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MEDICINSKI FAKULTET

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THE ETIOLOGY, TREATMENT AND OUTCOME OF DIABETIC KETOACIDOSIS AND HYPEROSMOLAR COMA

ETIOLOGIJA, TERAPIJA I ISHOD DIJABETESNE KETOACIDOZE I HIPEROSMOLARNE KOME

Dragana Melentijević [1], Teodora Beljić Živković [2, 3]

Summary

Diabetic ketoacidosis (DKA) and nonketotic hyperosmolar hyperglycemic state (NHOK) are acute complications of diabetes mellitus (DM). The aim was to investigate precipitating factors, therapy and the result of the treatment of DKA and NHOK.

The study included all the people who were admitted to the Clinical Department of Endocrinology, Diabetes and metabolism disease KBC "Zvezdara" in the state of DKA and NHOK between 2007 and 2010.

During this period we treated 56 people, aged 51.8 ± 18.8 years. DKA was diagnosed in 54 patients, whereas NHOK was diagnosed in two patients. Type 1 DM was present in 26 (46.42%) patients and type 2 in 30 (53.57%) patients.

In DKA average values of glucose were 32±8,85mmol / l, HbA1c -11.6±2,52%, pH 6.89±0,17, HCO3-7.40±5,03. In patients with NHOK average glucose was 60.35±15,14mmol / l,

HbA1c-11.2±1,7%,pH 7.43±0,1, HCO3-19, 8±5,23. There was a significant difference in the glucose level (t(54) =6.03,p<0.01) as well as in bicarbonate level (t(54)=3.72,p=0.01) between DKA and NHOK. The most common precipitating factors were: infection in 26 (46.42%) cases, inadequate therapy in 24 (42.85%) cases, myocardial infarction and cerebral stroke in 2 (3.57%) cases. All the patients with NHOK and 16 with DKA had previously been on oral antihyperglycemic drugs, while 29 patients with DKA had previously been on insulin therapy. Upon admission, the previous therapy was not in relation with the level of glucose and pH. Bicarbonate level was significantly higher in the group treated with oral therapy (t (43) = 2.16,p <0.05). The therapy was considered because of rehydration, fractionally giving boluses HM insulin, potassium compensation and treatment precipitating factors. Rehydration was achieved with an average of 5.6±1,65 liters on the first day,4.08±0,87l on the second day, and 3±0,01 liters on the third day of infusion solutions. The total daily dosage of insulin bolus HM on the first day was 81.04±27,97i.j, 59.64±17,60i.j. on the second day and 58.06±19,70i.j. on the third day. Maximum daily supplementation of potassium in the form of 7.4% KCL solution was 61±18,70mmol / l. Upon the end of the treatment, the total of 26 (46.42%) was discharged on basal – bolus human insulin therapy, 18 (32.14%) on the treatment of basal – bolus analogues, 11 (19.64%) on therapy insulin premix and metformin, while two (3.57%) were discharged on oral antihyperglycemic medications. The treatment outcome was successful in 50 people (89.3%), while death occurred in 6 (10.7%) cases. The most common complication was hypokalemia, present in 29 (51.78%) patients. Other complications were acute renal failure (3 people), acute respiratory distress syndrome (2 people), pulmonary edema (2 people) and gastrointestinal bleeding (1 person).

Despite education and available medications for diabetes, acute complications of diabetes can still occur and are sometimes accompanied by dangerous complications.

Key words: *diabetic ketoacidosis, nonketotic hyperosmolar hyperglycemic state*

Sažetak

Dijabetesna ketoacidoza (DKA) i hiperosmolarna neketonska hiperglikemijska koma (NHOK) su akutne komplikacije dijabetesa melitusa (DM).

Cilj rada bio je ispitivanje precipitirajućih faktora, terapije i ishoda lečenja osoba sa DKA i NHOK.

Ispitivanje je obuhvatilo sve osobe koje su primljene na Kliničko odeljenje za endokrinologiju, dijabetes i bolesti metabilizma KBC „Zvezdara" u stanju DKA i NHOK u periodu 2007 – 2010. godine.

U naznačenom priodu lečeno je 56 osoba, starosti 51,8±18,8 godina. DKA je dijagnostikovana kod 54, a NHOK kod 2 pacijenta. DM tip 1 bio je prisutan u 26 (46,42%), a tip 2 u 30 (53,57%) pacijenata.

