and Control/WHO Regional Office for Europe. Monkeypox, Joint Epidemiological overview, 27 July 2022.
6. Centres for Disease Control and Prevention. Monkeypox.

8. Reynolds MG, Yorita KL, Kuehnert MJ, Davidson WB, Huhn GD, Holman RC, et al. Clinical manifestations of human monkeypox influenced by route of infection. J Infect Dis 2006; 194(6):773-80. doi: 10.1086/505880
9. Minhaj FS, Ogale YP, Whitehill F, Schultz J, Foote M, Davidson W, et al. Monkeypox Outbreak – Nine States, May 2022. MMWR Morb Mortal Wkly Rep 2022; 71(23):764-9.
10. Bjekić M. Prevalencija sifilisa i HIV infekcije među muškarcima koji imaju seksualne odnose sa muškarcima testiranim u jednoj nevladinoj organizaciji u Beogradu. Zdravst Zašt 2022; 51(2):48-55. doi: 10.5937/zdravzast51-37819

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Available from: https://www.cdc.gov/poxvirus/monkeypox/index.html [Accessed on Avgust 3, 2022]

7. Petersen E, Kantele A, Koopmans M, Asogyn D, Yinke-Ogunleye A, Ihekweazu C, et al. Human monkeypox: epidemiologic and clinical characteristics, diagnosis and prevention. Infect Dis Clin North Am 2019; 33(4):1027-1043. doi: 10.1016/j.idc.2019.03.001
8. Reynolds MG, Yorita KL, Kuehnert MJ, Davidson WB, Huhn GD, Holman RC, et al. Clinical manifestations of human monkeypox influenced by route of infection. J Infect Dis 2006; 194(6):773-780. doi: 10.1086/505880
9. Minhaj FS, Ogale YP, Whitehill F, Schultz J, Foote M, Davidson W, et al. Monkeypox Outbreak – Nine States, May 2022. MMWR Morb Mortal Wkly Rep 2022; 71(23): 764-769.
10. Bjekic M. Prevalence of syphilis and HIV infection among men who have sex with men tested in one non-governmental organization in Belgrade. Health Care 2022; 51(2):48-55. doi: 10.5937/zdravzast51-37819

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SIGNALNI PUTEVI U KONTROLI EMBRIONALNOG RAZVOJA ENTERIČKOG NERVNOG SISTEMA

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SAŽETAK

Enterički nervni sistem (ENS) obezbeđuje intrinzičku inervaciju gastrointestinalnog trakta i predstavlja najveći i najkompleksniji deo perifernog nervnog sistema. Njegove funkcije su od vitalne važnosti i podrazumevaju kontrolu motiliteta digestivnog trakta, kontrolu sekrecije, kao i razmene tečnosti i elektrolita kroz sluznicu creva. Većinu ovih funkcija ENS je sposoban da obavlja potpuno autonomno. Proučavanje najčešće kongenitalne bolesti ENS, Hiršprungove bolesti, dalo je veliki doprinos u rasvetljavanju embrionalnog razvoja ENS. Ćelije ENS potiču najvećim delom od ćelija vagalnog, a nešto manjim delom od ćelija sakralnog regiona nervnog grebena. Ove ćelije migriraju duž primitivnog creva u suprotnim smerovima, kako bi konačno kolonizovali čitavo crevo. Procesi proliferacije, migracije, neuro-glijalne diferencijacije kroz koje prolaze prekursorske ćelije ENS, regulisani su brojnim signalnim putevima. Neki od najvažnijih molekula koji učestvuju u regulaciji pravilnog razvoja ENS su GDNF (*Glial Derived Neurotrophic Fatcor*) i njegov receptor RET (*REarranged during Transfection*), endotelin 3 i njegov receptor EDNRB (*endothelin receptor type B*), transkripcioni faktori SOX10 (*SRY-box transcription factor 10*), PHOX2B (*Paired-like Homeobox 2B*), morfogeni kao što su BMP 2 i 4 (*Bone Morphogenic Proteins*) i drugi. Iako su naša saznanja o kontroli razvoja ENS poslednjih godina značajno uvećana, kompleksnost strukture i funkcije ENS ostavlja dosta prostora za dalja istraživanja. U ovom preglednom radu prikazali smo dosadašnja znanja o najvažnijim regulatornim mehanizmima i signalnim putevima koji učestvuju u razvoju ENS.

Ključne reči: enterički nervni sistem, embrionalni razvoj, signalni putevi, Hiršprungova bolest

Uvod

Najveći broj ćelija enteričkog nervnog sistema (ENS) vodi poreklo od ćelija vagalnog dela nervnog grebena, u nivou 1-7. somita (1). Prekursorske ćelije sakralnog dela nervnog grebena, kaudalno od 28. somita takođe učestvuju u izgradnji ENS distalnih delova creva (2). Ćelije vagalnog dela nervnog grebena migriraju ventromedijalno kroz mezenhim somita, ulaze u region prednjeg creva i nastavljaju migraciju kaudalno kroz prednje, srednje i zadnje crevo (3). Nasuprot tome, ćelije sakralnog dela nervnog grebena imaju suprotan smer migracije. One ulaze u zid creva u predelu zadnjeg creva i započinju migraciju rostralno. Njihova migratorna putanja je kraća, tako da već u zadnjem crevu susreću prekursorske ćelije vagalnog porekla i završavaju svoju dalju migraciju (4). Na ovim migratornim putanjama, prekursorske ćelije prolaze i kroz procese proliferacije, diferencijacije u neurone ili glijalne ćelije ENS, formiranja ganglija ENS i njihovog povezivanja u pleksuse (5,6). O važnosti ovih procesa govore brojni razvojni poremećaji sa neadekvatnim brojem (hiper/hipoganglionoze) ili potpunim odsustvom ganglija ENS (aganglionoze), kakva je Hiršprungova bolest (7-10).

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SIGNALING PATHWAYS IN THE CONTROL OF EMBRYONIC DEVELOPMENT OF THE ENTERIC NERVOUS SYSTEM

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SUMMARY

The enteric nervous system (ENS) provides intrinsic innervation of the gastrointestinal tract and is the largest and most complex part of the peripheral nervous system. Its functions are vital for life and include control of motility of the digestive tract, secretion, as well as fluid and electrolyte exchange through the intestinal mucosa. ENS is capable of performing most of these functions completely autonomously. A large number of developmental and genetic studies of the most common congenital disease of the ENS, Hischsprung' s disease, has made a major contribution to the understanding of the embryonic development of the ENS. ENS cells raise from the vagal (mostly) and sacral region of the neural crest. These precursor cells migrate along the primitive gut in opposite directions, in order to colonize the entire gut. Proliferation, migration, neuro-glial differentiation, and other processes through which precursor cells of the ENS undergo, are regulated by various signaling pathways. Some of the most important molecules that participate in the regulation of the proper development of the ENS are GDNF (Glial Derived Neurotrophic Fatcor) and its receptor RET (REarranged during Transfection), endothelin 3 and its receptor EDNRB (endothelin receptor type B), transcription factors SOX10 (SRY-box transcription factor 10), PHOX2B (Paired-like Homeobox 2B), morphogens such as BMP 2 and 4 (Bone Morphogenic Proteins) and others. Although our knowledge about control of the development of the ENS has increased significantly in recent years, complexity of structure and function of the ENS requires further research. This review summarizes our current understanding of the most important regulatory mechanisms and signaling pathways involved in the development of the ENS.

Keywords: enteric nervous system, embryonic development, signaling pathways, Hirschsprung disease

Introduction

The majority of the enteric nervous system (ENS) is derived from vagal neural crest cells adjacent to somites 1-7 (1). Precursor cells of the sacral part of the neural crest, caudally from the somite 28 also take part in the development of ENS of distal parts of the gut (2). Vagal neural crest cells migrate ventromedially through the somite mesenchyme, enter the region of foregut and continue their migration caudally through the foregut, midgut and hindgut (3). Contrary to this, sacral neural crest cells migrate in opposite direction. They enter the gut wall in the region of hindgut and start their migration rostrally. Their migratory way is shorter, and therefore, they meet the vagal precursor cells in the hindgut and terminate their further migration (4). On these migratory pathways, precursor cells undergo the processes of proliferation, neuro-glial differentiation, formation of ganglia of the ENS and their interconnecting into plexuses (5, 6). Numerous developmental disorders with the inadequate number (hyper/hypoganglionosis) or complete absence of ENS ganglia (aganglionosis), such as Hirschsprung disease speak about the importance of these processes (7-10).

Dok je pravilan embrionalni razvoj zasigurno uslov za normalno funkcionisanje ENS, regulatorni mehanizmi koji učestvuju u razvoju ENS još uvek su u velikoj meri nepoznati. Ipak, ulaganjem velike istraživačke energije poslednjih godina, uloga nekih signalnih puteva u kontroli razvoja ENS je umnogome razjašnjena (11-13).

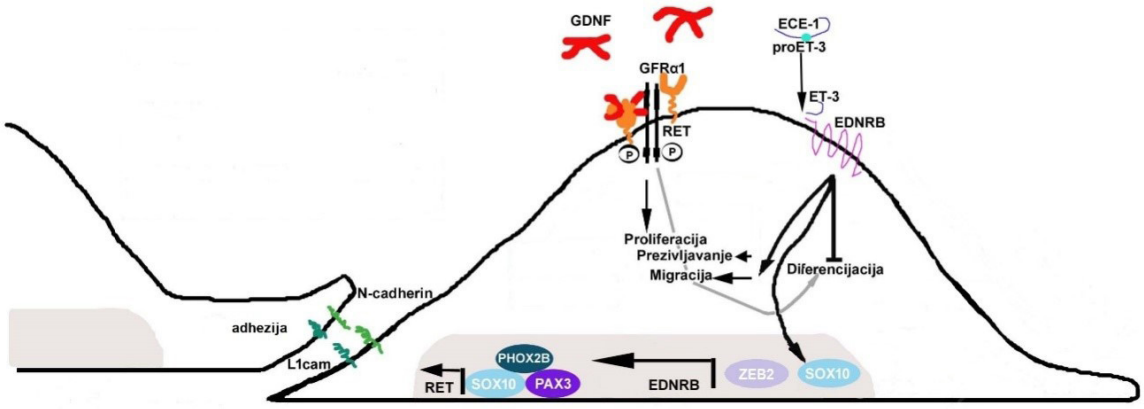
Cilj ovog rada jeste prikaz dosadašnjeg znanja o najvažnijim regulatornim mehanizmima i signalnim putevima koji učestvuju u kontroli embrionalnog razvoja ENS.

Metode

U ovom preglednom radu, korišćena je literatura dobijena pretraživanjem baze podataka MEDLINE. Literatura objavljena na engleskom jeziku, u poslednjih 10 godina, dobijena je pretraživanjem ključnih reči: enterički nervni sistem, embrionalni razvoj, signalni putevi, Hiršprungova bolest, RET, GDNF.

RET/GDNF

Najznačajniji i najbolje proučen signalni put koji učestvuje u kontroli embrionalnog razvoja ENS je RET (*REarranged during Transfection*)/ GDNF (*Glial Derived Neurotrophic Fatcor*) signalni put. Još pri prolasku prekursorskih ćelija poreklom od nervnog grebena kroz mezenhim somita, dešava se ushodna regulacija RET receptora posredovana retinoičnom kiselinom (14). Naime, mezenhimalne ćelije somita sekretuju povećanu količinu retinoične kiseline, koja aktivacijom svojih nuklearnih receptora, pre svega RARα (*Retinoic Acid Receptor alpha*), podstiče ekspresiju RET receptora na površini ćelija poreklom od nervnog grebena. Ovo će opredeliti prekursorske ćelije da migriraju ka budućem gastrointestinalnom traktu i daju ćelije ENS (15). Za ekrpesiju RET receptora na prekursorskim ćelijama ENS neophodna je aktivnost transkripcionih faktora SOX10 (*SRY-box transcription factor 10*), PHOX2B (*Paired-like Homeobox 2B*) i PAX3 (*Paired Box3*) (Slika 1) (13). RET receptor je transmembranski receptor sa tirozin kinaznom aktivnošću. Glavni ligand koji se vezuje za RET receptor je GDNF. GDNF se prethodno vezuje za koreceptor GFRα (*GDNF Family Receptor alpha*), lociran unutar lipidnih raftova ćelijske membrane, što indukuje privlačenje RET receptora, kao i formiranje i aktivaciju GDNF-GFRα-RET receptorskog kompleksa. Aktivacija RET receptora vodi autofosforilaciji intracelularnih domena i aktivaciji nishodnih signalnih puteva (16). Kao krajnji rezultat javlja se stimulacija preživljavanja, proliferacije i migracije prekursorskih ćelija ENS (17). Pored toga, dokazano je stimulativno dejstvo RET na diferencijaciju neurona *in vitro*, dok je uticaj RET na *in vivo* diferencijaciju još uvek predmet intenzivnog proučavanja (18). GDNF je eksprimiran u rastućem gradijentu



RET/GDNF signalni put stimuliše preživljavanje, proliferaciju, migraciju prekursorskih ćelija i diferencijaciju neurona ENS (*in vitro*), dok EDNRB signalni put ima iste efekte, izuzev na neuronalnu diferencijaciju, gde deluje inhibitorno. Na shemi su prikazani i adhezivni molekuli N-cadherin i L1CAM koji doprinose održavanju adekvatnog smera i brzine migracije prekursorskih ćelija tokom embrionalnog razvoja ENS. GDNF – *glial derived neurotrophic factor*; GFRα – *GDNF family receptor alpha*; RET – *rearranged during transfection receptor*; ECE-1 – *endothelin-converting enzyme*; ET-3 – endothelin 3; EDNRB – *endothelin receptor type B*; L1cam – *L1 cell adhesion molecule*; SOX10 – *SRY box transcription factor 10*; PHOX2B – *paired-like homeobox 2B*; PAX3 – *paired box 3*; ZEB2 – *zinc finger E-Box-binding homeobox 2*.

Slika 1. Uloga RET/GDNF i EDNRB signalnih puteva u kontroli razvoja enteričkog nervnog sistema (ENS).

While the normal embryonic development is certainly a condition necessary for normal functioning of ENS, regulatory mechanisms that take part in the ENS development are, to a great extent, unknown. However, a lot of energy has been devoted to research this topic in recent years, and therefore, the role of some signaling pathways in the control of ENS development has been explained (11-13).

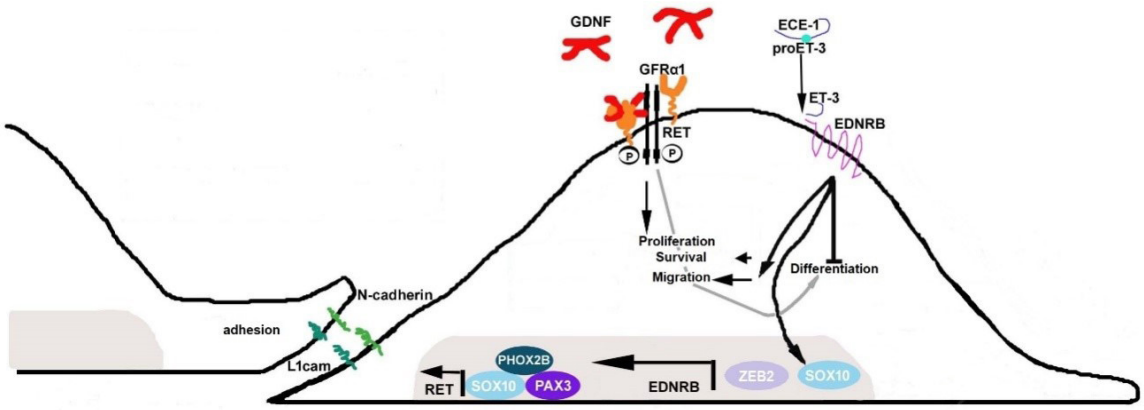
The aim of this review is to present the current knowledge about the regulatory mechanisms and signaling pathways that take part in the control of ENS development.

Materials and methods

In this review article, we used literature that was obtained through a search of MEDLINE database. The literature in the English language that has been published in the last 10 years was obtained by searching the following key words: enteric nervous system, embryonic development, signaling pathways, Hirschsprung disease, RET, GDNF.

RET/GDNF

RET (Rearranged during Transfection)/GDNF (Glial Derived Neurotrophic Factor) signaling pathway is regarded as the most important and most studied signaling pathway that participates in the control of the embryonic development of ENS. When precursor cells that are derived from the neural crest pass through the mesenchyme of somites, an upward regulation of RET receptors happens and it is mediated by retinoic acid (14). Namely, mesenchymal somite cells produce higher levels of retinoic acid, which by activating its nuclear receptors, first of all RARα (Retinoic Acid Receptor α), enhances the expression of RET receptor on the surface of cells derived from the neural crest. This will make precursor cells migrate towards the future gastrointestinal tract and give ENS cells (15). The activity of transcription factors SOX10 (SRY-box transcription factor 10), PHOX2B (Paired-like Homeobox 2B) and PAX3 (Paired box 3) is necessary for the expression of RET receptors on ENS precursor cells (Figure 1) (13). RET receptor is a transmembrane receptor with tyrosine kinase activity. The main ligand that is bound to RET receptor is GDNF. GDNF is previously bound to co-receptor GFRα (GDNF Family Receptor alpha) that is located within lipid rafts of the cell membrane, which induces the activation of RET receptor, as well as the formation and activation of GDNF-GFRα-RET receptor complex. The activation of RET receptor leads to the autophosphorylation of intracellular domains and activation of downward signaling pathways (16). The stimulation of survival, proliferation and migration of precursor ENS cells



The RET/GDNF signaling pathway stimulates survival, proliferation, migration of ENS precursor cells and differentiation of ENS neurons (in vitro), while the EDNRB signaling pathway has the same effects, except for neuronal differentiation, where it has inhibitory effects.Adhesive molecules, N-cadherin and L1CAM, which contribute to maintaining the adequate direction and speed of migration of precursor cells during the embryonic development of the ENS, are also shown.GDNF – glial derived neurotrophic factor; GFRα – GDNF family receptor alpha; RET – rearranged during transfection receptor; ECE-1 – endothelin-converting enzyme; ET-3 – endothelin 3; EDNRB – endothelin receptor type B; L1cam – L1 cell adhesion molecule; SOX10 – SRY box transcription factor 10; PHOX2B – paired-like homeobox 2B; PAX3 – paired box 3; ZEB2 – zinc finger E-Box-binding homeobox 2.

Figure 1. Role of RET/GDNF and EDNRB signaling pathways in the control of enteric nervous system (ENS) development

od prednjeg ka zadnjem crevu, pospešujući na taj način migraciju prekursorskih ćelija ENS u istom smeru (17). Međutim, studija Andersona i sar. iz 2007. pokazala je da prekursorske ćelije vagalnog porekla migriraju istom brzinom u rostralnom smeru kada su implantirane u sakralni region, čime opovrgavaju uticaj gradijenta GDNF na migraciju (19). Pored GDNF, RET na isti način mogu aktivirati i drugi neurotrofni faktori (neurturin, artemin i persefin) (20). Mutacije u genu za RET receptor pronađene su u čak 50% porodičnih slučajeva i oko 20-30% sporadičnih slučajeva Hiršprungove bolesti (12,21). Animalni modeli pokazali su da je uticaj RET receptora na pravilan razvoj ENS dozno zavisan. Kod homozigotnih isključenja RET gena javlja se potpuna intestinalna aganglionoza, dok se kod heterozigota ENS razvija pravilno. Kada je nivo ekspresije RET receptora na oko 1/3, javlja se distalna aganglionoza ograničena na rektum i deo debelog creva (22). Mutacije drugih molekula uključene u RET signalni put, kao što su GDNF, GFRα, SOX10, PHOX2B, dovode do gotovo istih fenotipa (21). Sa druge strane, povećana ekspresija i aktivnost RET receptora često je udružena sa MEN sindromima, u kojima se mogu sresti ganglioneuromi ENS (MEN2B) (21,23). Neke studije ukazuju da mutacije RET gena mogu istovremeno biti udružene sa Hiršprungovom bolešću i MEN sindromima, što može zvučati paradoksalno na osnovu dosadašnjeg znanja i zahteva dalje istraživanje (23). U studiji Soreta i sar. pokazana je uloga GDNF u postnatalnoj neurogenezi kod mišjeg modela Hiršprungove bolesti, što sugeriše potencijalnu terapijsku primenu GDNF (24).

Ostali signalni putevi

EDNRB (endothelin receptor type B) signalni put

EDNRB je receptor povezan sa G proteinom i eksprimiran na prekursorskim ćelijama ENS. Za njegovu ekspresiju važni su transkripcioni faktori SOX10 i ZEB2 (*Zinc finger E-Box-binding homeobox 2*) (25). Ligand koji se vezuje za ovaj receptor i aktivira ga je endotelin-3 (EDN3), kojeg produkuju mezenhimalne ćelije creva tokom embrionalnog razvoja. Glavna uloga EDNRB signalnog puta jeste inhibicija neuronalne diferencijacije, odnosno održavanje progenitorskog stanja prekursorskih ćelija ENS, kako bi se obezbedio dovoljan broj prekursorskih ćelija za kolonizaciju čitavog creva (26). Pored toga, ovaj signalni put ostvaruje uloge slične RET signalnom putu, a to su stimulacija proliferacije i migracije prekursorskih ćelija (27). Iz ovoga se zaključuje da RET i EDNRB signalni putevi imaju sinergistički efekat na migraciju i proliferaciju, a antagonistički kada je u pitanju diferencijacija prekursorskih ćelija ENS (26,27). Mutacije u genima za EDNRB, EDN3 ili endotelin konvertujući enzim (ECE - *endothelin converting enzyme*) koji stvara END3 iz prekursorskog proteina kod miševa, dovode do Hiršprungove bolesti, najčešće u sklopu *Waardenburg* sindroma tip IV. Ovaj sindrom se još manifestuje pigmentovanim promenama na koži i senzoneuralnom gluvoćom (21,28). Kod pacijenata sa Hiršprungovom bolešću dokazane su mutacije EDNRB signalnog puta, i javljaju se u oko 5% svih slučajeva (20).

Hedgehog (Hh) i Notch signalni put

Ova dva signalna puta međusobno su tesno povezana i ostvaruju dve važne funkcije u embrionalnom razvoju ENS, a to su održavanje progenitorskog statusa prekursorskih ćelija i stimulacija gliogeneze. Ipak, indirektno, uključeni su u gotovo sve faze ravoja ENS (29,30). Ihh (*indian hedgehog*) i Shh (*sonic hedgehog*) su glavni molekuli koji pripadaju Hh familiji. Oni su eksprimirani od strane mezenhimalnih ćelija primitivnog creva. Receptori za koje se vezuju ovi molekulli označeni su kao Ptch (*Patch*) receptori i eksprimirani su na prekursorskim ćelijama ENS koje putuju duž primitivnog creva. Krajnji rezultat stimulacije Ptch receptora je ekspresija Dll1 (*Delta-like canonical notch ligand 1*) proteina na površini ćelije. Ovaj ligand vezuje se za Notch receptor susedne prekursorske ćelije ENS, što indirektno, inhibirajući ekspresiju ASCL1 (*Achaete SCute Homolog 1*) gena, stimuliše ekspresiju SOX10 (31). Kao što je već pomenuto, SOX10 je bitan za održavanje progenitorskog statusa prekursorskih ćelija ENS pre svega preko EDNRB, ali je njegova ekspresija važna i za diferencijaciju prekursorskih ćelija u glija ćelije. Upravo ravnoteža između aktivnosti ASCL1 gena, koji promoviše neurogenezu, i SOX10, koji promoviše gliogenezu, ključna je za održavanje dovoljnog broja prekursorskih, odnosno diferentovanih ćelija (Slika 2) (32).

Bone Morphogenic Proteins (BMPs)

BMP2 i BMP4 imaju važnu ulogu u gotovo svim fazama embrionalnog razvoja ENS. Ovi proteini, koji pripadaju TGFβ superfamiliji faktora rasta,

appears as the final result (17). In addition, the stimulating effect of RET on the differentiation of neurons *in vitro* has been proved, whereas the influence of RET on *in vivo* differentiation is still the subject of intense research (18). GDNF is a growth factor expressed in an increasing gradient from the foregut towards the hindgut, thus enhancing the migration of ENS precursor cells in the same direction (17). However, the study of Anderson et al. from 2017 showed that precursor cells that are derived from vagal part migrate at the same speed in the rostral direction when they are implanted into the sacral region, thus denying the influence of gradient GDNF on the migration (19). In addition to GDNF, RET receptor may be activated in the same way by other neurotrophic factors (neurturin, artemin, and persephin) (20). Mutations in the gene for RET receptor have been found in 50% of family cases and in about 20-30% of sporadic cases of Hirschsprung disease (12,21). Animal models have shown that the influence of RET receptor on the normal development of ENS is dose-dependent. In the homozygous exclusion of RET gene, complete intestinal aganglionosis appears, while in heterozygous, the ENS develops normally. When the level of expression of RET receptor is about 1/3, distal aganglionosis appears and it is limited to the rectum and part of the distal colon (22). Mutations of other molecules involved in the RET signaling pathway, such as GDNF, GFRα, SOX10, PHOX2B lead to almost the same phenotypes (21). On the other hand, the increased expression and activity of RET receptor is often associated with MEN syndromes, in which ganglioneuromas of ENS may be seen (MEN2B) (21,23). Some studies indicate that the mutations of RET gene may simultaneously be associated with Hirschsprung disease and MEN syndromes, which may sound as a paradoxical based on the current knowledge and therefore it demands further research (23). In the study of Soret et al., the role of GDNF in the postnatal neurogenesis in a murine model of Hirschsprung disease has been shown, which suggests a potential therapeutic application of GDNF (24).

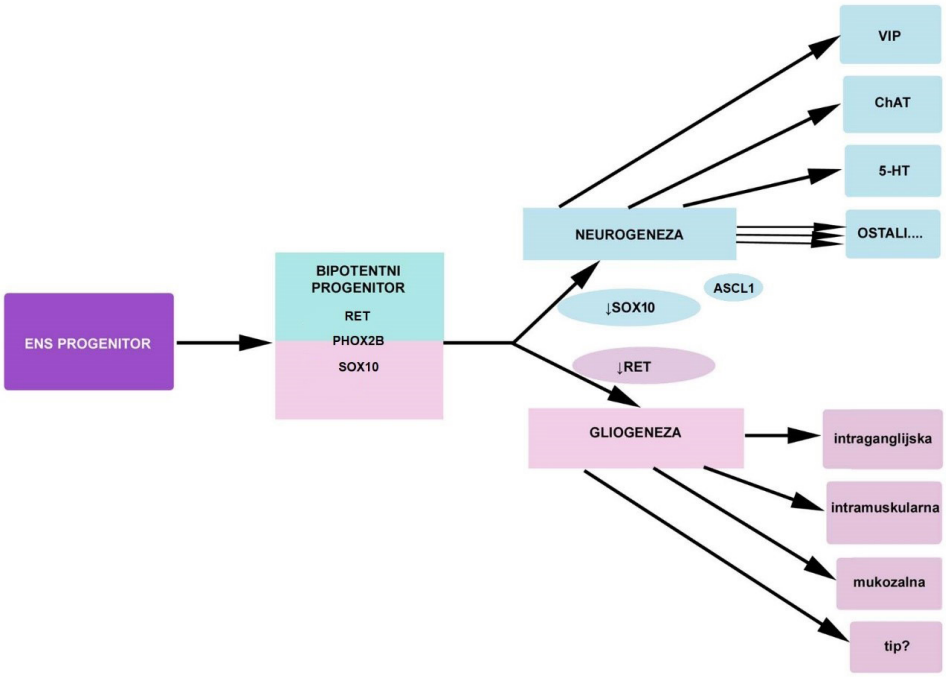
Other signaling pathways

EDNRB (endothelin receptor type B) signaling pathway

EDNRB is a G-protein-coupled receptor expressed by ENS precursor cells. Transcription factors SOX10 and ZEB2 (Zinc finger E-box-binding homeobox 2) are important for its expression (25). The ligand, which is bound to this receptor and which activates it, is endothelin-3 (EDN3) that is produced by mesenchymal intestinal cells during the embryonic development. The main role of EDNRB signaling pathway is the inhibition of neuronal differentiation, and consequently, the maintenance of progenitor state of precursor cells of the ENS, in order to secure the sufficient number of precursor cells for the colonization of the entire gut (26). In addition, this signaling pathway has roles similar to RET signaling pathway, like the stimulation of proliferation and migrations of precursor cells (27). Thus, one may conclude that RET and EDNRB signaling pathways have the synergistic effects on the migration and proliferation, while they have the antagonistic effects on the differentiation of ENS precursor cells (26,27). Mutations in genes for EDNRB, EDN3 or endothelin converting enzyme (ECE) that creates END3 from the precursor protein in mice lead to Hirschsprung disease, most frequently within Waardenburg syndrome type IV. This syndrome is also manifested by pigmented skin lesions and sensorineural hearing loss (21,28). In patients with Hirschsprung disease, mutations of EDNRB signaling pathway have been confirmed, and they appear in about 5% of cases (20).

Hedgehog (Hh) and Notch signaling pathway

These two signaling pathways are mutually interconnected and they accomplish two important functions in the embryonic development of the ENS: the maintenance of progenitor status of precursor cells and the stimulation of gliogenesis. However, they are indirectly involved in almost all stages of ENS development (29,30). *Ihh* (*Indian hedgehog*) and *Shh* (*sonic hedgehog*) are the main molecules that belong to Hh family. They are expressed by mesenchymal cells of the primitive gut. Receptors, which these molecules are marked as *Ptch* (*Patch*) receptors and they are expressed in precursor cells of the ENS that travel along the primitive gut.



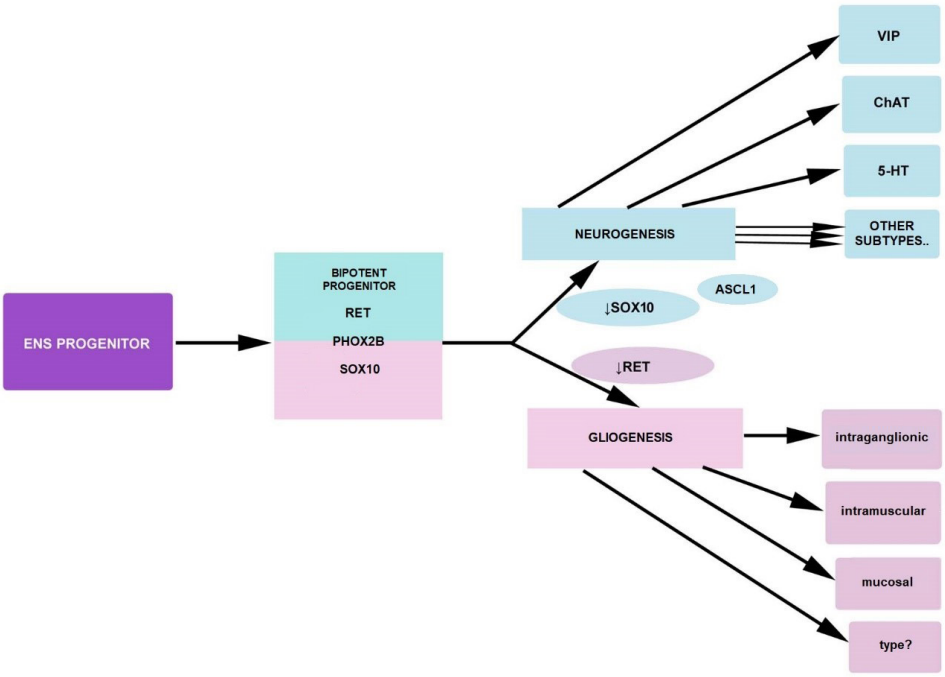
Jedan deo samoobnavljajućih progenitorskih ćelija ENS tokom razvoja daje bipotentne progenitore, koji su sposobni za dalju neuro-glijalnu diferencijaciju. U toku neurogeneze, ovi prekursori smanjuju ekspresiju SOX10 gena, dok održavaju ekspresiju RET. Suprotno se dešava tokom gliogeneze. PHOX2B ekspresija se održava u gotovo svim neuronima i nekim glija ćelijama ENS. Za sada je otkriven veliki broj različitih subtipova neurona u ENS (VIP, ChAT, 5-HT i dr.). Sa druge strane, raznolikost tipova glijalnih ćelija u ENS nije još uvek sasvim poznata, ali su glijalne ćelije morfološki sa sigurnošću prepoznate na nekoliko mesta u gastrointestinalnom traktu. VIP – vazoaktivni intestinalni peptid; ChAT – holin acetiltransferaza; 5-HT – serotonin.

Slika 2. Promena genske ekspresije u prekursorskim ćelijama enteričkog nervnog sistema (ENS) tokom neuro-glijalne diferencijacije.

vezuju se za BMP receptor. Aktivacijom receptora pokreće se nishodna kaskada i aktivacija signalnih molekula poznatih kao SMAD, koji se zatim translociraju u jedro i pokreću transkripciju brojnih gena (33). Pored pozitivne kontrole preživljavanja, migracije i proliferacije, važna je uloga, naročito BMP2, u diferencijaciji prekursorskih ćelija ENS u neurone. Kateholaminergički neuroni, zatim neuroni koji eksprimiraju nNOS i NPY diferenciraju se u prisustvu BMP2, dok uloga u diferencijaciji holinergičkih neurona i neurona koji eksprimiraju supstanciju P nije primećena. Ova diferencijacija ostvaruje se posredstvom SMAD1 (34). Dakle, BMPs zajedno sa GDNF doprinosi povećanju populacije neurona ENS. Ipak, pokazano je da BMPs nisu isključivo promoteri neuronalne, već i glijalne diferencijacije. Naime, za gliogenezu od velikog značaja su aktivacija ERBB3 receptora pomoću tzv. neuregulina, od kojih je najznačajniji GGF2 (*Glial Growth Factor 2*). Upravo pozitivnom regulacijom ovog puta, BMPs doprinose gliogenezi, odnosno uvećanju populacije glijalnih ćelija ENS (35). Inhibicijiom BMPs signalnog puta specifičnim antagonistima, kao što je nogin (*noggin*), dolazi do distalne hipoganglionoze i nepravilnog formiranja ganglija ENS. Ovim je potvrđena uloga BMPs u migraciji prekursorskih ćelija ENS, ali i gangliogenezi (36).

Semaforin 3A

Semaforin 3A i njegov receptor, neuropilin-1 (NRP1), označeni su kao negativni regulatori aksonske elongacije i sinaptogeneze (7,20). Poznato je da prekursorske ćelije ENS poreklom od sakralnog dela nervnog grebena ne ulaze u region zadnjeg creva sve dok on ne bude kolonizovan od strane prekursorskih ćelija vagalnog porekla. Pretpostavlja se da semaforin 3A doprinosi ovom odlaganju i sprečava prevremenu kolonizaciju zadnjeg creva (19). U susednim, ganglijskim delovima creva pacijenata sa Hiršprungovom bolešću pronađena je smanjena ekspresija sinapsina-1, koji je u negativnoj korelaciji sa ekspresijom semaforina 3A, što potencijalno može da bude objašnjenje postoperativnih komplikacija nakon uklanjanja aganglioznog dela creva ovih pacijenata(37). Dokazana



A number of ENS progenitor cells, during development, give rise to bipotent progenitor cells, which are capable of further neuro-glial differentiation.During neurogenesis, these precursors downregulate SOX10 gene expression, while maintaining RET expression. The opposite occurs during gliogenesis.PHOX2B expression is maintained in almost all neurons and some glial cells of the ENS.Large number of different neuron subtypes have been discovered in the ENS so far (VIP, ChAT, 5-HT, etc.). On the other hand, diversity of glial cell types in the ENS is still not completelyunderstood, but glial cells have been morphologically identified in several locations in the gastrointestinal tract. VIP – vasoactive intestinal peptide; ChAT – choline acetyltransferase; 5-HT – serotonin.

Figure 2. Gene expression alteration in precursor cells of the enteric nervous system (ENS) during neuro-glial differentiation

The final result of stimulation of Ptch receptors is the expression *Dll1* (*Delta-like canonical notch ligand 1*) protein on the cell surface. This ligand is bound to Notch receptor of the neighboring ENS precursor cell, which indirectly, stimulates the expression of SOX10 by inhibiting the expression of ASCL1 (*Achaete Scute Homolog 1*) gene. As it has already been mentioned, SOX10 is important for the maintenance of the progenitor status of precursor cells of the ENS, first of all through EDNRB, but also its expression is important for the differentiation of precursor cells into glial cells. Precisely this balance between the activity of ASCL1 gene, which promotes neurogenesis, and SOX10, which promotes gliogenesis, is of key importance for the maintenance of the sufficient number of precursor cells and differentiated cells (Figure 2) (32).

Bone Morphogenic Proteins (BMPs)

BMP2 and BMP4 have an important role in almost all phases of embryonic development of the ENS. These proteins, which belong to TGFß superfamily of growth factors, are bound to the BMP receptor. The activation of receptors instigates the downward cascade and the activation of signaling molecules known as SMAD, which are then translocated into the nucleus and trigger the transcription of numerous genes (33). In addition to the positive control of survival, migration and proliferation, especially BMP2 has an important role in the differentiation of ENS precursor cells into neurons. Catecholaminergic neurons, nNOS and NPY expressing neurons are differentiated in the presence of BMP2, while the role in the differentiation of cholinergic neurons and neurons expressing substance P has not been noticed. This differentiation is accomplished with the help of SMAD1 (34). Therefore, BPMs together with GDNF contribute to the increase in the population of neurons of the ENS. However, it has been shown that BPMs are not just promoters of neuronal, but also of glial differentiation. Namely, of great significance for gliogenesis is the activation of ERBB3 receptor with the help of the neuregulin, where GGF2 is the most important of them (Glial

je povezanost polimorfizama gena za semaforin 3A i pojave Hiršprungove bolesti (38).

Adhezioni molekuli

Tokom migracije, neophodno je da prekursorske ćelije ENS budu u kontaktu, kako bi se održali adekvatni smer i brzina migracije. Ove ćelije na svojoj površini eksprimiraju određene adhezione molekule kao što su N-kadherin, NCAM (*Neural Cell Adhesion Molecule*), kao i L1CAM (*L1 Cell Adhesion Molecule*) (Slika 1). Mutacije u genima za neki od ovih molekula mogu usporiti migraciju i potencirati nastanak distalne aganglionoze (39-41).

Zaključak

Razumevanje kontrole embrionalnog razvoja ENS značajno se poboljšalo u poslednjoj deceniji, kao posledica sve većeg interesovanja naučnika za ovaj problem i značajnim unapređenjem tehnologije u istraživanju. Otkrivanje velikog broja različitih signalnih puteva, genetskih i epigenetskih faktora uključenih u kontrolu procesa migracije, proliferacije i diferencijacije ćelija ENS doprinelo je i boljem razumevanju nekih poremećaja razvoja, pre svega Hiršprungove bolesti. Ipak, povezanost između različitih signalnih puteva i doprinos spoljašnjih faktora poremećajima razvoja samo su neki od budućih fokusa istraživanja u ovoj oblasti.

Konflikt interesa

Autori su izjavili da nema konflikta interesa.

Literatura

1. Yntema CL, Hammond WS. The origin of intrinsic ganglia of trunk viscera from vagal neural crest in the chick embryo. J Comp Neurol 1954; 101(2):515-41. doi: 10.1002/cne.901010212.
2. Burns AJ, Douarin NM. The sacral neural crest contributes neurons and glia to the post-umbilical gut: spatiotemporal analysis of the development of the enteric nervous system. Development 1998; 125(21):4335-47. doi: 10.1242/dev.125.21.4335.
3. Allan IJ, Newgreen DF. The origin and differentiation of enteric neurons of the intestine of the fowl embryo. Am J Anat 1980; 157(2):137-54. doi: 10.1002/aja.1001570203.
4. Hansen MB. The enteric nervous system I: organisation and calssification. Pharmacol Toxicol 2003; 92(3):105-13. doi: 10.1034/j.1600-0773.2003.t01-1-920301.x.
5. Druckenbrod NR, Epstein ML. The pattern of neural crest advance in the cecum and colon. Dev Biol 2005; 287(1):125-33. doi: 10.1016/j.ydbio.2005.08.040.
6. Đuknić M, Puškaš N, Labudović Borović M, Janković R. Poreklo ćelija enteričkog nervnog sistema i putevi migracije tokom embrionalnog razvoja. Zdravstvena zaštita 2022; 51(2):20-35. doi: 10.5937/zdravzast51-37799.
7. Obermayr F, Hotta R, Enomoto H, Young HM. Development and developmental disorders of the enteric nervous system. Nat Rev Gastroenterol Hepatol 2013; 10(1):43-57. doi: 10.1038/nrgastro.2012.234.
8. Janković R. Modern diagnostics of Hirschsprung disease and related disorders. Materia medica 2016; 32(2):1478-82.
9. Janković R. Analiza glija indeksa i Kahalovih ćelija u biopsijama debelog creva dece sa Hiršprungovom bolešću i srodnim oboljenjima [disertacija]. Beograd: Medicinski fakultet Univerziteta u Beogradu; 2016.
10. Jankovic R, Sindjic-Antunovic S, Lukac M, Vujovic D, Jevtic J, Skender-Gazibara M. Altered Distribution of Interstitial Cells of Cajal in Normoganglionic and Transitional Zone of Hirschsprung Disease and Their Clinical Significance. Central Eur J Paed 2020; 16(1):1-9. doi: 10.5457/p2005-114.251.
11. Rao M, Gershon MD. Enteric nervous system development: what could possibly go wrong? Nat Rev Neurosci 2018; 19(9):552-65. doi: 10.1038/s41583-018-0041-0.
12. Goldstein AM, Hofstra RM, Burns AJ. Building a brain in the gut: development of the enteric nervous system. Clin Genet 2013; 83(4):307-16. doi: 10.1111/cge.12054.
13. Nagy N, Goldstein AM. Enteric nervous system development: A crest cell's journey from neural tube to colon. Semin Cell Dev Biol 2017; 66:94-106. doi: 10.1016/j.semcdb.2017.01.006.
14. Simkin JE, Zhang D, Rollo BN, Newgreen DF. Retinoic acid upregulates ret and induces chain migration and population expansion in vagal neural crest cells to colonize the embryonic gut. PLoS One 2013; 8(5):e64077. doi: 10.1371/journal.pone.0064077.
15. Gao T, Wright-Jin EC, Sengupta R, Anderson JB, Heuckeroth RO. Cell-autonomous retinoic acid receptor signaling has stage-specific effects on mouse enteric nervous system. JCI Insight 2021; 6(10):e145854. doi: 10.1172/jci.insight.145854.
16. Kawai K, Takahashi M. Intracellular RET signaling pathways activated by GDNF. Cell Tissue Res 2020; 382(1) :113-123. doi: 10.1007/s00441-020-03262-1.
17. Mwizerwa O, Das P, Nagy N, Akbareian SE, Mably JD, Goldstein AM. Gdnf is mitogenic, neurotrophic, and chemoattractive to enteric neural crest cells in the embryonic colon. Dev Dyn 2011; 240(6):1402-11. doi: 10.1002/dvdy.22630.
18. Hearn CJ, Murphy M, Newgreen D. GDNF and ET-3 differentially modulate the numbers of avian enteric neural crest cells and enteric neurons in vitro. Dev Biol 1998; 197(1):93-105. doi: 10.1006/dbio.1998.8876.
19. Anderson RB, Bergner AJ, Taniguchi M, Fujisawa H, Forrai A, Robb L et al. Effects of different regions of the developing gut on the migration of enteric neural crest-derived cells: a role for Sema3A, but not Sema3F. Dev Biol 2007; 305(1):287-99. doi: 10.1016/j.ydbio.2007.02.020.

Growth Factor 2). With positive regulation of this pathway, BMPs contribute to gliogenesis and to the increase in the population of glial cells of the ENS (35). The inhibition of BMPs signaling pathway with specific antagonists, such as noggin, leads to the distal hypoganglionosis and abnormal formation of ganglia of the ENS. Thus, the role of BMPs in the migration of precursor cells of the ENS, as well as in gangliogenesis is confirmed (36).

Semaphorin 3A

Semaphorin 3A and its receptor, neuropilin-1 (NRP-1) are marked as negative regulators of axonal elongation and synaptogenesis (7,20). It is known that precursor cells of the ENS that are derived from the sacral part of the neural crest do not enter the region of hindgut until it is not colonized by vagal precursor cells. It is assumed that semaphorin 3A contributes to this postponement and prevents the preterm colonization of hindgut (19). In neighboring, ganglionic segment of intestines of patients with Hirschsprung disease, reduced expression of sinapsin-1 was found, which is in negative correlation with the expression of semaphorin 3A which may potentially explain the postoperative complications after the removal of aganglionic portion of intestines in these patients (37). The connection between the genetic polymorphism for semaphorin 3A and the appearance of Hirschsprung disease has been proved (38).

Adhesion molecules

During migration, in order to keep the adequate direction and speed of migration, precursor cells of the ENS should necessarily be in contact. These cells on their surface express certain adhesion molecules such as N-cadherin, NCAM (*Neural Cell Adhesion Molecule*), as well as L1CAM (*L1 Cell Adhesion Molecule*) (Figure 1). Mutation in genes for some of these molecules may slow down the migration and induce the appearance of distal aganglionosis (39-41).

Conclusion

Understanding the control of the embryonic development of the ENS has significantly improved in the last decade, which is the consequence of the fact that scientists have become increasingly interested in this problem and that advances have been made regarding the technology used in research. Detecting large numbers of different signaling pathways, genetic and epigenetic factors involved in the control of migration, proliferation and differentiation processes of ENS cells has contributed to better understanding of some developmental disorders, first of all, Hirschsprung disease. However, the interconnection between different signaling pathways and the contribution of extrinsic factors to developmental disorders are only some of future focus points of research in this field.

Competing interests

Authors declare no competing interests.

Literature

1. Yntema CL, Hammond WS. The origin of intrinsic ganglia of trunk viscera from vagal neural crest in the chick embryo. J Comp Neurol 1954; 101(2):515-41. doi: 10.1002/cne.901010212.
2. Burns AJ, Douarin NM. The sacral neural crest contributes neurons and glia to the post-umbilical gut: spatiotemporal analysis of the development of the enteric nervous system. Development 1998; 125(21):4335-47. doi: 10.1242/dev.125.21.4335.
3. Allan IJ, Newgreen DF. The origin and differentiation of enteric neurons of the intestine of the fowl embryo. Am J Anat 1980; 157(2):137-54. doi: 10.1002/aja.1001570203.
4. Hansen MB. The enteric nervous system I: organisation and calssification. Pharmacol Toxicol 2003; 92(3):105-13. doi: 10.1034/j.1600-0773.2003.t01-1-920301.x.
5. Druckenbrod NR, Epstein ML. The pattern of neural crest advance in the cecum and colon. Dev Biol 2005; 287(1):125-33. doi: 10.1016/j.ydbio.2005.08.040.
6. Đuknić M, Puškaš N, Labudović Borović M, Janković R. Poreklo ćelija enteričkog nervnog sistema i putevi migracije tokom embrionalnog razvoja. Zdravstvena zaštita 2022; 51(2):20-35. doi: 10.5937/zdravzast51-37799.
7. Obermayr F, Hotta R, Enomoto H, Young HM. Development and developmental disorders of the enteric nervous system. Nat Rev Gastroenterol Hepatol 2013; 10(1):43-57. doi: 10.1038/nrgastro.2012.234.
8. Janković R. Modern diagnostics of Hirschsprung disease and related disorders. Materia medica 2016; 32(2):1478-82.
9. Janković R. Analiza glija indeksa i Kahalovih ćelija u biopsijama debelog creva dece sa Hiršprungovom bolešću i srodnim oboljenjima [disertacija]. Beograd: Medicinski fakultet Univerziteta u Beogradu; 2016.
10. Jankovic R, Sindjic-Antunovic S, Lukac M, Vujovic D, Jevtic J, Skender-Gazibara M. Altered Distribution of Interstitial Cells of Cajal in Normoganglionic and Transitional Zone of Hirschsprung Disease and Their Clinical Significance. Central Eur J Paed 2020; 16(1):1-9. doi: 10.5457/p2005-114.251.

20. Diposarosa R, Bustam NA, Sahiratmadja E, Susanto PS, Sribudiani Y. Literature review: enteric nervous system development, genetic and epigenetic regulation in the etiology of Hirschsprung's disease. Heliyon 2021; 7(6):e07308. doi: 10.1016/j.heliyon.2021.e07308.
21. Amiel J, Lyonnet S. Hirschsprung disease, associated syndromes, and genetics: a review. J Med Genet 2001; 38(11):729-39. doi: 10.1136/jmg.38.11.729.
22. Uesaka T, Nagashimada M, Yonemura S, Enomoto H. Diminished Ret expression compromises neuronal survival in the colon and causes intestinal aganglionosis in mice. J Clin Invest 2008; 118(5):1890-8. doi: 10.1172/JCI34425.
23. Nagy N, Guyer RA, Hotta R, Zhang D, Newgreen DF, Halasy V et al. RET overactivation leds to concurrent Hirschsprung disease and intestinal ganglioneuromas. Development 2020; 147(21):dev190900. doi: 10.1242/dev.190900.
24. Soret R, Schneider S, Bernas G, Christophers B, Souchkova O, Charrier B et al. Glial Cell-Derived Neurotrophic Factor Induces Enteric Neurogenesis and Improve Colon Structure and Function in Mouse Models of Hirschsprung Disease. Gastroenterology 2020; 159(5):1824-38.e17. doi: 10.1053/j.gastro.2020.07.018.
25. Watanabe Y, Stanchina L, Lecerf L, Gacem N, Conidi A, Baral V et al. Differentiation of Mouse Enteric Nervous System Progenitor Cells Is Controlled by Endothelin 3 and Requires Regulation of Ednrb by SOX10 and ZEB2. Gastroenterology 2017; 152(5):1139-50. doi: 10.1053/j.gastro.2016.12.034.
26. Nagy N, Goldstein AM. Endothelin-3 regulates neural crest cell proliferation and differentiation in the hindgut enteric nervous system. Dev Biol 2006; 293(1):203-17. doi: 10.1016/j.ydbio.2006.01.032.
27. Barlow A, de Graaff E, Pachnis V. Enteric nervous system progenitors are coordinately controlled by the G protein-coupled receptor EDNRB and the receptor tyrosine kinase RET. Neuron 2003; 40(5):905-16. doi: 10.1016/s0896-6273(03)00730-x.
28. Read AP, Newton VE. Waardenburg syndrome. J Med Genet 1997; 34(8):656-65. doi: 10.1136/jmg.34.8.656.
29. Okamura Y, Saga Y. Notch signaling is required for the maintenance of enteric neural crest progenitors. Development 2008; 135(21):3555-65. doi: 10.1242/dev.022319.
30. Taylor MK, Yeager K, Morrison SJ. Physiological Notch signaling promotes gliogenesis in the developing peripheral and central nervous systems. Development 2007; 134(13):2435-47. doi: 10.1242/dev.005520.
31. Pawolski W, Schmidt MHH. Neuron-Glia Interaction in the Developing and Adult Enteric Nervous System. Cells 2021; 10(1): 47. doi: 10.3390/cells10010047.
32. Liu JA, Ngan ES. Hedgehog and Notch signaling in enteric nervous system development. Neurosignals 2014; 22(1):1-13. doi: 10.1159/000356305.
33. Chalazonitis A, Kessler JA. Pleiotropic effects of the bone morphogenetic proteins on development of the enteric nervous system. Dev Neurobiol 2012; 72(6):843-56. doi: 10.1002/dneu.22002.
34. Anitha M, Shahnavaz N, Qayed E, Joseph I, Gossrau G, Mwangi S et al. BMP2 promotes differentiation of nitrergic and catecholaminergic enteric neurons through a Smad1-dependent pathway. Am J Physiol Gastrointest Liver Physiol 2010; 298(3):G375-83. doi: 10.1152/ajpgi.00343.2009.
35. Chalazonitis A, D'Autréaux F, Pham TD, Kessler JA, Gershon MD. Bone morphogenetic proteins regulate enteric gliogenesis by modulating ErbB3 signaling. Dev Biol 2011; 350(1):64-79. doi: 10.1016/j.ydbio.2010.11.017.
36. Goldstein AM, Brewer KC, Doyle AM, Nagy N, Roberts DJ. BMP signaling is necessary for neural crest cell migration and ganglion formation in the enteric nervous system. Mech Dev 2005; 122(6):821-33. doi: 10.1016/j.mod.2005.03.003.
37. Gonzales J, Le Berre-Scoul C, Dariel A, Bréhéret P, Neunlist M, Boudin H. Semaphorin 3A controls enteric neuron connectivity and is inversely associated with synapsin 1 expression in Hirschsprung disease. Sci Rep 2020; 10(1):15119. doi: 10.1038/s41598-020-71865-3.
38. Wang LL, Zhang Y, Fan Y, Li H, Zhou FH, Miao JN, et al. SEMA3A rs7804122 polymorphism is associated with Hirschsprung disease in the Northeastern region of China. Birth Defects Res A Clin Mol Teratol 2012; 94(2):91-5. doi: 10.1002/bdra.22866.
39. Anderson RB, Turner KN, Nikonenko AG, Hemperly J, Schachner M, Young HM. The cell adhesion molecule L1 is required for chain migration of neural crest cells in the developingmouse gut. Gastroenterology 2006; 130:1221–32. doi:10.1053/j.gastro.2006.01.002.
40. Broders-Bondon F, Paul-Gilloteaux P, Carlier C, Radice GL, Dufour S. N-cadherin and β1-integrins cooperate during the development of the enteric nervous system. Dev Biol 2012; 364:178–91. doi: 10.1016/j.ydbio.2012.02.001.
41. Wallace AS, Schmidt C, Schachner M, Wegner M, Anderson RB. L1cam acts as a modifier gene during enteric nervous system development. Neurobiol Dis 2012; 40:622–33. doi: 10.1016/j.nbd.2010.08.006.

11. Rao M, Gershon MD. Enteric nervous system development: what could possibly go wrong? Nat Rev Neurosci 2018; 19(9):552-65. doi: 10.1038/s41583-018-0041-0.
12. Goldstein AM, Hofstra RM, Burns AJ. Building a brain in the gut: development of the enteric nervous system. Clin Genet 2013; 83(4):307-16. doi: 10.1111/cge.12054.
13. Nagy N, Goldstein AM. Enteric nervous system development: A crest cell's journey from neural tube to colon. Semin Cell Dev Biol 2017; 66:94-106. doi: 10.1016/j.semcdb.2017.01.006.
14. Simkin JE, Zhang D, Rollo BN, Newgreen DF. Retinoic acid upregulates ret and induces chain migration and population expansion in vagal neural crest cells to colonize the embryonic gut. PLoS One 2013; 8(5):e64077. doi: 10.1371/journal.pone.0064077.
15. Gao T, Wright-Jin EC, Sengupta R, Anderson JB, Heuckeroth RO. Cell-autonomous retinoic acid receptor signaling has stage-specific effects on mouse enteric nervous system. JCI Insight 2021; 6(10):e145854. doi: 10.1172/jci.insight.145854.
16. Kawai K, Takahashi M. Intracellular RET signaling pathways activated by GDNF. Cell Tissue Res 2020; 382(1) :113-123. doi: 10.1007/s00441-020-03262-1.
17. Mwizerwa O, Das P, Nagy N, Akbareian SE, Mably JD, Goldstein AM. Gdnf is mitogenic, neurotrophic, and chemoattractive to enteric neural crest cells in the embryonic colon. Dev Dyn 2011; 240(6):1402-11. doi: 10.1002/dvdy.22630.
18. Hearn CJ, Murphy M, Newgreen D. GDNF and ET-3 differentially modulate the numbers of avian enteric neural crest cells and enteric neurons in vitro. Dev Biol 1998; 197(1):93-105. doi: 10.1006/dbio.1998.8876.
19. Anderson RB, Bergner AJ, Taniguchi M, Fujisawa H, Forrai A, Robb L et al. Effects of different regions of the developing gut on the migration of enteric neural crest-derived cells: a role for Sema3A, but not Sema3F. Dev Biol 2007; 305(1):287-99. doi: 10.1016/j.ydbio.2007.02.020.
20. Diposarosa R, Bustam NA, Sahiratmadja E, Susanto PS, Sribudiani Y. Literature review: enteric nervous system development, genetic and epigenetic regulation in the etiology of Hirschsprung's disease. Heliyon 2021; 7(6):e07308. doi: 10.1016/j.heliyon.2021.e07308.
21. Amiel J, Lyonnet S. Hirschsprung disease, associated syndromes, and genetics: a review. J Med Genet 2001; 38(11):729-39. doi: 10.1136/jmg.38.11.729.
22. Uesaka T, Nagashimada M, Yonemura S, Enomoto H. Diminished Ret expression compromises neuronal survival in the colon and causes intestinal aganglionosis in mice. J Clin Invest 2008; 118(5):1890-8. doi: 10.1172/JCI34425.
23. Nagy N, Guyer RA, Hotta R, Zhang D, Newgreen DF, Halasy V et al. RET overactivation leds to concurrent Hirschsprung disease and intestinal ganglioneuromas. Development 2020; 147(21):dev190900. doi: 10.1242/dev.190900.
24. Soret R, Schneider S, Bernas G, Christophers B, Souchkova O, Charrier B et al. Glial Cell-Derived Neurotrophic Factor Induces Enteric Neurogenesis and Improve Colon Structure and Function in Mouse Models of Hirschsprung Disease. Gastroenterology 2020; 159(5):1824-38.e17. doi: 10.1053/j.gastro.2020.07.018.
25. Watanabe Y, Stanchina L, Lecerf L, Gacem N, Conidi A, Baral V et al. Differentiation of Mouse Enteric Nervous System Progenitor Cells Is Controlled by Endothelin 3 and Requires Regulation of Ednrb by SOX10 and ZEB2. Gastroenterology 2017; 152(5):1139-50. doi: 10.1053/j.gastro.2016.12.034.
26. Nagy N, Goldstein AM. Endothelin-3 regulates neural crest cell proliferation and differentiation in the hindgut enteric nervous system. Dev Biol 2006; 293(1):203-17. doi: 10.1016/j.ydbio.2006.01.032.
27. Barlow A, de Graaff E, Pachnis V. Enteric nervous system progenitors are coordinately controlled by the G protein-coupled receptor EDNRB and the receptor tyrosine kinase RET. Neuron 2003; 40(5):905-16. doi: 10.1016/s0896-6273(03)00730-x.
28. Read AP, Newton VE. Waardenburg syndrome. J Med Genet 1997; 34(8):656-65. doi: 10.1136/jmg.34.8.656.
29. Okamura Y, Saga Y. Notch signaling is required for the maintenance of enteric neural crest progenitors. Development 2008; 135(21):3555-65. doi: 10.1242/dev.022319.
30. Taylor MK, Yeager K, Morrison SJ. Physiological Notch signaling promotes gliogenesis in the developing peripheral and central nervous systems. Development 2007; 134(13):2435-47. doi: 10.1242/dev.005520.
31. Pawolski W, Schmidt MHH. Neuron-Glia Interaction in the Developing and Adult Enteric Nervous System. Cells 2021; 10(1): 47. doi: 10.3390/cells10010047.
32. Liu JA, Ngan ES. Hedgehog and Notch signaling in enteric nervous system development. Neurosignals 2014; 22(1):1-13. doi: 10.1159/000356305.
33. Chalazonitis A, Kessler JA. Pleiotropic effects of the bone morphogenetic proteins on development of the enteric nervous system. Dev Neurobiol 2012; 72(6):843-56. doi: 10.1002/dneu.22002.
34. Anitha M, Shahnavaz N, Qayed E, Joseph I, Gossrau G, Mwangi S et al. BMP2 promotes differentiation of nitrergic and catecholaminergic enteric neurons through a Smad1-dependent pathway. Am J Physiol Gastrointest Liver Physiol 2010; 298(3):G375-83. doi: 10.1152/ajpgi.00343.2009.
35. Chalazonitis A, D'Autréaux F, Pham TD, Kessler JA, Gershon MD. Bone morphogenetic proteins regulate enteric gliogenesis by modulating ErbB3 signaling. Dev Biol 2011; 350(1):64-79. doi: 10.1016/j.ydbio.2010.11.017.
36. Goldstein AM, Brewer KC, Doyle AM, Nagy N, Roberts DJ. BMP signaling is necessary for neural crest cell migration and ganglion formation in the enteric nervous system. Mech Dev 2005; 122(6):821-33. doi: 10.1016/j.mod.2005.03.003.

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37. Gonzales J, Le Berre-Scoul C, Dariel A, Bréhéret P, Neunlist M, Boudin H. Semaphorin 3A controls enteric neuron connectivity and is inversely associated with synapsin 1 expression in Hirschsprung disease. Sci Rep 2020; 10(1):15119. doi: 10.1038/s41598-020-71865-3.
38. Wang LL, Zhang Y, Fan Y, Li H, Zhou FH, Miao JN, et al. SEMA3A rs7804122 polymorphism is associated with Hirschsprung disease in the Northeastern region of China. Birth Defects Res A Clin Mol Teratol 2012; 94(2):91-5. doi: 10.1002/bdra.22866.
39. Anderson RB, Turner KN, Nikonenko AG, Hemperly J, Schachner M, Young HM. The cell adhesion molecule L1 is required for chain migration of neural crest cells in the developingmouse gut. Gastroenterology 2006; 130:1221–32. doi:10.1053/j.gastro.2006.01.002.
40. Broders-Bondon F, Paul-Gilloteaux P, Carlier C, Radice GL, Dufour S. N-cadherin and β1-integrins cooperate during the development of the enteric nervous system. Dev Biol 2012; 364:178–91. doi: 10.1016/j.ydbio.2012.02.001.
41. Wallace AS, Schmidt C, Schachner M, Wegner M, Anderson RB. L1cam acts as a modifier gene during enteric nervous system development. Neurobiol Dis 2012; 40:622–33. doi: 10.1016/j.nbd.2010.08.006.

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ULOGA METFORMINA U TERAPIJI NESITNOĆELIJSKOG KARCINOMA PLUĆA

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SAŽETAK

Nesitnoćelijski karcinom pluća (NSCLC) sačinjava 80-85% svih dijagnostikovanih formi karcinoma pluća. Konvencionalni terapijski modaliteti pokazuju malu uspešnost u lečenju uznapredovalih formi bolesti, što dovodi do razvoja novih lekova koji bi zajedno sa klasičnim hemioteraputicima pobošljali odgovor na postojeću terapiju. Jedan od takvih terapeutika je i antidijabetik metformin koji je pokazao obećavajuće rezultate tokom pretkliničkih i retrospektivnih studija. U ovom radu analizirane su prospektivne kliničke studije koje su ispitivale efekat lečenja karcinoma pluća pomoću metformina i konvencionalnih terapijskih pristupa, kao i pretkliničke studije koje opisuju mogući mehanizam dejstva metformina objavljene u *PubMed* bazi podataka u prethodnih 10 godina. U pojedinim prospektivnim kliničkim studijama uočene su naznake da terapija metforminom dovodi do poboljšanja opšte stope preživljavanja i produžetka perioda bez progresije bolesti. Međutim, ovakve studije su malobrojne i karakteriše ih nedovoljan broj ispitanika kao i smanjena komplijantnost prema terapiji metforminom. Pretkliničke studije ukazuju na citotoksični efekat metformina prema ćelijama NSCLC, aktivaciju apoptoze, kao i sinergizam sa radioterapijom, hemioterapeuticima i biološkom terapijom koja se primenjuje, ali su pokazani mehanizmi dejstva upitni uzimajući u obzir visoke koncentracije metformina koje se primenjuju *in vitro*. Na osnovu dostupnih podataka nije moguće sa sigurnošću proceniti da li metformin dovodi do poboljšanja efekta lečenja u poređenju sa konvencionalnim terapijskim pristupima, niti doneti jasan zaključak o ćelijskim mehanizmima kojima bi se ovakav efekat ostvario. Stoga je neophodno da buduća pretklinička istraživanja budu bolje dizajnirana u smislu mogućnosti translacije rezultata na *in vivo* okolnosti, a kliničke studije bolje kontrolisane kao i da obuhvate veći broj precizno odabranih ispitanika.

Ključne reči: karcinom pluća, metformin, klinička studija, molekularni mehanizam

Uvod

Karcinom pluća predstavlja vodeći uzrok mortaliteta u svetu, sačinjavajući 18% ukupnih smrtinih ishoda nastalih kao posledica maligniteta. Prema podacima Svetske zdravstvene organizacije (engl. *World Health Organization* - WHO) iz 2020. godine, sa 1,2 miliona slučajeva karcinom pluća je i najčešće dijagnostikovani tumor muškaraca preko 55 godina starosti (1). Najčešći faktor rizika i dalje predstavlja pušenje duvana, ali se kao etiološki faktori sa sve većom incidencijom pominju i izloženost industrijskim kancerogenima poput azbesta, arsena kao i policikličnih aromatičnih ugljovodonika (2). Ovakvim činjenicama u prilog ide i podatak da u proseku 20% osoba preminulih od karcinoma pluća predstavljaju nepušači (1).

Patohistološki, najčešći tipovi karcinoma pluća se mogu klasifikovati kao nesitnoćelijski (engl. *Non-small Cell Lung Cancer* – NSCLC) koji sačinjava 80-85% i ređe dijagnostikovani, sitnoćelijski karcinom pluća (engl. *Small Cell Lung Cancer* – SCLC) (3). Terapija izbora u ranim stadijumima bolesti (I-IIIa) je hirurška sa adjuvantnom hemioterapi-

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THE ROLE OF METFORMIN IN THE TREATMENT OF NON-SMALL CELL LUNG CANCER

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SUMMARY

Out of the all newly diagnosed lung cancers, non-small cell lung carcinoma (NSCLC) comprises 80-85%. When treating advanced stages of the disease, conventional therapy shows poor results, which implies that there is a need for new drugs that will improve the response to current therapy. Metformin, drug used to threat Diabetes mellitus showed promising results in preclinical and retrospective clinical studies. We have analyzed prospective clinical trials investigating the combined effect of conventional therapy and metformin in treating lung cancer, as well as preclinical studies investigating its possible mechanisms of action published in PubMed database in the last 10 years. Several studies indicated that combination therapy with metformin led to the improvement in categories like overal survival (OS) and progression-free survival (PFS). However, the number of studies is limited and is characterized by a low number of subjects, as well as by a reduced compliance in subjects using metformin. Preclinical studies suggest cytotoxic effects of metformin, activation of apoptosis, as well as synergistic effect with chemotherapeutics, radiotherapy and biological agents used. The relevance of determined results is questionable, taking into account high metformin concentrations used in vitro. Based on the clinical studies published in the last ten years, there is insufficient data to conclude whether metformin improves prognostic factors in comparison to the conventional therapy. It is also not clear which mechanisms are responsible for possible beneficial effects of metformin. Future preclinical studies thus have to be better designed in order to increase their translational potential, while clinical studies have to be better controlled with improved selection and higher number of subjects enrolled.

Keywords: lung cancer, metformin, clinical study, molecular mechanism

Introduction

Lung cancer is the leading cause of death worldwide, making up 18% of all deaths caused by malignancies. According to the data of the World Health Organization (WHO) for 2020, lung cancer was the most frequently diagnosed cancer in men older than 55 years, with 1.2 million cases occuring annualy (1). Tobacco use is still the most common risk factor, while etiological factors identified as risk factors include exposure to the industrial carcinogens such as asbestos, arsenic, as well as polycyclic aromatic hydrocarbons (2). This is supported by the fact that, on average, 20% of people who die of lung cancer are non-smokers (1).

Pathohistologically, the most common lung cancers are classified as NSCLC (Non-small Cell Lung Cancer), comprising 80-85% of all cases, while SCLC (Small Cell Lung Cancer) is more rarely diagnosed (3). The treatment of choice for early stages of NSCLC (I-IIIa) is surgical treatment with adjuvant chemotherapy, aimed at eliminating potential micrometastases. In advanced disease (stages IIIb-IV), standard treatment includes concomitant chemoradiotherapy, which is most frequently based on the application of platinum-based doublets in combination with radiotherapy (4,5). The specificities of treatment modalities

jom u cilju eliminacije potencijalnih mikrometastaza. Kod uznapredovale bolesti (stadijumi IIIb-IV) standardnu terapiju sačinjava konkomitantna hemioradioterapija, koja se najčešće zasniva na primeni platinskih dubleta u kombinaciji sa zračnom terapijom (4,5). Pojedinosti modaliteta lečenja zavise ne samo od stadijuma proširenosti, već i od histološke forme tumora, kao i od opšteg stanja samog pacijenta. U okolnostima kada ne postoje kontraindikacije, prednost se daje cisplatinu koji se najčešće kombinuje sa inhibitorima topoizomeraze II (etopozid), vinka alkaloidima (vinorelbin), antifolatima (pemetreksed) i nukleozidnim analozima (gemcitabin). Primena karboplatina je najčešća u kombinaciji sa lekovima koji inhibiraju polimeraziciju mikrotubula - paklitakselom u solubilnoj formi ili u obliku tzv. nab-paklitaksela koji je vezan za albumine. Radioterapija se primenjuje u ukupnoj dozi 60-65 Gy frakcionisano u 30-35 dnevnih zračenja, a prema preporukama u periodu ne dužem od 6-7 meseci (6). Pacijenti koji se nalaze u stadijumu metastatske bolesti (IV stadijum) se testiraju na prisustvo molekularnih markera na tumorskim ćelijama, u cilju potencijalne primene ciljane terapije (engl. *targeted therapy*) ili imunoterapije (5,6). Od najveće važnosti je inicijalna procena ekspresije PD-L1 proteina na tumorskim ćelijama, gde u slučaju pozitivnog nalaza u više od 50% analiziranog tkiva terapiju izbora kod ovih pacijenata predstavlja imunoterapija. U te svrhe primenjuje se anti-PD-1 agens pembrolizumab koji predstavlja humanizovano antitelo koje vezuje PD-1 protein eksprimiran na brojnim ćelijama imunskog sistema, blokirajući na taj način inhibitorno dejstvo PD-L1 tumorskih ćelija. Na ovaj način se povećava specifičan antitumorski imunski odgovor (7). Radi moguće primene ciljane terapije pacijenti se analiziraju na prisustvo tzv. onkogenih „*driver*" mutacija i u većini evropskih zemalja obavezno je testiranje gena EGFR, ALK, ROS1. U slučaju potvrde neke od mutacija u genu za receptor za epidermalni faktor rasta, EGFR, terapiju izbora predstavlja specifični EGFR inhibitor tirozin kinaze (TKI) poput erlotiniba, gefitiniba, ili afatiniba (8). Kod pacijenata sa prisustvom rearanžmana, tj. fuzije gena ALK koji kodira receptorsku tirozin kinazu primenjuje se neki od ALK inhibitora (krizotinib, ceritinib, alektinib) (9), dok je inhibitor izbora kod pacijenata sa rearanžmanom ROS1 gena (10), takođe inhibitor receptorske tirozin kinaze, krizotinib. Pored ovih mutacija, sve češće se ispituje i prisustvo mutacija u BRAF genu zbog mogućnosti primene specifičnih TKI, dabrafeniba ili trametiniba, lekova koji deluju sinergistički inhibirajući BRAF i MEK protein kinaze u protoonkogenom signalnom putu. Ciljana i imunoterapija je često praćena prethodno pomenutom platinskom dublet terapijom, dok se radioterapija kod ovih pacijenata primenjuje kao palijativna, tj. u cilju kontrole simptoma poput opstrukcije disajnih puteva, koštanih metastaza i slično (6).

Brojni mehanizmi dovode do stvaranja rezistencije na primenjenu hemio-radioterapiju što za posledicu ima veći stepen progresije bolesti i nastanak recidiva, čime se objašnjava i relativno loš uspeh terapije i pored primene različitih modaliteta, kao i relativno loša prognoza bolesti. Tako je petogodišnje preživljavanje pacijenata sa uznapredovalom formom karcinoma pluća svega 6-8 % (11). Upravo iz tog razloga sve se češće u lečenju maligniteta, uključujući NSCLC, ispituju lekovi čija primarna indikacija nije maligna bolest, ali koji bi potencijalno mogli da pokažu sinergistički efekat sa primenjenom hemioradioterapijom ili da povećaju osetljivost tumorskih ćelija na istu. U ovu grupu lekova se ubraja i antidijabetik metformin. Metformin predstavlja lek iz grupe bigvanida koji se primenjuje kao terapeutik izbora u lečenju *Diabetes mellitus*-a tipa II (DM tip II). Euglikemijski efekat ovog leka posledica je smanjenja apsorpcije glukoze na nivou intestinalnog trakta, inhibicije glukoneogeneze kao i povećanja osetljivosti insulinskog receptora (3). Prethodno sprovedene retrospektivne opservacione studije pokazale su da kod pacijenata koji boluju od NSCLC na kombinovanoj terapiji metforminom i hemioterapeuticima dolazi do značajnog poboljšanja efekta lečenja posmatrano kroz ukupno preživljavanje (engl. *overall survival* – OS), preživljavanje bez progresije bolesti (engl. *progression-free survival* – PFS) i objektivnu stopu odgovora na terapiju (engl. *objective response rate* – ORR) (12-17). Takođe, retrospektivno je pokazano i da metformin dovodi do poboljšanja 2-godišnjeg i 5-godišnjeg preživljavanja pacijenata nakon radioterapije NSCLC (18). Kako su pacijenti u ovim studijama bolovali od dijabetesa i uzimajući u obzir da su retrospektivne studije sklone pristrasnostima (19), u toku je veliki broj prospektivnih randomizovanih kontrolisanih kliničkih studija koje ispituju potencijalne benefite metformina kod pacijenata sa uznapredovalim NSCLC. Molekularni mehanizmi dejstva metformina kojima bi se mogao objasniti

depend not only on the stage of the disease development, but also on the histological form of the tumor, as well as on the patient's performance status. When there are no contraindications, an advantage is given to cisplatin, which is most frequently combined with topoisomerase II inhibitors (etoposide), vinca alkaloids (vinorelbine), antifolates (pemetrexed) and nucleoside analogs (gemcitabine). The application of carboplatine is most frequent in combination with drugs that inhibit the polymerization of microtubules – paclitaxel in a soluble form or in the form of nab-paclitaxel which is albumin-bound. Radiotherapy is applied in a total dose of 60-65 Gy divided into 30-35 daily fractions, and, according to the recommendations, no longer than 6-7 months (6). If the metastasis are present (stage IV), patients are tested for the presence of molecular markers on tumor cells, in order to potentially administer targeted therapy or immunotherapy (5,6). The initial estimation of expression of PD-L1 protein on tumor cells is of the greatest significance. If more than 50% of analyzed tissue sample is positive for PD-L1, the treatment of choice is immunotherapy – anti-PD-1 agent pembrolizumab. This humanized antibody binds lymphocytic PD-1 protein and by doing so blocks the inhibiting effect of tumor cells, PD-L1.

Thus, specific antitumor immune response is increased (7). In order to possibly administer targeted therapy, patients are analyzed for the presence of the so called oncogenic "driver" mutations and in most European countries EGFR, ALK, ROS1 testing is mandatory. If any of the mutations in the gene for the epidermal growth factor receptor, EGFR, are confirmed, the treatment of choice is specific inhibitor of EGFR tyrosine kinase (TKI) such as erlotinib, gefitinib, or afatinib (8). In patients with rearrangement (fusion) of ALK gene that encodes a receptor tyrosine kinase, ALK inhibitors are administered (crizotinib, ceritinib, alectinib) (9), while the inhibitor of choice in patients with the rearrangement of ROS1 gene (10) is also the inhibitor of a receptor tyrosine kinase, crizotinib. Besides these mutations, the presence of mutations in BRAF gene can also be examined due to the possibility of application of specific TKI, dabrafenib or trametinib. The targeted therapy and immunotherapy are often followed by the previously mentioned platinum-based doublets, while radiotherapy in these patients is applied as palliative, to control symptoms such as the airflow obstruction, bone metastasis etc. (6).

Numerous mechanisms lead to resistance to applied chemoradiotherapy, resulting in greater disease progression and the occurrence of relapse, and can be the explanation for relatively poor success of treatment and relatively poor prognosis although different modalities are used. Thus, five-year survival of patients with advanced stages of lung cancer is only 6-8% (11). Therefore, drugs whose primary indications do not include malignant diseases, but which could show synergism with chemoradiotherapy or increase the sensitivity of tumor cells to therapy, are examined more and more in the treatment of malignancies. The antidiabetic metformin belongs to this group of drugs. Metformin is a drug from the group of biguanides that is used as the treatment of choice in *Diabetes mellitus* type II (DM type II). The euglycemic effect of this drug is the consequence of decrease of glucose absorption in the intestinal tract, inhibition of gluconeogenesis, as well as the increase of insulin sensitivity (3). Previously conducted retrospective observational studies have shown that patients with NSCLC who use metformin in combination with chemotherapy, have significant improvement in terms of overall survival (OS), progression-free survival (PFS) and objective response rate (ORR) (12-17). Also, it has been shown retrospectively that metformin leads to the improvement of 2-year and 5-year survival of patients with NSCLC after radiotherapy (18). Since patients in these studies had diabetes and considering the fact that retrospective studies are prone to bias (19), many prospective randomized controlled studies that examine the potential benefits of metformin in patients with advanced NSCLC are currently underway. Molecular mechanisms of metformin effects, which could explain the favorable effect on NSCLC, have not been completely explained. Therefore, the aim of this study is the analysis of the results of prospective clinical studies that investigated the role of metformin in the treatment of NSCLC during the last 10 years, as well as the analysis of available literature that explains the possible mechanisms of antitumor effects of metformin *in vitro*.

povoljni efekat na NSCLC još uvek nisu u potpunosti razjašnjeni. Stoga je cilj ovog rada analiza rezultata prospektivnih kliničkih studija koje su se bavile ulogom metformina u terapiji NSCLC u proteklih 10 godina, kao i analiza dostupne literature koja objašnjava moguće mehanizme antitumorskog efekta metformina *in vitro*.

Metode

U cilju sumiranja aktuelnih podataka o ulozi metformina u terapiji pacijenata sa NSCLC u zavisnosti od primenjenih terapijskih modaliteta korišćena je literatura dobijena pretragom *PubMed* baze podataka poštujući pravila MeSH (engl. *Medical Subject Headings*) indeksiranja. Obuhvaćena su sva klinička ispitivanja koja su se bavila datom temom objavljena u periodu od januara 2012. do jula 2022. Literatura na engleskom jeziku selektovana je pretragom sledećih ključnih reči: metformin, karcinom pluća, kliničko ispitivanje. Ista baza podataka je korišćena i za prikaz rezultata koji se odnose na molekularne mehanizme antitumorskog dejstva metformina.

Metformin i hemioterapija NSCLC

U posmatranom periodu objavljeni su rezultati pet kliničkih studija nakon druge faze ispitivanja, a koje su uključivale pacijente u III i/ili IV stadijumu NSCLC koji nisu imali prethodno dijagnostikovan DM tip II kao i jedne studije koja je obuhvatala ovu grupu pacijenata (Tabela 1). Pet od šest spomenutih studija predstavlja rezultate kontrolisanih, randomizovanih, otvorenih kliničkih studija nakon IIa (20) i IIb (21-24) faze ispitivanja. Za razliku od njih studija *Parikh*-a i saradnika iz 2017. godine (25) za potrebe svog rada koristila je istorijsku kontrolu, što zbog poređenja različitih populacija ispitanika može uticati na ispravnost tumačenja rezultata.

Studije *Sayed*-a (20) i *Parikh*-a (25) analizirale su adjuvantnu ulogu metformina u citostatskoj terapiji (cisplatin/gemcitabin i karboplatin/pemetreksed) u uznapredovalim stadijuma NSCLC. Zajedničko za oba rada je da metformin ne dovodi do pogoršanja neželjenih efekata hemioterapije. Praćenjem parametara poput OS, PFS i ORR uočen je pozitivan trend rasta u grupi koja je u terapiji imala metformin. Izostanak statističke značajnosti u razlikama ovih prognostičkih parametara može se pripisati malom broju ispitanika koji su bili obuhvaćeni ovim istraživanjima (30, odnosno 14 pacijenata).

Dva velika istraživanja objavljena u julu 2021. godine bavila su se efektom metformina kod pacijenata sa lokalno uznapredovalim NSCLC, tokom i

Tabela 1. Rezultati prospektivnih kliničkih studija u kojima je ispitivan efekat metformina na prognozu pacijenata sa NSCLC na standardnoj hemioterapiji.

	Klinička studija	Dizajn studije	Broj ispitanika	Stadijum *NSCLC*	Ispitivana terapija	Rezultati
1	Sayed et al, 2015	randomizovana, otvorena, kontrolisana IIa faza kliničke studije	30	IV	gemcitabin, cisplatin	pozitivan trend rasta OS, PFS, ORR ali bez statistički značajne razlike
2	Parikh et al, 2017	otvorena, faza IIb kliničke studije sa istorijskom kontrolom	14	IIIb/IV	pemetreksed, karboplatin	
3	Marrone et al, 2018	otvorena, IIb faza kliničke studije sa istorijskom kontrolom	19	IIIb/IV	paklitaksel, karboplatin, bevacizumab	povećanje PFS i MOS u poređenju sa podacima iz literature
4	Skinner et al, 2021	randomizovana, otvorena, kontrolisana IIa faza kliničke studije	167	IIIa/IIIb	paklitaksel, karboplatin + radioterapija	pozitivan trend rasta OS i PFS ali bez statistički značajne razlike
5	OCOG-ALMERA (Tsakiridis et al, 2021)	randomizovana, multicentrična II faza kliničke studije	54	IIIa/IIIb	cisplatin + radioterapija	smanjenje OS i PFS kao i pogoršanje neželjenih efekata terapije
6	Lee et al, 2020	randomizovana, otvorena, kontrolisana IIb faza kliničke studije	164	IIIb/IV	gemcitabin, karboplatin	povećanje OS i PFS kod pacijenata sa većim

OS - ukupno preživljavanje; PFS - preživljavanje bez progresije bolesti; ORR - stopa odgovora na terapiju; MOS - medijana ukupnog preživljavanja; FDG – fluorodeoksiglukoza

Methods

In order to summarize current data about the role of metformin in the treatment of patients with NSCLC depending on the applied treatment modalities, literature was obtained through a search of PubMed database respecting the rules of MeSH (Medical Subject Headings) indexing. All clinical studies that were published between January 2012 and July 2022 were included in the analysis. The literature in the English language was selected by searching the following key words: metformin, lung cancer, clinical trial. The same database was used to present results related to molecular mechanisms of metformin's antitumor effects.

Metformin and chemotherapy in NSCLC

In the observed time period, the results of five clinical studies after phase II were published, and they included patients with NSCLC in the stage III and/or IV who did not have previously diagnosed DM type II, as well as the results of one study which included this group of patients (Table 1). Five out of six above mentioned studies present the results of controlled, randomized, open-label clinical trials after phase IIa (20) and IIb (21-24). On the other hand, the study of Parikh and associates from 2017 (25), used historical control, which may influence the accuracy of interpretation of the results due to the comparison of different populations of participants.

Studies of Sayed (20) and Parikh (25) analyzed the adjuvant role of metformin in cytostatic therapy (cisplatin/gemcitabine and carboplatine/pemetrexed) in advanced stages of NSCLC. In both studies, metformin did not cause worsening of side effects of chemotherapy. By observing the parameters such as OS, PFS and ORR, a positive trend of increase was noticed in the group that used metformin in the treatment. The absence of statistical significance regarding the difference between these prognostic factors may be attributed to the small number of participants that were included in these studies (30 and 14 patients, respectively).

Two large studies published in July 2021 investigated the effect of metformin in patients with locally advanced NSCLC, during and after concomitant chemoradiotherapy (CRT). OCOG-ALMERA study (23) is the first prospective clinical study that found significant worsening of side effects of chemoradiotherapy (cisplatin/etoposide), as well as distinctly worse results of treatment effects in the group of patients who used metformin (2000 mg/day). There are numerous limitations of this study, also stated by authors themselves, in particular the fact that only

Table 1. Results of the prospective clinical studies that investigated the effect of metformin on survival of NSCLC patients treated with standard chemotherapy

	Clinical study	Study design	Sample size	NSCLC stage	Lung cancer treatment	Results
1	Sayed et al, 2015	randomized, open-label, controlled phase IIa	30	IV	gemcitabin, cisplatin	increase in OS, PFS, ORR but statistically non-significant
2	Parikh et al, 2017	open-label, phase IIb with historical control	14	IIIb/IV	pemetrexed, carboplatin	
3	Marrone et al, 2018	open-label, phase IIb with historical control	19	IIIb/IV	paclitaxel, carboplatin, bevacizumab	increase in PFS and MOS in comparition with historical control
4	Skinner et al, 2021	randomized, open-label, controlled phase IIa	167	IIIa/IIIb	paklitaxel, carboplatin + radiotherapy	increase in OS and PFS but statistically non-significant
5	OCOG-ALMERA (Tsakiridis et al, 2021)	randomized, multicentric phase II	54	IIIa/IIIb	cisplatin + radiotherapy	decrease in OS, PFS and increased toxic events
6	Lee et al, 2020	randomized, open-label, controlled phase IIb	164	IIIb/IV	gemcitabin, carboplatin	increase in OS and PFS in patients with higher FDG uptake

OS - overall survival; PFS - progression- free survival; ORR - overall response rate; MOS - median overall survival; FDG - fluoro-deoxy-glucose

nakon konkomitantne hemioradioterapije (HRT). OCOG-ALMERA studija (23) predstavlja prvu prospektivnu kliničku studiju koja je uočila značajna pogoršanja neželjenih dejstava HRT (cisplatina/etopozid) kao i upadljivo lošije vrednosti efekata lečenja u grupi pacijenata koji su bili na terapiji metforminom (2000 mg/dnevno). Brojna su ograničenja ove studije koja navode i sami istraživači, od kojih se posebno ističe da je svega 56% pacijenata u ispitivanoj grupi primilo HRT po protokolu lečenja (u poređenju sa 77,8% u kontrolnoj grupi). Takođe, kod 20% pacijenata ispitivane grupe u potpunosti je prekinuta zračna terapija zbog pojave teških neželjenih efekata, dok su svi pacijenti kontrolne grupe primili predviđenu dozu zračenja (60-65 Gy). Drugo značajno ograničenje ispitivanja je činjenica da se radi o otvorenoj studiji u kojoj je ispitivana grupa bila na terapiji metforminom, dok kontrolna grupa nije uzimala placebo, što može uticati na pojavu neželjenih reakcija na lek kod ispitivane grupe.

Da su rezultati OCOG-ALMERA studije (23) najverovatnije posledica malog broja ispitanika i dodatno smanjene komplijantnosti prema terapiji, potvrđuje studija *Skinner*-a i saradnika iz 2021. godine (24) koja je obuhvatila 167 pacijenta na konkomitantnoj HRT (cisplatin/paklitaksel; 60-65Gy). Rezultati ove do sada najbronije prospektivne studije, pokazali su da prilikom adjuvantne primene metformina ne dolazi do povećanja učestalosti neželjenih efekata HRT, ali takođe da nema ni poboljšanja u parametrima preživljavanja i progresije bolesti. Potencijalno ograničenje ove studije je podatak da je svega 39% pacijenata ispitivane grupe uzimalo predviđenu dozu metformina (2000 mg/dnevno), dok je kod ostatka ove grupe tolerancija na lek i komplijantnost prema terapiji bila smanjena.

U radu *Lee* i saradnika iz 2020. godine (22) uočeno je da pacijenti sa skvamocelularnim tipom NSCLC čije tumorske promene preuzimaju više fluorodeoksiglukoze (FDG) prilikom PET snimanja, imaju više vrednosti OS i PFS nakon primene kombinovane terapije metforminom i hemioterapeuticima (karboplatin/gemcitabin). S obzirom da su ovim istraživanjem bili obuhvaćeni pacijenti sa i bez dijagnostikovanog DM, zaključeno je i da nema razlika u vrednostima prognostičkih parametara između ove dve populacije.

Istraživanje *Marrone*-a iz 2018. (21) koje je pored primene hemioterapeutika obuhvatalo i imunoterapiju anti-VEGF antitelom-bevacizumabom prekinuto je zbog izmene preporučenih terapijskih protokola. Rezultati ove studije pokazali su povećanje PFS kod pacijenata na terapiji metforminom, ali u poređenju sa podacima iz literature što samo po sebi ima upitan značaj.

Metformin i radioterapija *NSCLC*

Klinička studija *Chun*-a i saradnika (26) bavila se efektom metformina tokom hipofrakcionisane stereotaktičke radioterapije pacijenata u I i II stadijumu *NSCLC*. Efekti lečenja opisivani kroz PERCIST kriterijume u zavisnosti od stepena preuzimanja FDG tokom PET snimanja. Kod 70% pacijenata koji su bili na terapiji metforminom (2000 mg/dan) uočen je kompletan metabolički odgovor 6 meseci nakon radioterapije. Nažalost, placebo grupu je činio samo jedan pacijent tako da nije bilo moguće izvršiti adekvatnu komparativnu analizu.

Metformin i biološka terapija *NSCLC*

Istraživanje Arriete i saradnika iz 2019. godine (27) obuhvatalo je 139 ispitanika u odmaklim stadijumima adenokarcinoma pluća koji su bili pozitivni na EGFR mutaciju. U ovom radu pokazano je da kombinovana terapija metforminom i EGFR TKI (erlotinib, afatinib, gefitinib) dovodi do produžavanja PFS i OS. Međutim, u radu *Li* i saradnika objavljenom iste godine (28), uočeno je da nema razlike u prognostičkim faktorima kod pacijenata na kombinovanoj terapiji metforminom i gefitinibom. Diskrepance u ovim rezultatima potencijalno proističu iz razlike u dizajnu navedenih studija. Naime, studija *Li* i saradnika (28) predstavlja dvostruko-slepu randomizovanu kliničku studiju sa upotrebom placeba i dvostruko većim brojem ispitanika, što neminovno daje prednost pri tumačenju rezultata u odnosu na studiju koja je bila otvorenog tipa i nije bila kontrolisana placebom.

Kliničko ispitivanje koje se bavi efektima terapijske primene anti-PD1 antitela (nivolumaba) i metformina kod neoperabilnih NSCLC trenutno je u toku (registarski broj NCT03048500) (29).

Metformin i mogući mehanizmi citotoksičnog dejstva pokazani *in vitro*

Veliki je broj originalnih naučnih članaka koji su pokušali da objasne moguće mehanizme ćelijske smrti do koje dovodi metformin u ćelijama NSCLC, kao i aktivaciju signalnih puteva koji dovode do citotoksičnog efekta. Različiti rezultati koji se sreću u literaturi su verovatno posledica razlika u diza-

56% of patients in the examined group received chemoradiotherapy according to the protocol (in comparison to 77.8% in the control group). Also, in 20% of patients from the examined group, radiotherapy was stopped because of serious side effects, while all of the patients from the control group received the planned dose of radiotherapy (60-65 Gy). The second significant limitation of this study is the fact that it is an open-label study in which the examined group used metformin in the treatment, while the control group did not take placebo, which could influence the occurrence of side effects in the examined group.

The study of Skinner and associates from 2021 (24) that included 167 patients using concomitant CRT (cisplatin/paclitaxel; 60-65 Gy) confirmed that the results of OCOG-ALMERA study (23) were most probably the consequence of the small number of participants and reduced compliance with the therapy. The results of this prospective study, which has included most participants so far, have shown that the adjuvant treatment with metformin, does not increase the frequency of side effects of CRT, but at the same time the parameters of survival and disease progression do not improve. A potential limitation of this study is the fact that only 39% of patients from the examined group used metformin in the planned dose (2000 mg/day), while the tolerance to this medication and compliance with the therapy were reduced in the rest of the participants from this group.

In the study of Lee and associates from 2020 (22), it was found that patients with squamocellular NSCLC with high fluorodeoxyglucose (FDG) tumor uptake during PET imaging, had higher values of OS and PFS after the administration of combined therapy with metformin and chemotherapeutics (carboplatine/gemcitabine). Considering that this study included patients with and without DM, it was concluded that there were no differences regarding the values of prognostic factors between these two populations.

The study of Marrone from 2018 (21), in which immunotherapy with anti-VEGF antibody – bevacizumab was used in addition to chemotherapeutics, was ended due to the change in the recommended treatment protocols. The results of this study showed the increase in PFS in patients who used metformin, but in comparison with the literature data which in itself has questionable significance.

Metformin and radiotherapy in NSCLC

The clinical study of Chun and associates (26) assessed the effect of metformin during hypofractionated stereotactic radiotherapy of patients in stages I and II of NSCLC. The effects of the treatment were described through PERCIST criteria depending on the degree of FDG uptake during PET scanning. A complete metabolic response was noticed 6 months after radiotherapy in 70% of patients who used metformin in the treatment (2000 mg/day). Unfortunately, placebo group included only one patient and therefore, it was not possible to do an adequate comparative analysis.

Metformin and biological therapy of NSCLC

The study of Arriete and associates from 2019 (27) included 139 participants with advanced stages of adenocarcinoma who were EGFR-positive. It was shown that the combined therapy with metformin and EGFR-TKI (erlotinib, afatinib, gefitinib) led to the extension of PFS and OS. However, in the study of Li and associates that was published in the same year (28), it was found that there was no difference regarding prognostic factors between patients who used the combined therapy of metformin and gefitinib. The discrepancy between these results was potentially caused by difference regarding the studies' design. Namely, the study of Li and associates (29) was a double-blind randomized placebo-controlled trial with twice as many participants, which unavoidably gives advantage during the analysis of results in comparison with the study that was open-label and was not controlled by placebo.

The clinical trial that investigates the effects of therapeutic application of anti-PD1 antibody (nivolumab) and metformin in inoperable NSCLS is currently in progress (registration number NCT03048500) (28).

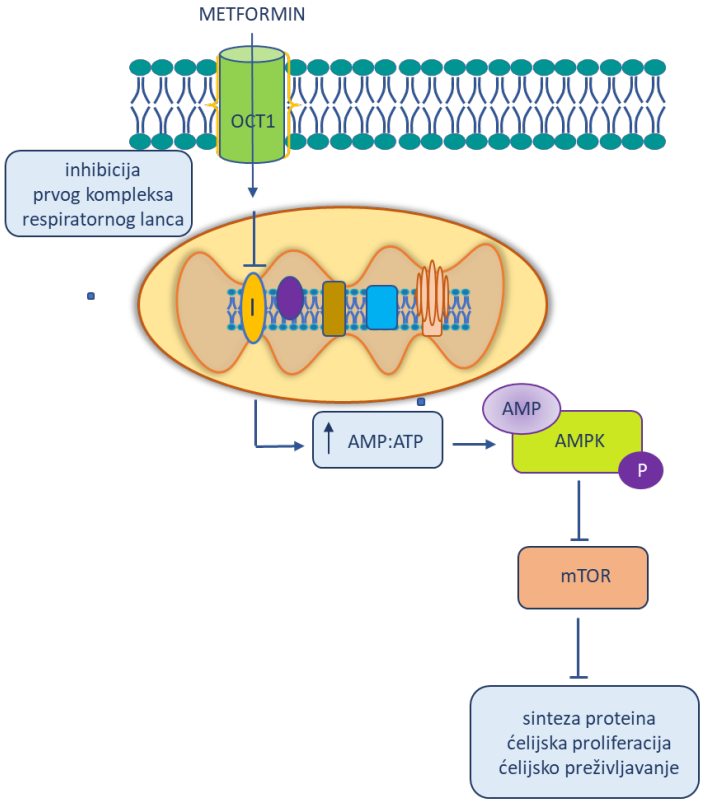
Metformin and the possible mechanisms of cytotoxic effects shown *in vitro*

Many original scientific articles have tried to explain the possible mechanisms of cell death caused by metformin in NSCLC cells, and the activation of signaling pathways which lead to the cytotoxic effect. Different results presented in literature are probably the result of differences in preclinical studies' design, primarily regarding the

jnu pretkliničkih studija, i to pre svega u pogledu odabira ćelijskih linija NSCLC (više od 10 različitih ćelijskih linija sa prisutnim ili odsutnim „*driver*" i drugim mutacijama), dužine trajanja tretmana kao i koncentracije metformina koja je korišćena.

Pokazano je u više istraživanja da sam metformin pokazuje citotoksični efekat prema ćelijama *NSCLC in vitro*, pri čemu se kao mehanizam ćelijske smrti opisuje apoptoza i to pokretanje spoljašnjeg puta praćeno aktivacijom kaspaze 8 (30). Takođe, opisuje se i pokretanje apoptoze koje je posledica smanjenja antiapoptotskog Bcl-2 i povećanja proapoptoskog Bax proteina, što aktivira mitohondrijalni, unutrašnji put pokretanja apoptoze (31). Iako ovi rezultati deluju međusobno isključujuće, aktivacija i spoljašnjeg i unutrašnjeg puta apoptoze, iako se ređe sreće, poznati je fenomen iz literature (32,33). Sa druge strane, neki od autora navode da metformin nema direktan citotoksičan efekat, već da dovodi do zaustavljanja progresije ćelijskog ciklusa u G1 fazi i time smanjuje proliferaciju ćelija karcinoma pluća (34,35).

Iako mehanizam ćelijske smrti do koje metformin dovodi nije u potpunosti jasan, veliki je broj istraživanja koja su ispitivala signalne puteve koji se pokreću kao posledica dejstva metformina, a bez ispitivanja modaliteta ćelijske smrti. Tako je pokazana aktivacija adenozin-monofosfatom aktivirane kinaze i posledična aktivacija GSK3β (36,37). Od ranije predloženi i u literaturi najčešće prikazivan molekularni mehanizam kojim metformin ostvaruje cititoksično dejstvo prema maligno izmenjenim ćelijama jeste efekat na energetski metabolizam koji podrazumeva inhibiciju I kompleksa respiratornog lanca mitohondrija. Smanjenje oskidativne fosforilacije tako dovodi do smanjene produkcije ATP-a i povećanja odnosa AMP:ATP, što dalje aktivira adenozin monofosfatom-aktiviranu kinazu (AMPK). Aktivacija nishodnog signalnog puta AMPK za posledicu ima inhibiciju PI3K/Akt/mTOR signalnog puta odgovornog za sintezu proteina, progresiju ćelijskog ciklusa i ćelijsku proliferaciju (38, 39) (Slika 1), što je i pokazano i na ćelijama NSCLC (34, 40). U suprotnosti sa ovim rezultatima je da metformin aktivira Akt i tako dovodi do citotoksičnog efekta nezavisnog od aktivacije AMPK (41). Zanimljivo je da aktivacija ćelijske smrti po tipu autofagije kao posledica dejstva metformina nije detaljnije ispitivana iako je poznato da je jedan od osnovnih mehanizama pokretanja autofagije



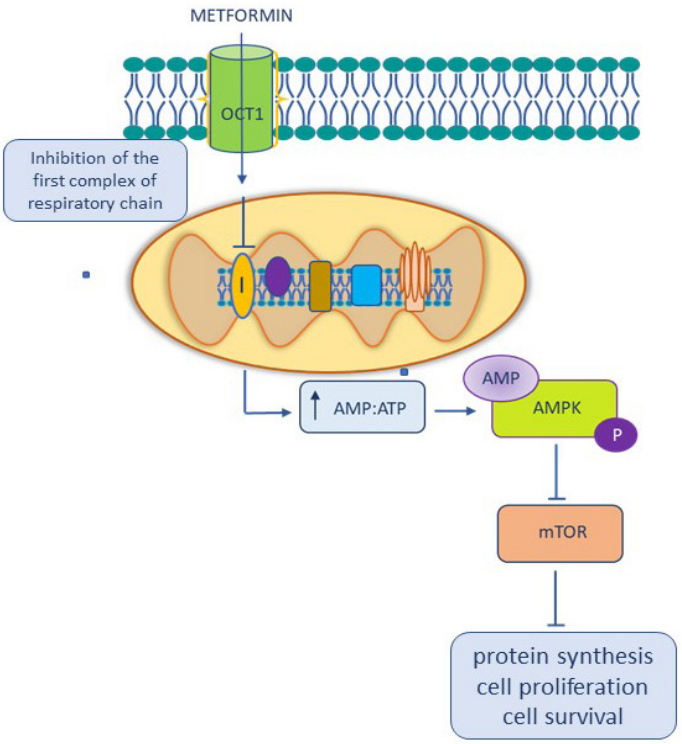
OCT1 – organski katjonski transporter 1; AMP – adenozin-monofosfat; ATP – adenozin-trifosfat; AMPK – adenozin-monofosfatom aktivirana kinaza; P – fosforilisan protein; mTOR – mehanistička meta rapamicina

Slika 1. Pretpostavljeni mehanizam citotoksičnog dejstva metformina aktivacijom AMPK

choice of cell lines of NSCLC (more than 10 different cell lines with the presence or absence of "driver" and other mutations), duration of treatment, as well as the concentration of metformin that was used.