U DKA prosečne vrednosti glikemije bile su 32±8,85mmol/l, HbA1c 11,6±2,52%, pH 6.89±0,17, HCO3- 7,40±5,03. Kod 2 pacijenata sa NHOK prosečna glikemija 60,35±15,14mmol/l, HbA1c 11,2±1,7%, pH 7,43±0,1, HCO3-19,8±5,23. Između DKA i NHOK bila je značajana razlika u visini glikemije (t(54)=6,03,p<0,01) i nivou bikarbonata (t(54)=3,72,p=0,01). Najčešći precipitirajući faktori bili su: infekcija 26(46,42%), neadekvatna terapija 24 (42,85%), infarkt miokarda i cerebrovaskularni insult 2(3,57%). Svi pacijenti sa NHOK i 16 sa DKA bilo je prethodno na oralnim antihiperglikemijskim lekovima, dok je 29 njih sa DKA bilo prethodno na insulinskoj terapiji. Predhodna terapija nije korelirala sa nivoom glikemije i pH na prijemu. Nivo bikarbonata je značajno bio veći u grupi lečenih oralnom terapijom (t(43)=2,16,p<0,05).

Lečenje je podrazumevalo rehidrataciju, frakcionirano davanje bolusnog HM insulina, nadoknadu kalijuma i lečenje precipitirajućeg faktora. Rehidratacija je ostvarena sa prosečno prvog dana 5,6±1,65litara, drugog dana 4,08±0,87, a trećeg 3±0,01 litara infuzionih rastvora. Ukupna dnevna doza bolusnog HM insulina prvog dana bila je 81,04±27,97 i.j, drugog 59,64±17,60 i.j, a trećeg 58,06±19,70. Maksimalna dnevna nadoknada kalijuma u obliku 7.4% rastvora KCL bila je 61±18,7 mmol/l, prvog dana. Po završetku lečenja, ukupno je 26 (46,42%) osoba otpušteno na bazal-bolusnoj terapiji humanim insulinom, 18 (32,14%) na bazal-bolusnoj terapiji analozima, 11 (19,64%) na terapiji premiks insulina i metformina, dok je dvoje (3,57%) otpušteno na oralnim antihiperglikemijskim lekovima. Ishod lečenja je bio upešan u 50 osoba (89,3%), smrtni ishod nastupio je u 6 (10.7%) osoba. Najčešća komplikacija bila je hipokalijemija, prisutna u 29 (51,78%) lečenih. Ostale komplikacije bile su: akutna bubrežna insuficijencija (3 osobe), akutni respiratorni distres sindrom (2 osobe), edem pluća (2 osobe) i gastrointestinalno krvavljenje (1 osoba).

Zaključimo, i pored edukacije i potpune dostupnosti lekova za šećernu bolest, akutne komplikacije dijabetesa se ipak dešavaju i praćene su ponekad opasnim komplikacijama.

Ključne reči: *dijabetesna ketoacidoza, hiperosmolarna koma*

Uvod

Dijabetesna ketoacidoza (DKA) i hiperosmolarna neketonska hiperglikemijska koma (NHOK) su akutne, potencijalno veoma opasne komplikacije dijabetes melitusa (DM). DKA se češće javlja kod dijabetes melitus tipa 1(1), a NHOK kod DM tip 2 tipično kod starijih pacijenata sa komorbiditetima, sa umerenim diabetesom, najčešće lečenim oralnim antihiperglikemijskim agensima koji neredovno uzimaju terapiju (2). Najčešći precipitirajući faktor za razvoj DKA je infekcija (najčešće urinarna, zatim respiratorna i na trećem mestu kožna infekcija) (3). Drugi uzroci su diskontinuirana ili neadekvatna insulinska terapija, kardiovaskularni događaji, moždani udar, hirurške intervencije, akutna alkoholisanost, akutni pankreatitis, upotreba nekih lekova. Lekovi kao što su kortikosteroidi, tiazidni diuretici, simpatikomimetici i pentamidini mogu precipitirati razvoj ketoacidoze (4).

Najčešći precipitirajući faktor za razvoj NHOK je infekcija, iznurenost, komorbiditet (CVI, demencija, infarkt miokarda, bubrežna slabost) ili socijalna situacija koja remeti unos vode. Kao precipitirajući faktor se navodi i upotreba pojedinih lekova (tiazidni diuretici, glikokortikoidi, fenitoin) (5).

U odnosu na težinu metaboličkog disbalansa i stepen poremećaja svesti, postoje tri oblika dijabetesne ketoacidoze. To su laka, umerena i teška ketoacidoza. (6). Njihove međusobne razlike prikazane su u Tabeli 1.