It has been shown in several studies that metformin has a cytotoxic effect on NSCLC cells *in vitro*, while apoptosis has been described as the mechanism of cell death, namely the triggering of extrinsic pathway accompanied by the activation of caspase 8 (30). The activation of apoptosis as the consequence of decrease in antiapoptotic Bcl-2 and increase in proapoptotic Bax protein which activates the mitochondrial, intrinsic pathway of apoptosis activation (31), has also been described. Although these results seem as mutually excluding, the activation of both the extrinsic and intrinsic pathways of apoptosis is, although rare, a phenomenon well-known from the literature (32,33). On the other hand, some of the authors state that metformin does not have a direct cytotoxic effect, but rather induces arrest in the G1 phase of the cell cycle, thus reducing the proliferation of lung cancer cells (34,35).

Although the mechanism of cell death caused by metformin is not completely clear, many studies have examined signaling pathways triggered by metformin, without investigating modalities of cell death. The activation of adenosine monophosphate-activated kinase was shown, as well as the resulting activation of GSK3ß (36, 37). The molecular mechanism most frequently presented in literature which has a cytotoxic effect on malignant cells, is the effect of metformin on energy metabolism, primarily the inhibition of mitochondrial respiratory-chain complex 1. Thus, reduced oxidative phosphorylation leads to the reduced production of ATP and increase in the AMP:ATP ratio, which further activates adenosine monophosphate-activated kinase (AMPK). The activation of AMPK downstream signaling results in the inhibition of PI3K/Akt/mTOR signaling pathway which is responsible for the protein synthesis, progression of cell cycle and cell proliferation (38, 39) (Picture 1), which has been confirmed in NSCLC cells (34,40). Contrary to these results, metformin can also activate Akt, leading to the AMPK-independent cytotoxic effect (41). It is interesting



OCT1 – organic cation transporter 1; AMP – adenosine-monophosphate; ATP – adenosine-triphosphate; AMPK – adenosine monophosphate activated protein kinase; P – phosphorilated protein; mTOR – mechanistic target of rapamycin

Figure 1. Suggested cytotoxic mechanism of metformin mediated by AMPK activation

aktivacija AMPK i posledična inhibicija PI3K/Akt/mTOR signalnog puta (42). Samo u jednom radu je pokazano da metformin pokreće apoptozu i istovremeno autofagiju koja je citotoksičnog karaktera (43). Važna činjenica koju treba napomenuti u vezi sa prethodno pomenutim studijama je da je u najvećem broju njih korišćena koncentracija metformina koja značajno prevazilazi koncentracije koje se mogu postići u krvotoku osoba koje su na terapiji metforminom. Naime, koncentracije ovog leka u laboratorijskim uslovima bile su stotinu i više puta veće (5-80 mM) u poređenju sa onim koje su registrovane u plazmi pacijenata na terapiji metforminom (20-50 µM). U *in vivo* uslovima ovako visoke koncentracije leka dovele bi do letalnih ishoda kao posledice teškog oblika laktatne acidoze. U svega jednom istraživanju koje je pokazalo citotoksični efekat metformina su korišćene klinički relevantne koncentracije (100 µM), a mehanizam je objašnjen aktivacijom AMPK i inhibicijom signalnog puta Akt/mTOR (44). Druga grupa autora je pak pokazala da ovaj lek u terapijskim koncentracijama (50 µM) nema citotoksični efekat na ćelije karcinoma pluća kao i da ne utiče na promenu potencijala mitohondrijalne membrane i produkciju ROS (45, 46). Dodatno, pokazano je da metformin ni na netumorskim ćelijama u niskim koncentracijama (µM) ne pokazuje očekivane efekte - inhibiciju respiratornog lanca i aktivaciju AMPK (47), čime se možda može objasniti nekonzistentnost u rezultatima između pretkliničkih tj. *in vitro*, i kliničkih studija.

U istraživanjima koja su ispitivala potencijalni sinergistički efekat metformina sa hemioterapeuticima koji se koriste u lečenju NSCLC, pokazano je da metformin povećava osetljivost ćelija rezistentnih na cisplatin, i to inhibicijom MAPK signalnog puta (48), aktivacijom unutrašnjeg puta apoptoze i oksidativnog stresa (49), inhibicijom mTOR nezavisno od AMPK (50), ili preko p53 (51). Takođe, opisano je i da u ovim ćelijama smanjuje nivo aspartata i NAD, čime se objašnjava povećanje citotoksičnosti (52). Sa druge strane, jedna studija je pokazala da zajednički tretman cisplatinom i metforminom modifikuje apoptozu aktivacijom Akt-a i pokretanjem autofagije koja onda dovodi do odloženog i smanjenog citotoksičnog dejstva cisplatina (53). Upravo ovakav, za tumorske ćelije protektivni mehanizam kojim se štite od dejstva toksičnih jedinjenja i na taj način stiču rezistenciju, i ranije je opisivan u literaturi (54). Svim studijama je zajedničko da su korišćene koncentracije metformina u opsegu između 2 i 20 mM.

Rezultati studije koja je ispitivala sinergističko dejstvo metformina i paklitaksela su pokazali da koncentracije metformina od 100 µM i veće dovode do inhibicije p38 komponente MAPK signalnog puta te se na taj način ostvaruje potencijalno klinički značajan efekat (55). Slično, tokom in vitro istraživanja pokazano je da metformin u terapijskim koncentracijama (50 µM) dovodi do potencijacije efekta paklitaksela stimulišući proces apoptoze u tumorskim ćelijama NSCLC (56). Kombinovana terapija metforminom i pemetreksedom takođe potencira apoptozu i zaustavlja ciklus ćelija karcinoma pluća u S fazi (57).

Uticaj metformina na zračnu terapiju ćelija karcinoma pluća je ređe ispitivan, ali je pokazano da metformin u visokim koncentracijama povećava radiosenzitivnost *NSCLC* ćelija i to preko smanjene transkripcije antioksidantnih proteina i zaustavljanja deobe u G2/M fazi ćelijskog ciklusa (58), dok je u drugom istraživanju pokazano da i koncentracije niže od 100 µM povećavaju osetljivost na radijaciju putem aktivacije AMPK i inhibicije Akt/mTOR signalnog puta (44). Takođe, postoje i podaci da metformin može povećati radiosenzitivišući efekat cisplatina, zavisno od tipa korišćene ćelijske linije NSCLC (59).

Jedini rad koji je pronađen pretragom literature, a koji se odnosi na primenu anti-PD1 pembrolizumaba i metformina (500 µM) na ćelije nesitnoćelijskog karcinoma pluća je pokazao povećanje citotoksičnog dejstva kao i povećanje aktivnosti citotoksičnih T limfocita prema tumorskim ćelijama u odnosu na primenu samog pembrolizumaba (60).

U *in vitro* studijama je ispitivan veći broj specifičnih i nespecifičnih tirozin kinaznih inhibitora, tj. uticaj metformina (koncentracije 1 mM i veće) na njihov antitumorski potencijal. U slučaju EGFR mutiranih NSCLC ćelija koje su rezistentne na TKI gefitinib i osimertinib, metformin dovodi do njihove resenzitizacije povećanjem apoptoze (61), ili aktivacijom AMPK i posledičnom inhibicijom ERK signalnog puta (62). Dokazana je pojačana apoptoza uz inhibiciju preuzimanja glukoze i smanjenje glikolize kod tretmana kombinacijom afatiniba i metformina (63). U slučaju stečene rezistencije na erlotinib, metformin doprinosi resenzitizaciji povećanjem ekspresije EGFR koji je hiperfosforilisan (64) te stoga dolazi do prekida prenosa proliferativnog signala. Dodatno, za gefitinib je pokazano da može

that autophagic cell death caused by metformin has not been examined in detail, although it is known that one of the main mechanisms of autophagy induction is the activation of AMPK and the resulting inhibition of PI3K/Akt/mTOR signaling pathway (42). In only one paper it has been shown that metformin activates apoptosis and simultaneously cytotoxic autophagy (43). An important fact related to previously mentioned studies is that the concentration of metformin used significantly exceeded the concentration that could be achieved in the bloodstream of patients who are treated with metformin. Namely, the concentrations used in laboratory setting were a hundred or more times higher (5-80 mM) in comparison to those measured in the plasma of patients who used metformin (20-50 μM). In *in vivo* conditions, such high concentrations of metformin would lead to lethal outcome as the consequence of severe forms of lactic acidosis. Only in one study the cytotoxic effect of clinically relevant concentrations (100 μM) of metformin was shown, while the mechanism was explained by the activation of AMPK and inhibition of Akt/mTOR signaling pathway (44). However, another group of authors have shown that therapeutic concentrations of metformin (50 μM) do not have the cytotoxic effect on the lung cancer cells, nor do they influence the mitochondrial membrane potential and production of ROS (45, 46). In addition, it has been shown that metformin in low concentrations (50 μM) does not have the expected effects on non-tumor cells – inhibition of respiratory chain and activation of AMPK (47), which can explain the inconsistency of results between preclinical (*in vitro*), and clinical studies.

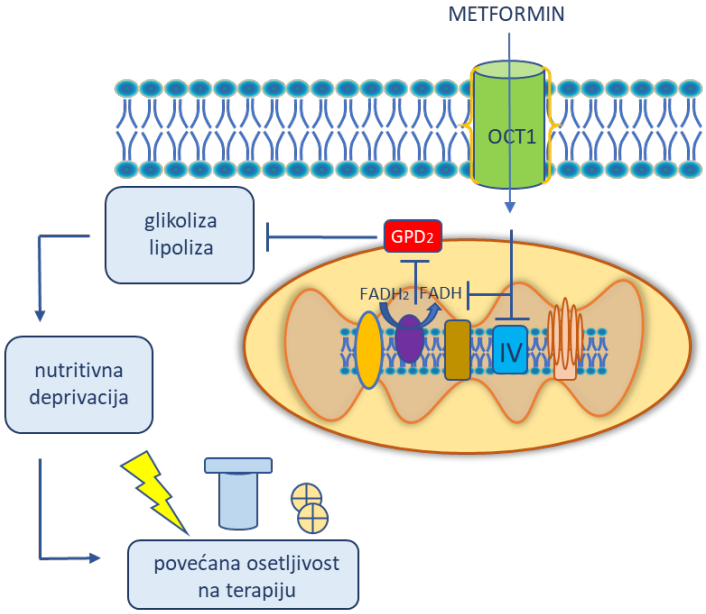
In studies that investigated the potential synergistic effect of metformin with chemotherapeutics used in the treatment of NSCLC, it has been shown that metformin increases the sensitivity of cells resistant to cisplatin, by inhibiting MAPK signaling pathway (48), activating the intrinsic pathway of apoptosis and oxidative stress (49), inhibiting mTOR independently of AMPK (50), or through p53 (51). In addition, metformin reduces the levels of aspartate and NAD in lung cancer cells, which can also be the explanation of increased cytotoxicity (52). On the other hand, one study has shown that the combined treatment with cisplatin and metformin modifies apoptosis by activating Akt and triggering autophagy, which then leads to the postponed and reduced effect of cisplatin (53). The same protective mechanism in which tumor cells protect themselves from the effect of toxic compounds and develop resistance, has been described in the literature before (54). Common for all these studies is that the concentrations of metformin used were between 2 and 20 mM.

The results of the study assessing the synergistic effect of metformin and paclitaxel have shown that concentrations of metformin of 100 μM and higher lead to the inhibition of p38 component of MAPK signaling pathway, and thus, the effect which is potentially clinically significant is achieved (55). Similarly, one *in vitro* study showed that metformin in therapeutic concentrations (50 μM) increases the potency of paclitaxel by stimulating the apoptotic process in NSCLC tumor cells (56). The combined therapy with metformin and pemetrexed also potentiates apoptosis and induces S phase cell cycle arrest (57).

The effect of metformin on radiotherapy of lung cancer cells has been rarely examined, but it has been shown that metformin in high concentrations increases radiosensitivity of NSCLC cells through the reduced transcription of antioxidant proteins and division arrest in G2/M phase of cell cycle (58), while in another study it has been shown that concentrations lower than 100 μM increase the sensitivity to radiation through AMPK activation and inhibition of Akt/mTOR signaling pathway (44). Also, there are data which show that metformin may increase the radiosensitizing effect of cisplatin, depending on the type of cell line of NSCLC (59).

The only paper which was found during the literature search which assessed the use of anti-PD1 agent pembrolizumab and metformin (500 μM) in the treatment of non-small cell lung cancer, showed the increase of cytotoxic effect, as well as the increase of activity of cytotoxic T-lymphocytes towards tumor cells in comparison to the use of pembrolizumab only (60).

In *in vitro* studies, a number of specific and non-specific tyrosine kinase inhibitors was investigated, examining the influence of metformin (concentrations 1 mM and higher) on their antitumor potential. In EGFR-mutated NSCLC cells that are resistant to TKIs gefitinib and osimertinib, metformin leads to their resensitization by increasing apoptosis (61), or activating AMPK and consequently inhibitiing ERK signaling pathway



OCT1 – organski katjonski transporter 1; GPD2 – glicerol-3-fosfat dehidrogenaza 2;
FAD – flavin-adenin dinukleotid; $FADH_2$ – dihidroflavin-adenin dinukleotid

Slika 2. Pretpostavljeni mehanizam citotoksičnog dejstva metformina inhibicijom GPD2

pokrenuti autofagiju u ćelijama karcinoma pluća koja je mogući mehanizam nastanka rezistencije, pri čemu dodatak metformina inhibicijom pokrenute autofagije povećava apoptozu (65).

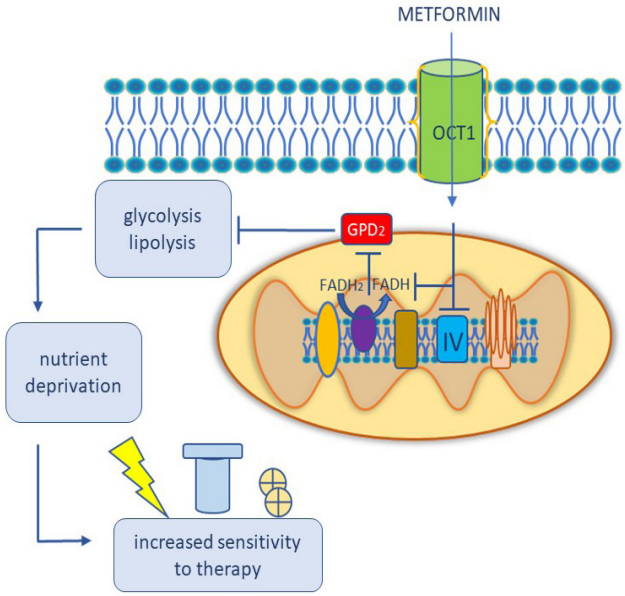
Rezultati studija koje su ispitivale dejstvo metformina na efekte krizotiniba kod ALK+ ćelija NSCLC su nekonzistentni - sa jedne strane je pokazano da metformin omogućava prevazilaženje rezistencije (66), dok druga studija ukazuje na to da ne postoji sinergistički efekat (67). Ćelije karcinoma pluća koje poseduju NRAS i BRAF mutacije su u slučaju tretmana metforminom i trametinibom pokazivale veći procenat smrtnosti i to inhibicijom Akt/mTOR od strane metformina i inhibicijom MAPK signalnog puta od strane trametiniba (40,68).

Novija istraživanja ukazuju da terapijske koncentracije metformina dovode do remećenja metaboličkog statusa tumorskih ćelija inhibicijom mitohondrijalne glicero-3-fosfat dehidrogenaze (GPD2) (69). Ovaj enzim posredstvom energetskih supstrata stimuliše procese poput glikolize i lipolize i kao takav se u većoj meri aktivira u tumorskim ćelijama zadovoljavajući povišene energetske zahteve neophodne za brzu deobu ovih ćelija (70). Samim tim inhibicija GPD2 od strane metformina potencijalno uvodi tumorsku ćeliju u nutritivnu deprivaciju, čineći je osetljivijom na efekat konvencionalnih terapijskih protokola (Slika 2). Uticaj metformina na GPD2 i eventualna povezanost sa citotoksičnim efektima na ćelijama NSCLC još uvek nije ispitivana.

Zaključak

Uprkos uvođenju novih terapijskih pristupa u lečenju uznapredovalih stadijuma NSCLC, veliki problem i dalje predstavljaju inicijalno neadekvatan odogovor kao i pojava rezistencije na terapiju što uslovljava rekurenciju i progresiju tumorskih promena. U cilju uvođenja lekova koji potencijalno mogu poboljšati odgovor na konvencionalne terapijske modalitete, antidijabetik metformin koji je tokom pretkliničkih i retrospektivnih opservacionih studija pokazao obećavajuće rezultate se ispituje u brojnim kliničkim studijama kao mogući dodatak terapiji. Ono što karakteriše većinu do sada sprovedenih prospektivnih studija je mali broj ispitanika i neadekvatna komplijantnost prema terapiji metforminom, verovatno uslovljena pojavom neželjenih efekata poput mučnine i dijareje, kao i činjenicom da se radi o grupi pacijenata koji nisu oboleli od *Diabetes mellitus*-a.

Kada su u pitanju molekularni mehanizmi kojima metformin potencijalno ostvaruje povoljne efekte na NSCLC, u literaturi se sreću različiti i povremeno suprotni rezultati. Sinergizam sa hemio- i radioterapijom je pokazan, dok su uključeni signalni putevi najčešće AMPK i Akt/mTOR. Veliki nedostatak većine pretkliničkih studija je genetička raznorodnost ćelijskih linija koje se koriste u eksperimentima, kao i nepostojanje konsenzusa oko odabira koncentracija metformina za *in vitro* istraživanja čije bi vrednosti bile primenljive i u *in vivo* studijama.



OCT1 – organic cation transporter 1; GPD2 – glycerol-3-dehydrogenase 2;
FAD – flavine-adenine dinucleotide; $FADH_2$ – dihydroflavine-adenine dinucleotide

Figure 2. Suggested cytotoxic mechanism of metformin mediated by GPD2 inhibition

(62). Increased apoptosis was shown with the inhibition of glucose uptake and reduction of glicolysis in the combined treatment with afatinib and metformin (63). In case of acquired resistance to erlotinib, metformin contributes to the resensitization by increasing the expression of EGFR that is hyperphosphorylated (64), and therefore, to the disruption of transduction of proliferative signal. In addition, it has been shown that gefitinib triggers autophagy in lung cancer cells, this possibly being the resistance mechanism, while the addition of metformin increases apoptosis by inhibiting triggered autophagy (65).

The results of studies which investigated the influence of metformin on the effects of crizotinib in ALK+ NSCLC cells are not consistent – on the one hand it has been shown that metformin makes it possible to overcome resistance (66), whereas another study points to the fact that there is no synergistic effect (67). Lung cancer cells with NRAS and BRAF mutations that were treated with metformin and trametinib showed decreased viability explained by Akt/mTOR inhibition by metformin and MAPK signaling pathway inhibition by trametinib (40, 68).

Latest studies have shown that therapeutic concentrations of metformin lead to the disruption of metabolic status of tumor cells via the inhibition of mitochondrial glycerol-3-phosphate dehydrogenase (GDP2) (69). This enzyme stimulates the processes such as glycolysis and lipolysis, and is significantly activated in tumor cells, satisfying the increased energy demands necessary for the fast division of these cells (70). Therefore, the inhibition of GPD2 caused by metformin potentially causes nutrient deprivation of tumor cells, making them more sensitive to the effect of conventional therapeutic protocols (Picture 2). The influence of metformin on GPD2 and potential link with the cytotoxic effects on NSCLC cells have not been examined yet.

Conclusion

Although new therapeutic approaches have been introduced in the treatment of advanced stages of NSCLC, the initially inadequate response to therapy, as well as the occurrence of resistance still presents a great problem, which causes the recurrence and disease progression. In order to use drugs that may potentially improve the response to conventional treatment modalities, the antidiabetic metformin, which has showed promising results in preclinical and retrospective observational studies, is being examined in numerous clinical studies as the possible addition to therapy. Prospective clinical studies, which have been conducted so far, are characterized by a small number of participants and inadequate compliance with metformin therapy, which is probably due to side effects such as nausea and

Na osnovu do sada objavljenih rezultata prospektivnih kliničkih i pretkliničkih studija, efekti primene metformina u nesitnoćelijskom karcinomu pluća i dalje nisu razjašnjeni te se ne može sa sigurnošću doneti zaključak da li metformin dovodi do promene efekata standardnog lečenja i koji su ćelijski mehanizmi ovih promena. Stoga je neophodno da buduće kliničke studije obuhvate veći broj ispitanika koji su jasno selektovani, kao i da iste budu osmišljene kao dvostruko slepe sa upotrebom placeba, jer bi se na taj način isključili različiti vidovi pristrasnosti. Dizajn pretkliničkih studija je potrebno unaprediti uz jasniji odabir ćelijskih linija koje se koriste, kao i odabir klinički relevantnih koncentracija metformina, a kako bi dobijeni rezultati imali veću šansu za ponovljivost u kliničkim okolnostima.

Konflikt interesa

Autori su izjavili da nema konflikta interesa.

Literatura

1. IARC. Cancer Incidence, Mortality and Prevalence Worldwide GLOBOCAN 2020. Available at: http://gco.iarc.fr/ (20.07.2022.)
2. Malhotra J, Malvezzi M, Negri E, Vecchia CL, Boffetta P. Risk factors for lung cancer worldwide. Eur Respir J 2016; 48: 889–902. doi: 10.1183/13993003.00359-2016.
3. Chen N, Zhou YS, Wang LC, Huang JB. Advances in metformin based metabolic therapy for non small cell lung cancer (Review). Oncol Rep 2022; 47(3):55. doi: 10.3892/or.2022.8266.
4. Postmus PE, Kerr KM, Oudkerk M, Senan S, Waller DA, Vansteenkiste J, et al. Early and locally advanced non-small-cell lung cancer (NSCLC): ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up†. Ann Oncol 2017; 28(Suppl 4):iv1-iv21. doi: 10.1093/annonc/mdx222.
5. Milašinović G. Nacionalni vodič dobre kliničke prakse: Karcinom pluća. Beograd: Republička stručna komisija za izradu i implementaciju vodiča dobre kliničke prakse, Ministarstvo zdravlja Republike Srbije; 2012.
6. Planchard D, Popat S, Kerr K, Novello S, Smit EF, Faivre-Finn C, et al. ESMO Guidelines Committee. Metastatic non-small cell lung cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol 2018; 29(Suppl 4):iv192-iv237. doi: 10.1093/annonc/mdy275.
7. Reck M, Rodriguez-Abreu D, Robinson AG, Hui R, Csőszi T, Fülöp A, et al. Pembrolizumab versus chemotherapy for PD-L1-positive non-small-cell lung cancer. N Engl J Med 2016; 375:1823–33. doi: 10.1056/NEJMoa1606774.
8. Mok T, Carbone D, Hirsch F. IASLC Atlas of EGFR testing in Lung cancer. North Fort Myers, Fl, USA: Editorial Rx press, 2017. Available at: http://wclc2017.iaslc.org/
9. Van der Wekken AJ, Pelgrim R, t Hart N, Werner N, Mastik MF, Hendriks L, et al. Dichotomous ALK-IHC is a better predictor for ALK inhibition outcome than traditional ALK-FISH in advanced non-small cell lung cancer. Clin Cancer Res 2017; 23:4251–8. doi: 10.1158/1078-0432.CCR-16-1631.
10. Lindeman NI, Cagle PT, Aisner DL, Arcila ME, Beasley MB, Bernicker EH, et al. Updated molecular testing guideline for the selection of lung cancer patients for treatment with targeted tyrosine kinase inhibitors: guideline from the college of American Pathologists, the International Association for the Study of Lung Cancer, and the Association for Molecular Pathology. J Thorac Oncol 2018; 13:323–58. doi: 10.5858/arpa.2017-0388-CP
11. American Cancer Society. Cancer Facts & Figures 2022. Atlanta: American Cancer Society; 2022.
12. Chuang MC, Yang YH, Tsai YH, Hsieh MJ, Lin YC, Lin CK, et al. Survival benefit associated with metformin use in inoperable non-small cell lung cancer patients with diabetes: A population-based retrospective cohort study. PLoS One 2018; 13(1):e0191129. doi: 10.1371/journal.pone.0191129.
13. Tian RH, Zhang YG, Wu Z, Liu X, Yang JW, Ji HL. Effects of metformin on survival outcomes of lung cancer patients with type 2 diabetes mellitus: a meta-analysis. Clin Transl Oncol 2016; 18:641-9. doi: 10.1007/s12094-015-1412-x
14. Wink KC, Belderbos JS, Dieleman EM, Rossi M, Rasch CR, Damhuis RA, et al. Improved progression free survival for patients with diabetes and locally advanced non-small cell lung cancer (NSCLC) using metformin during concurrent chemoradiotherapy Radiother Oncol 2016; 118:453-9. doi: 10.1016/j.radonc.2016.01.012
15. Wang JL, Tsai YT, Lin CH, Cidem A, Staniczek T, Chang GR, et al. Benefits of Metformin Combined with Pemetrexed-Based Platinum Doublets as a First-Line Therapy for Advanced Lung Adenocarcinoma Patients with Diabetes. Biomolecules 2021; 11(8):1252. doi: 10.3390/biom11081252.
16. Tan BX, Yao WX, Ge J, Peng XC, Du XB, Zhang R, et al. Prognostic influence of metformin as first-line chemotherapy for advanced nonsmall cell lung cancer in patients with type 2 diabetes. Cancer 2011; 117:5103-11. doi: 10.1002/cncr.26151
17. Chen H, Yao W, Chu Q, Han R, Wang Y, Sun J, et al. Synergistic effects of metformin in combination with EGFR-TKI in the treatment of patients with advanced non-small cell lung cancer and type 2 diabetes. Cancer Lett 2015; 369: 97-102. doi: 10.1016/j.canlet.2015.08.024
18. Rao M, Gao C, Guo M, Law BYK, Xu Y. Effects of metformin treatment on radiotherapy efficacy in patients with cancer and diabetes: a systematic review and meta-analysis. Cancer Manag Res 2018; 10:4881-90. doi: 10.2147/CMAR.S174535.
19. Sackett D. Bias in analytic research. J Chronic Dis 1979; 32(1):51-63. Available at: https://doi.org/10.1016/B978-0-08-024907-0.50013-4 (30.8.2022.)
20. Sayed R, Saad AS, El Wakeel L, Elkholy E, Badary O. Metformin Addition to Chemotherapy in Stage IV Non-

diarrhea, and to the fact that this group includes patients who do not have Diabetes Mellitus.

With regard to the molecular mechanisms of metformin that potentially have favorable effects on NSCLC, different and occasionally opposing results may be seen in literature. Synergistic effects with chemo- and radiotherapy have been shown, while most frequently involved signaling pathways are AMPK and Akt/mTOR. The significant limitation of most preclinical studies is genetic diversity of cell lines that are used in experiments, as well as the absence of consensus regarding the concentration of metformin used for *in vitro* studies, with levels that would be applicable in *in vivo* studies, as well.

According to the results of prospective clinical and preclinical studies that have been published so far, the effects of the use of metformin in non-small cell lung cancer have not been fully elucidated, and therefore it cannot be concluded with certainty whether metformin leads to the change of effects of standard treatment and what are the cell mechanisms of these changes. Therefore, future clinical studies should necessarily include more participants, with clear enrollment criteria, and they should be designed as double-blind with placebo controls, thus excluding different types of bias. Design of preclinical studies should be necessarily improved with the clearer selection of cell lines to be used, and the selection of clinically relevant concentrations of metformin, so that obtained results would have a greater chance to be repeated in clinical conditions.

Competing interests

Authors declare no competing interests.

Literature

1. IARC. Cancer Incidence, Mortality and Prevalence Worldwide GLOBOCAN 2020. Available at: http://gco.iarc.fr/ (20.07.2022.)
2. Malhotra J, Malvezzi M, Negri E, Vecchia CL, Boffetta P. Risk factors for lung cancer worldwide. Eur Respir J 2016; 48: 889–902. doi: 10.1183/13993003.00359-2016.
3. Chen N, Zhou YS, Wang LC, Huang JB. Advances in metformin based metabolic therapy for non small cell lung cancer (Review). Oncol Rep 2022; 47(3):55. doi: 10.3892/or.2022.8266.
4. Postmus PE, Kerr KM, Oudkerk M, Senan S, Waller DA, Vansteenkiste J, et al. Early and locally advanced non-small-cell lung cancer (NSCLC): ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up†. Ann Oncol 2017; 28(Suppl 4):iv1-iv21. doi: 10.1093/annonc/mdx222.
5. Milašinović G. Nacionalni vodič dobre kliničke prakse: Karcinom pluća. Beograd: Republička stručna komisija za izradu i implementaciju vodiča dobre kliničke prakse, Ministarstvo zdravlja Republike Srbije; 2012.
6. Planchard D, Popat S, Kerr K, Novello S, Smit EF, Faivre-Finn C, et al. ESMO Guidelines Committee. Metastatic non-small cell lung cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol 2018; 29(Suppl 4):iv192-iv237. doi: 10.1093/annonc/mdy275.
7. Reck M, Rodriguez-Abreu D, Robinson AG, Hui R, Csőszi T, Fülöp A, et al. Pembrolizumab versus chemotherapy for PD-L1-positive non-small-cell lung cancer. N Engl J Med 2016; 375:1823–33. doi: 10.1056/NEJMoa1606774.
8. Mok T, Carbone D, Hirsch F. IASLC Atlas of EGFR testing in Lung cancer. North Fort Myers, Fl, USA: Editorial Rx press, 2017. Available at: http://wclc2017.iaslc.org/
9. Van der Wekken AJ, Pelgrim R, t Hart N, Werner N, Mastik MF, Hendriks L, et al. Dichotomous ALK-IHC is a better predictor for ALK inhibition outcome than traditional ALK-FISH in advanced non-small cell lung cancer. Clin Cancer Res 2017; 23:4251–8. doi: 10.1158/1078-0432.CCR-16-1631.
10. Lindeman NI, Cagle PT, Aisner DL, Arcila ME, Beasley MB, Bernicker EH, et al. Updated molecular testing guideline for the selection of lung cancer patients for treatment with targeted tyrosine kinase inhibitors: guideline from the college of American Pathologists, the International Association for the Study of Lung Cancer, and the Association for Molecular Pathology. J Thorac Oncol 2018; 13:323–58. doi: 10.5858/arpa.2017-0388-CP
11. American Cancer Society. Cancer Facts & Figures 2022. Atlanta: American Cancer Society; 2022.
12. Chuang MC, Yang YH, Tsai YH, Hsieh MJ, Lin YC, Lin CK, et al. Survival benefit associated with metformin use in inoperable non-small cell lung cancer patients with diabetes: A population-based retrospective cohort study. PLoS One 2018; 13(1):e0191129. doi: 10.1371/journal.pone.0191129.
13. Tian RH, Zhang YG, Wu Z, Liu X, Yang JW, Ji HL. Effects of metformin on survival outcomes of lung cancer patients with type 2 diabetes mellitus: a meta-analysis. Clin Transl Oncol 2016; 18:641-9. doi: 10.1007/s12094-015-1412-x
14. Wink KC, Belderbos JS, Dieleman EM, Rossi M, Rasch CR, Damhuis RA, et al. Improved progression free survival for patients with diabetes and locally advanced non-small cell lung cancer (NSCLC) using metformin during concurrent chemoradiotherapy Radiother Oncol 2016; 118:453-9. doi: 10.1016/j.radonc.2016.01.012
15. Wang JL, Tsai YT, Lin CH, Cidem A, Staniczek T, Chang GR, et al. Benefits of Metformin Combined with Pemetrexed-Based Platinum Doublets as a First-Line Therapy for Advanced Lung Adenocarcinoma Patients with Diabetes. Biomolecules 2021; 11(8):1252. doi: 10.3390/biom11081252.
16. Tan BX, Yao WX, Ge J, Peng XC, Du XB, Zhang R, et al. Prognostic influence of metformin as first-line

Small Cell Lung Cancer: an Open Label Randomized Controlled Study. Asian Pac J Cancer Prev 2015; 16(15):6621-6. doi: 10.7314/apjcp.2015.16.15.6621.

21. Marrone KA, Zhou X, Forde PM, Purtell M, Brahmer JR, Hann CL, et al. A Randomized Phase II Study of Metformin plus Paclitaxel/Carboplatin/Bevacizumab in Patients with Chemotherapy-Naïve Advanced or Metastatic Nonsquamous Non-Small Cell Lung Cancer. Oncologist 2018; 23(7):859-65. doi: 10.1634/theoncologist.2017-0465.
22. Lee Y, Joo J, Lee YJ, Lee EK, Park S, Kim TS et al. Randomized phase II study of platinum-based chemotherapy plus controlled diet with or without metformin in patients with advanced non-small cell lung cancer. Lung Cancer 2021; 151:8-15. doi: 10.1016/j.lungcan.2020.11.011.
23. Tsakiridis T, Pond GR, Wright J, Ellis PM, Ahmed N, Abdulkarim B, et al. Metformin in Combination With Chemoradiotherapy in Locally Advanced Non–Small Cell Lung Cancer: The OCOG-ALMERA Randomized Clinical Trial. JAMA Oncol 2021; 7(9):1333–41. doi:10.1001/jamaoncol.2021.2328
24. Skinner H, Hu C, Tsakiridis T, Santana-Davila R, Lu B, Erasmus JJ, et al. Addition of Metformin to Concurrent Chemoradiation in Patients With Locally Advanced Non–Small Cell Lung Cancer: The NRG-LU001 Phase 2 Randomized Clinical Trial. JAMA Oncol 2021; 7(9):1324–32. doi:10.1001/jamaoncol.2021.2318
25. Parikh AB, Marrone KA, Becker DJ, Brahmer JR, Ettinger DS, Levy BP. A pooled analysis of two phase II trials evaluating metformin plus platinum-based chemotherapy in advanced non-small cell lung cancer. Cancer Treat Res Commun 2019; 20:100150. doi: 10.1016/j.ctarc.2019.100150.
26. Chun SG, Liao Z, Jeter MD, Chang JY, Lin SH, Komaki RU et al. Metabolic Responses to Metformin in Inoperable Early-stage Non-Small Cell Lung Cancer Treated With Stereotactic Radiotherapy: Results of a Randomized Phase II Clinical Trial. Am J Clin Oncol 2020; 43(4):231-5. doi: 10.1097/COC.0000000000000632.
27. Arrieta O, Barrón F, Padilla MS, Avilés-Salas A, Ramírez-Tirado LA, Arguelles Jiménez MJ, et al. Effect of Metformin Plus Tyrosine Kinase Inhibitors Compared With Tyrosine Kinase Inhibitors Alone in Patients With Epidermal Growth Factor Receptor-Mutated Lung Adenocarcinoma: A Phase 2 Randomized Clinical Trial. JAMA Oncol 2019; 5(11):e192553. doi: 10.1001/jamaoncol.2019.2553.
28. Li L, Liyan J, Yubo W, Yizhuo Z, Xiao-Ju Z, Guoming W, et al. Combination of Metformin and Gefitinib as First-Line Therapy for Nondiabetic Advanced NSCLC Patients with EGFR Mutations: A Randomized, Double-Blind Phase II Trial. Clin Cancer Res 2019; 25 (23): 6967–75. Available at: https://doi.org/10.1158/1078-0432.CCR-19-0437
29. Chae Y. Clinical trial: Nivolumab and Metformin Hydrochloride in Treating Patients With Stage III-IV Non-small Cell Lung Cancer That Cannot Be Removed by Surgery, NCT03048500. Available at: https://clinicaltrials.gov/ct2/show/results/NCT03048500 (26.07.2022.)
30. Liu S, Polsdofer EV, Zhou L, Ruan S, Lyu H, Hou D, et al. Upregulation of endogenous TRAIL-elicited apoptosis is essential for metformin-mediated antitumor activity against TNBC and NSCLC. Mol Ther Oncolytics 2021; 21:303-314. doi: 10.1016/j.omto.2021.04.012.
31. Zhou X, Liu S, Lin X, Xu L, Mao X, Liu J, et al. Metformin Inhibit Lung Cancer Cell Growth and Invasion in Vitro as Well as Tumor Formation in Vivo Partially by Activating PP2A. Med Sci Monit 2019; 25:836-46. doi: 10.12659/MSM.912059.
32. Kumar A, Malik F, Bhushan S, Sethi VK, Shahi AK, Kaur J, et al. An essential oil and its major constituent isointermedeol induce apoptosis by increased expression of mitochondrial cytochrome c and apical death receptors in human leukaemia HL-60 cells. Chem Biol Interact 2008; 171:332–47. doi: 10.1016/j.cbi.2007.10.003.
33. Isakovic AM, Petricevic SM, Ristic SM, Popadic DM, Kravic-Stevovic TK, Zogovic NS, et al. In vitro and in vivo antimelanoma effect of ethyl ester cyclohexyl analog of ethylenediamine dipropanoic acid. Melanoma Res 2018; 28(1):8-20. doi: 10.1097/CMR.0000000000000409.
34. Guo Q, Liu Z, Jiang L, Liu M, Ma J, Yang C, et al. Metformin inhibits growth of human non-small cell lung cancer cells via liver kinase B-1-independent activation of adenosine monophosphate-activated protein kinase. Mol Med Rep 2016; 13(3):2590-6. doi: 10.3892/mmr.2016.4830.
35. Jin DH, Kim Y, Lee BB, Han J, Kim HK, Shim YM, Kim DH. Metformin induces cell cycle arrest at the G1 phase through E2F8 suppression in lung cancer cells. Oncotarget 2017; 8(60):101509-19. doi: 10.18632/oncotarget.21552.
36. Zhou X, Liu S, Lin X, Xu L, Mao X, Liu J, et al. Metformin Inhibit Lung Cancer Cell Growth and Invasion in Vitro as Well as Tumor Formation in Vivo Partially by Activating PP2A. Med Sci Monit 2019; 25:836-46. doi: 10.12659/MSM.912059.
37. Luo Z, Zhu T, Luo W, Lv Y, Zhang L, Wang C. et al. Metformin induces apoptotic cytotoxicity depending on AMPK/PKA/GSK-3β-mediated c-FLIPL degradation in non-small cell lung cancer. Cancer Manag Res 2019; 11:681-9. doi: 10.2147/CMAR.S178688.
38. Owen MR, Doran E, Halestrap AP. Evidence that metformin exerts its anti-diabetic effects through inhibition of complex 1 of the mitochondrial respiratory chain. Biochem J 2000; 348 Pt 3:607-14.
39. El-Mir MY, Nogueira V, Fontaine E, Avéret N, Rigoulet M, Leverve X. Dimethylbiguanide inhibits cell respiration via an indirect effect targeted on the respiratory chain complex I. J Biol Chem 2000; 275(1):223-8. doi: 10.1074/jbc.275.1.223.
40. Ko E, Baek S, Kim J, Park D, Lee Y. Antitumor Activity of Combination Therapy with Metformin and Trametinib in Non-Small Cell Lung Cancer Cells. Dev Reprod 2020; 24(2):113-23. doi: 10.12717/DR.2020.24.2.113.
41. Jang SK, Hong SE, Lee DH, Kim JY, Kim JY, Hong J, et al Inhibition of AKT Enhances the Sensitivity of NSCLC Cells to Metformin. Anticancer Res 2021; 41(7):3481-7. doi: 10.21873/anticanres.15135.

chemotherapy for advanced nonsmall cell lung cancer in patients with type 2 diabetes. Cancer 2011; 117:5103-11. doi: 10.1002/cncr.26151

17. Chen H, Yao W, Chu Q, Han R, Wang Y, Sun J, et al. Synergistic effects of metformin in combination with EGFR-TKI in the treatment of patients with advanced non-small cell lung cancer and type 2 diabetes. Cancer Lett 2015; 369: 97-102. doi: 10.1016/j.canlet.2015.08.024
18. Rao M, Gao C, Guo M, Law BYK, Xu Y. Effects of metformin treatment on radiotherapy efficacy in patients with cancer and diabetes: a systematic review and meta-analysis. Cancer Manag Res 2018; 10:4881-90. doi: 10.2147/CMAR.S174535.
19. Sackett D. Bias in analytic research. J Chronic Dis 1979; 32(1):51-63. Available at: https://doi.org/10.1016/B978-0-08-024907-0.50013-4 (30.8.2022.)
20. Sayed R, Saad AS, El Wakeel L, Elkholy E, Badary O. Metformin Addition to Chemotherapy in Stage IV Non-Small Cell Lung Cancer: an Open Label Randomized Controlled Study. Asian Pac J Cancer Prev 2015; 16(15):6621-6. doi: 10.7314/apjcp.2015.16.15.6621.
21. Marrone KA, Zhou X, Forde PM, Purtell M, Brahmer JR, Hann CL, et al. A Randomized Phase II Study of Metformin plus Paclitaxel/Carboplatin/Bevacizumab in Patients with Chemotherapy-Naïve Advanced or Metastatic Nonsquamous Non-Small Cell Lung Cancer. Oncologist 2018; 23(7):859-65. doi: 10.1634/theoncologist.2017-0465.
22. Lee Y, Joo J, Lee YJ, Lee EK, Park S, Kim TS et al. Randomized phase II study of platinum-based chemotherapy plus controlled diet with or without metformin in patients with advanced non-small cell lung cancer. Lung Cancer 2021; 151:8-15. doi: 10.1016/j.lungcan.2020.11.011.
23. Tsakiridis T, Pond GR, Wright J, Ellis PM, Ahmed N, Abdulkarim B, et al. Metformin in Combination With Chemoradiotherapy in Locally Advanced Non–Small Cell Lung Cancer: The OCOG-ALMERA Randomized Clinical Trial. JAMA Oncol 2021; 7(9):1333–41. doi:10.1001/jamaoncol.2021.2328
24. Skinner H, Hu C, Tsakiridis T, Santana-Davila R, Lu B, Erasmus JJ, et al. Addition of Metformin to Concurrent Chemoradiation in Patients With Locally Advanced Non–Small Cell Lung Cancer: The NRG-LU001 Phase 2 Randomized Clinical Trial. JAMA Oncol 2021; 7(9):1324–32. doi:10.1001/jamaoncol.2021.2318
25. Parikh AB, Marrone KA, Becker DJ, Brahmer JR, Ettinger DS, Levy BP. A pooled analysis of two phase II trials evaluating metformin plus platinum-based chemotherapy in advanced non-small cell lung cancer. Cancer Treat Res Commun 2019; 20:100150. doi: 10.1016/j.ctarc.2019.100150.
26. Chun SG, Liao Z, Jeter MD, Chang JY, Lin SH, Komaki RU et al. Metabolic Responses to Metformin in Inoperable Early-stage Non-Small Cell Lung Cancer Treated With Stereotactic Radiotherapy: Results of a Randomized Phase II Clinical Trial. Am J Clin Oncol 2020; 43(4):231-5. doi: 10.1097/COC.0000000000000632.
27. Arrieta O, Barrón F, Padilla MS, Avilés-Salas A, Ramírez-Tirado LA, Arguelles Jiménez MJ, et al. Effect of Metformin Plus Tyrosine Kinase Inhibitors Compared With Tyrosine Kinase Inhibitors Alone in Patients With Epidermal Growth Factor Receptor-Mutated Lung Adenocarcinoma: A Phase 2 Randomized Clinical Trial. JAMA Oncol 2019; 5(11):e192553. doi: 10.1001/jamaoncol.2019.2553.
28. Li L, Liyan J, Yubo W, Yizhuo Z, Xiao-Ju Z, Guoming W, et al. Combination of Metformin and Gefitinib as First-Line Therapy for Nondiabetic Advanced NSCLC Patients with EGFR Mutations: A Randomized, Double-Blind Phase II Trial. Clin Cancer Res 2019; 25 (23): 6967–75. Available at: https://doi.org/10.1158/1078-0432.CCR-19-0437
29. Chae Y. Clinical trial: Nivolumab and Metformin Hydrochloride in Treating Patients With Stage III-IV Non-small Cell Lung Cancer That Cannot Be Removed by Surgery, NCT03048500. Available at: https://clinicaltrials.gov/ct2/show/results/NCT03048500 (26.07.2022.)
30. Liu S, Polsdofer EV, Zhou L, Ruan S, Lyu H, Hou D, et al. Upregulation of endogenous TRAIL-elicited apoptosis is essential for metformin-mediated antitumor activity against TNBC and NSCLC. Mol Ther Oncolytics 2021; 21:303-314. doi: 10.1016/j.omto.2021.04.012.
31. Zhou X, Liu S, Lin X, Xu L, Mao X, Liu J, et al. Metformin Inhibit Lung Cancer Cell Growth and Invasion in Vitro as Well as Tumor Formation in Vivo Partially by Activating PP2A. Med Sci Monit 2019; 25:836-46. doi: 10.12659/MSM.912059.
32. Kumar A, Malik F, Bhushan S, Sethi VK, Shahi AK, Kaur J, et al. An essential oil and its major constituent isointermedeol induce apoptosis by increased expression of mitochondrial cytochrome c and apical death receptors in human leukaemia HL-60 cells. Chem Biol Interact 2008; 171:332–47. doi: 10.1016/j.cbi.2007.10.003.
33. Isakovic AM, Petricevic SM, Ristic SM, Popadic DM, Kravic-Stevovic TK, Zogovic NS, et al. In vitro and in vivo antimelanoma effect of ethyl ester cyclohexyl analog of ethylenediamine dipropanoic acid. Melanoma Res 2018; 28(1):8-20. doi: 10.1097/CMR.0000000000000409.
34. Guo Q, Liu Z, Jiang L, Liu M, Ma J, Yang C, et al. Metformin inhibits growth of human non-small cell lung cancer cells via liver kinase B-1-independent activation of adenosine monophosphate-activated protein kinase. Mol Med Rep 2016; 13(3):2590-6. doi: 10.3892/mmr.2016.4830.
35. Jin DH, Kim Y, Lee BB, Han J, Kim HK, Shim YM, Kim DH. Metformin induces cell cycle arrest at the G1 phase through E2F8 suppression in lung cancer cells. Oncotarget 2017; 8(60):101509-19. doi: 10.18632/oncotarget.21552.
36. Zhou X, Liu S, Lin X, Xu L, Mao X, Liu J, et al. Metformin Inhibit Lung Cancer Cell Growth and Invasion in Vitro as Well as Tumor Formation in Vivo Partially by Activating PP2A. Med Sci Monit 2019; 25:836-46. doi: 10.12659/MSM.912059.
37. Luo Z, Zhu T, Luo W, Lv Y, Zhang L, Wang C. et al. Metformin induces apoptotic cytotoxicity depending on AMPK/PKA/GSK-3β-mediated c-FLIPL degradation in non-small cell lung cancer. Cancer Manag Res 2019; 11:681-9. doi: 10.2147/CMAR.S178688.

42. Li Y, Chen Y. AMPK and Autophagy. Adv Exp Med Biol 2019; 1206:85-108. doi: 10.1007/978-981-15-0602-4_4.
43. Nazim UM, Moon JH, Lee JH, Lee YJ, Seol JW, Eo SK, et al. Activation of autophagy flux by metformin downregulates cellular FLICE-like inhibitory protein and enhances TRAIL-induced apoptosis. Oncotarget. 2016; 7(17):23468-81. doi: 10.18632/oncotarget.8048.
44. Storozhuk Y, Hopmans SN, Sanli T, Barron C, Tsiani E, Cutz JC, et al. Metformin inhibits growth and enhances radiation response of non-small cell lung cancer (NSCLC) through ATM and AMPK. Br J Cancer 2013; 108(10):2021-32. doi: 10.1038/bjc.2013.187.
45. Ljubičić J, Pešić A, Pavlović K, Isaković A. Metformin effect on viability and mitochondrial status of tumor and non-tumor cell line. Oral presentation. Craiova Internatinal Medical Student's Congress, Craiova, Romania; 11-14 November 2021.
46. Isaković A, Ljubičić J, Pavlović K, Krako N, Misirlić-Denčić S. The effect of metformin on human non-small cell lung carcinoma cells: the role of mitochondria. Poster presentation. ENABLE Conference - The 3rd European Phd and Postdoc Symposium, Nijmegen, Netherlands; 13-15 November, 2019. Available at: https://www.irbbarcelona.org/ca/events/enable-conference-3rd-european-phd-and-postdoc-symposium.
47. Pavlovic K, Krako Jakovljevic N, Isakovic AM, Ivanovic T, Markovic I, Lalic NM. Therapeutic vs. Supra pharmacological Metformin Concentrations: Different Effects on Energy Metabolism and Mitochondrial Function in Skeletal Muscle Cells in vitro. Front Pharmacol 2022; 13:930308. doi: 10.3389/fphar.2022.930308.
48. Wang Y, Lin B, Wu J, Zhang H, Wu B. Metformin inhibits the proliferation of A549/CDDP cells by activating p38 mitogen-activated protein kinase. Oncol Lett. 2014 Sep;8(3):1269-1274. doi: 10.3892/ol.2014.2270. Epub 2014 Jun 19. PMID: 25120704; PMCID: PMC4114589.
49. Huang S, He T, Yang S, Sheng H, Tang X, Bao F, et al. Metformin reverses chemoresistance in non-small cell lung cancer via accelerating ubiquitination-mediated degradation of Nrf2. Transl Lung Cancer Res 2020; 9(6):2337-55. doi: 10.21037/tlcr-20-1072.
50. Morelli AP, Tortelli TC Jr, Pavan ICB, Silva FR, Granato DC, Peruca GF, et al. Metformin impairs cisplatin resistance effects in A549 lung cancer cells through mTOR signaling and other metabolic pathways. Int J Oncol 2021; 58(6):28. doi: 10.3892/ijo.2021.5208.
51. Tortelli TC, Tamura RE, de Souza Junqueira M, da Silva Mororó J, Bustos SO, Natalino RJM, et al. Metformin-induced chemosensitization to cisplatin depends on P53 status and is inhibited by Jarid1b overexpression in non-small cell lung cancer cells. Aging (Albany NY) 2021; 13(18):21914-40. doi: 10.18632/aging.203528.
52. Tully E, Bharti S, Woo J, Bhujwalla Z, Gabrielson E. Biguanide drugs enhance cytotoxic effects of cisplatin by depleting aspartate and NAD+ in sensitive cancer cells. Cancer Biol Ther 2021; 22(10-12):579-86. doi:10.1080/15384047.2021.1982599.
53. Xiao Z, Gaertner S, Morresi-Hauf A, Genzel R, Duell T, Ullrich A. et al. Metformin Triggers Autophagy to Attenuate Drug-Induced Apoptosis in NSCLC Cells, with Minor Effects on Tumors of Diabetic Patients. Neoplasia 2017; 19(5):385-95. doi: 10.1016/j.neo.2017.02.011.
54. Isakovic AM, Dulovic M, Markovic I, Kravic-Stevovic T, Bumbasirevic V, Trajkovic V. et al. Autophagy suppression sensitizes glioma cells to IMP dehydrogenase inhibition-induced apoptotic death. Exp Cell Res 2017; 350(1):32-40. doi: 10.1016/j.yexcr.2016.11.001.
55. Tseng SC, Huang YC, Chen HJ, Chiu HC, Huang YJ, Wo TY, et al Metformin-mediated downregulation of p38 mitogen-activated protein kinase-dependent excision repair cross-complementing 1 decreases DNA repair capacity and sensitizes human lung cancer cells to paclitaxel. Biochem Pharmacol 2013; 85(4):583-94. doi: 10.1016/j.bcp.2012.12.001.
56. Ljubičić J, Pešić A, Pavlović K, Isaković A. Impact of metformin on cytotoxic effect of paclitaxel, in vitro. Oral presentation. The16th YES Meeting, Porto, Portugal; 18th September, 2021.
57. Zhang Y, Feng X, Li T, Yi E, Li Y. Metformin synergistic pemetrexed suppresses non-small-cell lung cancer cell proliferation and invasion in vitro. Cancer Med 2017; 6(8):1965-75. doi: 10.1002/cam4.1133.
58. Sun X, Dong M, Gao Y, Wang Y, Du L, Liu Y, et al. Metformin increases the radiosensitivity of non-small cell lung cancer cells by destabilizing NRF2. Biochem Pharmacol 2022; 199:114981. doi: 10.1016/j.bcp.2022.114981.
59. Riaz MA, Sak A, Erol YB, Groneberg M, Thomale J, Stuschke M. Metformin enhances the radiosensitizing effect of cisplatin in non-small cell lung cancer cell lines with different cisplatin sensitivities. Sci Rep 2019; 9(1):1282. doi: 10.1038/s41598-018-38004-5.
60. Wang Z, Lu C, Zhang K, Lin C, Wu F, Tang X, et al. Metformin Combining PD-1 Inhibitor Enhanced Anti-Tumor Efficacy in STK11 Mutant Lung Cancer Through AXIN-1-Dependent Inhibition of STING Ubiquitination. Front Mol Biosci 2022; 9:780200. doi: 10.3389/fmolb.2022.780200.
61. Han R, Jia Y, Li X, Zhao C, Zhao S, Liu S, et al. Concurrent use of metformin enhances the efficacy of EGFR-TKIs in patients with advanced EGFR-mutant non-small cell lung cancer-an option for overcoming EGFR-TKI resistance. Transl Lung Cancer Res 2021; 10(3):1277-91. doi: 10.21037/tlcr-20-1153.
62. Li L, Wang T, Hu M, Zhang Y, Chen H, Xu L. Metformin Overcomes Acquired Resistance to EGFR TKIs in EGFR-Mutant Lung Cancer via AMPK/ERK/NF-κB Signaling Pathway. Front Oncol 2020; 10:1605. doi: 10.3389/fonc.2020.01605.
63. Barrios-Bernal P, Hernandez-Pedro N, Orozco-Morales M, Viedma-Rodríguez R, Lucio-Lozada J, Avila-Moreno F, et al. Metformin Enhances TKI-Afatinib Cytotoxic Effect, Causing Downregulation of Glycolysis, Epithelial-Mesenchymal Transition, and EGFR-Signaling Pathway Activation in Lung Cancer Cells. Pharmaceuticals (Basel) 2022; 15(3):381. doi: 10.3390/ph15030381.

38. Owen MR, Doran E, Halestrap AP. Evidence that metformin exerts its anti-diabetic effects through inhibition of complex 1 of the mitochondrial respiratory chain. Biochem J 2000; 348 Pt 3:607-14.
39. El-Mir MY, Nogueira V, Fontaine E, Avéret N, Rigoulet M, Leverve X. Dimethylbiguanide inhibits cell respiration via an indirect effect targeted on the respiratory chain complex I. J Biol Chem 2000; 275(1):223-8. doi: 10.1074/jbc.275.1.223.
40. Ko E, Baek S, Kim J, Park D, Lee Y. Antitumor Activity of Combination Therapy with Metformin and Trametinib in Non-Small Cell Lung Cancer Cells. Dev Reprod 2020; 24(2):113-23. doi: 10.12717/DR.2020.24.2.113.
41. Jang SK, Hong SE, Lee DH, Kim JY, Kim JY, Hong J, et al Inhibition of AKT Enhances the Sensitivity of NSCLC Cells to Metformin. Anticancer Res 2021; 41(7):3481-7. doi: 10.21873/anticanres.15135.
42. Li Y, Chen Y. AMPK and Autophagy. Adv Exp Med Biol 2019; 1206:85-108. doi: 10.1007/978-981-15-0602-4_4.
43. Nazim UM, Moon JH, Lee JH, Lee YJ, Seol JW, Eo SK, et al. Activation of autophagy flux by metformin downregulates cellular FLICE-like inhibitory protein and enhances TRAIL-induced apoptosis. Oncotarget. 2016; 7(17):23468-81. doi: 10.18632/oncotarget.8048.
44. Storozhuk Y, Hopmans SN, Sanli T, Barron C, Tsiani E, Cutz JC, et al. Metformin inhibits growth and enhances radiation response of non-small cell lung cancer (NSCLC) through ATM and AMPK. Br J Cancer 2013; 108(10):2021-32. doi: 10.1038/bjc.2013.187.
45. Ljubičić J, Pešić A, Pavlović K, Isaković A. Metformin effect on viability and mitochondrial status of tumor and non-tumor cell line. Oral presentation. Craiova Internatinal Medical Student's Congress, Craiova, Romania; 11-14 November 2021.
46. Isaković A, Ljubičić J, Pavlović K, Krako N, Misirlić-Denčić S. The effect of metformin on human non-small cell lung carcinoma cells: the role of mitochondria. Poster presentation. ENABLE Conference - The 3rd European Phd and Postdoc Symposium, Nijmegen, Netherlands; 13-15 November, 2019. Available at: https://www.irbbarcelona.org/ca/events/enable-conference-3rd-european-phd-and-postdoc-symposium.
47. Pavlovic K, Krako Jakovljevic N, Isakovic AM, Ivanovic T, Markovic I, Lalic NM. Therapeutic vs. Supra pharmacological Metformin Concentrations: Different Effects on Energy Metabolism and Mitochondrial Function in Skeletal Muscle Cells in vitro. Front Pharmacol 2022; 13:930308. doi: 10.3389/fphar.2022.930308.
48. Wang Y, Lin B, Wu J, Zhang H, Wu B. Metformin inhibits the proliferation of A549/CDDP cells by activating p38 mitogen-activated protein kinase. Oncol Lett. 2014 Sep;8(3):1269-1274. doi: 10.3892/ol.2014.2270. Epub 2014 Jun 19. PMID: 25120704; PMCID: PMC4114589.
49. Huang S, He T, Yang S, Sheng H, Tang X, Bao F, et al. Metformin reverses chemoresistance in non-small cell lung cancer via accelerating ubiquitination-mediated degradation of Nrf2. Transl Lung Cancer Res 2020; 9(6):2337-55. doi: 10.21037/tlcr-20-1072.
50. Morelli AP, Tortelli TC Jr, Pavan ICB, Silva FR, Granato DC, Peruca GF, et al. Metformin impairs cisplatin resistance effects in A549 lung cancer cells through mTOR signaling and other metabolic pathways. Int J Oncol 2021; 58(6):28. doi: 10.3892/ijo.2021.5208.
51. Tortelli TC, Tamura RE, de Souza Junqueira M, da Silva Mororó J, Bustos SO, Natalino RJM, et al. Metformin-induced chemosensitization to cisplatin depends on P53 status and is inhibited by Jarid1b overexpression in non-small cell lung cancer cells. Aging (Albany NY) 2021; 13(18):21914-40. doi: 10.18632/aging.203528.
52. Tully E, Bharti S, Woo J, Bhujwalla Z, Gabrielson E. Biguanide drugs enhance cytotoxic effects of cisplatin by depleting aspartate and NAD+ in sensitive cancer cells. Cancer Biol Ther 2021; 22(10-12):579-86. doi:10.1080/15384047.2021.1982599.
53. Xiao Z, Gaertner S, Morresi-Hauf A, Genzel R, Duell T, Ullrich A. et al. Metformin Triggers Autophagy to Attenuate Drug-Induced Apoptosis in NSCLC Cells, with Minor Effects on Tumors of Diabetic Patients. Neoplasia 2017; 19(5):385-95. doi: 10.1016/j.neo.2017.02.011.
54. Isakovic AM, Dulovic M, Markovic I, Kravic-Stevovic T, Bumbasirevic V, Trajkovic V. et al. Autophagy suppression sensitizes glioma cells to IMP dehydrogenase inhibition-induced apoptotic death. Exp Cell Res 2017; 350(1):32-40. doi: 10.1016/j.yexcr.2016.11.001.
55. Tseng SC, Huang YC, Chen HJ, Chiu HC, Huang YJ, Wo TY, et al Metformin-mediated downregulation of p38 mitogen-activated protein kinase-dependent excision repair cross-complementing 1 decreases DNA repair capacity and sensitizes human lung cancer cells to paclitaxel. Biochem Pharmacol 2013; 85(4):583-94. doi: 10.1016/j.bcp.2012.12.001.
56. Ljubičić J, Pešić A, Pavlović K, Isaković A. Impact of metformin on cytotoxic effect of paclitaxel, in vitro. Oral presentation. The16th YES Meeting, Porto, Portugal; 18th September, 2021.
57. Zhang Y, Feng X, Li T, Yi E, Li Y. Metformin synergistic pemetrexed suppresses non-small-cell lung cancer cell proliferation and invasion in vitro. Cancer Med 2017; 6(8):1965-75. doi: 10.1002/cam4.1133.
58. Sun X, Dong M, Gao Y, Wang Y, Du L, Liu Y, et al. Metformin increases the radiosensitivity of non-small cell lung cancer cells by destabilizing NRF2. Biochem Pharmacol 2022; 199:114981. doi: 10.1016/j.bcp.2022.114981.
59. Riaz MA, Sak A, Erol YB, Groneberg M, Thomale J, Stuschke M. Metformin enhances the radiosensitizing effect of cisplatin in non-small cell lung cancer cell lines with different cisplatin sensitivities. Sci Rep 2019; 9(1):1282. doi: 10.1038/s41598-018-38004-5.
60. Wang Z, Lu C, Zhang K, Lin C, Wu F, Tang X, et al. Metformin Combining PD-1 Inhibitor Enhanced Anti-Tumor Efficacy in STK11 Mutant Lung Cancer Through AXIN-1-Dependent Inhibition of STING Ubiquitination. Front Mol Biosci 2022; 9:780200. doi: 10.3389/fmolb.2022.780200.
61. Han R, Jia Y, Li X, Zhao C, Zhao S, Liu S, et al. Concurrent use of metformin enhances the efficacy of EGFR-TKIs in patients with advanced EGFR-mutant non-small cell lung

64. Wang X, Chen K, Yu Y, Xiang Y, Kim JH, Gong W, et al. Metformin sensitizes lung cancer cells to treatment by the tyrosine kinase inhibitor erlotinib. Oncotarget 2017; 8(65):109068-78. doi: 10.18632/oncotarget.22596.

65. Chen H, Wang Y, Lin C, Lu C, Han R, Jiao L, et al. Vorinostat and metformin sensitize EGFR-TKI resistant NSCLC cells via BIM-dependent apoptosis induction. Oncotarget 2017; 8(55):93825-38. doi: 10.18632/oncotarget.21225.

66. Li L, Wang Y, Peng T, Zhang K, Lin C, Han R, et al. Metformin restores crizotinib sensitivity in crizotinib-resistant human lung cancer cells through inhibition of IGF1-R signaling pathway. Oncotarget 2016; 7(23):34442-52. doi: 10.18632/oncotarget.9120.

67. Bland AR, Shrestha N, Bower RL, Rosengren RJ, Ashton JC. The effect of metformin in EML4-ALK+ lung cancer alone and in combination with crizotinib in cell and rodent models. Biochem Pharmacol 2021; 183:114345. doi: 10.1016/j.bcp.2020.114345.

68. Vujic I, Sanlorenzo M, Posch C, Esteve-Puig R, Yen AJ, Kwong A, et al. Metformin and trametinib have synergistic effects on cell viability and tumor growth in NRAS mutant cancer. Oncotarget 2015; 6(2):969-78. doi: 10.18632/oncotarget.2824.

69. LaMoia TE, Shulman GI. Cellular and Molecular Mechanisms of Metformin Action. Endocr Rev 2021; 42(1):77-96. doi: 10.1210/endrev/bnaa023.

70. Chowdhury SK, Gemin A, Singh G. High activity of mitochondrial glycerophosphate dehydrogenase and glycerophosphate-dependent ROS production in prostate cancer cell lines. Biochem Biophys Res Commun 2005; 333:1139–45.

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cancer-an option for overcoming EGFR-TKI resistance. Transl Lung Cancer Res 2021; 10(3):1277-91. doi: 10.21037/tlcr-20-1153.

62. Li L, Wang T, Hu M, Zhang Y, Chen H, Xu L. Metformin Overcomes Acquired Resistance to EGFR TKIs in EGFR-Mutant Lung Cancer via AMPK/ERK/NF-κB Signaling Pathway. Front Oncol 2020; 10:1605. doi: 10.3389/fonc.2020.01605.
63. Barrios-Bernal P, Hernandez-Pedro N, Orozco-Morales M, Viedma-Rodríguez R, Lucio-Lozada J, Avila-Moreno F, et al. Metformin Enhances TKI-Afatinib Cytotoxic Effect, Causing Downregulation of Glycolysis, Epithelial-Mesenchymal Transition, and EGFR-Signaling Pathway Activation in Lung Cancer Cells. Pharmaceuticals (Basel) 2022; 15(3):381. doi: 10.3390/ph15030381.
64. Wang X, Chen K, Yu Y, Xiang Y, Kim JH, Gong W, et al. Metformin sensitizes lung cancer cells to treatment by the tyrosine kinase inhibitor erlotinib. Oncotarget 2017; 8(65):109068-78. doi: 10.18632/oncotarget.22596.
65. Chen H, Wang Y, Lin C, Lu C, Han R, Jiao L, et al. Vorinostat and metformin sensitize EGFR-TKI resistant NSCLC cells via BIM-dependent apoptosis induction. Oncotarget 2017; 8(55):93825-38. doi: 10.18632/oncotarget.21225.
66. Li L, Wang Y, Peng T, Zhang K, Lin C, Han R, et al. Metformin restores crizotinib sensitivity in crizotinib-resistant human lung cancer cells through inhibition of IGF1-R signaling pathway. Oncotarget 2016; 7(23):34442-52. doi: 10.18632/oncotarget.9120.
67. Bland AR, Shrestha N, Bower RL, Rosengren RJ, Ashton JC. The effect of metformin in EML4-ALK+ lung cancer alone and in combination with crizotinib in cell and rodent models. Biochem Pharmacol 2021; 183:114345. doi: 10.1016/j.bcp.2020.114345.
68. Vujic I, Sanlorenzo M, Posch C, Esteve-Puig R, Yen AJ, Kwong A, et al. Metformin and trametinib have synergistic effects on cell viability and tumor growth in NRAS mutant cancer. Oncotarget 2015; 6(2):969-78. doi: 10.18632/oncotarget.2824.
69. LaMoia TE, Shulman GI. Cellular and Molecular Mechanisms of Metformin Action. Endocr Rev 2021; 42(1):77-96. doi: 10.1210/endrev/bnaa023.
70. Chowdhury SK, Gemin A, Singh G. High activity of mitochondrial glycerophosphate dehydrogenase and glycerophosphate-dependent ROS production in prostate cancer cell lines. Biochem Biophys Res Commun 2005; 333:1139–45.

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HIPERBARIČNA OKSIGENACIJA KAO PRETRETMAN I TRETMAN U ISHEMIJSKO-REPERFUZIJSKOM OŠTEĆENJU

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SAŽETAK

Ishemija tkiva podrazumeva nedovoljan dotok krvi u određeno područje tela. Prekid arterijskog snabdevanja krvlju dovodi do dizbalansa između metaboličkih potreba i potražnje i razvoja hipoksije tkiva. Hipoksija tkiva indukuje brojne metaboličke promene koje rezultuju inflamacijom, povećanim stvaranjem slobodnih kiseoničnih vrsta i smrću ćelije. Ukoliko se u ishemijskom tkivu uspostavi adekvatan protok krvi doći će do povećanja ćelijskog oštećenja što se označava kao ishemijsko-reperfuzijska povreda. Ishemija i ishemijsko-reperfuzijska povreda nalaze se u osnovni brojnih oboljenja široko zastupljenih u savremenom društvu, poput infarkta miokarda, cerebralnog insulta, akutnog bubrežnog oštećenja. Za sada ne postoji način da se utiče direktno na ćelijsku hipoksiju već je kliničko lečenje hipoksičnih stanja usmereno na modulaciju globalne hipoksemije i povećanje količine kiseonika rastvorenog u krvi. Hiperbarična oksigenacija (HBO) je tretman tokom kog bolesnik udiše 100% kiseonik pod pritiskom od najmanje 1,4 atmosfere. Iako je upotreba hiperbarične terapije zabeležena još u 17.veku, danas je ovaj tretman odobren za mali broj indikacija.

Ključne reči: ishemijsko-reperfuzijsko oštećenje, hiperbarična oksigenacija, hipoksija, ishemija.

Uvod

Ishemija tkiva podrazumeva nedovoljan dotok krvi u određeno područje tela. Uzroci mogu biti anatomski - zapušenje krvnog suda embolusom, ateroskleroza i tromboza aterosklerotskog plaka, kompresija krvnog suda otokom, tumorom, torzija vaskularne peteljke ili funkcionalni - hemoragijski šok, srčana insuficijencija, vaskularni spazam (1). Prekid arterijskog snabdevanja krvlju dovodi do disbalansa između metaboličkih potreba i potražnje i razvoja hipoksije tkiva. Usled hipoksije tkiva nastaje ćelijsko oštećenje koje u zavisnosti od trajanja ishemije može biti reverzibilno ili ireverzibilno (1-2). Reverzibilnost povrede zavisi od sposobnosti mitohondrija da proizvedu ATP (3). Ponovno uspostavljanje toka krvi u ishemijskom tkivu, paradoksalno, dovodi do povećanja ćelijskog oštećenja što se označava kao ishemijsko-reperfuzijska povreda (1,2,4,5). Ishemijsko-reperfuzijska povreda povezana je sa teškim kliničkim manifestacijama poput infarkta miokarda, infarkta mozga, akutnog bubrežnog oštećenjenja, kompartment sindroma. Napretkom medicine, u svakodnevnu kliničku praksu uvedene su savremene metode revaskularizacije ishemijskih područja poput perkutane angioplastike, bajpas hirurgije, transplantacije organa, te je značajno smanjena smrtnost od ishemijskih povreda (4). Međutim, sada u prvi plan dolazi ishemijsko-reperfuzijsko oštećenje, jer za sada ne postoji tretman koji smanjuje smrt ćelija izazvanu ovom povredom (6). Kako se hipoksija nalazi u osnovi ishemijskih povreda, korišćenje kiseonika u njihovom tretmanu nameće se kao logično rešenje. Za sada ne postoji način da se utiče direktno na ćelijsku hipoksiju, već je kliničko

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HYPERBARIC OXYGENATION AS THE PRETREATMENT AND THERAPY IN ISCHEMIA-REPERFUSION INJURY

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SUMMARY

Tissue ischemia means insufficient blood flow to a certain area of the body. Interruption of the arterial blood supply leads to an imbalance between metabolic supply and demand and the development of tissue hypoxia. Tissue hypoxia induces metabolic changes that result in inflammation, increased production of reactive oxygen species, and cell death. If adequate blood flow is established in the ischemic tissue, there will be an increase in cellular damage, which is referred to as ischemic-reperfusion injury. Ischemia and ischemia-reperfusion injury are at the root of numerous diseases widely present in modern society, such as myocardial infarction, cerebral insult, acute kidney injury. For now, there is no way to directly affect cellular hypoxia, but the clinical treatment of hypoxic conditions is aimed at modulating global hypoxemia and increasing the amount of oxygen dissolved in the blood. Hyperbaric oxygenation (HBO) is a treatment during which the patient breathes 100% oxygen under a pressure of at least 1.4 atmospheres. Although the use of hyperbaric therapy was recorded as early as the 17th century, today this treatment is approved for a few indications.

Key words: ischemia-reperfusion injury, hyperbaric oxygenation, hipoxia, ischemia.

Introduction

Tissue ischemia is the insufficient blood flow to a certain area of the body. The causes may be anatomical – the blockage of a blood vessel by an embolus, atherosclerosis and thrombosis of atherosclerotic plaque, compression of blood vessels caused by edema, tumor, torsion of vascular pedicle or functional – hemorrhagic shock, heart insufficiency, vascular spasm (1). The interruption of arterial blood supply leads to the imbalance between metabolic supply and demand and the development of tissue hypoxia. Tissue hypoxia induces cell damage, which can be reversible or irreversible depending on the duration of ischemia (1-2). The reversibility of injury depends on the ability of mitochondria to produce ATP (3). The restoration of blood flow in the ischemic tissue paradoxically leads to an increase in cellular damage, which is characterized as ischemia-reperfusion injury (1-2,4-5). Ischemia-reperfusion injury is associated with severe clinical manifestations such as myocardial infarction, stroke, acute kidney injury, compartment syndrome. With the advances made in the field of medicine, contemporary methods of revascularization of ischemic areas have been introduced into daily clinical practice, including percutaneous angioplasty, bypass surgery, organ transplantation, and therefore, the mortality due to ischemic injury has been significantly reduced (4). However, ischemia-reperfusion injury is now brought to the forefront because so far there have been no therapies which reduce cell death caused by this injury (6). Since hypoxia is at the root of ischemic injuries, the use of oxygen for the treatment is imposed as a logical solution. For now,

lečenje hipoksičnih stanja usmereno na modulaciju globalne hipoksemije i povećanje količine kiseonika rastvorenog u krvi (8,9).

Hiperbarična oksigenacija (HBO) je tretman tokom kog bolesnik udiše 100% kiseonik pod pritiskom od najmanje 1,4 atmosfere (10). Prva dokumentovana upotreba hiperbarične terapije zabeležena je 1662. godine od strane britanskog lekara *Henshaw*-a, koji je bolesnike smeštao u kontejner sa vazduhom pod pritiskom sa idejom da se povišeni pritisak koristi u tretmanu nekih akutnih, a sniženi u tretmanu hroničnih oboljenja. Dalje tokom istorije, terapija kiseonikom pod pritiskom korišćena je u različite svrhe – lečenje dekompresijske bolesti kod ronilaca u Drugom svetskom ratu, kao supstitucija elektrokonvulzivnoj terapiji kod shizofreničara, za povećanje senzitivnosti tumorskih ćelija pre radioterapije (11). Danas, je tretman HBO odobren od strane Američke asocijacije za hranu i lekove (engl. *Food and Drug Administration, FDA*) za trinaest indikacija, a neke od njih su: nezarastajuće rane, teške opekotine, nekrotišući fasciitis, gasna gangrena, kraš povreda, dekompresioni sindrom, trovanje ugljen monoksidom, teške anemija koje se ne mogu korigovati transfuzijom (12), (tabela 1). Korišćenje HBO u terapiji drugih oboljenja još uvek je u fazi ispitivanja (13,14). Cilj ovog istraživanja je da pruži uvid u do sada poznate i pokazane efekte pretretmana i tretmana hiperbaričnom oksigenacijom tokom lečenja ishemijsko-reperfuzijskih povreda.

Metode

U ovom preglednom radu, radi što preciznijeg i sveobuhvatnijeg prikaza efekata pretretmana i tretmana hiperbarične oksigenacije na ishemijsko-reperfuzijske povrede, korišćena je literatura dobijena pretraživanjem MEDLINE baze podataka uz pomoć servisa PUBMED. Literatura objavljena na engleskom jeziku, u poslednjih 10 godina, dobijena je pretraživanjem sledećih ključnih reči: ishemijsko-reperfuzijsko oštećenje, hiperbarična oksigenacija, pretretman, neuroprotektivni efekat, akutno bubrežno oštećenje, hipoksija, ishemija.

Ishemija i ishemijsko-reperfuzijska povreda

Ishemija tkiva nalazi se u osnovi brojnih oboljenja kao što su infarkt miokarda, ishemijska kardiomiopatija, cerebralni insult, akutno bubrežno oštećenje, komparment sindrom, hronične kožne ulceracije (4,5). U ishemijskim tkivima nedovoljan dotok kiseonika dovodi do metaboličkih promena

Tabela 1. Odobrene indikacije za tretman hiperbarčnom oksigenacijom

1.	Gasna embolija
2.	Anemija (teška anemija kada se transfuzija ne može koristiti)
3.	Opekotine (teške i velike opekotine)
4.	Trovanje ugljen monoksidom
5.	Kraš povrede
6.	Dekompresivni sindrom
7.	Gasna gangrene
8.	Gubitak sluha (potpuni gubitak sluha, koji se javlja iznenada i bez poznatog uzroka)
9.	Teške infekcije kože i kostiju
10.	Radijacione povrede
11.	Kožni transplantat sa rizikom od ishemije
12.	Gubitak vida (iznenadan i bezbolan gubitak vida na jednom oku zbog blokade krvotoka)
13.	Rane (nezarastajuće rane, dijabetični čirevi stopala)

there is no way to directly affect cellular hypoxia, but the clinical treatment of hypoxic conditions is aimed at modulating global hypoxemia and increasing the amount of oxygen dissolved in the blood (8,9).

Hyperbaric oxygenation is a treatment during which a patient breathes 100% oxygen under pressure of at least 1.4 absolute atmospheres. The first reported uses of hyperbaric therapy date back to 1662, when a British doctor named Henshaw put patients into the chamber with the air under pressure. He believed that the increased air pressure was used for the treatment of acute diseases, while low pressure was used to treat chronic diseases. Further, during history hyperbaric treatment was used for different purposes – the treatment of decompression sickness in divers during the Second World War, or as an alternative for electro-convulsive therapy in schizophrenia, and in order to increase sensitivity of tumor cells before radiotherapy (11). Today, HBO treatment has been approved by Food and Drug Agency of the United States of America for thirteen indications, including the following: non-healing wounds, severe burns, necrotizing fasciitis, gas gangrene, crush injury, decompression sickness, carbon monoxide poisoning, severe anemia when blood transfusion cannot be used (Table 1) (12). The use of HBO in the treatment of other diseases is still in the research phase (13,14). The aim of this study is to give insights into the effects of HBO pre-treatment and treatment in ischemia-reperfusion injuries, which have been presented so far.

Methods

In this review article, in order to present the effects of HBO pre-treatment and therapy on ischemia-reperfusion injuries in a precise and comprehensive way, we used literature that was obtained through a search of MEDLINE database with the help of PUBMED service. The literature has been published in the English language in the last ten years and it was obtained through a search of the following words: ischemia-reperfusion injury, hyperbaric oxygenation, preconditioning, neuroprotection, acute kidney injury.

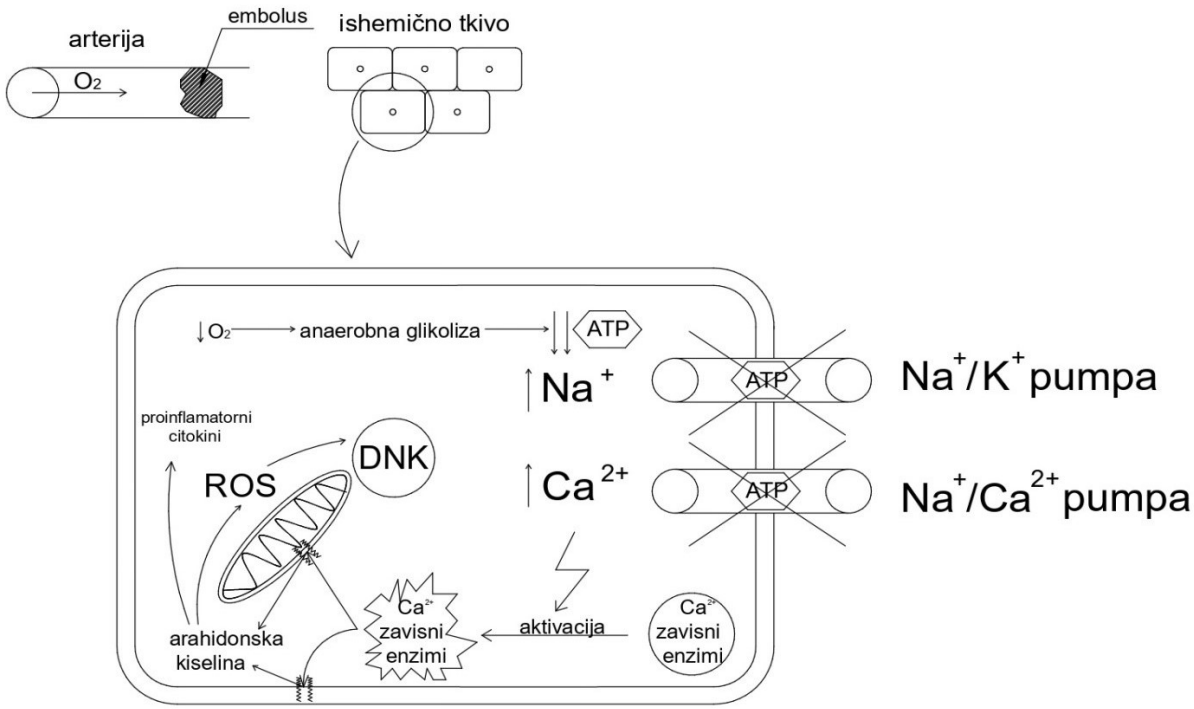
Ischemia and ischemia-reperfusion injury

Tissue ischemia is at the root of many diseases such as myocardial infarction, ischemic cardiomyopathy, cerebral insult, acute kidney injury, compartment syndrome, chronic skin ulcerations (4,5). In the ischemic tissues, the

Tabela 1. Approved indications for hyperbaric oxygenation therapy

1.	Air and gas bubbles in blood vessels
2.	Anemia (severe anemia whem blood transfusion cannot be used)
3.	Burns (severe and large burns treated at a specialized burn center)
4.	Carbon monoxide poisoning
5.	Crush injury
6.	Decompression sickness (diving risk)
7.	Gas gangrene
8.	Hearing loss (complete hearing loss that occurs suddenly and without any known cause)
9.	Infection of the skin and bone (severe)
10.	Radiation injury
11.	Skin graft flap at risk of tissue death
12.	Vision loss (when sudden and painless in one eye due to blockage of blood flow)
13.	Wounds (non-healing, diabetic foot ulcers)

– postepeno se smanjuje oksidativni metabolizam, a energija se dobija anaerobnom razgradnjom glukoze (2,5). Anaerobnim metabolizmom, proizvodi se manja količina energije, remeti se rad jonskih pumpi (natrijum-kalijumove, natrijum-kalcijumove, natrijum-vodonične) pa se u ćeliji nakupljaju kalcijum (Ca^{2+}), natrijum (Na^{+}) i vodonik (H^{+}), smanjuje se unutarćelijska pH, povećava se njena osmolarnost i ćelija bubri. Smanjenju unutarćelijske pH doprinosi i povećanje koncentracije NADH koji indukuje prelazak piruvata u laktat. Povećanje koncentracije Ca^{2+} aktivira Ca^{2+} zavisne ATP-aze, fosfolipaze koje oštećuju ćelijsku i mitohondrijsku membranu. Iz membrana se oslobađa arahidonska kiselina od koje nastaju proinflamatorni citokini i slobodne kiseonične vrste (engl. *Reactive oxygen species, ROS*) (2,5,15) (slika1). Pored povećanog stvaranja ROS-a, dodatan problem je smanjenje aktivnosti antioksidativnih enzima – superoksid dizmutaze, katalaze, glutation peroksidaze. Ovaj disbalans vodi ćeliju u smrt (15). Poseban vid ćelijske povrede predstavlja postishemijsko oštećenje. Naime, iako je tokom ishemije neophodna brza reperfuzija tkiva, paradoksalno, ponovno uspostavljanje krvotoka u tkivu u kom već postoji ćelijska povreda dovodi do povećanog stvaranja ROS-a posredstvom tri glavna sistema - sistema ksantin oksidaze, sistema NADPH oksidaze i sistema sintaze azot monoksida (engl. *Nitric oxide synthases, NOS*) (16). Ksantin oksidaza i ksantin dehidrogenaza su enzimi koji učestvuju u metabolizmu purina. Ksantin dehidrogenaza koristi NAD^{+} kao krajnji primalac elektrona, dok ksantin oksidaza koristi O_2 kao primalac elektrona. U ishemijskom tkivu, zbog smanjene količine ATP-a dolazi do pretvaranja ksantin dehidrogenaze u ksantin oksidazu. Tokom reperfuzije tkiva, novonastala ksantin oksidaza vrši pretvaranje hipoksantina u ksantin koristeći kiseonik kao krajnji primalac elektrona pri čemu nastaju superoksidni anjon i vodonik peroksid (17). U stanju hipoksije dolazi do pojačane aktivnosti hipoksijom indukovanog faktora 1 alfa (engl. *Hypoxia-inducible factor-1-alpha, HIF-1α*) koji aktivira enzime iz porodice NADPH oksidaza. Ovi enzimi kao krajnji primalac elektrona koriste kiseonik pri čemu nastaju superoksidni anjon i vodonik peroksid koji izazivaju oksidativni stres (18,19). Pozitivnom povratnom spregom, oksidativni stres dalje dovodi do povećane aktivnosti HIF- 1α. Ponovnim uspostavljanjem krvotoka u ishemijskom tkivu dolazi do oslobađanja brojnih hemijskih medijatora kao što su fosfolipaza A2, interferon γ, interleukin 1 β koji povećavaju aktivaciju NADPH oksidaza i dalje promovišu nastanak



Slika 1. Shematski prikaz ćelijskih promena u ishemijskom tkivu

insufficient oxygen flow leads to metabolic changes – oxidative metabolism is gradually reduced, while energy is obtained by anaerobic dissolution of glucose (2,5). Anaerobic metabolism produces the smaller amount of energy, disturbs the work of ion pumps (sodium-potassium, sodium-calcium, sodium-hydrogen), and therefore, calcium (Ca^{2+}), sodium (Na^{+}), and hydrogen (H^{+}) are accumulated in the cell, decreases cellular pH, thus causing its hyperosmolarity and cell swelling. The increase in the concentration of NADH, which induces the conversion of pyruvate to lactate, contributes to the decrease of cellular pH. The increase in Ca^{2+} concentration activates Ca^{2+} dependant ATP-ase, phospholipase that damage cell and mitochondrial membrane. Arachidonic acid is released from membranes, thus producing the proinflammatory cytokines and reactive oxygen species (ROS) (2,5,15) (Figure 1). In addition to the increased production of ROS, the decrease of antioxidative enzymes activities is also problematic – superoxide dismutase, catalase, glutathione peroxidase. This imbalance leads to cell death (15). A specific type of cell injury is post-ischemic injury. Namely, although fast tissue reperfusion is needed during ischemia, paradoxically, the restoration of blood flow in the already ischemic tissue leads to the increased production of ROS through three main systems – the xanthine oxidase system, the NADPH oxidase system, nitric oxide synthases system (NOS) (16). Xanthine oxidase and xanthine dehydrogenase are enzymes that take part in purine metabolism. Xanthine dehydrogenase uses NAD+ as the final electron acceptor, while xanthine oxidase uses O_2 as the electron acceptor. In the ischemic tissue, due to the reduced amount of ATP, xanthine dehydrogenase is shifted to xanthine oxidase. During the tissue reperfusion, the newly created xanthine oxidase induces hypoxanthine to form xanthine, using oxygen as the final electron acceptor, during which superoxide anion and hydrogen peroxide are released (17). In the hypoxic state, the activity of hypoxia-inducible factor-1-alpha (HIF-1α) increases and it activates enzymes from the family NADPH oxidase. These enzymes use oxygen as the final electron receptor, while superoxide anion and hydrogen peroxide, which cause oxidative stress, are released (18,19). Due to the positive feedback loop, oxidative stress further leads to the increased activity of HIF-1α. The restoration of blood flow in the ischemic tissue causes the release of numerous chemical mediators such as phospholipase A2, interferon γ, interleukin 1ß which increase the activation

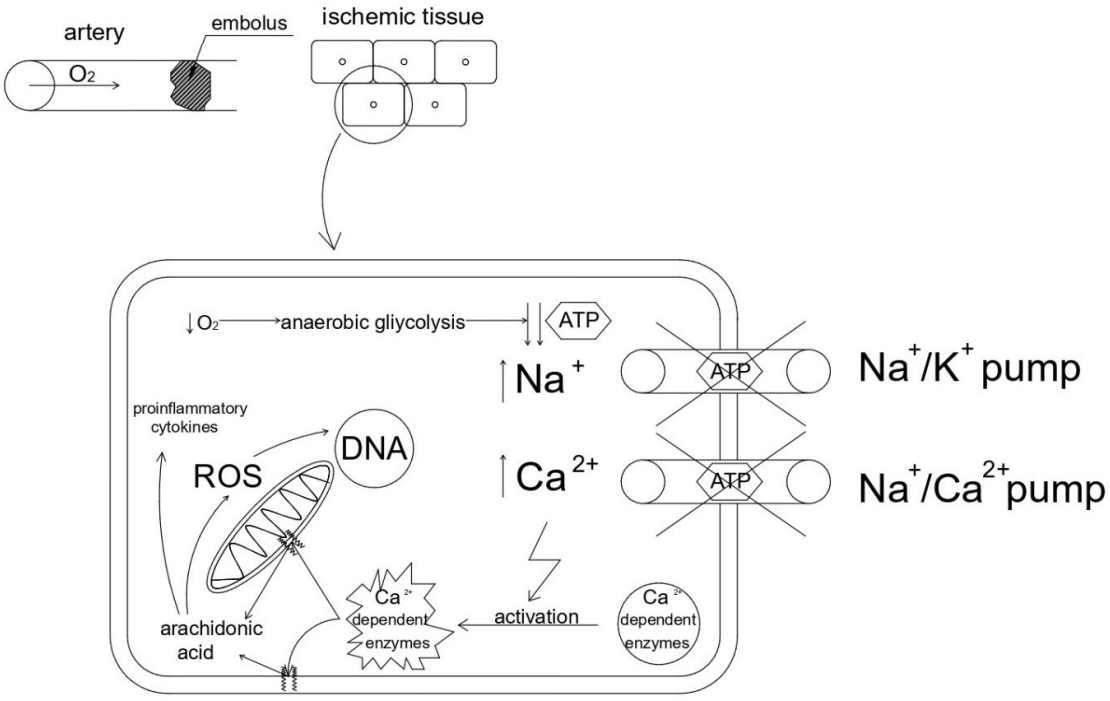


Figure 1. Tissue ischemia

slobodnih kiseoničnih radikala (20,21). Tri poznata tipa sintaze NOS – endotelni, neuronski i inducibilni vrše konverziju L-arginina u L-citrulin pri čemu se oslobađa azot monoksid (NO) koji ima antiinflamatornu ulogu. U stanju hipoksije, aktivnošću ovih enzima dolazi do stvaranja superoksidnog anjona koji promoviše oksidativni stres (22). Reaktivne kiseonične vrste oštećuju DNK, dovode do endotelne disfunkcije i pokretanja inflamacije. Inflamatorna kaskada i oksidativni stres dalje oštećuju ćelijske strukture što dovodi do smrti ćelije (15).

Hiperbarična oksigenacija

Hiperbarična oksigenacija (HBO) je tretman koji podrazumeva udisanje čistog, 100%-og kiseonika pod pritiskom od najmanje 1,4 atmosfere (ATM) (10). Ipak, za odobrene indikacije uglavnom se koriste pritisci veći od 2 ATM. Zbog toksičnosti koju izlaganje kiseoniku pod pritiskom ispoljava, gornja granica pritiska koja se u kliničkoj praksi primenjuje je 3 ATM. Trajanje tretmana je obično između 60 i 90 minuta. Povišen pritisak u komori je važan jer prema Henrijevom zakonu, povećanje pritiska gasa u vazduhu povećava njegovu rastvorljivost u tečnostima. Shodno ovom principu fizike, udisanjem kiseonika pod pritiskom povećava se njegova količina u krvi. Danas su u upotrebi komore dizajnirane da prime jednog bolesnika, kao i one koje istovremeno mogu primiti veći broj bolesnika (10,23,24).

Pretretman i tretman hiperbaričnom oksigenacijom

Tretman HBO može biti koristan u terapiji mnogih bolesti u čijoj osnovi se nalazi hipoksija. Pretretman i terapija HBO su pokazali posebno dobre efekte kod ishemijsko-reperfuzijskih povreda mozga i srca, a pozitivni efekti ostvareni su i u tretmanu ishemijsko-reperfuzijskog oštećenja bubrega. Kao što je napred pomenuto, udisanje kiseonika pod pritiskom povećava njegovu rastvorljivost u krvi (24). Ovo stanje hiperoksigenacije ima određene blagotvorne efekte – povećava se količina kiseonika koja se doprema do ishemijskog područja što je bitno za obnavljanje oksidativnog metabolizma, takođe povećanje količine kiseonika u krvi dovodi do vazokonstrikcije koja je važna, jer se smanjuje edem tkiva i poboljšava mikrocirkulacija (13,14,25-27). Na eksperimentalnim animalnim modelima, pokazano je da pretretman i tretman HBO ima blagotvorno dejstvo na očuvanje strukture i funkcije ćelija u različitim ishemijskim tkivima, a takođe se ostvaruje protektivni efekat od štetnih dejstava postishemijske reperfuzije (10,25-27). Studije pokazuju da pretretman i tretman HBO dovodi do hiperoksigenacije tkiva i stvaranja kiseoničnih „rezervoara“ koji ćeliju štite u stanjima iznenadne hipoksije, stimuliše proizvodnju antioksidanasa, obnavlja aerobni metabolizam čime se povećava proizvodnja ATP-a, smanjuje disfunkcija mitohondrija, te štiti ćelija od smrti (10,25-27). Nedavno objavljena eksperimentalna studija sprovedena na pacovima Wistar soja kojima je indukovano ishemijsko akutno bubrežno oštećenje, pokazala je da pretretman HBO dovodi do smanjenja lipidne peroksidacije u plazmi (28). Takođe, pretretman HBO, smanjuje nivoe uree i kreatinina i značajno poboljšava procenjenu brzinu glomerulske filtracije (engl. *estimated Glomerular filtration rate, eGFR*) kod jedinki kojima je indukovano akutno bubrežno oštećenje. U skladu sa ovim rezultatima je i statistički značajno smanjenje nivoa markera oštećenja bubrega 1 (engl. K*idney injury molecule-1, KIM-1*) u plazmi jedinki izlaganih ovom pretretmanu (28). Pretretman HBO doprinosi zaštiti od ishemijsko-reperfuzijskog oštećenja stimulacijom ekspresije hem oksigenaze 1, enzima koji igra važnu ulogu u regulaciji ćelijske proliferacije, diferencijacije, apoptoze, te štiti bubreg u akutnom bubrežnom oštećenju (29). Eksperimentalno ispitivanje izvedeno na pacovima kojima je indukovano ishemijsko-reperfuziono oštećenje jetre pokazalo je da se markeri oštećenja jetre smanjeno oslobađaju kod grupe jedinki koje su prethodno podvrgnute pretretmanu HBO. Takođe, u ovoj grupi primećeno je i poboljšanje mitohondrijskog disanja (30,31). Smanjenjem oštećenja mitohondrija smanjuje se i oslobađnje citohroma c i inhibira nastanak apoptoze (31). Pretretman HBO režnjeva kože pacova kojima je izazvana ishemijsko-reperfuziona povreda dovodi do smanjene ekspresije proapoptotskog proteina Bax, povećane ekspresije antiapoptotskog proteina Bcl-2 te se poboljšava preživljavanje ćelija u ishemijskom tkivu, a takođe dolazi i do poboljšanja mikrocirkulacije (32). Povećana ekspresija antiapoptotskog Bcl-2 proteina pokazana je i kod spontano hipertenzivnih i normotenzivnih pacova kojima je indukovano akutno bubrezno oštećenje (29).

Jedan od vodećih uzroka smrt u svetu je moždani udar, a čak 80% od svih cerebralnih in-

of NADPH oxidase and further promote the creation of reactive oxygen species (20,21). Three known types of NOS synthases – endothelial, neuronal and inducible convert L-arginine into L-citrulline, when nitric monoxide, which has an anti-inflammatory role, is released. In the hypoxic state, the activities of these enzymes induce the creation of superoxide anion which promotes oxidative stress (22). Reactive oxygen species damage DNA, lead to the endothelial dysfunction and instigate inflammation. The inflammatory cascade and oxidative stress further damage cell structures, thus leading to cell death (15).

Hyperbaric oxygenation

Hyperbaric oxygenation (HBO) is a treatment which includes breathing pure, 100% oxygen under pressure of at least 1.4 atmospheres (ATM). However, pressures higher than 2 ATM are used for approved indications. Due to the fact that exposure to oxygen under pressure may be toxic, the pressure applied in the clinical practice does not exceed 3 ATM. The duration of session is usually between 60 and 90 minutes. The increased pressure in the chamber is important because, according to Henry's law, the increase in pressure in the air causes higher dissolubility in fluids. In accordance with this principle of physics, inhaling oxygen under pressure increases its amount in the blood. Chambers, which are designed to accommodate one patient, are used today, as well as chambers that may accommodate multiple patients at the same time (10,23,24).

Hyperbaric oxygen pretreatment and therapy

The hyperbaric oxygen therapy may be useful in the treatment of many diseases with underlying hypoxia. The pretreatment and therapy using HBO have shown particularly good effects on ischemic-reperfusion injuries of heart and brain, while positive effects have been achieved in the treatment of ischemic-reperfusion injury of kidneys. As it has already been mentioned, inhaling oxygen under pressure increases its dissolubility in the blood (24). This state of hyperoxygenation has certain beneficial effects – the amount of oxygen that is brought to ischemic area increases, which is important for the restoration of oxidative metabolism. Also, the increase in the amount of blood leads to vasoconstriction which is important because tissue edema is reduced and microcirculation improves (13,14,25-27). It has been shown on experimental animal models that the HBO pretreatment and therapy have a beneficial effect on the maintenance of structure and function of cells in different ischemic tissues, while also a protective effect is achieved regarding the harmful effects of post-ischemic reperfusion (10,25-27). Studies have shown that HBO pretreatment and therapy lead to hyperoxygenation of tissues and creation of oxygen "reservoirs" that protect cells in case of sudden hypoxia, stimulate the production of antioxidants, restore the aerobic metabolism, thus increasing the production of ATP, reducing the mitochondrial dysfunction and protecting cells from cell death (10,25-27). A recently published experimental study, which was conducted on Wistar rats with induced ischemic acute kidney injury, has shown that HBO preconditioning leads to the reduction of lipid peroxidation in plasma (28). Also, HBO preconditioning reduces the levels of urea and creatinine and significantly improves the estimated glomerular filtration rate in induced acute kidney injury in rats. In accordance with these results is the statistically significant reduction of kidney injury marker (kidney injury molecule-1, KIM-1) in the plasma of rats exposed to this pretreatment (28). HBO pretreatment contributes to the protection from ischemic-reperfusion injury by stimulating the expression of hem oxygenase 1, the enzyme which has a significant role in the regulation of cell proliferation, differentiation, apoptosis, and therefore, protects the kidney in acute kidney injury (29). Experimental research of induced ischemic-reperfusion liver injury in rats has shown that markers of liver damage are released less in the group of rats that were previously exposed to HBO preconditioning. Also, the improvement of mitochondrial respiration was noticed in this group (30,31). The reduction of mitochondrial damage causes the decrease in cytochrome c release and inhibits apoptosis (31). HBO preconditioning of skin flaps in rats with induced ischemia-reperfusion injury leads to the reduced expression of pro-apoptotic protein Bax, increased expression of anti-apoptotic Bcl-2 and therefore the survival of cells improves in the ischemic tissue, as well as microcirculation (32). The increased expression of anti-apoptotic

sulta su ishemijskog tipa. Lečenje ovog entiteta je dosta ograničeno. Pokazalo se da HBO tretman pomaže u lečenju jer smanjuje cerebralni edem koji nastaje nakon infarkta mozga, poboljšava cerebralnu cirkulaciju i snabdevanje ishemijskog područja kiseonikom te ograničava gubitak periinfarktnog tkiva. Ovim tretmanom smanjuje se nivo proinflamatornih citokina, indukuje angiogeneza i regrutovanje progenitorskih ćelija u oštećene regione (33). Interesantno je da HBO tretman 7 dana nakon ishemijskog moždanog udara doprinosi boljem neurološkom oporavku (34).

U kliničkoj praksi, tretman HBO je značajan u terapiji dijabetesnog stopala jer utiče na mnoge komponente zapaljenja i regeneracije tkiva. Pojedine studije ukazuju da tretman HBO indukuje povećanje markera angiogeneze poput epidermalnog faktora raste (engl. *Epidermal growth factor, EGF*). Ovaj tretman takođe smanjuje incidencije anaerobnih infekcija i amputacije stopala (35).

Zaključak

Eksperimentalno je pokazano da pretretman i tretman HBO u velikoj meri koriste u lečenju ishemijsko-reperfuzijskih povreda. Stvaranje kiseoničnih „rezervoara“ u ishemijskom tkivu, obnavljanje oksidativnog metabolizma, poboljšanje mikrocirkulacije, stimulacija sinteze antioksidanasa, poboljšanje bubrežne funkcije, neuroprotektivni efekat, indukcija angiogeneze samo su neki od dobrobitnih efekata koje ova terapija pruža. Međutim, za sada ne postoji dovoljan broj kliničkih ispitivanja kojima bi se potvrdili blagotvorni efekti i ispitali rizici izlaganja kiseoniku pod pritiskom u humanoj populaciji. Iako postoji veliki broj oboljenja u čijoj osnovi se nalazi hipoksija i gde bi potencijalno mogla da se primeni terapija HBO, za sada postoji samo mali broj odobrenih indikacija za njeno korišćenje. Kako je potencijal ove terapije nedovoljno iskorišćen, u budućnosti nam je potreban veći broj studija i kliničkih ispitivanja za razvoj adekvatnih HBO protokola.

Konflikt interesa

Autori su izjavili da nema konflikta interesa.

Literatura

1. Atanacković M, Bacetić D, Basta-Jovanović G, Begić-Janeva A, Boričić I, Brašanac D, et al. Patologija. 5th ed.Beograd:Medicinski fakultet Univerziteta, Katedra za patologiju; 2015.
2. Pešić BČ. Patofiziologija: mehanizmi poremećaja zdravlja I deo. 1st ed. Beograd: Medicinski fakultet Univerziteta, CIBID; 2015.
3. Penttila A, Trump B. Studies on the modification of the cellular response to injury. Virchows Archiv B Cell Pathology 1975; 18(1):17-34.
4. Francis A, Baynosa R. Ischaemia-reperfusion injury and hyperbaric oxygen pathways: a review of cellular mechanisms. Diving Hyperb Med 2017; 47(2):110-7. doi: 10.28920/dhm47.2.110-117.
5. Sánchez E. Pathophysiology of ischemia-reperfusion injury and its management with hyperbaric oxygen (HBO): a review. J Emerg Crit Care Med 2019; 3:22. doi: 10.21037/jeccm.2019.04.03
6. Heusch G. Critical Issues for the Translation of Cardioprotection. Circ Res 2017; 120(9):1477-86. doi: 10.1161/CIRCRESAHA.117.310820.
7. Ibáñez B, Heusch G, Ovize M, Van de Werf F. Evolving therapies for myocardial ischemia/reperfusion injury. J Am Coll Cardiol 2015; 65(14):1454-71. doi: 10.1016/j.jacc.2015.02.032. PMID: 25857912.
8. Lee JW, Ko J, Ju C, Eltzschig HK. Hypoxia signaling in human diseases and therapeutic targets. Exp Mol Med 2019; 51(6):1-13. doi: 10.1038/s12276-019-0235-1.
9. Hentia C, Rizzato A, Camporesi E, Yang Z, Muntean DM, Săndesc D, et al. An overview of protective strategies against ischemia/reperfusion injury: The role of hyperbaric oxygen preconditioning. Brain Behav 2018; 8(5):e00959. doi: 10.1002/brb3.959.
10. García-Covarrubias L, Sánchez-Rodríguez EC. Hyperbaric oxygenation therapy, basic concepts. Gac Med Mex 2000; 136(1):45-56.
11. Živković M. Priručnik za hiperbaričnu medicinu. Beograd: Srpska zdravstvena organizacija; 2010.
12. FDA- Food and Drug Administration. Available at: www.fda.gov (Pristupljeno: 20.08.2022.).
13. Francis A, Baynosa R. Ischaemia-reperfusion injury and hyperbaric oxygen pathways: a review of cellular mechanisms. Diving Hyperb Med 2017; 47(2):110-7. doi: 10.28920/dhm47.2.110-117.
14. Wang L, Li W, Kang Z, Liu Y, Deng X, Tao H, et al. Hyperbaric oxygen preconditioning attenuates early apoptosis after spinal cord ischemia in rats. J Neurotrauma 2009; 26(1):55-66. doi: 10.1089/neu.2008.0538.
15. Wu MY, Yiang GT, Liao WT, Tsai AP, Cheng YL, Cheng PW, et al. Current Mechanistic Concepts in Ischemia and Reperfusion Injury. Cell Physiol Biochem 2018; 46(4):1650-67. doi: 10.1159/000489241.
16. Granger DN, Kvietys PR. Reperfusion injury and reactive oxygen species: The evolution of a concept. Redox Biol 2015; 6:524-51. doi: 10.1016/j.redox.2015.08.020.
17. Chung HY, Baek BS, Song SH, Kim MS, Huh JI, Shim KH, et al. Xanthine dehydrogenase/xanthine oxidase and oxidative stress. Age (Omaha) 1997; 20(3):127-40. doi: 10.1007/s11357-997-0012-2.
18. Paravicini TM, Touyz RM. NADPH oxidases, reactive oxygen species, and hypertension: clinical implications

Bcl-2 protein has been shown in spontaneously hypertensive and normotensive rats with induced acute kidney injury (29).

Stroke is one of the leading causes of death globally, and even 80% of all cerebral insults are ischemic-related. The treatment of this entity is very limited. It has been shown that HBO therapy helps in the treatment because it reduces cerebral edema, which develops during cerebral insult, it improves cerebral circulation and oxygen supply to the ischemic region, and therefore limits the loss of peri-infarct tissue. This treatment reduces the level of pro-inflammatory cytokines, induces angiogenesis and recruitment of progenitor cells into damaged regions (33). It is interesting that HBO treatment 7 days after ischemic cerebral insult contributes to a better neurological recovery (34).

In clinical practice, HBO therapy is important in the treatment of diabetes foot because it affects various components of inflammation and tissue regeneration. Some studies have shown that HBO therapy induces the increase in angiogenesis markers such as epidermal growth factor (EGF). This therapy also reduces the incidence of anaerobic infections and foot amputations (35).

Conclusion

Experiments have shown that HBO pre-treatment and therapy are used to a large extent in the treatment of ischemia-reperfusion injury. The creation of oxygen “reservoirs” in ischemic tissue, restoration of oxidative metabolism, improvement of microcirculation, stimulation of synthesis of antioxidants, improvement of renal function, neuroprotective effect, induction of angiogenesis are some of beneficial effects provided by this therapy. However, the number of clinical trials is not sufficient to prove the beneficial effects and investigate risks of exposure to oxygen under pressure in human population. Although there is a great number of diseases with underlying hypoxia, in which HBO therapy could potentially be used, few indications have been approved so far for its use. As the potential of this therapy has not been sufficiently used, more studies and clinical trials will be necessary in the future for the development of adequate HBO protocols.

Competing interests

Authors declare no competing interests.

Literature

1. Atanacković M, Bacetić D, Basta-Jovanović G, Begić-Janeva A, Boričić I, Brašanac D, et al. Patologija. 5th ed.Beograd:Medicinski fakultet Univerziteta, Katedra za patologiju; 2015.
2. Pešić BČ. Patofiziologija: mehanizmi poremećaja zdravlja I deo. 1st ed. Beograd: Medicinski fakultet Univerziteta, CIBID; 2015.
3. Penttila A, Trump B. Studies on the modification of the cellular response to injury. Virchows Archiv B Cell Pathology 1975; 18(1):17-34.
4. Francis A, Baynosa R. Ischaemia-reperfusion injury and hyperbaric oxygen pathways: a review of cellular mechanisms. Diving Hyperb Med 2017; 47(2):110-7. doi: 10.28920/dhm47.2.110-117.
5. Sánchez E. Pathophysiology of ischemia-reperfusion injury and its management with hyperbaric oxygen (HBO): a review. J Emerg Crit Care Med 2019; 3:22. doi: 10.21037/jeccm.2019.04.03
6. Heusch G. Critical Issues for the Translation of Cardioprotection. Circ Res 2017; 120(9):1477-86. doi: 10.1161/CIRCRESAHA.117.310820.
7. Ibáñez B, Heusch G, Ovize M, Van de Werf F. Evolving therapies for myocardial ischemia/reperfusion injury. J Am Coll Cardiol 2015; 65(14):1454-71. doi: 10.1016/j.jacc.2015.02.032. PMID: 25857912.
8. Lee JW, Ko J, Ju C, Eltzschig HK. Hypoxia signaling in human diseases and therapeutic targets. Exp Mol Med 2019; 51(6):1-13. doi: 10.1038/s12276-019-0235-1.
9. Hentia C, Rizzato A, Camporesi E, Yang Z, Muntean DM, Săndesc D, et al. An overview of protective strategies against ischemia/reperfusion injury: The role of hyperbaric oxygen preconditioning. Brain Behav 2018; 8(5):e00959. doi: 10.1002/brb3.959.
10. García-Covarrubias L, Sánchez-Rodríguez EC. Hyperbaric oxygenation therapy, basic concepts. Gac Med Mex 2000; 136(1):45-56.
11. Živković M. Priručnik za hiperbaričnu medicinu. Beograd: Srpska zdravstvena organizacija; 2010.
12. FDA- Food and Drug Administration. Available at: www.fda.gov (Cited: 20.08.2022.).
13. Francis A, Baynosa R. Ischaemia-reperfusion injury and hyperbaric oxygen pathways: a review of cellular mechanisms. Diving Hyperb Med 2017; 47(2):110-7. doi: 10.28920/dhm47.2.110-117.
14. Wang L, Li W, Kang Z, Liu Y, Deng X, Tao H, et al. Hyperbaric oxygen preconditioning attenuates early apoptosis after spinal cord ischemia in rats. J Neurotrauma 2009; 26(1):55-66. doi: 10.1089/neu.2008.0538.
15. Wu MY, Yiang GT, Liao WT, Tsai AP, Cheng YL, Cheng PW, et al. Current Mechanistic Concepts in Ischemia and Reperfusion Injury. Cell Physiol Biochem 2018; 46(4):1650-67. doi: 10.1159/000489241.
16. Granger DN, Kvietys PR. Reperfusion injury and reactive oxygen species: The evolution of a concept. Redox Biol 2015; 6:524-51. doi: 10.1016/j.redox.2015.08.020.

and therapeutic possibilities. Diabetes Care 2008; 31(Suppl 2):S170-80. doi: 10.2337/dc08-s247.

19. Sedeek M, Nasrallah R, Touyz RM, Hébert RL. NADPH oxidases, reactive oxygen species, and the kidney: friend and foe. J Am Soc Nephrol 2013; 24(10):1512-8. doi: 10.1681/ASN.2012111112.
20. Carden DL, Granger DN. Pathophysiology of ischaemia-reperfusion injury. J Pathol 2000; 190(3):255-66. doi:10.1002/(SICI)10969896(200002)190:3<255:AID-PATH526>3.0.CO;2-6.
21. Granger DN, Korthuis RJ. Physiologic mechanisms of postischemic tissue injury. Annu Rev Physiol 1995; 57:311-32. doi: 10.1146/annurev.ph.57.030195.001523.
22. Förstermann U, Sessa WC. Nitric oxide synthases: regulation and function. Eur Heart J 2012; 33(7):829-37. doi: 10.1093/eurheartj/ehr304.
23. Kirby JP, Snyder J, Schuerer DJE, Peters JS, Bochicchio GV. Essentials of Hyperbaric Oxygen Therapy: 2019 Review. Mo Med 2019; 116(3):176-9.
24. Ortega MA, Fraile-Martinez O, García-Montero C, Callejón-Peláez E, Sáez MA, Álvarez-Mon MA, et al. A General Overview on the Hyperbaric Oxygen Therapy: Applications, Mechanisms and Translational Opportunities. Medicina (Kaunas) 2021; 57(9):864. doi: 10.3390/medicina57090864.
25. Hentia C, Rizzato A, Camporesi E, Yang Z, Muntean DM, Săndesc D, Bosco G. An overview of protective strategies against ischemia/reperfusion injury: The role of hyperbaric oxygen preconditioning. Brain Behav 2018; 8(5):e00959. doi: 10.1002/brb3.959.
26. Hu SL, Feng H, Xi GH. Hyperbaric oxygen therapy and preconditioning for ischemic and hemorrhagic stroke. Med Gas Res 2016; 6(4):232-6. doi: 10.4103/2045-9912.196907.
27. Hu Q, Manaenko A, Matei N, Guo Z, Xu T, Tang J, et al. Hyperbaric oxygen preconditioning: a reliable option for neuroprotection. Med Gas Res. 2016 Apr 4;6(1):20-32. doi: 10.4103/2045-9912.179337.
28. Kovacevic S, Ivanov M, Brkic P, Vajic UJ, Mihailovic-Stanojevic N, Nesovic-Ostojic J et al. Hyperbaric oxygen preconditioning and the role of NADPH oxidase inhibition in postischemic acute kidney injury induced in spontaneously hypertensive rats. PLoS One 2020; 15(1):e0226974. doi: 10.1371/journal.pone.0226974.
29. Nesovic Ostojic J, Ivanov M, Mihailovic-Stanojevic N, Karanovic D, Kovacevic S, Brkic P, et al. Hyperbaric Oxygen Preconditioning Upregulates Heme OxyGenase-1 and Anti-Apoptotic Bcl-2 Protein Expression in Spontaneously Hypertensive Rats with Induced Postischemic Acute Kidney Injury. Int J Mol Sci 2021; 22(3):1382. doi: 10.3390/ijms22031382.
30. Losada DM, Jordani ME, Jordani MC, Piccinato MA, Fina CF, Feres O, et al. Should preconditioning hyperbaric oxygenation protect the liver against ischemia-reperfusion injury? An experimental study in a rat model. Transplant Proc 2014; 46(1):56-62. doi: 10.1016/j.transproceed.2013.10.044.
31. Losada DM, Chies AB, Feres O, Chaib E, D'Albuquerque LA, Castro-e-Silva O. Effects of hyperbaric oxygen therapy as hepatic preconditioning in rats submitted to hepatic ischemia/reperfusion injury. Acta Cir Bras 2014; 29(Suppl 2):61-6. doi: 10.1590/s0102-8650201400140012.
32. Xiao YD, Liu YQ, Li JL, Ma XM, Wang YB, Liu YF, et al. Hyperbaric oxygen preconditioning inhibits skin flap apoptosis in a rat ischemia-reperfusion model. J Surg Res 2015; 199(2):732-9. doi: 10.1016/j.jss.2015.06.038.
33. Sunami K, Takeda Y, Hashimoto M, Hirakawa M. Hyperbaric oxygen reduces infarct volume in rats by increasing oxygen supply to the ischemic periphery. Crit Care Med 2000; 28(8):2831-6. doi: 10.1097/00003246-200008000-00025.
34. Hu SL, Feng H, Xi GH. Hyperbaric oxygen therapy and preconditioning for ischemic and hemorrhagic stroke. Med Gas Res 2016; 6(4):232-6. doi: 10.4103/2045-9912.196907.
35. Sharma R, Sharma SK, Mudgal SK, Jelly P, Thakur K. Efficacy of hyperbaric oxygen therapy for diabetic foot ulcer, a systematic review and meta-analysis of controlled clinical trials. Sci Rep 2021; 11(1):2189. doi: 10.1038/s41598-021-81886-1

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17. Chung HY, Baek BS, Song SH, Kim MS, Huh JI, Shim KH, et al. Xanthine dehydrogenase/xanthine oxidase and oxidative stress. Age (Omaha) 1997; 20(3):127-40. doi: 10.1007/s11357-997-0012-2.
18. Paravicini TM, Touyz RM. NADPH oxidases, reactive oxygen species, and hypertension: clinical implications and therapeutic possibilities. Diabetes Care 2008; 31(Suppl 2):S170-80. doi: 10.2337/dc08-s247.
19. Sedeek M, Nasrallah R, Touyz RM, Hébert RL. NADPH oxidases, reactive oxygen species, and the kidney: friend and foe. J Am Soc Nephrol 2013; 24(10):1512-8. doi: 10.1681/ASN.2012111112.
20. Carden DL, Granger DN. Pathophysiology of ischaemia-reperfusion injury. J Pathol 2000; 190(3):255-66. doi:10.1002/(SICI)10969896(200002)190:3<255:AID-PATH526>3.0.CO;2-6.
21. Granger DN, Korthuis RJ. Physiologic mechanisms of postischemic tissue injury. Annu Rev Physiol 1995; 57:311-32. doi: 10.1146/annurev.ph.57.030195.001523.
22. Förstermann U, Sessa WC. Nitric oxide synthases: regulation and function. Eur Heart J 2012; 33(7):829-37. doi: 10.1093/eurheartj/ehr304.
23. Kirby JP, Snyder J, Schuerer DJE, Peters JS, Bochicchio GV. Essentials of Hyperbaric Oxygen Therapy: 2019 Review. Mo Med 2019; 116(3):176-9.
24. Ortega MA, Fraile-Martinez O, García-Montero C, Callejón-Peláez E, Sáez MA, Álvarez-Mon MA, et al. A General Overview on the Hyperbaric Oxygen Therapy: Applications, Mechanisms and Translational Opportunities. Medicina (Kaunas) 2021; 57(9):864. doi: 10.3390/medicina57090864.
25. Hentia C, Rizzato A, Camporesi E, Yang Z, Muntean DM, Săndesc D, Bosco G. An overview of protective strategies against ischemia/reperfusion injury: The role of hyperbaric oxygen preconditioning. Brain Behav 2018; 8(5):e00959. doi: 10.1002/brb3.959.
26. Hu SL, Feng H, Xi GH. Hyperbaric oxygen therapy and preconditioning for ischemic and hemorrhagic stroke. Med Gas Res 2016; 6(4):232-6. doi: 10.4103/2045-9912.196907.
27. Hu Q, Manaenko A, Matei N, Guo Z, Xu T, Tang J, et al. Hyperbaric oxygen preconditioning: a reliable option for neuroprotection. Med Gas Res. 2016 Apr 4;6(1):20-32. doi: 10.4103/2045-9912.179337.
28. Kovacevic S, Ivanov M, Brkic P, Vajic UJ, Mihailovic-Stanojevic N, Nesovic-Ostojic J et al. Hyperbaric oxygen preconditioning and the role of NADPH oxidase inhibition in postischemic acute kidney injury induced in spontaneously hypertensive rats. PLoS One 2020; 15(1):e0226974. doi: 10.1371/journal.pone.0226974.
29. Nesovic Ostojic J, Ivanov M, Mihailovic-Stanojevic N, Karanovic D, Kovacevic S, Brkic P, et al. Hyperbaric Oxygen Preconditioning Upregulates Heme OxyGenase-1 and Anti-Apoptotic Bcl-2 Protein Expression in Spontaneously Hypertensive Rats with Induced Postischemic Acute Kidney Injury. Int J Mol Sci 2021; 22(3):1382. doi: 10.3390/ijms22031382.
30. Losada DM, Jordani ME, Jordani MC, Piccinato MA, Fina CF, Feres O, et al. Should preconditioning hyperbaric oxygenation protect the liver against ischemia-reperfusion injury? An experimental study in a rat model. Transplant Proc 2014; 46(1):56-62. doi: 10.1016/j.transproceed.2013.10.044.
31. Losada DM, Chies AB, Feres O, Chaib E, D'Albuquerque LA, Castro-e-Silva O. Effects of hyperbaric oxygen therapy as hepatic preconditioning in rats submitted to hepatic ischemia/reperfusion injury. Acta Cir Bras 2014; 29(Suppl 2):61-6. doi: 10.1590/s0102-8650201400140012.
32. Xiao YD, Liu YQ, Li JL, Ma XM, Wang YB, Liu YF, et al. Hyperbaric oxygen preconditioning inhibits skin flap apoptosis in a rat ischemia-reperfusion model. J Surg Res 2015; 199(2):732-9. doi: 10.1016/j.jss.2015.06.038.
33. Sunami K, Takeda Y, Hashimoto M, Hirakawa M. Hyperbaric oxygen reduces infarct volume in rats by increasing oxygen supply to the ischemic periphery. Crit Care Med 2000; 28(8):2831-6. doi: 10.1097/00003246-200008000-00025.
34. Hu SL, Feng H, Xi GH. Hyperbaric oxygen therapy and preconditioning for ischemic and hemorrhagic stroke. Med Gas Res 2016; 6(4):232-6. doi: 10.4103/2045-9912.196907.
35. Sharma R, Sharma SK, Mudgal SK, Jelly P, Thakur K. Efficacy of hyperbaric oxygen therapy for diabetic foot ulcer, a systematic review and meta-analysis of controlled clinical trials. Sci Rep 2021; 11(1):2189. doi: 10.1038/s41598-021-81886-1

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FAKTORI KOJI DOPRINOSE BOLJEM OPORAVKU OD ANEMIJE OSOBAMA NA HEMODIJALIZI PRILIKOM PRIMENE REKOMBINANTNOG ERITROPOETINA

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SAŽETAK

Uvod/Cilj: Anemija se najčešće javlja kod bolesnika na hroničnom programu hemodijalize i uzrok je smanjenog kvaliteta života. Cilj studije je bio da identifikuje faktore koji doprinose osobama na hemodijalizi bolju kontrolu anemije primenom rekombinantnog eritropoetina.
Metod: Studijom preseka su 2017. godine, bile obuhvaćene 52 osobe na hemodijalizi iz Kliničkog centra Crne Gore. Ispitanicu su podeljini na one kod kojih je posle 3 meseca od primene rekombinantnog eritropoetina došlo do porasta hemoglobina na zadovoljavajuće vrednosti (110 g/l ili više) (grupa 1) i one kod kojih je vrednost hemoglobina i dalje bila niža od 110 g/l (grupa 2). Od svih ispitanika prikupljeni su podaci iz istorija bolesti. U statističkoj analizi podataka korišćen je t-test.
Rezultati: Posle tromesečne primene terapije eritropoetinom samo kod 21 bolesnika (40,4%) je postignuta ciljna vrednost hemoglobina od 110 g/l ili više (grupa 1), a kod 59,6% vrednosti hemoglobina su bile niže od 110 g/l. Ispitanici kod kojih nisu postignute ciljne vrednosti hemoglobina su primali značajno veće prosečne doze eritropoetina i imali veći indeks telesne mase nego ispitanici kod kojih su postignute ciljne vrednosti hemoglobina. Između ispitivanih grupa nije bilo značajne razlike u odnosu na odgovor na eritropoetin, dužinu trajanja terapije eritropoetinom, dužinu trajanja dijalize, starost ispitanika, debljinu kožnog nabora, obim nadlaktice, vrednosti transferina, broj eritrocit, vrednosti albumina, hemoglobina, hamatokrita i gvožđja.
Zaključak: Mogući faktori koji doprinose lošijoj kontroli anemije su više doze eritropoetina i veći stepen uhranjenosti procenjen prema indeksu telesne mase. Neophodna su dalja istraživanja u cilju pronalaženja faktora koji bi doprineli odklanjanju anemije kod osoba na hemodijalizi, jer nelečena anemija može dovesti do brojnih nepovoljnih ishoda (loš kvalitet života, kardiovaskularne bolesti, cerebrovaskularni insult, smanjenje opšte stope preživljavanja i drugo).

Ključne reči: hemodijaliza, eritropoetin, hronična bubrežna insuficijencija

Uvod

Anemija se definiše kao koncentracija hemoglobina u krvi manja od 120 g/l kod žena i manja od 130 g/l kod muškaraca (1). Ona je prateća pojava kod bolesnika na hroničnom programu hemodijalize i uzrok je smanjenog kvaliteta života. Multicentrične studije sprovedene na osobama sa hroničnom bubrežnom insuficijencijom (HBI) su pokazale da je nedostatak eritropoetina ključni faktor za nastanak anemije kod ovih osoba (2-7).

Većina studija pokazuje da primena eritropoetina supkutano ima „štedeći efekat" (8,9), pri čemu se optimalna vrednost hematokrita postiže manjim dozama eritropoetina (10). Postoji više studija koje govore o prednosti supkutane u odnosu na intravensku primenu eritropoetina u terapiji anemije kod ovih bolesnika, a to su: smanjenje potrebne doze eritropoetina, manji bol pri aplikovanju koji opisuju bolesnici, i smanjenje troškova lečenja (11,12). Efikasnost terapije eritropoetinom zavisi od adekvatne doze, učestalosti primene i načina davanja. Uobičajeno je da se daje u dozi 20-50 IJ/kg telesne mase, tri puta nedeljno, a zatim, uko-

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FACTORS CONTRIBUTING TO THE RECOVERY FROM ANEMIA IN HEMODIALYSIS PATIENTS DURING THE ADMINISTRATION OF RECOMBITANT ERYTHROPOIETIN

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SUMMARY

Introduction/Aim: Anemia is the commonest complication in patients on a chronic hemodialysis program and is the cause of reduced quality of life. The aim of this study was to identify the factors that contribute to the better control of anemia with the help of recombinant erythropoietin in persons undergoing hemodialysis.
Methods: The cross-sectional study was conducted in 2017 and it included 52 persons on hemodialysis at the Clinical Center of Montenegro. The participants were divided into those, in whom hemoglobin values increased to satisfactory values (110 g/l or more) after three months of application of recombinant erythropoietin (group 1), and those, in whom hemoglobin values were lower than 110 g/l (group 2). Data were collected from the medical history of all participants. T-test was used for the statistical analysis of data.
Results: After the three-month administration of erythropoietin, the target value of hemoglobin of 110 g/l or more (group 1) was achieved in only 21 patients (40.4%), while hemoglobin values were below 110 g/l in 59.6% of patients. Participants, in whom target values of hemoglobin were not achieved, received significantly higher average doses of erythropoietin and they had higher body mass index in comparison to participants, in whom the target values were achieved. There was no significant difference between the examined groups regarding the response to erythropoietin, duration of erythropoietin therapy, duration of hemodialysis, participants' age, skin-fold thickness, upper arm width, transferrin values, number of erythrocytes, value of albumin, hemoglobin, hematocrit and iron.
Conclusion: Possible factors that contribute to worse control of anemia are higher doses of erythropoietin and greater level of nutritional status estimated according to the body mass index. Further research is necessary aimed at finding factors that would contribute to the elimination of anemia in persons on hemodialysis, because non-treated anemia may lead to numerous unfavorable outcomes (poor quality of life, cardiovascular diseases, cerebrovascular insult, decreased survival rate etc.).

Key words: hemodialysis, erythropoietin, chronic kidney insufficiency

Introduction

Anemia is defined as the concentration of hemoglobin lower than 120 g/l in women and lower than 130 g/l in men (1). It is the accompanying complication in patients on a chronic hemodialysis program and it is the cause of the poor quality of life. Multicentric studies that included persons with chronic kidney insufficiency have shown that the lack of erythropoietin is a key factor for the development of anemia in these persons (2-7).

Most studies have shown that the subcutaneous administration of erythropoietin has a "saving effect" (8,9), while the optimal value of hematocrit is achieved with smaller doses of erythropoietin (10). There are several studies that speak about the advantages of subcutaneous application of erythropoietin in comparison to intravenous application in the treatment of anemia in these patients, and they include the following: reduction of the necessary dose of erythropoietin, less painful injection described by patients, and reduced costs of treatment (11,12). The efficacy of treatment with erythropoietin

liko nije dostignut ciljni hematokrit, svake četvrte nedelje doza se povećava za 25-96% (13). Ukoliko su potrebne doze veće od 150 IJ/kg telesne mase, tri puta nedjeljno, smatra se da postoji rezistencija prema eritropoetinu.

Prema terapijskom vodiču (14), za korekciju anemije kod HBI neophodno je davanje eritropoetina supkutano u dozi 80-120 IJ/kg telesne težine nedeljno (podeljeno u 2-3 doze) ili intravenski 120-180 IJ/kg telesne težine nedeljno (podeljeno u 3 doze). Ciljna vrednost hematokriat treba da bude od 33 do 36%, a hemoglobina od 11 do 12 g/dl. Optimalni način korekcije podrazumeva porast vrednosti hematokrita za 4-6% tokom 4 nedelje (a ciljne vrednosti unutar 2-3 meseca). Varijabilnost vrednosti hemoglobina treba tretirati kroz prilagođavanje doze, imajući u vidu ciljni opseg hemoglobina od 10g/dl (6,2 mmol/l) do 12 g/dl (7,5 mmol/l) (16). „Održavanje nivoa hemoglobina iznad 12g/dl (7,5 mmol/l) treba izbegavati (14). Ako je brzina porasta hemoglobina veća od 2g/dl (1,25 mmol/l) tokom jednog meseca ili se rastući nivo hemoglobina približava 12g/dl (7,45 mmol/l), dozu treba smanjiti za 25%. Ukoliko nivo hemoglobina nastavlja da raste, terapiju treba prekinuti dok nivo hemoglobina ne počne da opada, kada terapiju treba ponovo započeti u dozi koja je 25% niža od prethodno primenjene doze."

Terapija eritropoetinom se deli u dve faze: faza korekcije i faza održavanja. U fazi korekcije vrši se supkutana primena eritropoetina (15). „Inicijalna doza je 3 × 20 IJ/kg telesne težine nedeljno. Ova doza se može povećavati svake 4 nedelje za 3 × 20 IJ/kg telesne težine nedeljno, ako povećanje hemoglobina nije adekvatno (< 0,25 g/dl nedeljno). Ova nedeljna doza se može podeliti u dnevne doze. Maksimalna doza ne sme da pređe 720 IJ/kg telesne težine nedeljno. U fazi održavanja da bi se hemoglobin održao na nivou između 10 i 12 g/dl, doziranje se inicijalno smanjuje na polovinu prethodno date doze. Posle toga se doza podešava u intervalima od jedne do dve nedelje individualno za svakog bolesnika (doza održavanja) (13)."

Terapija eritropoetinom je uobičajeno dugoročna terapija. Podaci o šemi doziranja jednom nedeljno zasnivaju se na kliničkim ispitivanjima sa trajanjem terapije od 24 nedelje (16). U kliničkim ispitivanjima (17) primećen je povećan rizik od smrti i ozbiljnih kardiovaskularnih događaja kada je ciljna vrednost hemoglobina, postignuta lekovima za stimulaciju eritropoeze, bila viša od 12 g/dl (7,5 mmol/l). Kontrolisana klinička ispitivanja nisu pokazala značajne koristi koje bi se mogle pripisati primeni eritropoetina, kada se koncentracija hemoglobina poveća iznad nivoa neophodnog za kontrolu simptoma anemije i izbegavanje transfuzije krvi (18-20).

Na osnovu rezultata kliničkih ispitivanja kojima je obuhvaćeno 1725 pacijenata, očekuje se da približno 8% pacijenata koji se leče eritropoetinom ima neka neželjena dejstva (21). Najčešće neželjeno dejstvo tokom terapije eritropoetinom jeste povećanje krvnog pritiska i pogoršanje već postojeće hipertenzije (5). Terapija eritropoetinom se vrlo dobro podnosi, kako subjektivno, tako i objektivno i ima bezbroj prednosti u odnosu na transfuzije krvi, čija primena može biti praćena brojnim neželjenim reakcijama.

Cilj ove studije preseka je bio da se identifikuju faktori koji kod osoba na hemodijalizi tokom primene rekombinantnog eritropoetina doprinose njihovom boljem oporavku od anemije.

Metod

Ovom studijom preseka obuhvaćene su 52 osobe na hemodijalizi koje su bile hospitalizovane na Klinici za urologiju i Klinici za nefrologiju, Kliničkog centra Crne Gore. Svi ispitanici su primali humani rekombinantni eritropoetin tokom poslednja 3 meseca. Ispitanici su podeljeni u dve grupe na osnovu odgovora anemije na primenu eritropoetina tokom poslednja 3 meseca. Prvu grupu činile su osobe na hemodijalizi sa postignutim ciljnim hemoglobinom od 110 g/l ili više (grupa 1), a drugu grupu osobe na hemodijalizi sa ciljnim hemoglobinom nižim od 110 g/l (grupa 2). Od svih ispitanika prikupljeni su podaci iz istorija bolesti. Za sve ispitanike prikupljeni su podaci koji se odnose na uzrast, dužinu trajanja hemodijalize, dužinu primene eritropoetina, doza eritropoetina, odgovor na dozu eritropoetina, vrednosti eritrocita, hematokrita i albumina. Istraživanje je odobreno od strane etičkog komiteta Kliničkog centra Crne Gore. U statističkoj analizi podataka korišćen je Studentov t-test.

Rezultati

Studija preseka je obuhvatila 52 osobe koje su na hemodijalizi i koje su lečene od anemije primenom eritropoetina (tabela 1). Posle primene terapije eritropoetinom samo kod 21 bolesnika (40,4%)

depends on the adequate dose, the frequency of administration and manner of administration. It is usually administered in a dose 20-50 IU/kg of body weight, three times a week, and then, if the target hematocrit is not achieved, every fourth week the dose is increased for 25-96% (13). If the necessary doses are higher than 150 IU/kg, three times a week, it is deemed that it is the resistance to erythropoietin.

According to the treatment protocol (14), in order to treat anemia in chronic kidney insufficiency, erythropoietin should be administered subcutaneously in a dose 80-120 IU/kg (divided into 2-3 doses) or intravenously 120-180 IU/kg a week (divided into 3 doses). Target values of hematocrit should be 33-36%, and of hemoglobin 11-12 g/dl. The optimal correction means the increase in hematocrit values for 4-6% during 4 weeks (while target values within 2-3 months). The variability of hemoglobin values should be treated through dose adjustment, considering the target range of hemoglobin from 10g/dl (6.2 mmol/l) to 12 g/dl (7.5 mmol/l) (16). "The maintenance of hemoglobin level above 12g/dl (7.5 mmol/l) should be avoided (14). If hemoglobin value rises more than 2g/dl (1.25 mmol/l) within one month or the increasing hemoglobin reaches 12 g/dl (7.45 mmol/l), a dose should be reduced for 25%. If the level of hemoglobin continues to increase, the therapy should be interrupted until the hemoglobin level begins to decrease, and then the therapy should be reintroduced in a dose which is 25% lower than the previously administered dose."

The treatment with erythropoietin is divided into two phases: the correction phase and maintenance phase. In the correction phase, erythropoietin is administered subcutaneously (15). "The initial dose is 3 × 20 IU/kg per week. This dose may be increased every fourth week for 3 × 20 IU/kg per week if the hemoglobin increase is not adequate (< 0.25 g/dl a week). This weekly dose may be divided into daily doses. Maximal dose must not exceed 720 IU/kg per week. In the maintenance phase, in order to keep hemoglobin at the level between 10 and 12 g/dl, the dose is initially reduced to one half of the previously administered dose. After that, the dose is adjusted within one to two weeks individually for each patient (maintenance dose) (13)."

The erythropoietin treatment is usually a long-term treatment. Data on the scheme of doses once a week are based on the clinical trials in which therapy lasts 24 weeks (16). In the clinical trials (17), it was noticed that the risk of mortality and severe cardiovascular events increased when the target value of hemoglobin, which was achieved with the help of medications that stimulate erythropoiesis, was higher than 12 g/dl (7.5 mmol.l). Controlled clinical trials did not show significant benefits that could be attributed to the use of erythropoietin, when the concentration of hemoglobin increased above the level necessary for the control of the symptoms of anemia and avoidance of blood transfusion (18-20).

According to the results of clinical trials, which included 1725 patients, it is expected that approximately 8% of patients who are treated with erythropoietin will have some side effects (21). The most common side effect during the erythropoietin treatment is the elevation of blood pressure and worsening of already existing hypertension (5). The treatment with erythropoietin is well-tolerated, subjectively and objectively, and it has numerous advantages in comparison to blood transfusion, whose application may be accompanied by numerous side effects.

The aim of this study was to identify factors that contribute to the better recovery from anemia in patients on hemodialysis during the administration of recombinant erythropoietin.

Method

This cross-sectional study included 52 patients on hemodialysis, who were hospitalized at the Clinic of Urology and Clinic of Nephrology at the Clinical Center of Montenegro. All participants received human recombinant erythropoietin during the previous three months. The participants were divided into two groups according to their response to the application of erythropoietin during the last three months. The first group included persons on hemodialysis with the achieved target hemoglobin of 110 g/l or higher (group 1), while the second group included persons on hemodialysis with the target hemoglobin lower than 110 g/l (group 2). Data were collected from the medical history of all participants. The collected data related to age, duration of hemodialysis, duration of administration of erythropoietin, dose of erythropoietin, response to the dose of erythropoietin, values of erythrocytes, hematocrit and albumin. The study

Tabela 1. Demografske, kliničke i terapijske karakteristike ispitanika na hemodijalizi koji primaju tromesečnu terapiju eritropoetina u cilju lečenja anemije

Varijabla	Grupa 1 (n=21) AS ± SD	Grupa 2 (n=31) AS ± SD	t-test	p vrednost
Starost (godine)	56,71 ± 13,24	57,32 ± 11,97	-0,172	0,864
Dužina trajanja hemodijalize (godine)	6,10 ± 5,47	5,24 ± 4,71	0,604	0,549
Dužina trajanja terapije EPO (godine)	5,67 ± 5,58	5,07 ± 4,52	0,427	0,549
Doza EPO (IJ/kg telesne mase)	121,65 ± 63,17	176,44 ± 92,78	-2,358	0,022
Odgovor na terapiju EPO (IJ/kg telesne mase)	1,28 ± 0,87	0,95 ± 1,31	1,000	0,322
ITM (kg/m^2)	21,93 ± 3,10	24,18 ± 5,26	-1,924	0,050
Debljina kožnog nabora (mm)	18,44 ± 21,72	19,82 ± 11,04	-0,300	0,766
Obim nadlaktice (mm)	25,39 ± 3,74	26,44 ± 3,54	-0,987	0,329
Transferin (mg/dl)	0,61 ± 0,34	0,81 ± 0,74	-1,114	0,271
Broj eritrocita (x 10^{12})	3,43 ± 0,39	3,29 ± 0,57	0,768	0,438
Albumini (g/dl)	36,48 ± 3,73	36,28 ± 2,35	0,168	0,868
Hemoglobin (g/l)	95,02 ± 8,41	91,58 ± 15,23	0,804	0,428
Hematokrit (l/l)	0,29 ± 0,028	0,28 ± 0,05	0,854	0,400
Gvožđe (mmol/l)	8,68 ± 3,99	7,68 ± 3,27	0,735	0,468

AR- aritmetička sredina; SD- standardna devijacija; grupa 1 (osobe na hemodijalizi sa vrednostima hemoglobina ≥ 110 g/l i više); grupa 2 (osobe na hemodijalizi sa vrednostima hemoglobina nižim od 110 g/l); EPO – eritropoetin; ITM – indeks telesne mase.

je postignuta ciljana vrednost hemoglobina od 110 g/l ili više (grupa 1), a kod 59,6% ciljne vrednosti hemoglobina nisu postignute (grupa 2). Ispitanici grupe 2 su primali značajno veće prosečne doze eritropoetina i imali nešto veći indeks telesne mase (ITM) nego ispitanici grupe 2. Između ispitivanih grupa nije bilo značajne razlike u odnosu na odgovor na eritropoetin, dužinu trajanja terapije eritropoetinom, dužinu trajanja hemodijalize, starost ispitanika, debljinu kožnog nabora, obim nadlaktice, vrednosti transferina, broj eritrocita, vrednosti albumina, hemoglobina, hamatokrita i gvožđa.

Diskusija

Cilj ovog istraživanja je bio da se ispitaju faktori koji utiču na oporavak od anemije osoba na hemodijalizi, a koje su na terapiji rekombinantnim eritropoetinom, kako bi se eventualnim uklanjanjem ovih faktora olakšao oporavak od anemije. Prema rezultatima našeg istraživanja postoji značajna razlika u prosečnoj dozi rekombinantnog eritropoetina između osoba na hemodijalizi kod kojih je postignuta ciljna vrednost hemoglobuna (grupa 1) i onih kod kojih nije (grupa 2). Prosečna doza primanog eritropoetina za prvu grupu ispitanika (121,65 ± 63,17 IJ/kg) je bila u okviru preporučenih i za supkutanu i za intravensku primenu,

Table 1. Demographic, clinical and treatment characteristics of participants on hemodialysis who receive a three-month erythropoietin treatment aimed at treating anemia

Variable	Group 1 (n=21) AS ± SD	Group 2 (n=31) AS ± SD	t-test	p vrednost
Age (years)	56.71 ± 13.24	57.32 ± 11.97	-0.172	0.864
Duration of hemodialysis (years)	6.10 ± 5.47	5.24 ± 4.71	0.604	0.549
Duration of EPO therapy (years)	5.67 ± 5.58	5.07 ± 4.52	0.427	0.549
Dose of EPO (IU/kg body weight)	121.65 ± 63.17	176.44 ± 92.78	-2.358	0.022
Response to EPO therapy (IU/kg body weight)	1.28 ± 0.87	0.95 ± 1.31	1.000	0.322
BMI (kg/m^2)	21.93 ± 3.10	24.18 ± 5.26	-1.924	0.050
Skin-fold thickness (mm)	18.44 ± 21.72	19.82 ± 11.04	-0.300	0.766
Upper arm width (mm)	25.39 ± 3.74	26.44 ± 3.54	-0.987	0.329
Transferrin (mg/dl)	0.61 ± 0.34	0.81 ± 0.74	-1.114	0.271
Number of erythrocytes (x 10^{12})	3.43 ± 0.39	3.29 ± 0.57	0.768	0.438
Albumin (g/dl)	36.48 ± 3.73	36.28 ± 2.35	0.168	0.868
Hemoglobin (g/l)	95.02 ± 8.41	91.58 ± 15.23	0.804	0.428
Hematocrit (l/l)	0.29 ± 0.028	0.28 ± 0.05	0.854	0.400
Iron (mmol/l)	8.68 ± 3.99	7.68 ± 3.27	0.735	0.468

AM- arithmetic mean; SD- standard deviation; group 1 (persons on hemodialysis with haemoglobin values ≥ 110 g/l and higher); group 2 (persons on hemodialysis with haemoglobin values lower than 110 g/l); EPO – erythropoietin; BMI – body mass index.

was approved by the Ethical Committee of the Clinical Center of Montenegro. Student's t-test was used in the statistical analysis of data.

Results

The cross-sectional study included 52 persons on hemodialysis who were treated due to anemia with the administration of erythropoietin (Table 1). After the administered erythropoietin, the target hemoglobin value of 110 g/l or higher was achieved only in 21 patients (40.4%) (group 1), while in 59.6% of patients the target hemoglobin values were not achieved (group 2). The participants from group 2 received significantly higher average doses of erythropoietin and had a little bit higher body mass index (BMI) than participants in group 2. There was no significant difference between the examined groups regarding their response to erythropoietin, duration of treatment with erythropoietin, duration of hemodialysis, participants' age, thickness of skin folds, upper arm width, transferrin values, number of erythrocytes, value of albumin, hemoglobin, hematocrit and iron.

Discussion

The aim of this study was to investigate factors that affect the recovery from anemia in persons undergoing hemodialysis, and who received

dok su za drugu grupu (176,44 ± 92,78 IJ/kg) bile u okviru dozvoljenih za intravensku primenu. Vrednosti eritropoetina za drugu grupu ispitanika su iznad preporučene doze i za supkutanu i za intravensku primenu, što može da ukaže da kod ovih ispitanika postoji rezistencija anemije na lek. Ukoliko su potrebne doze eritropoetina veće od 150 IJ/kg, tri puta nedjeljno, smatra se da postoji rezistencija prema eritropoetinu (14,22). Anemija rezistentna na preporučene doze eritropoetina (22), uz dovoljnu količinu gvožđa i vitamina, često ukazuje na neadekvatnu dijalizu, nekontrolisani hiperparatireoidizam, trovanje aluminijumom, hronični gubitak krvi ili hemolizu i propratnu hemoglobinopatiju, malnutriciju, hroničnu infekciju, multipli mijelom ili druge malignitete. Terapija eritropoetinom se vrlo dobro podnosi, kako subjektivno, tako i objektivno, i ima mnogo prednosti u odnosu na transfuzije krvi, čija primena može biti praćena brojnim neželjenim posledicama. Transfuzije krvi mogu doprineti supresiji eritropoeze u HBI, zato što povećavaju rizik od hepatitisa, hemosideroze i odbacivanja transplantata, pa ih treba izbegavati dok bolesnik ne odgovori na terapiju eritropoetinom i osoba ne ispolji simptomatologiju (22). Korekcija anemije dovodi do poboljšanja kardijalne hemodinamike, poboljšanja fizičke kondicije i radnog kapaciteta. Iako postoje razlike u prosečnoj dozi eritropoetina između ispitivanih grupa, to nije uticalo da se dobiju značajne razlike između grupa u odnosu na vrednosti eritrocita, hemoglobina i hematokrita.

U našem istraživanju, nije bilo značajne razlike u odnosu na uzrast između osoba na hemodijalizi sa postignutim ciljnim vrednostima hemoglobina (56,71 ± 13,24 godina) i onih kod kojih ciljna vrednost nije ostvarena (57,32 ± 11,97 godina). Rezultati brojnih studija pokazuju da su osobe koje se leče dijalizom i kod nas i u svetu sve starije osobe (23-25).

Uočava se da su osobe na hemodijalizi koje su postigle adekvatan odgovor na terapiju eritropoetinom (grupa 1) imale značajno niži indeks telesne mase nego osobe bez adekvatnog odgovora na terapiju eritropoetinom (grupa 2), iako su obe grupe ispitanika pripadale grupi normalno uhranjenih osoba (vrednosti indeksa telesne mase za normalno uhranjene se kreću od 18,5 do 24,9 kg/m^2). U studiji *El-Kannishy* i saradnika, multicentrična studija koja je objedinila podatke 9 centara za hemodijalizu u Egiptu, pokazala je, takođe, da su gojazne osobe (indeks telesne mase ≥ 30 kg/m^2) koje su na hemodijalizi ređe (25,3 %) primenom eritropoetina ostvarivale ciljne vrednosti hemoglobina (10,0–11,5 g/dL) nego negojazne osobe (27,3 %), ali uočena razlika nije bila statistički značajna (26). Između ovih grupa nije bilo značajne razlike u prosečnim vrednostima serumskog feritina i indeksu zasićenosti transferinom, ali je nedeljna doza eritropoetina bila značajno niža kod gojaznih nego negojaznih osoba. U većini do sada sprovedenih istraživanja ukazuje se da je stopa incidencije i prevalencija gojaznih mnogo veća među osobama na hemodijalizi (preko 30%), nego u opštoj populaciji (7,28), što zahteva istraživanja o vezi između gojaznosti, anemije i odgovora na eritropoetin kod osoba na hemodijalizi. U zemljama u razvoju, anemija kod osoba na hemodijalizi može biti pogoršana ishranom, kao i većom učestalošću zaraznih bolesti.

Nedostaci ovog istraživanja odnose se na sprovođenje same studije preseka (u ovim studijama nismo sigurni šta je uzrok, a šta posledica) i nemoćunosti dobijanja podataka za veći broj varijabli koje mogu da se dovedu u vezu sa adekvatnim odgovorom anemije na terapiju eritropoetinom. Takođe, ovo istraživanje je sprovedeno na malom broju ispitanika. Zahvaljujući ovom istraživanju, otvorene su mogućnosti za dalja istraživanja u ovoj oblasti. Rezultati istraživanja pokazuju da rezistencija na terapiju eritropoetinom i veći indeks telesne mase mogu biti razlog za neadekvatan odgovor anemije kod osoba na hemodijalizi.

Zaključak

Neophodna su dalja istraživanja u ovoj oblasti, posebno kliničke studije, koje bi ukazale na sve faktore koji onemogućuvaju adekvatan odgovor anemije na terapiju hematopoetinom.

Konflikt interesa

Autori su izjavili da nema konflikta interesa.

Literatura

1. Jacović S, Jovanović M, Hamzagić N, Pavlović R, Petrović D. Rezistencija na eritropoetin kod bolesnika na hemodijalizi. Acta Facultatis Medicae Naissensis 2019; 36(1):5-14. doi: 10.2478/afmnai-2019-0001
2. Sosnin DY, Khovaeva YB, Podyanova AI, Syromyatnikova TN, Nenasheva OY. [Eritropoetin as laboratory index of the degree of respiratory insufficiency in chronic obstructive pulmonary diseases.]. Klin Lab Diagn 2018; 63(11):691-5. doi: 10.18821/0869-2084-2018-63-11-691-695.

recombinant erythropoietin, so that the recovery from anemia might be improved with the possible elimination of these factors. According to the results of our research, there is a significant difference in the average dose of recombinant erythropoietin between persons on hemodialysis, in whom the target hemoglobin value was achieved (group 1), and those patients, in whom the target value was not achieved (group 2). The average dose of administered erythropoietin for the first group of participants (121.65 ± 63.17 IU/kg) was within the recommended range for both the subcutaneous and intravenous application, while in the second group of participants (176.44 ± 92.78 IU/kg), it was within the range allowed for the intravenous application. The values of erythropoietin for the second group of participants were above the recommended dose for both the subcutaneous and intravenous application, which may indicate that there is resistance of anemia to this medication in these patients. If the necessary doses of erythropoietin are higher than 150 IU/kg three times a week, it is deemed to be the resistance to erythropoietin (14,22). Anemia which is resistant to the recommended doses of erythropoietin (22), with the sufficient amount of iron and vitamins, often points to the inadequate dialysis, uncontrolled hyperthyroidism, poisoning with aluminum, chronic blood loss or hemolysis and accompanying hemoglobinopathy, malnutrition, chronic infection, multiple myeloma, or other malignancies. The therapy of erythropoietin is often well-tolerated, subjectively and objectively, and it has numerous advantages in comparison to blood transfusion, whose application may be accompanied by numerous side effects. Blood transfusion may contribute to the suppression of erythropoiesis in chronic kidney disease, because it increases the risk of hepatitis, hemosiderosis, and transplant rejection, and therefore, it should be avoided until anemia responds to the therapy with erythropoietin and the symptoms appear (22). The correction of anemia leads to the improvement of cardiovascular hemodynamics, physical strength and work capacity. Although there are differences in the average dose of erythropoietin between the examined groups, it did not influence obtaining significant difference between the groups regarding the values of erythrocytes, hemoglobin and hematocrit.

In our study, there was no significant difference regarding the age between persons on hemodialysis with the achieved target values of hemoglobin (56.71 ± 13.24 years) and those in whom the target value was not achieved (57.32 ± 11.97). The results of numerous studies have shown that persons undergoing dialysis belong to the group of elderly in the world and in our country, as well (23-25).

It has been noticed that persons on hemodialysis, who achieved an adequate response to treatment with erythropoietin (group 1) had a significantly lower body mass index than persons who did not respond adequately to erythropoietin (group 2), although both groups of participants belonged to the group of persons with normal body weight (values of body mass index for normal weight range from 18.5 to 24.9 kg/m^2). In the study of El-Kannishy and associates, which is a multicentric study which coalesced data from 9 centers for hemodialysis in Egypt, showed that overweight persons (body mass index > 30 kg/m^2) on hemodialysis achieved more rarely (25.3%) the target values of hemoglobin (10.0 - 11.5 g/dL) than persons who were not overweight (27.3%), but the difference was not statistically significant (26). There was no significant difference between these groups regarding the values of serum ferritin, and transferrin saturation index, but the weekly dose of erythropoietin was significantly lower in overweight persons than in persons who were not overweight. In most of the research studies, which have been conducted so far, the incidence and prevalence rate was higher in persons on hemodialysis (more than 30%) than in the general population (7,28), which requires further research about the relationship between obesity, anemia and response to erythropoietin in persons on hemodialysis. In developing countries, anemia in persons on hemodialysis may be worsened because of the diet and higher frequency of infectious diseases.

The limitations of this research relate to the characteristics of cross-sectional studies (in these studies we are not certain about causes and consequences) and the impossibility of obtaining data for more variables that may be linked to the adequate response of anemia to erythropoietin therapy. Also, this study included a small number of participants. Thanks to this research, new possibilities have been opened for further research in this field. The results of research showed that the resistance to erythropoietin and higher body

3. Sakaguchi Y, Hamano T, Wada A, Masakane I. Types of Erythropoietin-Stimulating Agents and Mortality among Patients Undergoing Hemodialysis. J Am Soc Nephrol 2019; 30(6):1037-48. doi: 10.1681/ASN.2018101007
4. Georgianos PI, Agarwal R. Systolic and diastolic hypertension among patients on hemodialysis: Musings on volume overload, arterial stiffness, and erythropoietin. Semin Dial 2019; 32(6):507-12. doi: 10.1111/sdi.12837
5. Aapro M, Krendyukov A, Schiestl M, Gascón P. Epoetin Biosimilars in the Treatment of Chemotherapy-Induced Anemia: 10 Years' Experience Gained. BioDrugs 2018; 32(2):129-35. doi: 10.1007/s40259-018-0262-9
6. Nafar M, Samavat S, Khoshdel A, Alipour-Abedi B. Anemia Evaluation and Erythropoietin Dose Requirement Among Hemodialysis Patients: a Multicenter Study. Iran J Kidney Dis 2017;11(1):56-65. PMID: 28174354.
7. Mimran A. Renal effects of antihypertensive agents in parenchimal renal disease and renovascular hypertension. J Cardiovasc Pharmacol 1992; 19(suppl 6): 45-50. doi: 10.1097/00005344-199219006-00009.
8. Veys N, Dhondt A, Lameire N. Pain at the injection site of subcutaneously administered erythropoietin: phosphate-buffered epoetin alpha compared to citrate-buffered epoetin alpha and epoetin beta. Clin Nephrol 1998; 49(1):41-4.
9. Weiss LG, Clyne N, Divino Fihlho J, Frisenette-Fich C, Kurkus J, Svensson B. The efficacy of once weekly compared with two or three times weekly subcutaneous epoetin beta: results from a randomized controlled multicentre trial. Swedish Study Group. Nephrol Dial Transplant 2000; 15(12):2014-9. doi: 10.1093/ndt/15.12.2014.
10. Locatelli F, Andrulli S, Memoli B, Maffei C, Del Vecchio L, Aterini S, et al. Nutritional-inflammation status and resistance to erythropoietin therapy in haemodialysis patients. Nephrol Dial Transplant 2006; 21(4):991-8. doi: 10.1093/ndt/gfk011
11. Besarab A, Reyes CM, Hornberger J. Meta-analysis of subcutaneous versus intravenous epoetin in maintenance treatment of anemia in hemodialysis patients. Am J Kidney Dis 2002; 40(3):439-46. doi: 10.1053/ajkd.2002.34881
12. Goldsmith D and O' Donoghue D. Optimizing hemoglobin control with erythropoesis-stimulating agent: results of the Subcutaneus Erythropoetin Control (SEC) study. Presented at ASN Philadelphia, USA; 2005.
13. Ebben JP, Gilbertson DT, Foley RN, Collins AJ. Hemoglobin level variability: associations with comorbidity, intercurrent events, and hospitalizations. Clin J Am Soc Nephrol 2006; 1(6):1205-10. doi: 10.2215/CJN.01110306
14. KDOQI. Clinical practice guidelines and clinical practice recommendations for anemia in chronic kidney disease in adults. Am J Kidney Dis 2006; 47(5 Suppl 3):16-85. doi: 10.1053/j.ajkd.2006.03.011.
15. Petković N, Ristić S, Stošović M, Đukanović L. Razlike u odgovoru bolesnika lečenih hemodijalizama na humani rekombinovani eritropoetin. Medicinski pregled 2013; 66(3-4):130-7. doi: 10.2298/MPNS1304130P
16. Drüeke TB, Locatelli F, Clyne N, Eckardt KU, Macdougall IC, Tsakiris D, et al. CREATE Investigators. Normalization of hemoglobin level in patients with chronic kidney disease and anemia. N Engl J Med 2006; 355(20):2071-84. doi: 10.1056/NEJMoa062276
17. Ifudu O, Feldmann J, Friedman EA. The intensity of haemodialysis and the response to erythropoietin in patients with end-stage renal disease. N Engl J Med 1996; 334:420-5. doi: 10.1056/NEJM199602153340702
18. Sowade B, Sowade O, Mocks J, Franke W, Warnke H. The safety of treatment with recombinant human erythropoeitin in clinical use: a review-controlled studies. Int J Med 1998; 1: 303-14. doi: 10.3892/ijmm.1.2.303.
19. Choukroun G. Pain at the injection site: Results of the first cross-over study comparing epoetin beta and darbepoetin alfa administered subcutaneously in healthy volunteers. Presented at XLII ERA-ADTA congress, Istambul, Turkey; 2005.
20. Schmitt CP, Nau B, Brummer C, Rosenkranz J, Schaefer F. Increased injection pain with darbepoetin-alpha compared to epoetin-beta in paediatric dialysis patients. Nephrol Dial Transplant 2006; 21(12):3520-4. doi: 10.1093/ndt/gfl479
21. Stosevic M. Uporedna analiza indeksa adekvatnosti dijalize upotrebom kliničkih parametara pri raznim nivoima dijaliziranosti kod bolesnika na hemodijalizi. Doktorska disertacija, Medicinski fakultet Univerziteta u Beogradu; 2002.
22. Djukanovic L, Lezaic V. Liječenje anemije kod bolesnika sa hroničnom insuficijencijom bubrega rekombinantnim eritropoetinom. Srp arh 1996; 124:93-7.
23. Ratković M. Procjena kvaliteta liječenja bolesnika sa terminalnom bubrežnom insuficijencijom hemodijalizama. Doktorska disertacija, Medicinski fakultet Univerziteta u Beogradu; 2002.
24. Valderrabano F. Quality of life benefits of early anaemia treatment. Nephrol Dial Transplant 2000; 15(Supll 3):23-8. doi: 10.1093/oxfordjournals.ndt.a027972
25. Priyadarshi A, Shapiro JI. Erythropoietin resistance in the treatment of the anemia of chronic renal failure. Semin Dial 2006; 19(4):273-8. doi: 10.1111/j.1525-139X.2006.00172.x
26. El-Kannishy GM, Megahed AF, Tawfik MM, El-Said G, Zakaria RT, Mohamed NA, et al. Obesity may be erythropoietin dose-saving in hemodialysis patients. Kidney Res Clin Pract 2018; 37(2):148-56. doi: 10.23876/j.krcp.2018.37.2.148.
27. Kramer HJ, Saranathan A, Luke A, Durazo-Arvizu RA, Guichan C, Hou S, Cooper R. In-creasing body mass index and obesity in the incident ESRD population. J Am Soc Neph-rol 2006; 17:1453–9. doi: 10.1681/ASN.2005111241.
28. Lorenzo V, Martin M, Rufino M, Sanchez E, Jiménez A, Hernández D, Torres A. High prevalence of overweight in a stable Spanish hemodialysis population: a cross sectional study. J Ren Nutr 2003; 13:52–9. doi: 10.1053/jren.2003.50004.

mass index may be the reason for the inadequate response of anemia in persons on hemodialysis.

Conclusion

Further research in this field is necessary, especially clinical trials, which would point to all factors that make it impossible to adequately respond to the therapy of hematopoietin in anemia.

Competing interests

Authors declare no competing interests.

Literature

1. Jacović S, Jovanović M, Hamzagić N, Pavlović R, Petrović D. Rezistencija na eritropoetin kod bolesnika na hemodijalizi. Acta Facultatis Medicae Naissensis 2019; 36(1):5-14. doi: 10.2478/afmnai-2019-0001
2. Sosnin DY, Khovaeva YB, Podyanova AI, Syromyatnikova TN, Nenasheva OY. [Eritropoetin as laboratory index of the degree of respiratory insufficiency in chronic obstructive pulmonary diseases.]. Klin Lab Diagn 2018; 63(11):691-5. doi: 10.18821/0869-2084-2018-63-11-691-695.
3. Sakaguchi Y, Hamano T, Wada A, Masakane I. Types of Erythropoietin-Stimulating Agents and Mortality among Patients Undergoing Hemodialysis. J Am Soc Nephrol 2019; 30(6):1037-48. doi: 10.1681/ASN.2018101007
4. Georgianos PI, Agarwal R. Systolic and diastolic hypertension among patients on hemodialysis: Musings on volume overload, arterial stiffness, and erythropoietin. Semin Dial 2019; 32(6):507-12. doi: 10.1111/sdi.12837
5. Aapro M, Krendyukov A, Schiestl M, Gascón P. Epoetin Biosimilars in the Treatment of Chemotherapy-Induced Anemia: 10 Years' Experience Gained. BioDrugs 2018; 32(2):129-35. doi: 10.1007/s40259-018-0262-9
6. Nafar M, Samavat S, Khoshdel A, Alipour-Abedi B. Anemia Evaluation and Erythropoietin Dose Requirement Among Hemodialysis Patients: a Multicenter Study. Iran J Kidney Dis 2017;11(1):56-65. PMID: 28174354.
7. Mimran A. Renal effects of antihypertensive agents in parenchimal renal disease and renovascular hypertension. J Cardiovasc Pharmacol 1992; 19(suppl 6): 45-50. doi: 10.1097/00005344-199219006-00009.
8. Veys N, Dhondt A, Lameire N. Pain at the injection site of subcutaneously administered erythropoietin: phosphate-buffered epoetin alpha compared to citrate-buffered epoetin alpha and epoetin beta. Clin Nephrol 1998; 49(1):41-4.
9. Weiss LG, Clyne N, Divino Fihlho J, Frisenette-Fich C, Kurkus J, Svensson B. The efficacy of once weekly compared with two or three times weekly subcutaneous epoetin beta: results from a randomized controlled multicentre trial. Swedish Study Group. Nephrol Dial Transplant 2000; 15(12):2014-9. doi: 10.1093/ndt/15.12.2014.
10. Locatelli F, Andrulli S, Memoli B, Maffei C, Del Vecchio L, Aterini S, et al. Nutritional-inflammation status and resistance to erythropoietin therapy in haemodialysis patients. Nephrol Dial Transplant 2006; 21(4):991-8. doi: 10.1093/ndt/gfk011
11. Besarab A, Reyes CM, Hornberger J. Meta-analysis of subcutaneous versus intravenous epoetin in maintenance treatment of anemia in hemodialysis patients. Am J Kidney Dis 2002; 40(3):439-46. doi: 10.1053/ajkd.2002.34881
12. Goldsmith D and O' Donoghue D. Optimizing hemoglobin control with erythropoesis-stimulating agent: results of the Subcutaneus Erythropoetin Control (SEC) study. Presented at ASN Philadelphia, USA; 2005.
13. Ebben JP, Gilbertson DT, Foley RN, Collins AJ. Hemoglobin level variability: associations with comorbidity, intercurrent events, and hospitalizations. Clin J Am Soc Nephrol 2006; 1(6):1205-10. doi: 10.2215/CJN.01110306
14. KDOQI. Clinical practice guidelines and clinical practice recommendations for anemia in chronic kidney disease in adults. Am J Kidney Dis 2006; 47(5 Suppl 3):16-85. doi: 10.1053/j.ajkd.2006.03.011.
15. Petković N, Ristić S, Stošović M, Đukanović L. Razlike u odgovoru bolesnika lečenih hemodijalizama na humani rekombinovani eritropoetin. Medicinski pregled 2013; 66(3-4):130-7. doi: 10.2298/MPNS1304130P
16. Drüeke TB, Locatelli F, Clyne N, Eckardt KU, Macdougall IC, Tsakiris D, et al. CREATE Investigators. Normalization of hemoglobin level in patients with chronic kidney disease and anemia. N Engl J Med 2006; 355(20):2071-84. doi: 10.1056/NEJMoa062276
17. Ifudu O, Feldmann J, Friedman EA. The intensity of haemodialysis and the response to erythropoietin in patients with end-stage renal disease. N Engl J Med 1996; 334:420-5. doi: 10.1056/NEJM199602153340702
18. Sowade B, Sowade O, Mocks J, Franke W, Warnke H. The safety of treatment with recombinant human erythropoeitin in clinical use: a review-controlled studies. Int J Med 1998; 1: 303-14. doi: 10.3892/ijmm.1.2.303.
19. Choukroun G. Pain at the injection site: Results of the first cross-over study comparing epoetin beta and darbepoetin alfa administered subcutaneously in healthy volunteers. Presented at XLII ERA-ADTA congress, Istambul, Turkey; 2005.
20. Schmitt CP, Nau B, Brummer C, Rosenkranz J, Schaefer F. Increased injection pain with darbepoetin-alpha compared to epoetin-beta in paediatric dialysis patients. Nephrol Dial Transplant 2006; 21(12):3520-4. doi: 10.1093/ndt/gfl479
21. Stosevic M. Uporedna analiza indeksa adekvatnosti dijalize upotrebom kliničkih parametara pri raznim nivoima dijaliziranosti kod bolesnika na hemodijalizi. Doktorska disertacija, Medicinski fakultet Univerziteta u Beogradu; 2002.
22. Djukanovic L, Lezaic V. Liječenje anemije kod bolesnika sa hroničnom insuficijencijom bubrega rekombinantnim eritropoetinom. Srp arh 1996; 124:93-7.

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23. Ratković M. Procjena kvaliteta liječenja bolesnika sa terminalnom bubrežnom insuficijencijom hemodijalizama. Doktorska disertacija, Medicinski fakultet Univerziteta u Beogradu; 2002.
24. Valderrabano F. Quality of life benefits of early anaemia treatment. Nephrol Dial Transplant 2000; 15(Supll 3):23-8. doi: 10.1093/oxfordjournals.ndt.a027972
25. Priyadarshi A, Shapiro JI. Erythropoietin resistance in the treatment of the anemia of chronic renal failure. Semin Dial 2006; 19(4):273-8. doi: 10.1111/j.1525-139X.2006.00172.x
26. El-Kannishy GM, Megahed AF, Tawfik MM, El-Said G, Zakaria RT, Mohamed NA, et al. Obesity may be erythropoietin dose-saving in hemodialysis patients. Kidney Res Clin Pract 2018; 37(2):148-56. doi: 10.23876/j.krcp.2018.37.2.148.
27. Kramer HJ, Saranathan A, Luke A, Durazo-Arvizu RA, Guichan C, Hou S, Cooper R. In-creasing body mass index and obesity in the incident ESRD population. J Am Soc Neph-rol 2006; 17:1453–9. doi: 10.1681/ASN.2005111241.
28. Lorenzo V, Martin M, Rufino M, Sanchez E, Jiménez A, Hernández D, Torres A. High prevalence of overweight in a stable Spanish hemodialysis population: a cross sectional study. J Ren Nutr 2003; 13:52–9. doi: 10.1053/jren.2003.50004.

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INTOLERANCIJA NA HRANU

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SAŽETAK

Intolerancija na hranu je neimunološki odgovor indukovan hranom ili komponentom hrane u dozi koja se normalno toleriše. Obuhvata pseudoalergijske i farmakološke efekte uzrokovane: salicilatima, biogenim aminima, sulfitima, natrijum glutamatom, bojama i konzervansima, zaslađivačima, ili enzimopatijama. U okviru ovog preglednog rada prikazana je patofiziologija, kliničke manifestacije, dijagnostikovanje i lečenje najučestalijih intolerancija na hranu. Prietraživanje literature je sprovedeno korišćenjem sledećih ključnih reči: intolerancija, hrana, aditivi, ugljeni hidrati i gluten u okviru *PubMed*, *Emabase*, *Scopus*, *SCIndex* i Hrčak za period od 2001. do 2022. godine. Na osnovu pregleda literature može se konstatovati da nedostatak standardizovanih testova uslovljava nesklad između percipirane prevalencije štetnih efekata povezanih s hranom, koji su izuzetno česti, i stvarne prevalencije neimunoloških reakcija na hranu unutar ovih događaja. Intolerancija na hranu se manifestuju u prvom redu gastrointerstinalnim, a zatim i ekstrainterstinalnim (neurološkim, kardiovaskularnim, respiratornim i dermatološkim) znakovima i simptomima. Dijagnoza zahteva detaljnu anamnezu, fizikalni pregled, kao i vođenje dnevnika ishrane i pojave simptoma, sprovođenje eliminacijske dijete i dvostruko slepih placebom kontrolisanih oralnih ekspozicijskih testova na hranu. Lečenje podrazumeva modifikaciju ishrane, suplementaciju i lečenje osnovnog stanja kod osoba sa sekundarnom intolerancijom.

Ključne reči: intolerancija, hrana, aditivi, enzimopatije

Uvod

Neželjene reakcije na hranu podrazumevaju svaku abnormalnu reakciju nakon uzimanja hrane (1). Uključuju intoleranciju na hranu, alergiju na hranu i averziju na hranu (1). Intolerancija na hranu je neimunološki odgovor indukovan hranom ili komponentom hrane u dozi koja se normalno toleriše (1,2). Obuhvata pseudoalergijske i farmakološke efekte uzrokovane: salicilatima, biogenim aminima, sulfitima, natrijum glutamatom, bojama i konzervansima, zaslađivačima, ili enzimopatijama (3). Intolerancija nezavisna od domaćina podrazumeva odgovor na prirodne sastojke hrane (vazoaktivni amini i salicilati) ili aditive u hrani (glutamat, sulfiti i benzoati) (4). Neimunološka preosetljivost na laktozu, fruktozu, fermentabilne oligosaharide, poliole i gluten predstavlja intoleranciju zavisnu od domaćina (4).

Nedostatak standardizovanih testova uslovljava nesklad između percipirane prevalencije štetnih efekata povezanih s hranom, koji su izuzetno česti, i stvarne prevalencije neimunoloških reakcija na hranu unutar ovih događaja (5-7). Dvostruko slepa, placebom kontrolisana istraživanja utvrdila su prevalenciju intolerancije na hranu od svega 1,8% (6). S druge strane, prevalencija samoprijavljene „bolesti“ ili „nelagode“ uzrokovane unosom određene hrane u epidemiološkim istraživanjima iznosi do 31,1% (5,7).

Intolerancija na hranu se obično karakteriše odloženim početkom simptoma (nastaju nakon nekoliko sati ili dana od uzimanja hrane) i prolongiranom simptomatskom fazom (4,8). Česta je intolerancija na nekoliko namirnica, ili skupina namirnica istovremeno (4). Količina unesene

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FOOD INTOLERANCE

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SUMMARY

Food intolerance is a non-immunological response induced by a food or food component in a dose that is normally tolerated. It includes pseudo allergic and pharmacological effects caused by: salicylates, biogenic amines, sulphites, sodium glutamate, colours and preservatives, sweeteners, or enzymopathies. The pathophysiology, clinical manifestations, diagnosis and treatment of the most common food intolerances have been presented in this review article. The literature search was done with the help of the following keywords: intolerance, food, additives, carbohydrates and gluten within PubMed, Embase, Scopus, SCIndeks and Hrčak databases. According to the literature, it may be argued that the lack of standardized tests accounts for the discrepancy between the perceived prevalence of food-related adverse effects, which are extremely common, and the actual prevalence of non-immunological reactions to food within these events. Food intolerance is manifested primarily by gastrointestinal and then extraintestinal (neurological, cardiovascular, respiratory and dermatological) signs and symptoms. Diagnosis requires a detailed medical history, physical examination, as well as keeping a diet and symptom diary, implementing an elimination diet and double-blind placebo-controlled oral food exposure tests. Treatment includes dietary modification, supplementation and treatment of the underlying condition in persons with secondary intolerance.

Key words: intolerance, food, additives, enzymopathy

Introduction

Adverse food reactions are defined as any abnormal reaction following the ingestion of food (1). They include food intolerance, food allergy and food aversion (1). Food intolerance is a non-immunological response induced by a food or food component in a dose that is normally tolerated (1,2). It includes pseudoallergic and pharmacological effects caused by: salicylates, biogenic amines, sulfites, sodium glutamate, colorants, preservatives, sweeteners, or enzymopathies (3). Host-independent intolerance involves the reaction to natural ingredients (vasoactive amines and salicylates) or additives in food (glutamate, sulfites and benzoates) (4). Non-immunologic hypersensitivity to lactose, fructose, fermentable oligosaccharides, polyols, and gluten is host-dependant intolerance (4).

The lack of standardized tests accounts for the discrepancy between the perceived prevalence of food-related adverse effects, which are extremely common, and the actual prevalence of non-immunological reactions to food within these events (5-7). Double-blind, placebo-controlled studies have found the prevalence of intolerance to food of 1.8% (6). On the other hand, the prevalence of self-reported “disease” or “discomfort” caused by the intake of certain foods in epidemiological studies amounts to 31.1% (5,7).

Food intolerance is usually characterized by a delay in symptom onset (they appear a few hours or days after the intake) and a prolonged symptomatic phase (4,8). Intolerance to several foods is common, as well as to food groups simultaneously. The amount of food is often

hrane neretko je direktno povezana sa ozbiljnošću simptoma (4,8).

Intolerancija na hranu se manifestuju u prvom redu gastrointerstinalnim (bol u trbuhu, nadimanje i dijareja), a zatim neurološkim (vrtoglavica i glavobolja) i kardiovaskularnim znakovima i simptomima (palpitacija, tahikardija, hipotenzija) (4). Respiratorne (hronična rinoreja, kijanje, dispneja) i dermatološke manifestacije (svrab, crvenilo lica i/ili tela, urtikarija) i angioedem su veoma retki (4).

U nedostatku standardizovanih testova dijagnoza intolerancije na hranu zahteva detaljnu anamnezu, fizikalni pregled, kao i vođenje dnevnika ishrane i pojave simptoma, sprovođenje eliminacione dijete i dvostruko slepih placebom kontrolisanih oralnih ekspozicijskih testova na hranu (8,9).

Cilj rada je da se kroz pregled literature analizira patofiziologija, klinička manifestacija, postavljanje dijagnoze i lečenje najučestalijih intolerancija na hranu.

Metode

Literatura je pretražena korišćenjem ključnih reči: intolerancija, hrana, aditivi, ugljeni hidrati i gluten. Pretraživanje je sprovedeno za period od 2001. godine do 2022. godine u okviru sledećih baza podataka: *PubMed*, *Emabase*, *Scopus*, *SCIndex* i Hrčak. Zbog ograničenog broja dostupnih studija u pretraživanju baza nisu korišteni dostupni filteri. Nakon pročitanih sažetaka, radovi su detaljnije proučeni te su isključeni oni koji ne odgovaraju postavljenom cilju istraživanja.

Intolerancije na hranu nezavisne od domaćina

Hiljade različitih jedinjenja s potencijalnom farmakološkom aktivnošću prisutno je u hrani (4). To mogu biti prirodni sastojci hrane, kao što su vazoaktivni amini (npr. histamin) i salicilati, ili aditivi u hrani, kao što su glutamati (npr. mononatrijum glutamat), sulfiti i benzoati (4).

Histamin (2-[4-imidazolil]etilamin) je bioaktivni heterociklični diamin sa imidazolnim prstenom i etilaminom (10). Glavni put stvaranja histamina u hrani je dekarboksilacija histidina delovanjem enzima bakterijskog porekla, L-histidin dekarboksilaze (10). Samim tim, visoke koncentracije histamina se nalaze uglavnom u proizvodima mikrobne fermentacije, kao što su stari sir, kiseli kupus, vino i prerađeno meso ili u mikrobiološki pokvarenoj hrani (11).

Intolerancija na histamin (enteralna histaminoza ili osetljivost na histamin iz hrane) opterećuje 1-3% populacije (11). Nastaje usled poremećene degradacije ili povećane dostupnosti histamina (Tabela 1) (11).

Smanjen kapacitet degradacije histamina u crevima uzrokovan genetski uslovljenim ili stečenim poremećajem aktivnosti diamin oksidaze (engl. *diamine oxidase* - DAO) predstavlja vodeći uzrok intolerancije na histamin (10). Genetski uslovljena intolerancija podrazumeva jednonukleotidne polimorfizme u genu (promotorskoj regiji gena) koji kodira DAO (10). Stečenu intoleranciju mogu indukovati gastrointestinalne bolesti, hronična bubrežna insuficijencija, virusni hepatitis, uznapredovala ciroza jetre, hronična urtikarija, kao i kompetitivna inhibicija DAO (drugi amini, alkohol ili lekovi), kao i nedostatak kofaktora DAO (vitamin B6, vitamin C, bakar i cink) (10-12).

Povećanu dostupnost histamina uzrokuju endogena hiperprodukcija (alergijska reakcija, mastocitoza, inefekcija bakterijama, gastrointestinalno krvarenje, drugi biogeni amini, 1-karnozin) ili povećan egzogeni unos histidina ili histamina (hrana ili alkohol) (Tabela 1) (9-12).

Intolerancija na histamin se manifestuje širokim spektrom nespecifičnih gastrointestinalnih i ekstraintestinalnih simptoma i znakova (10-12). Tipični simptomi uključuju gastrointerstinalne manifestacije (postprandijalnu punoću, bol u trbuhu, konstipaciju, dijareju), kijanje, rinoreju, začepljenost nosa, glavobolju, dismenoreju, hipotoniju, aritmiju, urtikariju, pruritus, crvenilo i astmu (10-11).

Dijagnoza zahteva isključivanje prisustva alergije na hranu i lekove, mastocitoze, psihosomatske bolesti, kao i prisustvo dva ili više tipičnih simptoma intolerancije na histamin, kao i njihovo poboljšanje ili remisiju nakon dijete s niskim sadržajem histamina ili upotrebe antihistaminika (10-12). Terapija podrazumeva dijetu sa niskim sadržajem histamina uz eventualnu upotrebu antihistaminika, cinka, vitamina B6, vitamina C i stabilizatora mastocita (10-12).

Intolerancija na salicilate predstavlja neimunološki odgovor indukovan salicilnom kiselinom i njenim derivatima (13,14). U različitim koncentracijama derivati salicilne kiseline su prisutni u nesteroidnim antiinflamatornim lekovima (acetilsalicilna kiselina, diflunisal, natrijum salicilat, salsalat, sulfasalazin), hrani (pasulj, karfiol, kiselo

directly linked to the severity of symptoms (4,8).

Food intolerance is primarily manifested as gastrointestinal symptoms (abdominal pain, bloating, diarrhea), neurological (dizziness, headache), and cardiovascular signs and symptoms (palpitations, tachycardia, hypotension) (4). Respiratory (chronic rhinorrhea, sneezing, dyspnea) and dermatological manifestations (itching, flushing of the face and/or body, urticaria) and angioedema are very rare (4).

Due to the lack of standardized tests, diagnosis requires a detailed anamnesis, physical examination, as well as keeping a diet and symptom diary, implementing an elimination diet and double-blind placebo-controlled oral food exposure tests (8,9).

The aim of this paper is to analyze the pathophysiology, clinical manifestations, diagnosis and treatment of the most common food intolerances through the review of literature.

Methods

The literature was searched with the help of the following key words: intolerance, food, additives, carbohydrates and gluten. The search was conducted for the period 2001 to 2022 within the following databases: PubMed, Emabase, Scopus, SCIndex and Hrcak. Due to the limited number of available studies, available filters were not used during the search. Abstracts were read first, and then studies were analyzed in more detail and those that did not correlate with the aim of the research were excluded.

Host-independent food intolerance

Thousands of different chemicals with potential pharmacological activity are present in food (4). They can be natural food chemicals, such as vasoactive amines (e.g. histamine) and salicylates, or food additives, such as glutamates (e.g. monosodium glutamate), sulfites and benzoates (4).

Histamine (2-[4-imidazolyl]ethylamine) is a bioactive heterocyclic diamine with an imidazole ring and ethylamine (10). The main route for histamine formation in food is the decarboxylation of histidine through the action of L-histidine decarboxylase, an enzyme of bacterial origin (10). Therefore, high concentrations of histamine are present mainly in products of microbial fermentation, such as aged cheese, sauerkraut, wine and processed meat, or in microbially spoiled food (11).

Histamine intolerance (enteral histaminosis or sensitivity to histamine in food) affects 1-3% of the population (11). It appears due to an impaired histamine degradation and increased availability of histamine (Table 1) (11).

The reduced capacity of histamine degradation in the intestines caused by genetically conditioned or acquired disorder of DAO activity (diamine oxidase-DAO) is the leading cause of histamine intolerance (10). Intolerance, which has a genetic origin, includes single-nucleotide polymorphisms in the DAO encoding gene (in the promoter region of the gene) (10). The acquired intolerance may be induced by gastrointestinal diseases, chronic kidney disease, hepatitis, advanced liver cirrhosis, chronic urticaria, as well as competitive DAO inhibition (other amines, alcohol, and medications) and the lack of DAO co-factors (vitamin B6, vitamin C, copper and zinc) (10-12).

The increased availability of histamine is caused by endogenous hyperproduction (allergic reaction, mastocytosis, bacterial infection, gastrointestinal bleeding, other biogenic amines, l-karnosine), or the increased intake of histidine or histamine (food or alcohol) (Table 1) (9-12).

Histamine intolerance is manifested by a wide range of non-specific gastrointestinal and extraintestinal signs and symptoms (10-12). Typical symptoms include gastrointestinal manifestations (postprandial fullness, abdominal pain, constipation, diarrhea), sneezing, rhinorrhea, nasal congestion, headache, dysmenorrheal, hypotonia, arrhythmia, urticaria, pruritus, flushing and asthma (10-11).

Diagnosis requires the exclusion of allergy to food and medications, mastocytosis, psychosomatic disease, as well as the presence of two or more typical symptoms of histamine intolerance, and their improvement or remission after diet with the low contents of histamine or the use of antihistamines (10-12). The treatment involves the diet with the low contents of histamine and the possible use of antihistamines, zinc, vitamin B6, vitamin C and mast cell stabilizers (10-12).

Salicylate intolerance is a non-immunological reaction induced by the salicylic acid and its derivatives (13,14). Derivatives of salicylic acid are present in different concentrations in non-steroidal anti-inflammatory drugs (acetylsalicylic acid, diflunisal, sodium salicylate, salsalate, sulfasalazin),

Tabela 1. Izvori histamina i mogući uzroci povećanja koncentracije u organizmu (12)

Prirodno proizveden histamin, uglavnom u mastocitima	
Namirnice sa visokim koncentracijama histamina	Paradajz, patlidžan, spanać, riba, piletina, uskladišteno meso, fermentisana hrana (sirevi, kobasice, kiseli kupus, vino, pivo, šampanjac...)
Namirnice koje oslobađaju histamin	Ananas, banane, agrumi, jagode, orasi, papaja, paradajz, sladić, začini, mahunarke, kakao, alkohol, riba, plodovi mora, svinjetina, belance
Aditivi koji oslobađaju histamina	Boje, konzervansi, stabilizatori, pojačivači ukusa, arome
Proizvodnja histamina indukovana kvascem i bakterijama	Namirnice sa održivim kvascem – kiselo testo, svež hleb
Supstance koje smanjuju aktivnost DAO u hrani	Alkohol
Lekovi koji smanjuju aktivnost DAO	Verapamil, propafenon, cefuroksim, cefotiam, klavulanska kiselina, doksiciklin, izoniazid, framicetin, metamizol, amitriptilin, diazepam, haloloperidol, prometazin, cimetidin, dihidralazin, hlorokin, aminofilin, teofilin, furosemide, N-acetilcistein, ambroksol, alkuronijum, pancuronijum, d –tubokurarin, akriflavinijum hlorid, hinidin
Lekovi koji oslobađaju histamin	Morfijum, petidin, kodein, metamizol, acetilsalicilna kiselina, d-cikloserin, hlorokin, pentamidin, dobutamin, verapamil, alprenolol, kodein, amilorid, kontrasna sredstva koje sadrže jod, mezokain, prokain, markain, prilokain d–tubokurarin, barbiturati, tiopental
Lekovi koji inaktiviraju piridoksin	Hidralazin, d-ciklosporin, izoniazid, hormonska kontracepcija
Alergijska reakcija	Oslobađanje histamina iz mastocita posredovano IgE
Lekovi koje potenciraju oslobađanje histamina posredovano IgE	Acetilsalicilna kiselina, diklofenak, flurbiprofen, indometacin, ketoprofen, mefenamin, naproksen…
Infekcija, trauma, šok	

DAO - diamin oksidaza

povrće, jagode, šljive, lubenice, maline, ananas, kikiriki, badem, grožđe, heljda, zob, kukuruz, kobasice, umaci i gotova jela, voćni sokovi, čaj, pivo, liker, rum, vino, začinsko bilje i začini), konzervansima i bojama (13-16).

Intolerancija na salicilate nastaje usled snažne inhibicije ciklooksigenaze tipa 1 (engl. *cyclooxygenase type 1* - COX-1) i posledičnog smanjenja proizvodnje prostaglandina, prostaciklina i tromboksana, kao i povećane dostupnosti leukotriena A4 (13,16). Manifestuje se, u prvom redu, rinosinuzitisom, polipozom nosa i sinusa, ili bronhijalnom astmom (17). Istovremeno postojanje intolerancije na nesteroidne antiinfllamatorne lekove, polipoze i astme označava se kao „trijada“ (17). Osim toga, mogu biti prisutni utrikarija, kolitis, ili dijareja (17). Anafilaktični šok je veoma redak (17).

Dijagnostička obrada započinje anamnezom koja ima za cilj da utvrdi moguću povezanost upotrebe derivata salicilne kiseline i simptoma intolerancije (17). Test izloženosti ili provokacije predstavlja zlatni standard u dijagnozi intolerancije (17). U slučaju kada je provokacija neprihvatljiva (ozbiljnost očekivane reakcije) ili kontraindikovana (infekcija ili bronhijalna astma) koriste se funkcionalni testovi (merenje količine leukotriena oslobođenih iz bazofilnih leukocita, merenje membranskog proteina CD63 povezanog s lizozimoma, prošireni funkcionalni eikozanoidni test) (17). Najpouzdaniji oblik profilakse i terapije je prekid upotrebe preparata salicilne kiseline (17).

Prehrambeni aditiv je supstanca poznatog hemijskog sastava koja se uobičajeno ne upotrebljava kao hrana sama za sebe, niti je tipičan sastojak hrane, a dodaje se namenski radi promene tehnoloških i organoleptičkih svojstava hrane, što dovodi ili se može očekivati da dovede do toga da on sam ili njegov sekundarni proizvod direktno ili indirektno postaje sastojak te hrane (18,19). Sulfiti, benzoati i glutamati se mogu naći u gotovo svim vrstama hrane, pića i nekim lekovima (Tabela 2) (18,19).

Prevalencija samoprijavljenih intolearancija na navedene prehrambene aditive u odraslih iznosi 0,01-0,23% (21).

Table 1. Sources of histamine and possible causes of increase of concentration in organism (12)

Naturally produced histamine, mostly in mast cells	
Histamine naturally occurring	Tomatoes, eggplant, spinach, fish, chicken and stored meat, fermented food (cheeses, sausages, sauerkraut, wine, beer, champagne...)
Histamine liberators	Pineapple, bananas, citrus fruits, strawberries, nuts, papaya, tomatoes, liquorice, spices, legumes, cocoa, alcohol, fish, seafood, pork, egg white
Histamine liberators of additives	Colourants, preservatives, stabilisers, taste enhancers, flavourings
Histamine production induced by yeast and bacteria	Foods with viable yeast – sourdough, fresh bread
Substances decreasing DAO activity in food	Alcohol
Medication decreasing DAO activity in food	Verapamil, propafenone, cefuroxime, cefotiame, acidum clavulanicum, doxycyclinum, isoniazid, framycetin, metamizole, amitriptiline, diazepam, haloperidol, promethazine, cimetidine, dihydralazine, chloroquin, aminophylline, theophylline, furosemide, N-acetylcysteine, alcuronium, pancuronium, d-tubocurarin, acriflavinium chloride
Histamine liberators in medication	Morfijum, petidin, kodein, metamizol, acetilsalicilna kiselina, d -cikloserin, hlorokin, pentamidin, dobutamin, verapamil, alprenolol, kodein, amilorid, kontrasna sredstva koje sadrže jod , mezokain, prokain, markain, prilokain d –tubokurarin, barbiturati, tiopental
Pyridoxine inactivating drugs	Hydralazine d-cyklosporine, isoniazid, hormonal contraception
Allergic reaction	IgE-mediated histamine release from mast cells
Medication potentiating allergic IgE-mediated histamine release	Acetylsalicylic acid, diclofenac, flurbiprofen, indomethacin, ketoprofen, mefenamin, naproxen
Infection, trauma, shock	

DAO - diaminooxidase

in food (beans, cauliflower, sour vegetables, strawberries, plums, watermelons, raspberries, pineapple, peanuts, almond, grapes, buckwheat, oat, corn, sausages, sauces, ready-made meals, fruit juices, tea, beer, liqueur, rum, wine, herbs, spices), preservatives and colorants (13-16).

Salicylate intolerance is induced by a marked inhibition of cyclooxygenase type 1 (COX-1) and the resulting diminished production of prostaglandin, prostacyclin and thromboxan, as well as the increased availability of leukotriene A4 (13,16). It is manifested, first of all, by rhinosinusitis, nasal and sinus polyps, or bronchial asthma (17). The simultaneous existence of intolerance to non-steroidal anti-inflammatory drugs, polyposis and asthma is marked as a "triad" (17). In addition, urticaria, colitis or diarrhea may appear (17). Anaphylactic shock is very rare (17).

Diagnostic analysis starts with anamnesis aimed at establishing the possible connection between the derivatives of salicylic acid and symptoms of intolerance (17). Exposure testing or provocation testing is a gold standard in the diagnostics of intolerance (17). When provocation is not acceptable (the severity of expected reaction) or if it is contraindicated (infection or bronchial asthma), functional tests are used (measurement of the quantity of leukotriene liberated from the basophilic leukocytes, measurement of membrane protein CD63 associated with lysosomes, extended functional eicosanoid test) (17).

The most reliable form of prophylaxis and treatment is the elimination of salicylic acid products (17). Food additive is a substance that has known chemical composition, which is not used as food alone, and it is not a typical food ingredient, but it is added to food in order to change its technological and organoleptic properties. Therefore, it may be expected that the food additive alone or its secondary product directly or indirectly becomes the food ingredient (18,19).

Sulfites, benzoates and glutamates may be found in almost all kinds of food, drinks, and some medications (Table 2) (18,19).

Tabela 2. Namirnice i lekovi sa visokim sadržajem sulfita, mononatrijum glutamata i benzoata (18,20)

Namirnice	Sulfiti	Benzoati	Mononatrijum glutamat
Meso, živina i plodovi mora	Kozice, jastog, sušeni bakalar, rakovi štapići, lignje, pljeskavica od mesa, kobasice	Jela sa ljutim sosom, gotova jela koja sadrže benzoate	Riblji sos
Mleko i jaja		Jogurt, sir	Parmezan
Voće	Sušene kajsije, smokve, suhe šljive, datulje, suhe banana, kandirano voće sušeni kokos, ribizla	Brusnice, borovnice, suve šljive, papaja, sušeno voće, avokado	
Povrće, orašasti plodovi, semenke i slane grickalice	Sušene pečurke i druge gljive, smrznuti, konzervisani ili vakuumirani krompir, pomfrit, instant kaše, njoke, kroketi od krompira, vegetarijanski hamburgeri i kobasice, šparoge u konzervi, mahune, pasulj, kesteni,	Bundeva, mahunarke, soja pasulj, sojino brašno, brokoli, spanać, pečeni pasulj i paradajzu u ljutom sosu, pečeni orasi, čips (osim gotovih usoljenih), grickalice od krompira ili kukuruza,	Pečurke, spanać, slane grickalice, čips
Začini i ostalo	Umak od hrena, karamel boja	Kari u prahu, aleva paprika, mješavina začina, muškatni oraščić, karanfilić, cimet, čokolada, kakao, kečap, soja sos, preliv za salatu, krema za salatu, majonez, džem, kiseli krastavci	Supe, temeljac, umaci, premazi, gotova jela, soja sos, sos od crnog pasulja, sos od ostriga, paradajz sos, instant pirinač i jela sa rezancima
Piće	Jabukovača, vino, pivo, kašasti i gazirani sokovi, sok od grožđa, sok od narandže, kola	Čaj, kašasti i gazirani sokovi, pivo, žestoka pića, žestoka pića sa dodatkom začina	
Lekovi	Isopterenalin, isoproksiharimetrin, dopamin, lokalni anestetici, propofol, aminoglikozidni antibiotici, metoklopramid, doksiciklin, vitamini B kompleks, topikalne antifugalne i kortikosteroidne kreme		Natrijumfenilacetat/ natrijum benzoat

S obzirom na značajne varijacije u simptomima i ozbiljnosti reakcije postoji nekoliko patogenetskih mehanizama intolerancije na sulfite (20,22).

Udisanje sumpor dioksida, nastalog iz unesenih sulfita u toploj kiseloj sredini usta i želuca, može uzrokovati respiratorne simptome (20,22). Nedostatak sulfit oksidaze i posledična prekomerna akumulacija sulfita uzrokuje holinergički posredovanu bronhokonstrikciju (20,22). Sulfiti uzrokuju degranulaciju mastocita (u odsustvu IgE) te oslobađanje histamina i drugih medijatora intolerancije (20,22). Osim toga, smanjuju dostupnost prostaglandina i povećavaju koncentraciju leukotriena (21,22).

Hipersulfatinemija može uzrokovati inhibiciju enzima uključenih u sintezu/aktivaciju neurotransmitera nukleus akumbensa (23). Sulfiti narušavaju integritet mitohondrijalne membrane neurona (smanjuju proizvodnju adenozin tri fosfata i povećavaju koncentraciju reaktivnih vrsta kiseonika) (24,25).

Intolerancija na sulfite se predominantno manifestuju respiratornim simptomima (20,22). Mogu se javiti i dermatološki i gastrointestinalni znaci i simptomi (20,22). Izbegavanje sulfita u intolerantnih osoba može ublažiti tinitus, hiperakuziju, kao i manifestacije bolesti povezanih sa smanjenom dopaminergičkom ili serotonergičkom aktivnošću (23).

Konzumiranje visoke doze mononatrijum glutamata na prazan želudac u malog broja ljudi uzrokuje pojavu „sindroma kineskog restorana“ koji se karakteriše glavoboljom, crvenilom lica, utrnulošću gorneg dela tela, glave i vrata, opštom slabošću, palpitacijom, urtikarijom, bolom u sto-

Table 2. Foods and medications with high levels of sulfites, monosodium glutamate and benzoate (18,20)

Foods	Sulphites	Benzoates	Monosodium glutamate
Meat, poultry and seafood	Prawns, lobster, dried salt cod, crab sticks, squid, meat burger, sausages	Dishes with a spicy sauce, ready to eat meals containing benzoates	Fish sauce
Milk and eggs		Yoghurt, cheese	Parmesan cheese
Fruits	Dried apricots, sultanas, figs, prunes, dates, dried banana, candied, glace fruit desiccated coconut, currants	Cranberries, bilberries, prunes, papaya, dried fruit, avocado	
Vegetables, nuts, seeds and savoury snacks	Dried mushrooms and other fungi, frozen, tinned or vacuum packed potatoes, french fries, instant mash, gnocchi, potato cakes, potato croquettes, vegetarian burgers and sausages, tinned asparagus, broad beans, french beans, chestnuts	Pumpkin, kidney beans, soy beans, soy flour, broccoli, spinach, baked beans, tomato in spicy sauce, dry roasted and spicy nuts, crisps (except ready salted), potato or corn snacks	Mushrooms, spinach, savoury snacks, crisps
Condiments and miscellaneous	Horseradish sauce, caramel colouring	Curry powder, allspice, mixed spice, nutmeg, clove, cinnamon, chocolate, cocoa, ketchup, soy sauce, Worcestershire sauce, salad dressing, salad cream, mayonnaise, jam, pickles	Soups, stock, gravy, coatings, ready-meals, soy sauce, black bean sauce, oyster sauce, tomato sauce, miso, marmite, instant rice and noodle dishes
Drinks	Cider, wine, beer, fruit squash and cordials, soft drinks, grape juice, fruit juice drinks, cola drinks	Tea, squash, cordial, carbonated drinks, milkshake syrup, beer, ready-to-drink alcohol and mixers, spirits with added spices	
Medication	Isoprenaline, isoproterenol, isoetharine, phenylephrine, dexamethasone and injectable corticosteroids, dopamine, local anaesthetics, propofol, aminoglycoside antibiotics, metoclopramide, doxycycline and vitamin B complex, Topical anti-fungal and corticosteroid creams		Sodium phenylacetate/ sodium benzoate

The prevalence of self-reported intolerance to the above mentioned food additives in adults is 0.01-0.23% (21).

Considering significant variations in the symptoms and severity of reactions, there are several pathogenetic mechanisms of intolerance to sulfites (20,22).

The inhalation of sulphur dioxide from the ingested sulfites in the warm acidic environments of the mouth and stomach may cause respiratory symptoms (20,22). The lack of sulfite oxidase and the resulting excess accumulation of sulfites cause the cholinergic-mediated bronchoconstriction (20,22). Sulfites cause degranulation of mast cells (in the absence of IgE), and the liberation of histamines and other mediators of intolerance (20,22). In addition, they reduce the availability of prostaglandin and increase the concentration of leukotriene (21,22).

Hypersulfitemia may cause the inhibition of enzymes involved in the synthesis/activation of neurotransmitters nucleus accumbens (23). Sulfites disturb the integrity of mitochondrial membrane of neurons (reduce the production of adenosine 3 phosphate and increase the concentration of reactive forms of oxygen) (24,25).

Intolerance to sulfites is predominantly manifested by respiratory symptoms (20,22).

maku i odgođenim angioedemom (26-28). Predloženo je nekoliko etioloških mehanizama uključujući nedostatak vitamina B6, hipernatrijemiju, intoksikaciju histaminom, vazokonstrikciju, gastroezofagealni refluks, povećanu produkciju intermedijera Krepsovog ciklusa (28). U nedostatku dokaza koji potvrđuju bilo koji od navedenih, tačan uzrok i dalje nije poznat (28).

Kratkotrajno izlaganje benzoatima može izazvati iritaciju očiju, kože i respiratornog trakta, (29). Produžen kontakt rezultuje ekcemom, urtikarijom i perzistentnim rinitisom (29,30). Upotreba visokih doza benzoata može uzrokovati promenu sekrecije želudačne sluzi i razvoj ulkusa, te disfunkciju jetre i bubrega i hiperaktivnost (29). Zbog dokazane interkalacije u DNK natrijum benzoat se smatra genotoksičnim (31).

Dijagnoza intolerancija zahteva vođenje dnevnika ishrane i pojave simptoma, sprovođenje eliminacijske dijete te ponovno postepeno uvođenje u ishranu uz praćenje simptoma (17). Najpouzdaniji oblik profilakse i terapije je prekid upotrebe aditiva (17).

Intolerancije na hranu zavisne od domaćina

Laktoza je disaharid izgrađen od D-galaktoze i D-glukoze (32-35). Prisutan je u mlečnim proizvodima (32-35).

Intoleranciju na laktozu predstavlja nemogućnost apsorpcije laktoze u crevima uzrokovana smanjenjem ili gubitkom aktivnosti enzima laktaze (32,33). Intolerancija na laktozu ima visoku prevalenciju koja se kreće između 57% i 65% (34). Najčešće je prisutna kod adolescenata i mladih odraslih osoba (33).

Intolerancija laktoze može biti primarna (postepeni pad aktivnosti laktaze sa starenjem), stečena (u prisustvu gastroenteritisa, celijakije, Kronove bolesti, ulceroznog kolitisa, upotrebe antibiotika, hemoterapije, ozlede sluznice crieva), kongenitalna (odsustvo ili pad aktivnosti enzima laktaze u novorođenčeta koji se nasleđuje autozomno recesivno) i razvojna (nedonoščad sa nerazvijenim crevima) (33). Neapsorbovana laktoza u crevima povećava sadržaj vode u lumenu creva što uzrokuje osmotsku dijareju (33,35). Dodatni priliv tečnosti uzrokuju bakterije debelog creva koje fermentišu laktozu u kratkolančane masne kiseline i gasove (vodonik, ugljen dioksid i metan) (33,35).

Simptomi i znakovi inetolerancije na laktozu manifestuje se u periodu od 30 minuta do 2 sata nakon uzimanja mlečnih proizvoda (33). Njihova ozbiljnost zavisi od količine unete laktoze, preostale funkcije laktaze, enteričkog mikrobiona i vremena prolaska hrane kroz tanko crevo (33,35). Uobičajeni znakovi i simptomi uključuju: bol u stomaku, nadutost, dijareju, zatvor, mučninu i povraćanje (33,35,36). Ekstrainterstinalni simptomi (otežana koncentracija, glavobolja, bol u kostima i mišićima, depresija, anksioznost, promene na sluznici usta i poremećaj srčanog ritam) su retko prisutni (35). Dijagnoza intolerancije na laktozu se postavlja na osnovu anamneze, fizikalnog pregleda, funkcionalnih testova (izdisajni test na vodonik, test tolerancije na mleko, test kiselosti stolice, test tolerancije na laktozu), sprovođenja eliminacijske dijete, biopsije creva i genotipizacije (33,35).

Lečenje podrazumeva modifikaciju ishrane, suplementaciju laktazom i lečenje osnovnog stanja kod osoba sa sekundarnim nedostatkom laktaze (33,37).

Fruktoza je prirodno prisutna u raznim namirnicama (36-39). Osim toga, fruktoza se proizvodi iz kukuruza (kukuruzni sirup s visokim sadržajem fruktoze je prisutan u bezalkoholna pićima i zaslađivačima hrane) (Tabela 3) (36-39).

Intolerancija na fruktozu se manifestuje u obliku nasledne ili stečene intolerancije na fruktozu (38,39).

Naslednu intoleranciju na fruktozu uzrokuje nedostatak enzima fruktoza-1-fosfat aldolaze, i posledična akumulacija fruktoza-1-fosfata u jetri (40). Fruktoza-1-fosfat inhibira fosforilazu, enzim glikogenolize, što dovodi do laktacidoze i hipoglikemije (40). Osim toga, konzumiranje fruktoze u intolerantnih osoba indukuje hipofasfatemiju, oštećenje proksimalnih tubula bubrega i jetre (40). Ozbiljnost simptoma je proporcionalna količini unete fruktoze (40). Manje količine uzrokuju povraćanje, bol u trbuhu, dijareju i hipoglikemiju (40). U ranom dojenačkom dobu velike količine unete fruktoze mogu rezultovati šokom, akutnim zatajenjem jetre i bubrega i smrću (40). Dijagnoza nasledne intolerancije na fruktozu se postavlja uz pomoć genetskog testa koji je komercijalno dostupan (40). Terapija podrazumeva izbegavanje fruktoze u ishrani (40). Stečena intolerancija na fruktozu nastaje kao posledica malapsorpcije uzrokovane promenama u proteinu za transport glukoze 5 i 2 (eng. *Glucose Transporter 5 and Glucose Transporter 2* - GLUT 5 i GLUT 2) (38-42).

Dermatological and gastrointestinal signs and symptoms may appear, as well (20,22). The avoidance of sulfites in persons who are intolerant may alleviate tinnitus, hyperacusis, and manifestations of disease connected with the reduced dopaminergic and serotonergic activity (23).

The consumption of higher doses of monosodium glutamate on an empty stomach may cause in the small number of people the "syndrome of Chinese restaurant", which is manifested by headache, flushing, numbness of upper part of the body, head and neck, weakness, palpitation, urticaria, abdominal pain and prolonged angioedema (26-28). A few etiological mechanisms were recommended including the lack of vitamin B6, hypernatremia, intoxication with histamine, vasoconstriction, gastroesophageal reflux, the increased production of Crebs cycle intermediate (28). Due to the lack of evidence, which would prove any of the above mentioned, the actual cause is still not known (28).

A short-term exposure to benzoates may cause the irritation of eyes, skin, respiratory tract (29). The prolonged contact results in eczema, urticaria and persistent rhinitis (29,30). The use of high doses of benzoates may cause the change of the secretion of stomach mucosa and the development of ulcus, and dysfunction of liver and kidneys and hyperactivity (29). Due to the proved intercalation in DNA, sodium benzoate is deemed to be genotoxic (31).

The diagnosis of intolerance requires keeping a diary of diet and symptoms, implementing the elimination diet, and gradual introduction of ingredients into the diet together with observing the symptoms (17). The most reliable form of prophylaxis and treatment is the elimination of additives (17).

Intolerance to host-dependent food

Lactose is a disaccharide that consists of D-galactose and D-glucose (32-35). It is present in dairy products (32-35).

Lactose intolerance is the inability of absorption of lactose in the intestines caused by the deficiency or loss of the enzyme lactase (32,33). Lactose intolerance has high prevalence which ranges between 57% and 65% (34). It is most common in adolescents and young adults (33).

Lactose intolerance can be primary (a gradual decline in lactase enzyme activity with increasing age), secondary (caused by gastroenteritis, celiac disease, Crohn disease, ulcerative colitis, antibiotics, chemotherapy, injury to intestinal mucosa), congenital (a decrease or absence of lactase enzyme activity since birth due to autosomal recessive inheritance) and developmental (newborns with immature intestines) (33). The unabsorbed lactose within the bowel results in an influx of fluid into the bowel lumen resulting in osmotic diarrhea (33,35). The additional influx of fluids is caused by colonic bacteria that ferment the lactose thus producing short-chain fatty acids and gas (hydrogen, carbon dioxide, and methane) (33,35).

Signs and symptoms of lactose intolerance manifest 30 minutes to 2 hours after ingesting dairy products (33). The severity of symptoms depends on the amount of consumed lactose, the residual lactase function, and the small bowel transit time (33,35). Common signs and symptoms include the following: abdominal pain, abdominal bloating, diarrhea, constipation, nausea and vomiting (33,35,36). Extraintestinal symptoms (loss of concentration, headache, muscle pain, joint pain, mouth ulcers and arrhythmia) are rarely present (35). The diagnosis of lactose intolerance is established based on anamnesis, physical examination and functional tests (hydrogen breath test, milk tolerance test, stool acidity test, lactose tolerance test, dietary elimination, small bowel biopsy, and genotyping) (33,35).

The treatment of lactose intolerance consists of dietary modification, lactase supplementation, and treating an underlying condition in people with secondary lactase deficiency (33,37).

Fructose is naturally present in various foods (36-39). Also, fructose is produced from corn (corn syrup with the high amount of fructose is present in many non-alcoholic drinks and sweeteners) (Table 3) (36-39).

Fructose intolerance is manifested as hereditary or acquired fructose intolerance (38,39).

Hereditary fructose intolerance is caused by the lack of the enzyme fructose-1-phosphate aldolase, and the resulting accumulation fructose-1-phosphate in the liver (40). Fructose-l-phosphate inhibits phosphorylase, the enzyme of glycogenolysis, which causes lactacidosis and hypoglycemia (40). Also, the consumption of fructose in intolerant people induces

Tabela 3. Namirnice sa visokim sadržajem fruktoze (41)

Kategorija	Namirnice
Voće	Svo voće (osim avokada, brusnice, limete, dinje, limuna, ananasa, jagoda, mandarina, banane), voćni sokovi, sušeno voće i voće konzervirano u soku ili sirupu.
Povrće	Artičoka, šparoge, brokoli, praziluk, šampinjoni, bamija, luk, grašak, crvena paprika, proizvodi od paradajza (pasta, kečap, konzervirani paradajz).
Žitarice	Namirnice sa pšenicom kao glavnim sastojkom (pšenični hleb, testenina, kus-kus), žitarice sa dodatkom suvog voća, žitarice sa dodatkom kukuruznog sirupa s visokim sadržajem fruktoze.
Meso	Marinirano ili prerađeno meso
Mlečni proizvodi	Mlečni proizvodi sa dodatkom kukuruznog sirupa s visokim sadržajem fruktoze (jogurt, aromatizovano mleko...)

GLUT 5 je jedini specifični transporter fruktoze koji pasivno prenosi fruktozu sa apikalne membrane enterocita (predominantno u duodenumu i proksimalnom jejunumu) (42). GLUT 2 predstavlja pomoćni transporter koji ima nizak afinitet za fruktozu (u stanju je transportovati i druge monosaharide kao što su glukoza i galaktoza) (40). Apsorpcija fruktoze u tankom crevu je ograničena, značajan broj odraslih osoba ne može apsorbovati više od 25 g/dan (42).

Smanjena aktivnost transportera frukoze uzrokuje povećano osmotsko opterećenje lumena, povećanu fermentaciju bakterija debelog creva, narušava gastrointestinalni motilitet i vodi promeni u crevnoj flori (42).

Stečena intolerancija na fruktozu se manifestuje nadutošću, bolom u stomaku, mučninom i dijarejom (41). Dijagnoza se postavlja izdisajnim testom na vodonik i/ili metan (od 1,5 do 3 sata nakon konzumiranja fruktoze) (40). Najpouzdaniji oblik profilakse i terapije je prekid upotrebe fruktoze (38-41).

Polioli predstavljaju posebnu grupu alkohola koji nastaju katalitičkom hidrogenacijom ugljenih hidrata (42,43). Polioli se nalaze u određenom voću (jabuka, kajsija, avokado, kupina, trešnja, nektarina, kruška, suve šljive, grožđice), povrću (karfiol) i gljivama (42,43). Osim toga oni se koriste kao veštački zaslađivači (u zamenu za saharozu jer daju manje kalorija po gramu) u mnogim industrijskim proizvodima (bombone, žvakaće gume, sladoledi, peciva, pekarski proizvodi i čokolade) (42,43). Polioli se mogu naći u pasti za zube i vodicama za ispiranje usta (41). Agencija za hranu i lekove (engl. *Food and Drug Administration* - FDA) Sjedinjenih Američkih Država je odobrila upotrebu osam različitih poliola, koji uključuju eritritol, hidrogenizovane hidrolizate skroba, izomalt, laktitol, maltitol, manitol, sorbitol i ksilitol (43).

Intolerancija na poliole nastaje kao rezultat promena u crevnom mikrobiomu (43). Manifestuje se nadimanjem, bolovima u stomaku i dijarejom (42,43). Simptomi se povećavaju sa količinom unetih poliola i konzumiranjem drugih ugljenih hidrata (43). Dijagnoza intolerancije na poliole se postavlja izdisajnim testom (polioli koji se dobro apsorbuju poput sorbitola) ili sprovođenjem eliminacione dijete (polioli koji se slabo apsorbuju poput manitola) (44).

Galaktani predstavljaju polimere galaktoze (42). Prisutni su u mahunarkama poput pasulja, leblebija i proizvodima od soje, prokelju, orašastim plodovima i kupusu (42). Ove namirnice su neretko deo vegetarijanske ishrane, indijske i meksičke kuhinje (42).

Intolerancija na galaktane nastaje kao posledica povećane fermentacije bakterija debelog creva (45). Simptomi uključuju nadimanje i grčeve u stomaku (45). Dijagnoza i terapija su slične ostalim fermentabilnim ugljenim hidratima (42).

Gluten predstavlja protein za skladištenje semena visoke molekularne težine koji se obično nalazi u žitaricama kao što su pšenica, ječam i raž (1,46). On hrani seme tokom cvetanja i klijanja, čime doprinose uspešnoj reprodukciji vrste (46). Gluten je kompozitni protein, sastavljen od glutenina i prolamina (46). Doprinosi kvalitetu testa i predstavlja sastavnu komponentu velikog broja namirnica koje sadrže pšenicu, uključujući hleb, žitarice i testeninu (1,46).

Intolerancija na gluten (necelijakijska osetljivost na gluten) podrazumeva sindrom kojeg karakterišu interstinalni i ekstraintestinalni simptomi povezani s konzumiranjem hrane koja sadrži

Table 3. Foods to contain high levels of fructose

Category	Food
Fruits	All fruits not on the allowed list, especially juices, dried fruits (such as prunes, raisins or dates) and fruits canned in juice or syrup)
Vegetables	Artichoke, asparagus, broccoli, chutney, leeks, mushrooms, okra, onions, peas, red pepper, shallots, tomato paste, tomato products (canned tomatoes, ketchup)
Cereals	Foods with wheat as a major ingredient (wheat bread, pasta, couscous), grains with added dried fruit, grains with added fructose
Meats	Marinated or processed meats
Dairy Products	Dairy product with fructose (yogurts, and flavored milks...)

hypophosphatemia, damage of proximal tubules of kidneys and liver (40). The severity of symptoms is proportional to the amount of ingested fructose (40). Smaller quantities cause vomiting, abdominal pain, diarrhea and hypoglycemia (40). In breastfed infants, great amounts of fructose may result in shock, acute kidney and liver failure and death (40).

Diagnosis of hereditary fructose intolerance is established with the help of genetic test which is commercially available (40). The treatment includes avoidance of fructose in the diet (40).

Acquired fructose intolerance appears as a consequence of malabsorption caused by the changes in the proteins glucose transporter 5 and glucose transporter 2 (GLUT 5 and GLUT 2) (38-42).

GLUT 5 is the only specific transporter of fructose that transports fructose passively from the apical membrane of enterocytes (predominantly in the duodenum and proximal jejunum) (42). GLUT 2 is an assistant transporter which has a low affinity with fructose (it may transport other monosaccharides such as glucose and galactose) (40). The absorption of fructose in the small intestines is limited, and a significant number of people cannot absorb more than 25 g/day (42).

The reduced activity of fructose transporters causes an increased osmotic load of the lumen, increased fermentation of bacteria in the colon, it harms the gastrointestinal motility and leads to the change in the intestinal flora (42).

Acquired intolerance to fructose is manifested as abdominal bloating, abdominal pain, nausea and diarrhea (41). Diagnosis is established with the help of hydrogen and/or methane breath test (1.5 to 3 hours after fructose consumption) (40). The most reliable form of prophylaxis and treatment is the elimination of fructose from the diet (38-41).

Polyols belong to the specific group of alcohols that are produced by hydrogenation of carbohydrates (42,43). Polyols are present in certain fruits (apple, apricot, avocado, blackberry, cherry, nectarine, pear, prunes, raisins), vegetables (cauliflower) and mushrooms (42,43). In addition, they are used as artificial sweeteners (instead of sacharose because they have fewer calories per gram) in many industrial products (candies, chewing gum, ice-cream, pastry, baked goods and chocolate) (42,43). Polyols may be found in toothpaste and mouthwash (41). The American Food and Drug Agency has approved the use of eight different polyols, including the following: erythritol, hydrogenated starch hydrolysates, isomalt, lactitol, maltitol, mannitol, sorbitol and xylitol (43).

Intolerance to polyols is the result o changes in the gut microbiota (43). It is manifested as abdominal bloating, pain and diarrhea (42,43). The symptoms increase with the amount of ingested polyols and consumption of other carbohydrates (43). Diagnosis of polyols intolerance is established with the help of breath test (polyols that are absorbed well such as sorbitol) or with the elimination diet (polyols that are not absorbed well such as manitol) (44).

Galactans are polymers of galactose (42). They are present in legumes such as beans, chick-pea, soy products, broccoli, nuts and cabbage (42). These foods are often part of vegetarian diet, of Indian and Mexican cuisine (42).

Intolerance to galactans is the result of increased fermentation of bacteria in the colon (45). The symptoms include abdominal bloating and cramps in the stomach (45). Diagnosis and treatment are similar to other fermentable carbohydrates (42).

gluten kod osoba kod kojih je isključena celijakija i alergija na gluten (1,47). Prevalencije samoprijavljene intolerancije na gluten iznosi 0,5-13,0% (45). Patofiziologija nije u potpunosti jasna (1,46,47). Predloženi mehanizmi uključuju crevnu disfunkciju te promenu crevnog mikrobioma (1,46,47).

Intolerancija na gluten može uzrokovati bol u trbuhu, dijareju, gubitak težine, glavobolju, umor, malaksalost, bol u mišićima, ponavljajuće oralne ulceracije i depresiju (47). U nedostatku seroloških i patohistoloških kriterija za postavljanje dijagnoze dvostruko slepo, placebom kontrolisano ispitivanje predstavlja zlatni standard u postavljanju dijagnoze (47). Terapija zahteva isključivanje glutena iz ishrane što neretko smanjuje nutritivnu adekvatnost i predstavlja socio-ekonomski teret (1,46-50). Obećavajuća većina terapija testiranih u kliničkim ispitivanjima (probiotici, modulatori crevne barijere, endopeptidaze, inhibitori transglutaminaze 2) pokazala je važna ograničenja (49).

Zaključak

Intolerancije na hranu predstavljaju zanimljivo i nedovoljno istraženo područje. Postoji značajna diskrepancija između prevalencije intolerancije na hranu utvrđene dvostruko slepim, placebom kontrolisanim istraživanjima i samoprijavljenih „bolesti“ ili „nelagode“' uzrokovanih unosom određene hrane.

Intolerancija na hranu se manifestuju širokim spektrom nespecifičnih gastrointestinalnih i ekstraintestinalnih simptoma i znakova. S druge strane, patogenetski mehanizmi određenih intolerancija na hranu, u prvom redu intolerancije na hranu nezavisni od domaćina nisu poznati.

Za mnoge intolerancije na hranu ne postoji pouzdan dijagnostički biomarker. Zbog toga se u postavljanju dijagnoze neretko koristi pristup pokušaja i greške koji podrazumeva uklanjanje jedinjenja s potencijalnom farmakološkom aktivnošću iz ishrane na kratak period, i njihovo postepeno ponovo uvođenje u ishranu. Odabir komponenti hrane za uklanjanje iz ishrane može se temeljiti na anamnestičkim podacima, kliničkim manifestacijama, i, gde je primenjivo, genetskim varijacijama.

Najpouzdaniji oblik profilakse i terapije intolerancija na hranu predstavlja isključivanje uzročnih komponenti iz ishrane. Međutim, promene u ishrani mogu uzrokovati smanjenje nutritivne adekvatnosti. Stoga je potrebna edukacija obolelih i, po mogućnosti, primena strategija za poboljšanje tolerancije na komponente hrane koja bi smanjila nivo neophodnih ograničenja u ishrani.

Konflikt interesa

Autori su izjavili da nema konflikta interesa.

Literatura

1. Tuck CJ, Biesiekierski JR, Schmid-Grendelmeier P, Pohl D. Food Intolerances. Nutrients 2019; 11(7):1684. doi: 10.3390/nu11071684.
2. Lomer MCE. Review article: the aetiology, diagnosis, mechanisms and clinical evidence for food intolerance. Aliment Pharmacol Ther 2015; 41:262-75. doi: 10.1111/apt.13041.
3. Zopf Y, Baenkler HW, Silbermann A, Hahn EG, Raithel M. The differential diagnosis of food intolerance. Dtsch Arztebl Int 2009; 106(21):359-69; quiz 369-70; p following 370. doi: 10.3238/arztebl.2009.0359.
4. Gargano D, Appanna R, Santonicola A, De Bartolomeis F, Stellato C, Cianferoni A et al. Food Allergy and Intolerance: A Narrative Review on Nutritional Concerns. Nutrients 2021; 13(5):1638. doi: 10.3390/nu13051638
5. Jansson-Knodell C, White M, Lockett C, Xu H, Shin A. High prevalence of food intolerances among US internet users. Public Health Nutrition 2021; 24(3):531-5. Dostupno na: https://doi.org/10.1017/S1368980020003298
6. Young E, Stoneham MD, Petruckevitch A, Barton J, Rona R. A population study of food intolerance. Lancet 1994; 343(8906):1127-30. doi: 10.1016/s0140-6736(94)90234-8.
7. Woods R, Abramson M, Bailey M, Walters EH. International prevalences of reported food allergies and intolerances. Comparisons arising from the European Community Respiratory Health Survey (ECRHS) 1991–1994. Eur J Clin Nutr 2001; 55:298–304. doi: 10.1038/sj.ejcn.1601159.
8. Tivković R. Dijetetika. Medicinska naklada, Zagreb; 2002.
9. Lugović-Mihić L, Šešerko A, Duvančić T, Šitum M, Mihić J. Intolerancija na histamin.koje su moguće posljedice na koži. Acta Med Croatica 2012; 66:375-81.
10. Comas-Basté O, Sánchez-Pérez S, Veciana-Nogués MT, Latorre-Moratalla M, Vidal-Carou MDC. Histamine Intolerance: The Current State of the Art. Biomolecules 2020; 10(8):1181. doi: 10.3390/biom10081181
11. Maintz L, Novak N. Histamine and histamine intolerance. Am J Clin Nutr 2017; 85(5):1185–96. doi: 10.1093/ajcn/85.5.1185.
12. Kovacova-Hanuskova E, Buday T, Gavliakova S, Plevkova J. Histamine, histamine intoxication and intolerance. Allergol Immunopathologia 2015; 43(5):498-506. DOI: 10.1016/j.aller.2015.05.001
13. Raithel M, Baenkler HW, Naegel A, Buchwald F, Schultis HW, Backhaus B et al. Significance of salicylate intolerance in diseases of the lower gastrointestinal tract. J Physiol Pharmacol 2005; 56(Suppl 5):89-102.
14. Kęszycka PK, Lange E, Gajewska D. Effectiveness of

Gluten is a seed storage protein of high molecular weight usually found in grains such as wheat, barley and rye (1,46). It feeds the seed during flourishing and sprouting, thus contributing to the reproduction of species (46). Gluten is a composite protein, made of glutenin and prolamin. It contributes to the quality of dough and it is an ingredient of numerous foods that contain wheat, including bread, cereals, and pasta (1,46).

Gluten intolerance (non-celiac gluten sensitivity) is a disorder that is characterized by intestinal and extraintestinal symptoms associated with the consumption of food that contains gluten in persons, in whom celiac disease and allergy to wheat were excluded (1,47). The prevalence of self-reported intolerance to gluten amounts to 0.5-13.0% (45). The pathophysiology is not completely clear (1,46,47). The proposed mechanisms include intestinal dysfunction, and change in gut microbiome (1,46,47).

Intolerance to gluten may cause abdominal pain, diarrhea, weight loss, headache, fatigue, weakness, muscle pain, repeated oral ulcerations, and depression (47). Due to the lack of serological and pathohistological criteria necessary to make diagnosis, a double-blind, placebo-controlled examination is a gold standard of diagnostics (47). The treatment requires the elimination of gluten from the diet, which often reduces the nutritive adequacy and is a socio-economic burden (1,46-50). The majority of promising therapies tested in clinical trials (probiotics, modulation of gut barrier, endopeptidase, inhibitors of transglutaminase 2) have shown significant limitations (49).

Conclusion

Food intolerance is an interesting and insufficiently studied field of research. There is a significant discrepancy between the prevalence of food intolerance established by double-blind, placebo-controlled trials and self-reported “diseases” or “discomfort” caused by the ingestion of certain foods.

Food intolerance is manifested as a wide range of non-specific intestinal and extraintestinal signs and symptoms. On the other hand, pathogenetic mechanisms of certain food intolerances, first of all host-independent intolerance, are still not clear.There are no reliable diagnostic biomarkers for many food intolerances. Therefore, a trial-and-error approach is used, which means that food constituents with a potential pharmacological activity are reduced for a short period and then gradually reintroduced into the diet. The selection of components to be removed from the diet may be grounded on anamnestic data, clinical manifestations, and when it is applicable, by genetic variations.

The most reliable form of prophylaxis and treatment of food intolerances is the elimination of causative food components from the diet. However, changes in the diet may cause the reduction of nutritional adequacy. Therefore, the education of patients is necessary and possibly the implementation of strategies to improve tolerance to food components which would reduce the level of necessary limitations in the diet.

Competing interests

Authors declare no competing interests.

Literature

1. Tuck CJ, Biesiekierski JR, Schmid-Grendelmeier P, Pohl D. Food Intolerances. Nutrients 2019; 11(7):1684. doi: 10.3390/nu11071684.
2. Lomer MCE. Review article: the aetiology, diagnosis, mechanisms and clinical evidence for food intolerance. Aliment Pharmacol Ther 2015; 41:262-75. doi: 10.1111/apt.13041.
3. Zopf Y, Baenkler HW, Silbermann A, Hahn EG, Raithel M. The differential diagnosis of food intolerance. Dtsch Arztebl Int 2009; 106(21):359-69; quiz 369-70; p following 370. doi: 10.3238/arztebl.2009.0359.
4. Gargano D, Appanna R, Santonicola A, De Bartolomeis F, Stellato C, Cianferoni A et al. Food Allergy and Intolerance: A Narrative Review on Nutritional Concerns. Nutrients 2021; 13(5):1638. doi: 10.3390/nu13051638
5. Jansson-Knodell C, White M, Lockett C, Xu H, Shin A. High prevalence of food intolerances among US internet users. Public Health Nutrition 2021; 24(3):531-5. Dostupno na: https://doi.org/10.1017/S1368980020003298
6. Young E, Stoneham MD, Petruckevitch A, Barton J, Rona R. A population study of food intolerance. Lancet 1994; 343(8906):1127-30. doi: 10.1016/s0140-6736(94)90234-8.
7. Woods R, Abramson M, Bailey M, Walters EH. International prevalences of reported food allergies and intolerances. Comparisons arising from the European Community Respiratory Health Survey (ECRHS) 1991–1994. Eur J Clin Nutr 2001; 55:298–304. doi: 10.1038/sj.ejcn.1601159.
8. Tivković R. Dijetetika. Medicinska naklada, Zagreb; 2002.
9. Lugović-Mihić L, Šešerko A, Duvančić T, Šitum M, Mihić J. Intolerancija na histamin.koje su moguće posljedice na koži. Acta Med Croatica 2012; 66:375-81.

Personalized Low Salicylate Diet in the Management of Salicylates Hypersensitive Patients: Interventional Study. Nutrients 2021; 13(3):991. doi: 10.3390/nu13030991

15. Duthie GG, Wood AD. Natural salicylates: foods, functions and disease prevention. Food Funct 2011; 2(9):515-20. doi: 10.1039/c1fo10128e.
16. Katzung BG, Masters SB, Trevor AJ. Temeljna i klinička farmakologija. Zagreb, Medicinska naklada; 2011.
17. Baenkler HW. Salicylate intolerance: pathophysiology, clinical spectrum, diagnosis and treatment. Dtsch Arztebl Int 2008; 105(8):137-42. doi: 10.3238/arztebl.2008.0137
18. Skypala IJ, Williams M, Reeves L, Meyer R, Venter C. Sensitivity to food additives, vaso-active amines and salicylates: a review of the evidence. Clin Transl Allergy 2015; 5:34. doi: 10.1186/s13601-015-0078-3
19. Jandrić-Kočić MC. Stvarni i uočeni rizik: aditivi za hranu. Medicinski glasnik Specijalne bolnice za bolesti štitaste žlezde i bolesti metabolizma 'Zlatibor' 2021; 26(82):50-67. Dostupno na: https://scindeks.ceon.rs/article.aspx?artid=1821-19252182050J
20. Vally H, Misso NL. Adverse reactions to the sulphite additives. Gastroenterol Hepatol Bed Bench 2012; 5(1):16-23.
21. Martinis I. Nutritivna alergija. Medix 2004; 10(52):86-8.
22. Skypala IJ, Williams M, Reeves L, Meyer R, Venter C. Sensitivity to food additives, vaso-active amines and salicylates: a review of the evidence. Clin Transl Allergy 2015; 5:34. doi: 10.1186/s13601-015-0078-3
23. Kronberg MT. Sulfite intolerance: A cause of tinnitus? Bioscience Hypotheses 2008; 1(4):185-8.
24. Zhang X, Shoba Vincent A, Halliwell B, Ping Wong K. A Mechanism of Sulfite Neurotoxicity. JBC 2004; 279(41):43035–45. doi: https://doi.org/10.1074/jbc.M402759200
25. Marshall KA, Reist M, Jenner P, Halliwell B. The neuronal toxicity of sulfite plus peroxynitrite is enhanced by glutathione depletion: implications for Parkinson's disease. Free Radic Biol Med 1999; 27(5-6):515-20. doi: 10.1016/s0891-5849(99)00094-5.
26. Taliaferro PJ. Monosodium glutamate and the Chinese Restaurant Syndrome: a review of food additive security. J Environ Health 1995; 57(10):8.
27. Bawaskar HS, Bawaskar PH, Bawaskar PH. Chinese Restaurant Syndrome. Indian J Crit Care Med 2017; 21(1):49-50. doi: 10.4103/0972-5229.198327
28. Geha RS, Beiser A, Ren C, Patterson R, Greenberger PA, Grammer LC et al. Review of Alleged Reaction to Monosodium Glutamate and Outcome of a Multicenter Double-Blind Placebo-Controlled Study. J Nutrition 2000; 130(4):1058S–62S. doi: 10.1093/jn/130.4.1058S
29. Shahmohammadi M, Javadi M, Nassiri-Asl M. An Overview on the Effects of Sodium Benzoate as a Preservative in Food Products. Biotech Health Sci 2016; 3(3):e35084. doi: 10.17795/bhs-35084
30. Pacor ML, Di Lorenzo G, Martinelli N, Mansueto P, Rini GB, Corrocher R. Monosodium benzoate hypersensitivity in subjects with persistent rhinitis. Allergy 2004; 59(2):192-7. doi: 10.1046/j.1398-9995.2003.00380.x.
31. Linke BGO, Casagrande TAC, Cardoso LAC. Food additives and their health effects: A review on preservative sodium benzoate. Afr J Biotechnology 2018; 17(10):306-10. doi: 10.5897/AJB2017.16321
32. Vonk RJ, Reckman GA , Harmsen HJ, Priebe MG. Probiotics and Lactose Intolerance. In: Rigobelo EC editor. Probiotics [Internet]. London: Intech Open; 2012. Dostupno na: https://www.intechopen.com/chapters/39620
33. Malik TF, Panuganti KK. Lactose Intolerance. In: StatPearls [Internet]. Treasure Island (FL): StatPearls; 2022. Dostupno na: https://www.ncbi.nlm.nih.gov/books/NBK532285/
34. Catanzaro R, Sciuto M, Marotta F. Lactose intolerance: An update on its pathogenesis, diagnosis, and treatment. Nutr Res 2021; 89:23-34. doi: 10.1016/j.nutres.2021.02.003
35. Storhaug CL, Fosse SK, Fadnes LT. Country, regional, and global estimates for lactose malabsorption in adults: a systematic review and meta-analysis. Lancet Gastroenterol Hepatol 2017; 2(10):738-46. doi: 10.1016/S2468-1253(17)30154-1.
36. Misselwitz B, Butter M, Verbeke K, Fox MR. Update on lactose malabsorption and intolerance: pathogenesis, diagnosis and clinical management. Gut 2019; 68:2080-91. doi: 10.1136/gutjnl-2019-318404
37. Forsgård RA. Lactose digestion in humans: intestinal lactase appears to be constitutive whereas the colonic microbiome is adaptable. Am J Clin Nutr 2019; 110(2):273–9. doi: 10.1093/ajcn/nqz104.
38. Latulippe ME, Skoog SM. Fructose malabsorption and intolerance: effects of fructose with and without simultaneousglucoseingestion.CritRevFoodSciNutr2011; 51(7):583-92. doi: 10.1080/10408398.2011.566646.
39. Wilder-Smith CH, Li X, Ho SS, Leong SM, Wong RK, Koay ES et al. Fructose transporters GLUT5 and GLUT2 expression in adult patients with fructose intolerance. United European Gastroenterol J 2014; 2(1):14-21. doi: 10.1177/2050640613505279.
40. Langdon D, Stanley C, Sperling M. Hypoglycemia in the Infant and Child. 4th edition of Sperling's Pediatric Endocrinology; 2013.
41. Fedewa A, Rao SS. Dietary fructose intolerance, fructan intolerance and FODMAPs. Curr Gastroenterol Rep 2014; 16(1):370. doi: 10.1007/s11894-013-0370-0.
42. Zugasti Murillo A, Estremera Arévalo F, Petrina Jáuregui E. Dieta pobre en FODMAPs (fermentable oligosaccharides, disaccharides, monosaccharides and polyols) en el síndrome de intestino irritable: indicación y forma de elaboración. Endocrinol Nutr 2016; 63:132–8. doi: 10.1016/j.endonu.2015.10.009
43. Lenhart A, Chey WD. A Systematic Review of the Effects of Polyols on Gastrointestinal Health and Irritable Bowel Syndrome. Adv Nutr 2017; 8(4):587-96. doi: 10.3945/an.117.015560.
44. Barrett JS, Gibson PR. Fermentable oligosaccharides, disaccharides, monosaccharides and polyols (FODMAPs)

10. Comas-Basté O, Sánchez-Pérez S, Veciana-Nogués MT, Latorre-Moratalla M, Vidal-Carou MDC. Histamine Intolerance: The Current State of the Art. Biomolecules 2020; 10(8):1181. doi: 10.3390/biom10081181
11. Maintz L, Novak N. Histamine and histamine intolerance. Am J Clin Nutr 2017; 85(5):1185–96. doi: 10.1093/ajcn/85.5.1185.
12. Kovacova-Hanuskova E, Buday T, Gavliakova S, Plevkova J. Histamine, histamine intoxication and intolerance. Allergol Immunopathologia 2015; 43(5):498-506. DOI: 10.1016/j.aller.2015.05.001
13. Raithel M, Baenkler HW, Naegel A, Buchwald F, Schultis HW, Backhaus B et al. Significance of salicylate intolerance in diseases of the lower gastrointestinal tract. J Physiol Pharmacol 2005; 56(Suppl 5):89-102.
14. Kęszycka PK, Lange E, Gajewska D. Effectiveness of Personalized Low Salicylate Diet in the Management of Salicylates Hypersensitive Patients: Interventional Study. Nutrients 2021; 13(3):991. doi: 10.3390/nu13030991
15. Duthie GG, Wood AD. Natural salicylates: foods, functions and disease prevention. Food Funct 2011; 2(9):515-20. doi: 10.1039/c1fo10128e.
16. Katzung BG, Masters SB, Trevor AJ. Temeljna i klinička farmakologija. Zagreb, Medicinska naklada; 2011.
17. Baenkler HW. Salicylate intolerance: pathophysiology, clinical spectrum, diagnosis and treatment. Dtsch Arztebl Int 2008; 105(8):137-42. doi: 10.3238/arztebl.2008.0137
18. Skypala IJ, Williams M, Reeves L, Meyer R, Venter C. Sensitivity to food additives, vaso-active amines and salicylates: a review of the evidence. Clin Transl Allergy 2015; 5:34. doi: 10.1186/s13601-015-0078-3
19. Jandrić-Kočić MC. Stvarni i uočeni rizik: aditivi za hranu. Medicinski glasnik Specijalne bolnice za bolesti štitaste žlezde i bolesti metabolizma 'Zlatibor' 2021; 26(82):50-67. Dostupno na: https://scindeks.ceon.rs/article.aspx?artid=1821-19252182050J
20. Vally H, Misso NL. Adverse reactions to the sulphite additives. Gastroenterol Hepatol Bed Bench 2012; 5(1):16-23.
21. Martinis I. Nutritivna alergija. Medix 2004; 10(52):86-8.
22. Skypala IJ, Williams M, Reeves L, Meyer R, Venter C. Sensitivity to food additives, vaso-active amines and salicylates: a review of the evidence. Clin Transl Allergy 2015; 5:34. doi: 10.1186/s13601-015-0078-3
23. Kronberg MT. Sulfite intolerance: A cause of tinnitus? Bioscience Hypotheses 2008; 1(4):185-8.
24. Zhang X, Shoba Vincent A, Halliwell B, Ping Wong K. A Mechanism of Sulfite Neurotoxicity. JBC 2004; 279(41):43035–45. doi: https://doi.org/10.1074/jbc.M402759200
25. Marshall KA, Reist M, Jenner P, Halliwell B. The neuronal toxicity of sulfite plus peroxynitrite is enhanced by glutathione depletion: implications for Parkinson's disease. Free Radic Biol Med 1999; 27(5-6):515-20. doi: 10.1016/s0891-5849(99)00094-5.
26. Taliaferro PJ. Monosodium glutamate and the Chinese Restaurant Syndrome: a review of food additive security. J Environ Health 1995; 57(10):8.
27. Bawaskar HS, Bawaskar PH, Bawaskar PH. Chinese Restaurant Syndrome. Indian J Crit Care Med 2017; 21(1):49-50. doi: 10.4103/0972-5229.198327
28. Geha RS, Beiser A, Ren C, Patterson R, Greenberger PA, Grammer LC et al. Review of Alleged Reaction to Monosodium Glutamate and Outcome of a Multicenter Double-Blind Placebo-Controlled Study. J Nutrition 2000; 130(4):1058S–62S. doi: 10.1093/jn/130.4.1058S
29. Shahmohammadi M, Javadi M, Nassiri-Asl M. An Overview on the Effects of Sodium Benzoate as a Preservative in Food Products. Biotech Health Sci 2016; 3(3):e35084. doi: 10.17795/bhs-35084
30. Pacor ML, Di Lorenzo G, Martinelli N, Mansueto P, Rini GB, Corrocher R. Monosodium benzoate hypersensitivity in subjects with persistent rhinitis. Allergy 2004; 59(2):192-7. doi: 10.1046/j.1398-9995.2003.00380.x.
31. Linke BGO, Casagrande TAC, Cardoso LAC. Food additives and their health effects: A review on preservative sodium benzoate. Afr J Biotechnology 2018; 17(10):306-10. doi: 10.5897/AJB2017.16321
32. Vonk RJ, Reckman GA , Harmsen HJ, Priebe MG. Probiotics and Lactose Intolerance. In: Rigobelo EC editor. Probiotics [Internet]. London: Intech Open; 2012. Dostupno na: https://www.intechopen.com/chapters/39620
33. Malik TF, Panuganti KK. Lactose Intolerance. In: StatPearls [Internet]. Treasure Island (FL): StatPearls; 2022. Dostupno na: https://www.ncbi.nlm.nih.gov/books/NBK532285/
34. Catanzaro R, Sciuto M, Marotta F. Lactose intolerance: An update on its pathogenesis, diagnosis, and treatment. Nutr Res 2021; 89:23-34. doi: 10.1016/j.nutres.2021.02.003
35. Storhaug CL, Fosse SK, Fadnes LT. Country, regional, and global estimates for lactose malabsorption in adults: a systematic review and meta-analysis. Lancet Gastroenterol Hepatol 2017; 2(10):738-46. doi: 10.1016/S2468-1253(17)30154-1.
36. Misselwitz B, Butter M, Verbeke K, Fox MR. Update on lactose malabsorption and intolerance: pathogenesis, diagnosis and clinical management. Gut 2019; 68:2080-91. doi: 10.1136/gutjnl-2019-318404
37. Forsgård RA. Lactose digestion in humans: intestinal lactase appears to be constitutive whereas the colonic microbiome is adaptable. Am J Clin Nutr 2019; 110(2):273–9. doi: 10.1093/ajcn/nqz104.
38. Latulippe ME, Skoog SM. Fructose malabsorption and intolerance: effects of fructose with and without simultaneous glucose ingestion. Crit Rev Food Sci Nutr 2011;51(7):583-92.doi:10.1080/10408398.2011.566646.
39. Wilder-Smith CH, Li X, Ho SS, Leong SM, Wong RK, Koay ES et al. Fructose transporters GLUT5 and GLUT2 expression in adult patients with fructose intolerance. United European Gastroenterol J 2014; 2(1):14-21. doi: 10.1177/2050640613505279.
40. Langdon D, Stanley C, Sperling M. Hypoglycemia in the Infant and Child. 4th edition of Sperling's Pediatric Endocrinology; 2013.

and nonallergic food intolerance: FODMAPs or food chemicals? Therap Adv Gastroenterol 2012; 5(4):261-8. doi: 10.1177/1756283X11436241.

45. Caballero B, Allen L, Prentice A. Encyclopedia of Human Nutrition Reference Work, Third Edition. Elsevier Academic Press, San Diego, CA; 2013.
46. Diez-Sampedro A, Olenick M, Maltseva T, Flowers M. A Gluten-Free Diet, Not an Appropriate Choice without a Medical Diagnosis. J Nutr Metab 2019; 2019:2438934. doi: 10.1155/2019/2438934.
47. Barbaro MR, Cremon C, Stanghellini V, Barbara G. Recent advances in understanding non-celiac gluten sensitivity. F1000Res 2018; 7:F1000 Faculty Rev-1631. doi: 10.12688/f1000research.15849.1.
48. Nucera E, Aruanno A, Ianiro G, Cammarota G, Gasbarrini A, Schiavino D. Wheat desensitization treatment in patients with gluten sensitivity. Postepy Dermatol Alergol 2018; 35(3):320-2. doi: 10.5114/ada.2018.76229
49. Serena G, D'Avino P, Fasano A. Celiac Disease and Non-celiac Wheat Sensitivity: State of Art of Non-dietary Therapies. Front Nutr 2020; 7:152. doi: 10.3389/fnut.2020.00152
50. Roszkowska A, Pawlicka M, Mroczek A, Bałabuszek K, Nieradko-Iwanicka B. Non-Celiac Gluten Sensitivity: A Review. Medicina (Kaunas) 2019; 55(6):222. doi: 10.3390/medicina55060222.

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41. Fedewa A, Rao SS. Dietary fructose intolerance, fructan intolerance and FODMAPs. Curr Gastroenterol Rep 2014; 16(1):370. doi: 10.1007/s11894-013-0370-0.
42. Zugasti Murillo A, Estremera Arévalo F, Petrina Jáuregui E. Dieta pobre en FODMAPs (fermentable oligosaccharides, disaccharides, monosaccharides and polyols) en el síndrome de intestino irritable: indicación y forma de elaboración. Endocrinol Nutr 2016; 63:132–8. doi: 10.1016/j.endonu.2015.10.009
43. Lenhart A, Chey WD. A Systematic Review of the Effects of Polyols on Gastrointestinal Health and Irritable Bowel Syndrome. Adv Nutr 2017; 8(4):587-96. doi: 10.3945/an.117.015560.
44. Barrett JS, Gibson PR. Fermentable oligosaccharides, disaccharides, monosaccharides and polyols (FODMAPs) and nonallergic food intolerance: FODMAPs or food chemicals? Therap Adv Gastroenterol 2012; 5(4):261-8. doi: 10.1177/1756283X11436241.
45. Caballero B, Allen L, Prentice A. Encyclopedia of Human Nutrition Reference Work, Third Edition. Elsevier Academic Press, San Diego, CA; 2013.
46. Diez-Sampedro A, Olenick M, Maltseva T, Flowers M. A Gluten-Free Diet, Not an Appropriate Choice without a Medical Diagnosis. J Nutr Metab 2019; 2019:2438934. doi: 10.1155/2019/2438934.
47. Barbaro MR, Cremon C, Stanghellini V, Barbara G. Recent advances in understanding non-celiac gluten sensitivity. F1000Res 2018; 7:F1000 Faculty Rev-1631. doi: 10.12688/f1000research.15849.1.
48. Nucera E, Aruanno A, Ianiro G, Cammarota G, Gasbarrini A, Schiavino D. Wheat desensitization treatment in patients with gluten sensitivity. Postepy Dermatol Alergol 2018; 35(3):320-2. doi: 10.5114/ada.2018.76229
49. Serena G, D'Avino P, Fasano A. Celiac Disease and Non-celiac Wheat Sensitivity: State of Art of Non-dietary Therapies. Front Nutr 2020; 7:152. doi: 10.3389/fnut.2020.00152
50. Roszkowska A, Pawlicka M, Mroczek A, Bałabuszek K, Nieradko-Iwanicka B. Non-Celiac Gluten Sensitivity: A Review. Medicina (Kaunas) 2019; 55(6):222. doi: 10.3390/medicina55060222.

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DASH DIJETA U PREVENCIJI I LEČENJU ARTERIJSKE HIPERTENZIJE

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SAŽETAK

Procenjuje se da od arterijske hipertenzijeu Republici Srbiji boluje skoro polovina odraslog stanovništva. Adekvatnom dijetoterapijom može se uspešno kontrolisati i sprečiti razvoj arterijske hipertenzije i njenih komplikacija. Dijetetskipristupi za zaustavljanje hipertenzije (engl. *Dietary Approaches to Stop Hypertension* – DASH) predstavljaju jedan od najefikasnijih načina ishrane u kontroli hipertenzije, dovode do prosečnog smanjenja arterijskog krvnog pritiska za 4 do 15 mmHg, postizanja optimalne telesne mase i lipida u serumu. DASH dijeta promoviše pre svega racionalnu i uravnoteženu ishranu. Kako bi se principi DASH dijete približili pacijentima, neophodno je adekvatno angažovanje nutricioniste - dijetetičara u radu sa visokorizičnim pacijentima. U radu su predstavljene praktične preporuke za implementaciju DASH dijete i priložene su ilustracije koje mogu da se koriste za unapređenje savetodavnog rada sa pacijentima na nivou primarne zdravstvene zaštite.

Ključne reči: hipertenzija, nutricionista, dijetetičar, edukacija, DASH dijeta

Uvod

Kardiovaskularne bolesti (KVB) predstavljaju značajan javnozdravstveni problem širom sveta i u Srbiji. Bolesti srca i krvnih sudova sa učešćem od 47,3% u svim uzrocima smrti, vodeći su uzrok umiranja odraslog stanovništva u Republici Srbiji. Tokom 2020. godine u Srbiji je zbog kardiovaskularnih bolesti život izgubilo preko 55 hiljada osoba (1,2).

Arterijska hipertenzija predstavlja najčešći oblik KVB koja se manifestuje sistolnim krvnim pritiskom ≥ 140 mmHg i/ili dijastolnim pritiskom ≥ 90 mmHg (3). Prema istraživanju zdravlja stanovništva u Srbiji (2019), hipertenzija je evidentirana kod skoro polovine (46%) odraslog stanovništva (4). Za postavljanje dijagnoze arterijske hipertenzije, neophodno je registrovati povišene vrednosti krvnog pritiska u dva odvojena merenja (3,4).

Poznato je da nepravilna ishrana ugrožava vitalni kapacitet srca i krvnih sudova. Deficitarna ishrana dovodi do slabljenja srčanog mišića i usporenog protoka krvi (5). Sa druge strane, prekomeran unos zasićenih masti, prostih šećera i soli, kao i energetski suficit, dovodi do ubrzane ateroskleroze i povećanja rizika za nastanak hipertenzije, oštećenja vida, bubrega, tromboze, infarkta miokarda, cerebrovaskularnog insulta i dekompenzacije srca (6). Prevencija kardiovaskularnih faktora rizika, od kojih je većina povezana sa načinom ishrane, smanjuju rizik za nastanak hipertenzije i drugih KVB (7).

Cilj ovog rada je sagledavanje aktuelnih preporuka za prevenciju arterijske hipertenzije dijetoterapijom i značaja nutricioniste-dijetetičara u medicinskoj nutritivnoj prevenciji na nivou primarne zdravstvene zaštite.

Lečenje arterijske hipertenzije

Terapijski ciljevi u lečenju hipertenzije, zavise od uzrasta pacijenta, prisutnih komorbiditeta i uspešnosti tretmana. Ciljne vrednosti krvnog pritis-

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DASH DIET IN THE PREVENTION AND TREATMENT OF ARTERIAL HYPERTENSION

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SUMMARY

It is estimated that almost half of the adult population suffers from arterial hypertension in the Republic of Serbia. The development of arterial hypertension and its complications can be successfully controlled and prevented. The Dietary Approaches to Stop Hypertension, that is, the DASH diet is one of the most efficient dietary eating patterns in the control of hypertension. It leads to an average reduction in blood pressure by 4 to 15 mmHg, supports the optimization of body mass and lipids in the blood serum. The DASH diet promotes rational and balanced diet. In order to bring principles of the DASH diet closer to patients, it is necessary to adequately engage a nutritionist - dietician in working with high-risk patients. The paper presents practical recommendations for the implementation of the DASH diet and includes illustrations that serve to improve counseling work with patients in primary health care.

Key words: hypertension, nutritionist, dietitian, education, DASH diet

Introduction

Cardiovascular diseases (CVD) represent a significant public health problem worldwide, and in Serbia, as well. Disorders of the heart and blood vessels are the leading cause of death of adults in the Republic of Serbia, representing 47.3% of all deaths. In 2020, more than 55 thousand people died from cardiovascular diseases in Serbia (1,2).

Arterial hypertension is the most frequent form of CVDs, which is manifested by systolic blood pressure > 140 mmHg and/or diastolic pressure > 90 mmHg (3). According to the population health research in Serbia (2019), hypertension was registered in almost half (46%) of adult population (4). In order to establish the diagnosis of arterial hypertension, it is necessary to register increased values of blood pressure in two separate measurements (3,4).

It is known that the irregular diet jeopardizes the vital capacity of heart and blood vessels. Nutritional deficiency leads to the weakening of heart muscle and slowing down of blood flow (5). On the other hand, the excessive intake of saturated fats, simple sugars and salts, as well as excess energy lead to the accelerated atherosclerosis and the increased risk of hypertension, vision damage, kidney damage, thrombosis, myocardial infarction, cerebrovascular insult and cardiac decompensation (6). The prevention of cardiovascular risk factors, the majority of which is associated with diet, reduces the risk of hypertension and cardiovascular diseases (7).

The aim of this paper is to perceive current recommendations for the prevention of arterial hypertension with the help of diet therapy, as well as the significance of a nutritionist – dietician in medical nutritional prevention at the level of primary healthcare.

The treatment of arterial hypertension

The goals of therapy in the treatment of hypertension depend on patients' age, present comorbidities and the successfulness of the

ka za hipertenzivne bolesnike prvenstveno se odnose na snižavanje krvnog pritiska ispod 140/90 mmHg. Za osobe mlađe od 65 godina preporučeno je sniženje sistolnog krvnog pritiska na 120-129 mmHg, dok kod starijih osoba ciljna vrednost sistolnog krvnog pritiska treba da bude u opsegu 130-139 mmHg. Vrednost dijastolnog krvnog pritiska za sve pacijente treba da iznosi ispod 80 mmHg (8).

Pored adekvatne farmakološke terapije, u lečenju arterijske hipertenzije koriste se nefarmakološke mere (3). U nefarmakološke mere lečenja hipertenzije spadaju adekvatna ishrana, smanjenje nivoa stresa, adekvatna fizička aktivnost, postizanje optimalne telesne mase, kao i eliminisanje drugih faktora rizika poput konzumiranja alkohola i duvana.

Promena stila života jeste prva karika u lečenju prema algoritmu lečenja hipertenzije evropskog Društva za kardiologiju (engl. *Europen Society of Cardiology*). Sprovodi se počev od utvrđenih prehipertenzivnih vrednosti krvnog pritiska sa ciljem da se spreči dalje povećanje vrednosti krvnog pririska i da se smanji kardiovaskularni rizik (8).

Prevencija arterijske hipertenzije adekvatnom ishranom

Adekvatna, uravnotežena i raznovrsna ishrana može doprineti prevenciji arterijske hipertenzije ili stabilizaciji, kao i sprečavanju komplikacija. Takođe, uravnotežena ishrana doprinosi postizanju optimalne telesne mase i prevenciji drugih masovnih nezaraznih oboljenja.

Medicinska nutrititvna prevencija KVB podrazumeva uravnoteženu i racionalnu ishranu baziranu pretežno na namirnicama biljnog porekla. Osnov ishrane treba da čine integralne žitarice, voće i povrće, posebno leguminoze odnosno visok unos dijetnih vlakana, flavonoida, fenola i drugih fitohemikalija. Potrebno je ograničiti unos soli, rafinisanih žitarica, crvenog mesa, mesnih prerađevina, slatkiša i grickalica, jer je prekomeran unos ovih namirnica povezan sa češćom pojavom KVB i cerebrovaskularnih oboljenja (6,9,10). U cilju nutritivne prevencije KVB preporučuje se unos mesa, peradi i ribe uz izbegavanje pripremanja ovih namirnica prženjem. Unos mono- i poli-nezasićenih masnih kiselina putem ribe, maslinovog ulja i orašastog voća je važan princip nutritivne prevencije KVB.

Najpoznatiji način ishrane koji se preporučuje u okviru prevencije i kontrole hipertenzije jeste dijetetski pristup za zaustavljanje hipertenzije (engl. *Dietary Approaches to Stop Hypertension* - DASH), odnosno DASH dijeta (9,11).

DASH piramida ishrane

Značajna komponenta DASH dijete jeste ograničen unos kuhinjske soli tj. natrijum-hlorida i to najviše 2g dnevno za osobe sa potvrđenom hipertenzijom, odnosno do 5 g za ostalu populaciju. Procenjuje se da se svakodnevnom ishranom unosi prosečno oko 15-20 g soli dnevno. Važan izvor kuhinjske soli su namirnice koje sadrže velike količine skrivene soli, kao što su suhomesnati i konzervisani proizvodi (11-13).

Samo polovina osoba sa visokim krvnom pritiskom je osetljiva na smanjen unos soli (6,9,10,13). U polovini slučajeva redukcija unosa soli nije efikasna u snižavanju povišenog krvnog pritiska. Pridržavanjem navedenih preporuka mogu se eliminisati faktori rizika drugih srčanih i metaboličkih oboljenja. Smanjenje unosa soli za 1 g dnevno smanjuje rizik za razvoj KVB za četvrtinu (6,9,10,13).

Obilje dijetnih vlakana, kalijuma i magnezijuma, koji se unose putem DASH ishrane pomaže vezivanju dela holesterola u cirkulaciji i podstiče peristaltiku creva, dok redukcija unosa soli sprečava zadržavanje prekomerne količine tečnosti u telu. Sve navedeno rezultira smajenjem krvnog pritiska i prekomerne telesne mase dugoročno (6,9,11-13). Prevalencija hipertenzije je dva puta češća kod prekomerno uhranjenih nego kod normalno uhranjenih osoba. Smanjivanjem telesne mase dolazi do rasterećivanja srca i krvnih sudova. Arterijski krvni pritisak može se sniziti za 2-4 mmHg po izgubljenom kilogramu telesne mase (6).

Pored adekvatanog izbora namirnica, važno je da obroci budu redovni, količinski manji, a češći kako bi se tokom dana obezbedio dovoljan energetski unos i iskoristljivost hranljivih materija bez naglog opterećivanja organizma (14).

Ilustrovani principi DASH dijete koji su pogodni za savetodavni rad i pripremu edukativnog materijala za pacijente prikazani su na slici 1 (6,9,11-13). Preporuke su bazirane za energetski unos od 2000 kalorija dnevno.

Uticaj DASH dijete na krvni pritisak, glikemiju i lipidni status

Studije su pokazale da DASH dijeta ima uočljive pozitivne efekte već nakon 14 dana od početka

treatment. Targeted values of blood pressure for hypertensive patients primarily refer to lowering blood pressure below 140/90 mmHg. It is recommended that systolic blood pressure should be lowered to 120-129 mmHg in people younger than 65 years, while in older people the targeted value of systolic blood pressure should be within the range 130-139 mmHg. The value of diastolic blood pressure for all patients should be below 80 mmHg (8).

An addition to adequate pharmacotherapy, non-pharmacological measures are used in the treatment of arterial hypertension (3). Non-pharmacological measures related to the treatment of hypertension include adequate diet, reducing stress levels, adequate physical activity, achieving optimal body mass, as well as the elimination of other risk factors such as alcohol consumption and tobacco use.

Lifestyle changes are the first step in the treatment according to the treatment algorithm for hypertension of the European Society of Cardiology. It is conducted starting from the established pre-hypertensive values of blood pressure aimed at preventing further increase in the values of blood pressure and decreasing cardiovascular risk (8).

The prevention of arterial hypertension with the help of adequate diet

An adequate, balanced and varied diet may contribute to the prevention of arterial hypertension or stabilization, as well as to the prevention of complications. Also, a balanced diet contributes to achieving the optimal body mass and prevention of other major non-communicable diseases.

The medical nutritional prevention of CVDs means the balanced and rational diet, which is mainly a plant-based diet. This diet should be made up of whole grains, fruit and vegetables, especially legumes, that is, the high intake of dietary fibers, flavonoids, phenols and other phytochemicals. The intake of salt, refined grains, red meat, meat products, sweets and snacks should be limited, because the excessive intake of these products is associated with the more frequent occurrence of CVDs and cerebrovascular diseases (6,9,10). The intake of meat, poultry and fish, which should not be fried, is recommended with the aim of nutritional preventing of CVD. The intake of mono- and poly-unsaturated fatty acids via fish, olive oil, and nuts is the principle of nutritional prevention of CVD.

The most famous dietary eating pattern which is recommended within the prevention and control of hypertension is a dietary approach to stop hypertension, that is, DASH diet (9,11).

DASH food pyramid

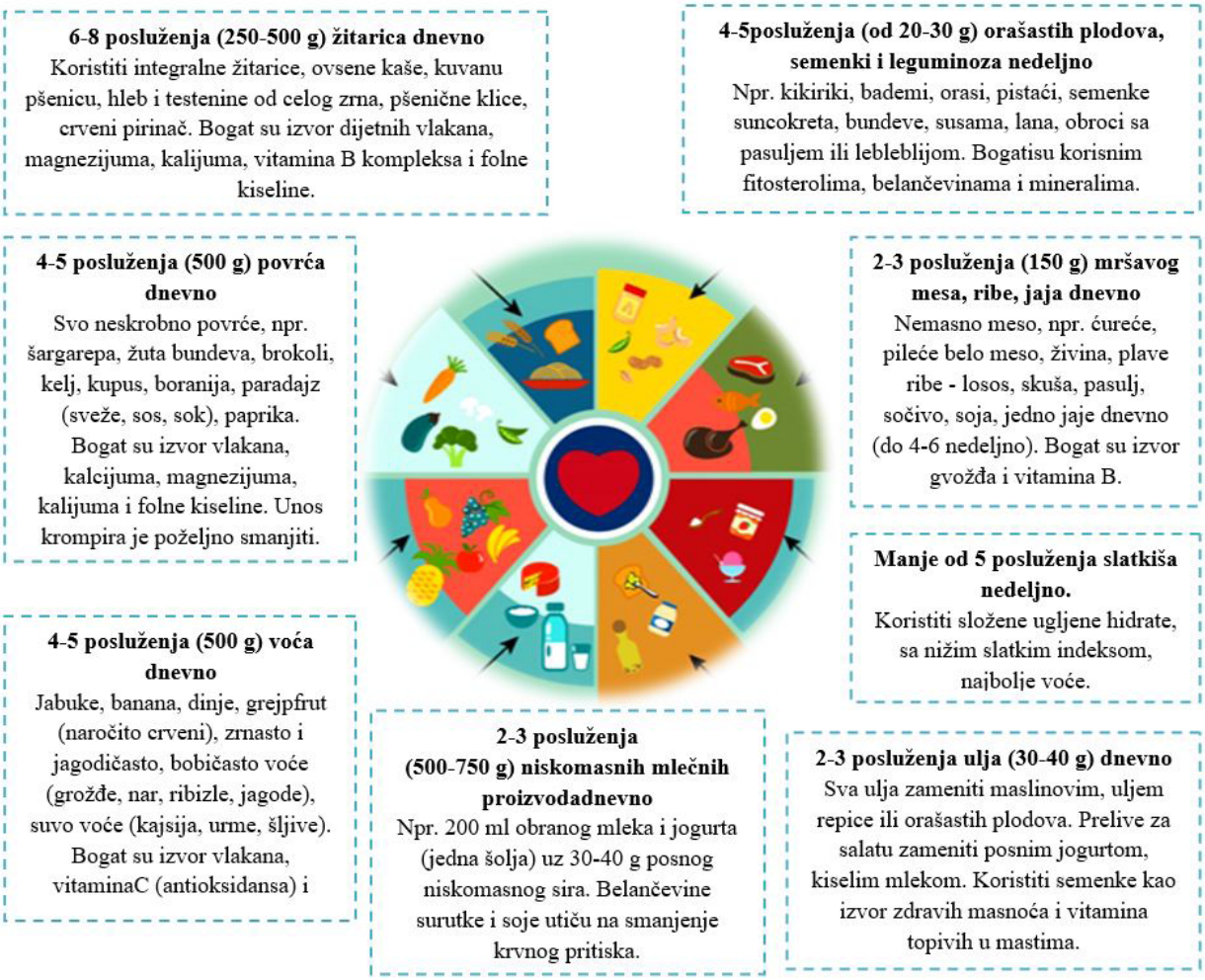
A significant component of DASH diet is the limited intake of table salt, that is, sodium-chloride up to 2 g a day for persons with the confirmed hypertension, that is, 5 g for the rest of the population. It is estimated that about 15-20 g of salt are taken every day. An important source of table salt is food rich in high amounts of hidden salt, such as cured meat products and preserved products (11-13).

Only one half of persons with hypertension is sensitive to the reduced intake of salt (6,9,10,13). In 50% of cases, the reduction of salt intake is not efficient in lowering high blood pressure. By respecting the above mentioned recommendations, risk factors for other cardiac and metabolic diseases may be eliminated. By reducing the salt intake for 1 g a day, the risk of CVD is reduced by 25% (6,9,10,13).

The abundance of dietary fibers, potassium and magnesium, which are taken via DASH diet, help by binding to cholesterol particles in the circulation and enhance the intestinal peristalsis, while the reduction of salt intake prevents excess water retention. All the above mentioned results in lowering of blood pressure and excessive body weight on a long-term basis (6,9,11-13). The prevalence of hypertension is two times more frequent in overweight than in persons with normal weight. The reduction of body weight leads to relaxing of heart and blood vessels. Arterial blood pressure may be lowered for 2-4 mmHg for each lost kilogram of body mass (6).

In addition to the adequate selection of food, meals should be regular, smaller in quantity, and more frequent in order to secure a sufficient energy intake during the day and usability of nutrients without sudden burdening the body (14).

The illustrated examples of DASH diet which are suitable for counseling work and preparation of educational materials for patients are presented in figure 1 (6,9,11-13). The recommendations are based on the energy intake of 2000 calories a day.



Slika 1. Principi DASH dijete
(Preuzeto i adaptirano: https://bariel-med.com/bmc-medical-quicktips/)

primene (9). Primena DASH dijete može dovesti do snižavanja krvnog pritiska za 8-14 mmHg, smanjenja telesne mase, snižavanja nivoa ukupnog holesterola u krvi (7).

Ispitivanja o uticaju DASH dijete i smanjenog unosa natrijuma (od 50-150 mmol/dan pri energetskom unosu od 2100 kcal), ukazuju da DASH dijeta rezultira smanjenjem sistolnog krvnog pritiska za 5,10 ili čak 20 mmHg. Kod većeg stepena hipertenzije (≥ 150 mmHg) veće je i smanjenje sistolnog krvnog pritiska po uvođenju DASH dijete u poređenju sa konvencionalnom ishranom (15).

Meta-analiza o efektima DASH dijete pokazuje da pomenuti način ishrane dovodi do prosečnog smanjenja nivoa krvnog pritiska od 3,5 mmHg kod normotenzivnih i kod osoba sa hipertenzijom. Zaključak pomenute studije je da DASH dijeta ima veći uticaj na smanjenje sistolnog i dijastolnog krvnog pritiska kod osoba mlađih od 50 godina u odnosu na stariju populaciju. Značajnije smanjenje sistolnog i dijastolnog krvnog pritiska evidentirano je u studijama u kojima je dnevni unos natrijuma bio iznad 2400 mg/dan u odnosu na studije sa niskim unosom natrijuma (ispod 2400 mg) (16).

U studiji koja je upoređivala efekte tronedeljne konvencionalne ishrane, standardne DASH dijete i modifikovane HF-DASH dijete (dijeta sa niskim sadržajem ugljenih hidrata i visokim sadržajem masti), utvrđeno je da se kod ispitanika na DASH i HF-DASH dijeti sistolni krvni pritisak smanjio za prosečnih 4 mmHg, a dijastolni za 0,9 mmHg. Kod ispitanika na DASH dijeti značajno se smanjio LDL-holesterol i HDL-holesterol u poređenju sa ispitanicima koji su se hranili konvencionalno. Kod ispitanika na HF-DASH dijeti značajno se smanjio nivo triglicerida, koncentracija VLDL čestica i povećao nivo HDL-holesterola u poređenju sa ispitanicima na DASH dijeti, što ukazuje da ishrana bogata mastima i siromašna ugljenim hidratima može imati pozitivan uticaj na nivo arterijskog krvnog pritiska bez značajnog povećanja LDL-holesterola (Tabela 1) (17).

Figure 1. The principles of the DASH diet
(Retrived and adapted from: https://bariel-med.com/bmc-medical-quicktips/)

The influence of the DASH diet on blood pressure, glycemia and lipid status

Studies have shown that the DASH diet has visible positive effects within 14 days of starting the plan (9). The application of the DASH diet may lead to lowering of blood pressure for 8-14 mmHg, reduction of body mass, lowering of the level of total cholesterol in blood (7).

The investigation of the influence of DASH diet and reduced intake of sodium (from 50-150 mmol/day during the energy intake of 2100 kcal) has shown that the DASH diet results in the lowering of systolic blood pressure for 5, 10 or even 20 mmHg. In case of higher hypertension (>150 mmHg), lowering of systolic blood pressure is greater after the introduction of DASH diet in comparison to conventional diet (15).

A meta-analysis of the effects of the DASH diet has shown that it leads to the average lowering of blood pressure level of 3.5 mmHg in normotensive persons and in persons with hypertension. The conclusion of the above mentioned study is that the DASH diet has a greater influence on the lowering of systolic and diastolic blood pressure in persons younger than 50 years in comparison to the elderly. A more significant lowering of systolic and diastolic blood pressure was recorded in studies, in which a daily intake of sodium was above 2400 mg/day in comparison to studies with the low intake of sodium (below 2400 mg) (16).

In a study that compared the effects of a three-week conventional diet, the standard DASH diet and modified HF-DASH diet (diet with the low amount of carbohydrates and high contents of fat), it has been found that in participants on the DASH and HF-DASH diet, systolic blood pressure was lowered for 4 mmHg on average, while diastolic for 0.9 mmHg. In participants who were on the DASH diet, LDL-cholesterol and HDL-cholesterol were significantly lower in comparison to participants who used a conventional diet. In participants on

Tabela 1. Nutritivni sastav konvencionalne ishrane, DASH i HF-DASH dijete i njihov uticaj na nivo krvnog pritiska i lipoproteinski status (preuzeto i adaptirano prema referenci broj 17)

	HF-DASH dijeta	DASH dijeta	Konvencionalna ishrana
Ugljeni hidrati (% od dnevnog unosa)	43	55	47
Belančevina (% od dnevnog unosa)	18	17	14
Masti (% od dnevnog unosa)	40	27	38
Sistolni krvni pritisak (mmHg)	125,0	125,4	128,8
Dijastolni krvni pritisak (mmHg)	79,0	78,3	81,2
Ukupan holesterol (mmol/L)	4,53	4,51	4,76
LDL-holesterol (mmol/L)	2,65	2,60	2,81
HDL-holesterol (mmol/L)	1,36	1,32	1,40
Trigliceridi (mmol/L)	1,15	1,32	1,20

Uočen je pozitivan uticaj DASH dijete u kontrolisanju dijabetesa i hiperglikemije, koja je značajan faktor rizika za razvoj i progresiju KVB (18-20).

Nutrijenti u kontrolisanju arterijske hipertenzije

Postoji širok spektar mikronutrijenta i namirnica čiji povećan ili smanjen unos ima uticaj na pojavu KVB. Iako su poznati kardioprotektivni efekti pojednih nutrijenata i namirnica, većina autora ostaje bez konačnog zaključka o njihovim efektima na prevenciju KVB zbog nemogućnosti kontrolisanja iskoristljivosti nutrijenata u organizmu.

Flavonoidi, koji se nalaze u voću i povrću, zelenom i crnom čaju, doprinose snižavanju arterijskog krvnog pritiska verovatno putem antioksidativnog dejtva (21). Resveratrol i flavonoidi prisutni u semenkama grožđa i mesu, i kvercetin prisutan u luku, borovnicama i jabukama, mogu znatno da utiču na stabilizaciju krvnog pritiska, ali i na prevenciju KVB (22). Istraživanja su pokazala da antioksidansi iz grožđa utiču na smanjenje sistolnog i dijastolnog arterijskog pritiska za 5-6%, dok se ukupan nivo holesterola u krvi smanjuje za 14% (23).

Omega-3 masne kiseline imaju antiinflamatorno dejstvo i utiču na funkciju endotela krvnih sudova čime se poboljšava vazodilatacija kod osoba sa metaboličkim sindromom. Pregledni radovi pokazuju da povećanje unosa omega-3 polinezasićenih masnih kiselina doprinosi smanjenju ukupnih triglicerida u krvi i rizika od smrtnosti usled koronarne bolesti srca (24). Rastvorljiva dijetna vlakna, koja se nalaze u ovsenim pahuljicama, jagodama, sočivu, pasulju i mnogim drugim namirnicama, vezuju holesterol smanjujući rizik od hiperlipidemije i KVB. Svakodnevni unos rastvorljivih dijetnih vlakana dovodi do snižavanja ukupnog holesterola u krvi. Dijetna vlakna imaju ulogu u apsorpciji u tankom crevu smanjujući glikemijski indeks namirnica i pospešuju peristaltiku u debelom crevu gde dolazi do njihove fermentacije (25). Visok unos kalijuma može doprineti smanjenju arterijskog krvnog pritiska za 2-4 mmHg. Kalijum se nalazi u svežem voću i povrću poput blitve, spanaća, kelja, peršuna, šampinjona, banana, trešanja, dinje i grožđa (26). Veliki broj biljnih čajeva, poput čaja od peršuna ili maslačka, deluje diuretski što doprinosi i smanjenju arterijskog krvnog pritiska. Salate od rukole i celera imaju slično dejstvo. Konzumacija cvekle u vidu salate ili soka dovodi do smanjenja nivoa holesterola u krvi za 40% (27). Fitoestrogeni su materije biljnog porekla koji u organizmu izazivaju efekte slične estrogenu. Najpoznatiji izvor pomenutih materija su izoflavoni iz soje. Umeren unos utiče na smanjenje rizika od KVB i malignih bolesti (28). Vitamin D iz ribe i jaja ima ulogu u regulaciji povišenog krvnog pritiska. Povišen homocistein u plazmi, koji je povezan sa pojavom periferne arterijske bolesti, može značajno da se smanji putem DASH dijete bogate povrćem i folatima (29).

Uloga nutricioniste-dijetetičara u prevenciji i lečenju arterijske hipertenzije

Nutricionisti-dijetetičari imaju značajnu ulogu u prevenciji i lečenju masovnih nezaraznih oboljenja posebno na nivou primarne zdravstvene zaštite. Zajedno sa medicinskim sestrama, nutri-

Table 1. Nutrient composition of conventional diet, DASH and HF-DASH diet and influence on blood pressure level and lipoprotein status (retrieved and adapted from reference number 17)

	HF-DASH diet	DASH diet	Conventional diet
Carbohydrates (% of daily intake)	43	55	47
Protein (% of daily intake)	18	17	14
Fats (% of daily intake)	40	27	38
Systolic blood pressure (mmHg)	125.0	125.4	128.8
Diastolic blood pressure (mmHg)	79.0	78.3	81.2
Total Cholesterol (mmol/L)	4.53	4.51	4.76
LDL-cholesterol (mmol/L)	2.65	2.60	2.81
HDL-cholesterol (mmol/L)	1.36	1.32	1.40
Triglycerides (mmol/L)	1.15	1.32	1.20

the HF-DASH diet the level of triglycerides was significantly lower, as well as the concentration of VLDL particles, while the level of HDL-cholesterol increased in comparison to the participants on the DASH diet, which indicates that the diet rich in fats and lower in carbohydrates may have a positive effect on the level of arterial blood pressure without a significant increase in LDL cholesterol (Table 1) (17).

A positive influence of the DASH diet was noticed regarding the control of diabetes and hyperglycemia, which is a significant risk factor for the development and progression of CVD (18-20)

Nutrients in the control of arterial hypertension

There is a wide range of micronutrients and foods, whose increased or reduced intake has influence on the occurrence of CVD. Although cardio-protective effects of certain nutrients and foods are known, most authors have not made final conclusions on their effects on the prevention of CVDs due to the impossibility of controlling the usability of nutrients in the body.

Flavonoids that are present in fruits and vegetables, green and black tea contribute to lowering of arterial blood pressure probably via their antioxidative effect (21). Resveratrol and flavonoid present in grape seeds and meat, quercetin present in onions, blueberries and apples, may significantly affect the stabilization of blood pressure, as well as the prevention of CVD (22). The research has shown that antioxidants from grapes influence lowering of systolic and diastolic blood pressure by 5-6%, while the total level of cholesterol is reduced for 14% (23).

Omega-3 fatty acids have anti-inflammatory effect and influence the endothelial function of blood vessels, thus enhancing the vasodilatation in persons with metabolic syndrome. Review articles show that the increase of the intake of omega-3 polyunsaturated fatty acids contributes to the lowering of total triglycerides in blood and the risk of CVD mortality (24). Soluble dietary fibers, which are present in oat meals, strawberries, lentils, beans and other foods, bind to cholesterol thus reducing the risk of hyperlipidemia and CVDs. Daily intake of soluble dietary fibers leads to the lowering of total cholesterol in blood. Dietary fibers have a role in the absorption in small intestines, thus reducing the glycemic index of foods and enhancing peristalsis in the colon, where their fermentation happens 925). The high intake of potassium may contribute to the lowering of arterial blood pressure for 2-4 mmHg. Potassium is present in fresh fruit and vegetables such as chard, spinach, Savoy cabbage, parsley, mushrooms, bananas, sour cherries, melon and grapes (26). A large number of teas, such as tea made of parsley or dandelion, have a diuretic effect, thus contributing to the lowering of arterial blood pressure. Salads, which are made of arugula and celery, have a similar effect. The consumption of beetroot in the form of salad or juice leads to the lowering of cholesterol level in blood by 40% (27). Phytoestrogens are plant compounds

Tabela 2. Preporučene namirnice kod povišenog krvnog pritiska prema DASH dijeti (13,14,32)

Namirnice	Preporučeno	Umereno	Izbegavati
Žitarice i proizvodi	Integralne žitarice, ovas, ječam, raž, pšenica, heljda, integralni pirinač, proso, palenta. Integralne testenine i neslan crni hleb.	Bele/rafinisane žitarice (beli pirinač, testenina, hleb od belog brašna). Dve kriške hleba sadrže preko 1,5 g kuhinjske soli.	Pekarski proizvodi, lisnata testa, peciva, proizvodi od belog brašna sa dodatkom jaja, punomasnog mleka ili maslaca.
Voće	Sveže, sezonsko voće ili smrznuto. Trešnje, višnje, kajsije, jabuke i ostalo voće. Bogato je vitaminom C, vlaknima.	Voće sa puno šećera (ananas, krompir, lubenica). Konzervisano, suvo ili ušećereno voće, marmelade.	Prženo voće, voće pripremljen sa slatkom pavlakom.
Povrće	Sveže sezonsko povrće, ili kuvano, dinstano, smrznuto povrće. Krompir je dobar izvor kalijuma, a siromašan natrijumom.	Konzervisano povrće (isprati pre upotrebe). Npr. 100 g konzervisanog graška sadrži 0,7 g soli, dok sveži grašak ne sadrži so.	Prženo i pohovano povrće, povrće pripremljen sa pavlakom, sirom, maslacem. Npr. konzerviran paradajz u proseku sadrži 2,2 g soli u pola šoljice proizvoda.
Mleko i proizvodi	Obrano mleko (manje od 2% mlečne masti), fermentisani mlečni proizvodi, jogurt, kefir, sveži posni sir, zrnasti sir, surutka. Iako mlečni proizvodi sadrže natrijum, vredan su izvor belančevina.	Četvrt masni sirevi (15–25 % m.m.). Npr. mozzarella, koja u 60 g proizvoda sadrži 0,7 g soli.	Punomasno mleko i fermentisani mlečni proizvodi, punomasni, zreli, slani sirevi, mlečni sladoled. Npr. 60 g feta sira sadrži 2,3 g soli.
Meso, riba, jaja	Pileće i ćureće belo meso - bez kožice. Sve vrste (plave, morske) nemasne ribe - sardele, skuše; kuvana jaja, belance jajeta.	Plodovi mora (školjke, rakovi, lignje), mršava svinjetina, govedina, teletina, jagnjetina. 4-5 jaja nedeljno, uključujući i one koje se koriste za pripremu jela. Pileće, ćureće šunke, npr. u 60g* proizvoda sadrže 0,85 g soli. **60 g predstavlja prosečno posluženje ovih proizvoda*	Masno meso –svinjetina, teletina, jagnjetina, govedina, iznutrice, perad sa kožicom. Prženo, pohovano meso/ riba, i meso pripremljeno u dubokom ulju/masti. Pržena jaja, kajgana. Suhomesnati proizvodi. Npr. 60 g pršute sadrži 3,2 g soli; dok čajna salama u 60 g sadrži 2,4 g soli, dve viršle (130 g proizvoda) sadrže 3,1 g soli.
Mahunarke	Grašak, pasulj, sočiva, leblebije, soja pripremljeni sa malo ulja u obliku variva i salata (ukoliko ne izazivaju nadutost).	Konzervisane mahunarke (isprati pre upotrebe).	Mahunarke pripremljene u varivu sa zaprškom, masnim mesom i sa puno ulja/masti.
Orašasto voće i semenke	Bademi, orasi, lešnik, semenke bundeve, lana, suncokreta.		Ušećereno i usoljeno orašasto voće i semenke.
Ulja i masti	Maslinovo i bundevino ulje.	Suncokretovo, repičino i ostala biljna ulja, meki „*light*" margarin sa nižim procentom masti.	Životinjska i svinjska mast, slanina, salo, čvarci, maslac, pavlaka, tvrdi margarin, majonez, palmino i kokosovo ulje.
Napici	Voda, nezaslađeni biljni i voćni čajevi.	Prirodni sokovi od voća i povrća, kafa, pivo i vino.	Zaslađeni gazirani i negazirani napici, vitaminski napici u prahu, alkohol, naročito žestoka pića.
Slatkiši	Med i voće.	Smrznuti jogurt sa bobičastim voćem, domaći voćni sladoled pripremljen od obranog mleka, integralni kolač sa svežim sirom i/ili voćem, voćni frape ili tamna čokolada.	Industrijski proizvedeni keksi, krekeri, kolači, džemovi, mlečna čokolada, bombone, pekarske pite i krofne (svi proizvodi sa puno šećera, od punomasnog mleka, sa dodatkom jaja, margarina, pavlake ili kokosa).
Začini	Beli i crni luk, paprika, peršun, majoran, mirođija, kim, lan, korijander, kurkuma, muskatni oraščići, cimet, majčina dušica, nana, đumbir itd.		So i industrijske mešavine začina (vegeta i sl.). Promeniti način doziranja soli. Npr. jednom kafenom kašičicom dozira se 5 g soli, prstohvatom oko 0,5 g, a vrhom noža 0,25 g soli.

Table 2. Recommended foods for high blood pressure according to the DASH diet (13,14,32)

Food	Recommended	Moderate	Avoid
Cereals and products	Whole grains, oats, barley, rye, wheat, buckwheat, whole grain rice, millet, polenta. Integral pasta and unsalted brown bread.	White/refined grains (white rice, pasta, white flour bread). cTwo slices of bread contain over 1.5 g of table salt.	Bakery products, puff pastry, pastries, products made from white flour with the addition of eggs, whole milk or butter.
Fruit	Fresh, seasonal fruit or frozen. Cherries, sour cherries, apricots, apples and other fruits. It is rich in vitamin C, fiber.	Fruits with a lot of sugar (pineapple, potato, watermelon). Canned, dried or candied fruit, marmalades.	Fried fruit, fruit prepared with sweet cream.
Vegetables	Fresh seasonal vegetables, or boiled, stewed, frozen vegetables. Potatoes are a good source of potassium, but poor in sodium.	Canned vegetables (wash before use). For example. 100 g of canned peas contain 0.7 g of salt, while fresh peas do not contain salt.	Fried and fried vegetables, vegetables prepared with sour cream, cheese, butter. For example. canned tomatoes contain an average of 2.2 g of salt in half a cup of the product.
Milk and products	Skimmed milk (less than 2% m.m.), fermented milk products, yogurt, kefir, fresh cottage cheese, granulated cheese, whey. Although dairy products contain sodium, they are a valuable source of protein.	A quarter of fat cheeses (15–25 % m.m.). For example. mozzarella, which contains 0.7 g of salt in 60 g of the product.	Whole milk and fermented milk products, full-fat, mature, salty cheeses, milk ice cream. For example. 60 g of feta cheese contains 2.3 g of salt.
Meat, fish, eggs	Chicken and turkey white meat - without skin. All types of (blue, sea) lean fish - sardines, mackerel; boiled eggs, egg whites.	Seafood (shellfish, crabs, squid), lean pork, beef, veal, lamb. 4-5 eggs per week, including those used for cooking. Chicken, turkey hams, e.g. 60 g* of product contain 0.85 g of salt. **60 g represents an average serving of these products*	Fatty meat - pork, veal, lamb, beef, offal, poultry with skin. Fried, fried meat/fish, and meat prepared in deep oil/fat. Fried eggs, scrambled eggs. Cured meat products, e.g. 60 g of prosciutto contains 3.2 g of salt; while tea salami in 60 g contains 2.4 g of salt, two hot dogs (130 g of product) contain 3.1 g of salt
Legumes	Peas, beans, lentils, chickpeas, soybeans prepared with a little oil in the form of stews and salads (as long as they do not cause bloating). Canned legumes (rinse before use).	Canned legumes (rinse before use).	Legumes prepared in a stew with sauce, fatty meat and a lot of oil/fat.
Oils and fats	Bademi, orasi, lešnik, semenke bundeve, lana, suncokreta.		Ušećereno i usoljeno orašasto voće i semenke.
	Olive and pumpkin oil.	Sunflower, rapeseed and other vegetable oils, soft "light" margarine with a lower percentage of fat.	Animal and pork fat, bacon, lard, crackers, butter, sour cream, hard margarine, mayonnaise, palm and coconut oil.
Soups	Vegetable soups and tender meat soups prepared without oil, with a small amount of salt or spices.		Fatty meat soups, vegetable cream soups prepared with whole milk or cream.
Drinks	Water, unsweetened herbal and fruit teas.	Natural fruit and vegetable juices, coffee, beer and wine.	Sweetened carbonated and non-carbonated drinks, powdered vitamin drinks, alcohol, especially spirits.
Spices	Garlic and onion, paprika, parsley, marjoram, dill, cumin, flax, coriander, turmeric, nutmeg, cinnamon, thyme, mint, dill, ginger, etc.		Salt and industrial mixtures of spices (vegetables, etc.). Change the method of dosing salt. For example. 5 g of salt is dosed with one coffee spoon, about 0.5 g with a pinch, and 0.25 g of salt with the tip of a knife.

cionisti-dijetetičari sprovode edukaciju pacijenata o adekvatnoj ishrani i promeni stila života na nivou primarne i sekunadrne zdravstvene zaštite.

Jedan od osnovnih zadataka nutricioniste-dijetetičara je da pomoću nutritivne procene utvrdi navike u ishrani, stanje uhranjenosti i druge faktore rizika za nastanak KVB. Nakon nutritivne procene odnosno identifikovanih faktora rizika, potrebno je utvrditi probleme povezane sa ishranom i planirati nutritivnu intervenciju. U dogovoru sa pacijentom potrebno je odrediti individualne ciljeve lečenja, dati savet i motivaciju za postepenu promenu stila života (30).

Edukacija o pravilnoj ishrani primenjuje se kroz individualno i grupno savetovanje u savetovalištima za ishranu na nivou primarne zdravstvene zaštite. To su mesta gde se pružaju saveti o pravilnoj ishrani, energetskoj vrednosti namirnica, dnevnim energetskim potrebama, energetskom i nutritivnom sastavu namirnica, sastavljanju optimalnog obroka, termičkoj obradi namirnica i uticaju fizičke aktivnosti na energetski bilans (31).

Nutricionista – dijetetičar treba da ponudi različite mogućnosti za promenu životnih navika i motiviše pacijente da prihvate promene u ishrani.

Primer edukativnog materijala za bolesnike sa primerima preporučenih namirnica prema principima DASH dijete su prikazani u Tabeli 2.

Zaključak

Arterijska hipertenzija, jedna od najčešćih KVB, predstavlja faktor rizika za razvoj akutnog infarkta miokarda i cerebrovaskularnog insulta, bolesti koje predstavljaju vodeće uzroke umiranja odraslih osoba u svetu. Redovnom, umerenom i racionalnom ishranom, kao i fizičkom aktivnošću, moguće je sprečiti komplikacije arterijske hipertenzije.

Jedan od najpoznatijih načina ishrane koji ima za cilj smanjenje arterijskog krvnog pritiska kao i normalizovanje lipidnog statusa jeste DASH dijeta. Osnovni principi DASH dijete podrazumevaju povećan unos svežeg voća i povrća, dijetnih vlakana iz žitarica od celog zrna, smanjenje unosa soli, prostih šećera, zamenu životinjske masti biljnim uljima. Pored navedenih principa, za kontrolu arterijskog krvnog pritiska važno je održavanje optimalne telesne mase, redovno upražnjavanje fizičke aktivnosti, kao i prestanak pušenja i ograničen unos alkohola.

Uloga nutricioniste-dijetetičara u prevenciji i lečenju arterijske hipertenzije podrazumeva nutritivnu procenu, procenu faktora rizika i edukaciju viskorizične iopšte populacije o pravilnoj ishrani, fizičkoj aktivnosti i promenama drugih životnih navika.

Konflikt interesa

Autori su izjavili da nema konflikta interesa.

Literatura

1. Registar za akutni koronarni sindrom. Izveštaj br.15. Beograd: Institut za javno zdravlje Srbije „Dr Milan Jovanović Batut“; 2021. Dostupno na: https://www.batut.org.rs/download/publikacije/AKS2020.pdf.
2. Zdravstveno-statistički godišnjak Rrepublike Srbije, 2020. Beograd: Institut za javno zdravlje Srbije „Dr Milan Jovanović Batut“; 2021. Dostupno na: https://www.batut.org.rs/download/publikacije/pub2020.pdf.
3. Republička stručna komisija za izradu i implementaciju vodiča dobre kliničke prakse. Nacionalni vodič dobre kliničke prakse za dijagnostikovanje i lečenje arterijske hipertenzije. Beograd; 2012.
4. Istraživanje zdravlja stanovništva Srbije 2019. godine. Beograd: Institut za javno zdravlje Srbije „Dr Milan Jovanović Batut“; 2021. Dostupno na: https://www.batut.org.rs/download/publikacije/ZdravljeStanovnistva2019.pdf.
5. Webb JG, Kiess MC, Chan-Yan CC. Malnutrition and the heart. CMAJ 1986; 135(7):753-8.
6. Mandić M. Dijetoterapija. Osijek: Sveučilište u Osijeku, Prehrambeno-tehnološki fakultet; 2014.
7. Gabrić ID. Prehrana i kardiovaskularno zdravlje. Medicus [Kardiologija danas] 2016;25(2): 227-34.
8. 2018 ESC/ESH preporuke za lečenje arterijske hipertenzije. Radna grupa za lečenje arterijskehipertenzije Evropskog udruženja kardiologa (ESC) i Evropskog udruženja za hipertenziju (ESH); 2018. Dostupno na: http://uksrb.rs/uploads/1542909443-2018%20ESC%20ESH%20preporuke%20za%20le%C4%8Denje%20arterijske%20hipertenzije.pdf.
9. Nikolić M. Dijetetika. WUS Austria: Medicinski fakultet, Niš; 2008.
10. Vasiljević N, Dragović R, Stojanović S. Značaj ishrane u prevenciji i terapiji hipertenzije. Opšta medicina 2004; 10(3-4):148-52.
11. Mayo Clinic staff [Internet]. DASH diet: Healthy eating to lower your blood pressure. Mayo Clinic; 2022. Dostupno na: https://www.mayoclinic.org/healthy-lifestyle/nutrition-and-healthy-eating/in-depth/dash-diet/art-20048456.
12. UK Healthcare [Internet]. Gill Heart; Vascular Institute. DASH Diet; 2018. Dostupno na: https://ukhealthcare.uky.edu/gill-heart-vascular-institute/patient-resources/general-health/dash.

that are similar to estrogens. The most famous source of the above mentioned compounds are soy isoflavones. A moderate intake helps to lower the risk of CVD and malignant diseases (28). Vitamin D from fish and eggs influences the regulation of high blood pressure. An elevated plasma homocysteine, which is associated with the occurrence of peripheral arterial disease, may significantly be reduced with the help of the DASH diet rich in vegetables and pholates (29).

The role of a nutritionist-dietician in the prevention and treatment of arterial hypertension

Nutritionists-dieticians have a significant role in the prevention and treatment of major non-communicable diseases, especially in primary healthcare. Together with nurses, nutritionists-dieticians educate patients about the adequate diet and lifestyle change at the level of primary and secondary healthcare.

One of the main tasks of a nutritionist-dietician is to assess eating habits with the help of nutritional estimates, as well as the weight status and other risk factors important for the occurrence of CVDs. After the nutritional assessment, that is, identified risk factors, it is necessary to establish diet-related problems and plan the nutritional intervention. Together with the patient, it is necessary to determine individual goals of the treatment, give advice and motivate patients to change lifestyles (30).

Education about the healthy diet is applied through individual and group counseling in nutrition counseling offices in primary healthcare. These are places when one may get advice on healthy diet, the energy value of food, daily energy requirements, energy and nutrient composition of foods, optimal meal plan, thermal processing of foods and influence of physical activity on energy expenditure (31).

A nutritionist-dietitian should offer different possibilities for lifestyle change and motivate patients to accept changes in their diet.

An example of educational material for patients with the examples of recommended foods according to the principles of the DASH diet are shown in Table 2.

Conclusion

Arterial hypertension, one of the most common CVDs, is a risk factor of myocardial infarction and cerebrovascular insult, which are the leading causes of death of adults globally. A regular, balanced and rational diet, as well as physical activity may prevent the complications of arterial hypertension.

One of the most known dietary eating patterns, whose goal is the reduction of arterial blood pressure, as well as the normalization of lipid status, is the DASH diet. The main principles of the DASH diet include the increased intake of fresh fruit and vegetables, dietary fibers from whole grains, the reduction of salt intake, simple sugars intake and the use of vegetable oil instead of animal fat. In addition to the above mentioned principles, in order to control arterial blood pressure, it is important to maintain optimal body mass, regular physical activity, as well as to stop smoking and limit the intake of alcohol.

The role of a nutritionist-dietician in the prevention and treatment of arterial hypertension includes a nutritional assessment, the assessment of risk factors, as well as the education of population at risk and general population about the healthy diet, physical activity and change of other life habits.

Competing interests

Authors declare no competing interests.

Literature

1. Registar za akutni koronarni sindrom. Izveštaj br.15. Beograd: Institut za javno zdravlje Srbije „Dr Milan Jovanović Batut“; 2021. Dostupno na: https://www.batut.org.rs/download/publikacije/AKS2020.pdf.
2. Zdravstveno-statistički godišnjak Rrepublike Srbije, 2020. Beograd: Institut za javno zdravlje Srbije „Dr Milan Jovanović Batut“; 2021. Dostupno na: https://www.batut.org.rs/download/publikacije/pub2020.pdf.
3. Republička stručna komisija za izradu i implementaciju vodiča dobre kliničke prakse. Nacionalni vodič dobre kliničke prakse za dijagnostikovanje i lečenje arterijske hipertenzije. Beograd; 2012.
4. Istraživanje zdravlja stanovništva Srbije 2019. godine. Beograd: Institut za javno zdravlje Srbije „Dr Milan Jovanović Batut“; 2021. Dostupno na: https://www.batut.org.rs/download/publikacije/ZdravljeStanovnistva2019.pdf.
5. Webb JG, Kiess MC, Chan-Yan CC. Malnutrition and the heart. CMAJ 1986; 135(7):753-8.

13. Martinis I, Lasić M, Oreč I, Pugelnik J. Dijetopterapija arterijske hipertenzije. Priručnik za bolesnike. Dubrava (Hrvatska): Klinička bolnica Dubrava; 2022. [citirano 21.05.2022]. Dostupno na: http://www.kbd.hr/fileadmin/Arhiva/Dokumenti/Dijetopterapija_arterijske_hipertenzije.pdf.
14. Katačić R. Dijetoterapija hipertenzije. Istarska Županija, Opća bolnica Pula; 2020. [Internet]. Dostupno na: https://zdrava-sana.istra-istria.hr/media/filer_public/fd/32/fd3289ee-2455-4db0-998a-458e6918aa01/dijetoterapija_hipertenzije.pdf.
15. Filippou CD, Tsioufis CP, Thomopoulos CG, Mihas CC, Dimitriadis KS, Sotiropoulou LI, et al. Dietary Approaches to Stop Hypertension (DASH) Diet and Blood Pressure Reduction in Adults with and without Hypertension: A Systematic Review and Meta-Analysis of Randomized Controlled Trials. Adv Nutr 2020; 11(5):1150-60. doi: 10.1093/advances/nmaa041.
16. Juraschek SP, Miller ER, Weaver CM, Appel LJ. Effects of Sodium Reduction and the DASH Diet in Relation to Baseline Blood Pressure. J Am Coll Cardiol 2017; 70(23):2841-8. doi: 10.1016/j.jacc.2017.10.011.
17. Chiu S, Bergeron N, Williams PT, Bray GA, Sutherland B, Krauss RM. Comparison of the DASH (Dietary Approaches to Stop Hypertension) diet and a higher-fat DASH diet on blood pressure and lipids and lipoproteins: a randomized controlled trial. Am J Clin Nutr 2016;103(2): 341-7. doi: 10.3945/ajcn.
18. Doheny K. Mediterranean Edges Out DASH for Best Diet of 2019 [Internet]. Medscape, WebMD, New York [uploaded Jan 2019, cited Jan 2019]. Available from: https://www.medscape.com/viewarticle/907133?nlid=126970_2823&src=in Patients with Type 2 Diabetes and Uncontrolled Hypertension: A Randomized Controlled Trial. J Clin Hypertens (Greenwich) 2015; 17(11): 895-901.
19. Drehmer M, Odegaard AO, Schmidt MI, Duncan BB, Cardoso LO, Matos SMA, et al. Brazilian dietary patterns and the dietary approaches to stop hypertension (DASH) diet-relationship with metabolic syndrome and newly diagnosed diabetes in the ELSA-Brasil study. Diabetol Metab Syndr 2017; 9:13.
20. Kooshki A, Hoseini B L. Phytochemicals and Hypertension. Shiraz E-Med J 2014; 15(1):e19738. doi: 10.17795/semj19738.
21. Edwards RL, Lyon T, Litwin SE, Rabovsky A, Symons JD, Jalili T. Quercetin reduces blood pressure in hypertensive subjects. J Nutr 2007; 137(11):2405-11. doi: 10.1093/jn/137.11.2405.
22. Jiménez JP, Serrano J, Tabernero M, Arranz S, Díaz-Rubio ME, García-Diz L, Goñi I, Saura-Calixto F. Effects of grape antioxidant dietary fiber in cardiovascular disease risk factors. Nutrition 2008; 24(7-8):646-53. doi: 10.1016/j.nut.2008.03.012.
23. Abdelhamid AS, Brown TJ, Brainard JS, Biswas P, Thorpe GC, Moore HJ, et al. Omega-3 fatty acids for the primary and secondary prevention of cardiovascular disease. Cochrane Database Syst Rev 2020; 3(3):CD003177. doi: 10.1002/14651858.CD003177.pub5.
24. Mladenović I, Mladenović D. Nutritivne karakteristike, fiziološki efekti i zdravstveni značaj dijetnih vlakana. Zdravstvena zaštita 2020; 49(1):47-53. doi: 10.5937/ZZ2001047M.
25. Alibabić V, Mujić I. Pravilna prehrana i zdravlje. Rijeka: Veleučilište u Rijeci; 2016.
26. Tapsell LC, Hemphill I, Cobiac L, Patch CS, Sullivan DR, Fenech M, et al. Health benefits of herbs and spices: the past, the present, the future. Med J Aust 2006; 185(S4):S1-S24. doi: 10.5694/j.1326-5377.2006.tb00548.x.
27. Đulančić N. Toksikologija hrane (interna skripta). Sarajevo: Univerzitet u Sarajevu; 2016.
28. Onyemelukwe OU, Maiha BB. Relationship between plasma homocysteine and blood pressure in hypertensive Northern-Nigerians. Afr Health Sci 2020; 20(1):324-37. doi: 10.4314/ahs.v20i1.38.
29. Mitchell LJ, Ball LE, Ross LJ, Barnes KA, Williams LT. Effectiveness of Dietetic Consultations in Primary Health Care: A Systematic Review of Randomized Controlled Trials. J Acad Nutr Diet 2017; 117(12):1941-62. doi: 10.1016/j.jand.2017.06.364.
30. Ivanišević K, Vuković Z, Mančinković D. Sestrinska edukacija o pravilnoj prehrani osoba oboljelih od šećerne bolesti. Sestrinski Glasnik 2014;19: 109-15.
31. Gabrić ID, Škoro V. Bolesti srca i krvnih žila – prehrana [Internet]. Klinički bolnički centar Sestre Milosrdnice, Zagreb. [citirano 21.05.2022]. Dostupno na: https://www.kbcsm.hr/za-pacijente/upute-o-prehrani-pacijenata-oboljelih-od-kardiovaskularnih-bolesti/prehrana-bolesnika/prehrana-kod-bolesti-srca-i-krvnih-zila/.

6. Mandić M. Dijetoterapija. Osijek: Sveučilište u Osijeku, Prehrambeno-tehnološki fakultet; 2014.
7. Gabrić ID. Prehrana i kardiovaskularno zdravlje. Medicus [Kardiologija danas] 2016;25(2): 227-34.
8. 2018 ESC/ESH preporuke za lečenje arterijske hipertenzije. Radna grupa za lečenje arterijskehipertenzije Evropskog udruženja kardiologa (ESC) i Evropskog udruženja za hipertenziju (ESH); 2018. Dostupno na: http://uksrb.rs/uploads/1542909443-2018%20ESC%20ESH%20preporuke%20za%20le%C4%8Denje%20arterijske%20hipertenzije.pdf.
9. Nikolić M. Dijetetika. WUS Austria: Medicinski fakultet, Niš; 2008.
10. Vasiljević N, Dragović R, Stojanović S. Značaj ishrane u prevenciji i terapiji hipertenzije. Opšta medicina 2004; 10(3-4):148-52.
11. Mayo Clinic staff [Internet]. DASH diet: Healthy eating to lower your blood pressure. Mayo Clinic; 2022. Dostupno na: https://www.mayoclinic.org/healthy-lifestyle/nutrition-and-healthy-eating/in-depth/dash-diet/art-20048456.
12. UK Healthcare [Internet]. Gill Heart; Vascular Institute. DASH Diet; 2018. Dostupno na: https://ukhealthcare.uky.edu/gill-heart-vascular-institute/patient-resources/general-health/dash.
13. Martinis I, Lasić M, Oreč I, Pugelnik J. Dijetopterapija arterijske hipertenzije. Priručnik za bolesnike. Dubrava (Hrvatska): Klinička bolnica Dubrava; 2022. [citirano 21.05.2022]. Dostupno na: http://www.kbd.hr/fileadmin/Arhiva/Dokumenti/Dijetopterapija_arterijske_hipertenzije.pdf.
14. Katačić R. Dijetoterapija hipertenzije. Istarska Županija, Opća bolnica Pula; 2020. [Internet]. Dostupno na: https://zdrava-sana.istra-istria.hr/media/filer_public/fd/32/fd3289ee-2455-4db0-998a-458e6918aa01/dijetoterapija_hipertenzije.pdf.
15. Filippou CD, Tsioufis CP, Thomopoulos CG, Mihas CC, Dimitriadis KS, Sotiropoulou LI, et al. Dietary Approaches to Stop Hypertension (DASH) Diet and Blood Pressure Reduction in Adults with and without Hypertension: A Systematic Review and Meta-Analysis of Randomized Controlled Trials. Adv Nutr 2020; 11(5):1150-60. doi: 10.1093/advances/nmaa041.
16. Juraschek SP, Miller ER, Weaver CM, Appel LJ. Effects of Sodium Reduction and the DASH Diet in Relation to Baseline Blood Pressure. J Am Coll Cardiol 2017; 70(23):2841-8. doi: 10.1016/j.jacc.2017.10.011.
17. Chiu S, Bergeron N, Williams PT, Bray GA, Sutherland B, Krauss RM. Comparison of the DASH (Dietary Approaches to Stop Hypertension) diet and a higher-fat DASH diet on blood pressure and lipids and lipoproteins: a randomized controlled trial. Am J Clin Nutr 2016;103(2): 341-7. doi: 10.3945/ajcn.
18. Doheny K. Mediterranean Edges Out DASH for Best Diet of 2019 [Internet]. Medscape, WebMD, New York [uploaded Jan 2019, cited Jan 2019]. Available from: https://www.medscape.com/viewarticle/907133?nlid=126970_2823&src=in Patients with Type 2 Diabetes and Uncontrolled Hypertension: A Randomized Controlled Trial. J Clin Hypertens (Greenwich) 2015; 17(11): 895-901.
19. Drehmer M, Odegaard AO, Schmidt MI, Duncan BB, Cardoso LO, Matos SMA, et al. Brazilian dietary patterns and the dietary approaches to stop hypertension (DASH) diet-relationship with metabolic syndrome and newly diagnosed diabetes in the ELSA-Brasil study. Diabetol Metab Syndr 2017; 9:13.
20. Kooshki A, Hoseini B L. Phytochemicals and Hypertension. Shiraz E-Med J 2014; 15(1):e19738. doi: 10.17795/semj19738.
21. Edwards RL, Lyon T, Litwin SE, Rabovsky A, Symons JD, Jalili T. Quercetin reduces blood pressure in hypertensive subjects. J Nutr 2007; 137(11):2405-11. doi: 10.1093/jn/137.11.2405.
22. Jiménez JP, Serrano J, Tabernero M, Arranz S, Díaz-Rubio ME, García-Diz L, Goñi I, Saura-Calixto F. Effects of grape antioxidant dietary fiber in cardiovascular disease risk factors. Nutrition 2008; 24(7-8):646-53. doi: 10.1016/j.nut.2008.03.012.
23. Abdelhamid AS, Brown TJ, Brainard JS, Biswas P, Thorpe GC, Moore HJ, et al. Omega-3 fatty acids for the primary and secondary prevention of cardiovascular disease. Cochrane Database Syst Rev 2020; 3(3):CD003177. doi: 10.1002/14651858.CD003177.pub5.
24. Mladenović I, Mladenović D. Nutritivne karakteristike, fiziološki efekti i zdravstveni značaj dijetnih vlakana. Zdravstvena zaštita 2020; 49(1):47-53. doi: 10.5937/ZZ2001047M.
25. Alibabić V, Mujić I. Pravilna prehrana i zdravlje. Rijeka: Veleučilište u Rijeci; 2016.
26. Tapsell LC, Hemphill I, Cobiac L, Patch CS, Sullivan DR, Fenech M, et al. Health benefits of herbs and spices: the past, the present, the future. Med J Aust 2006; 185(S4):S1-S24. doi: 10.5694/j.1326-5377.2006.tb00548.x.
27. Đulančić N. Toksikologija hrane (interna skripta). Sarajevo: Univerzitet u Sarajevu; 2016.
28. Onyemelukwe OU, Maiha BB. Relationship between plasma homocysteine and blood pressure in hypertensive Northern-Nigerians. Afr Health Sci 2020; 20(1):324-37. doi: 10.4314/ahs.v20i1.38.
29. Mitchell LJ, Ball LE, Ross LJ, Barnes KA, Williams LT. Effectiveness of Dietetic Consultations in Primary Health Care: A Systematic Review of Randomized Controlled Trials. J Acad Nutr Diet 2017; 117(12):1941-62. doi: 10.1016/j.jand.2017.06.364.
30. Ivanišević K, Vuković Z, Mančinković D. Sestrinska edukacija o pravilnoj prehrani osoba oboljelih od šećerne bolesti. Sestrinski Glasnik 2014;19: 109-15.
31. Gabrić ID, Škoro V. Bolesti srca i krvnih žila – prehrana [Internet]. Klinički bolnički centar Sestre Milosrdnice, Zagreb. [citirano 21.05.2022]. Dostupno na: https://www.kbcsm.hr/za-pacijente/upute-o-prehrani-pacijenata-oboljelih-od-kardiovaskularnih-bolesti/prehrana-bolesnika/prehrana-kod-bolesti-srca-i-krvnih-zila/.

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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.).

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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.

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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 cases, the abstract should consist of three parts: Introduction/Aim, Case report and Conclusion, and in

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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.

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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).

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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.

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).

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Acknowledgment
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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.

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