Osnovna terapijska mera u lečenju stanja DKA je rehidratacija. Daje se 0,9% NaCl u količini od 10-20 ml/kg/TT/čas (7). Nadoknada kalijuma (K) je druga osnovna mera u terapiji DKA. Nivo K se prati na 2-4 časa i nadoknada ne bi trebalo da bude brža od 40 ml 7,4% KCl na 1 čas (u slučaju K<3,5 mmol/l) (8). Nadoknada insulina je na trećem mestu po prioritetu. Insulinska terapija kod DKA je 0.1i.j./kg/čas i.v. u 500 ml rastvora. Insulin se daje u drugi litar rastvora, pošto je započeta nadoknada kalijuma (38). Ukoliko je pH<7.2 može se dodati 100 ml 4,2% bikarbonata tokom 30 min. Ukoluko je pH<7,0 nadoknada bikarbonata se ostvaruje sa 100 ml 8,4% rastvorom natrijum bikarbonata putem najšire igle (9).

Najčešće komplikacije DKA su hipokalijemija i hipoglikemija, cerebrovaskularni insult, akutni respiratorni distres sindrom i edem pluća, akutna bubrežna insuficijencija (10), hipofosfatemija, akutna dilatacija želuca, tromboembolije. Smrtnost u DKA je 0,5-2% (43).

Terapija NHOK se zasniva na brzoj rehidrataciji. U prvih dva sata daje se 2-3 litra fiziološkog rastvora (11). Nadoknada kalijuma je izuzetno važna. Ukoliko je K < 3,5 mmol/l ne daje se insulin. Nadiknada K vrši se rastvorom 7,4% KCl 40 ml u prvi litar rastvora u toku prvog sata, potom 20 ml na litar rastvora na sat vremena, sve do normalizacije. Kratkodelujući insulin (regularni HM insulin) se dodaje u količini od 0.1i.j./kg/h. (12).

Najčešće komlikacije koje prate stanje hiperosmolarne neketonske hiperglikemijske kome su rabdomioliza, DIK , tromboembolija (13), akutna renalna insuficijencija i edem pluća (14). Smrtnost u DKA je 0,5-2% (15).

Cilj rada

Primarni cilja ovog rada je ispitivanje precipitirajućih faktora, terapije i ishoda lečenja u osoba sa dijabetesnom ketoacidozom i hiperosmolarnom hiperglikemijskom neketonskom komom. Sekundarni cilj ispitivanja je poređenje uticaja predhodne terapije i glikoregulacije na ozbiljnost metaboličkog poremećaja, dalje lečenje i pojavu komplikacija.

Metod

Ovom studijom preseka, obuhvaćeni su pacijenti koji su u periodu 2007. do 2010. godine hospitalizovani na Klinički odeljenju za endokrinologiju, dijabetes i bolesti metabilizma KBC Zvezdara. Laboratorijski kriterijumi za dijagnozu dijabetesne ketoacidoze na hitnom internističkom prijemu bili su glikemija >16 mmol/l , pH<7,3, HCO3<15 mmol/l, osmolalnost >300 mOsmo/l. Kriterijumi za dijagnozu hiperosmolarne neketonske hiper-

Tabela 1. Oblici dijabetesne ketoacidoze;
Table 1. Forms of diabetic ketoacidosis

	Blaga Mild	Umerena Moderate	Teška Serious
Glikemija mMol Glycemia mMol	>15	>15	>15
Arterijski pH Arterial pH	7.25-7.30	7.00-7.24	<7.00
Bikarbonati mEq/l Bicarbonates mEq/l	15-18	10-15	< 10
Ketoni/ urin Ketones/urine	+	+	+
Ketoni/ serum Ketones/serum	+	+	+
Plazma EOsm Plasma EOsm	Varijabilna Variable	Varijabilna Variable	Varijabilna Variable
Anjonska praznina Anion gap	>10	>12	>12
Poremećaj svesti Disturbance of consciousness	Normalna svest Normal consciousness	Pomućena svest Confused consciousness	Koma Coma

glikemijske kome na hitnom internističkom prijemu bili su glikemija >40 mmol /l, pH >7,3, HCO3 >20 mmol/l, osmolalnost >335 mOsmo/l. Podaci su dobijeni iz istorija bolesti bolesnika koji su u navedenom periodu lečeni. U toku hospitalizacije pacijentima je određivana osnovna biohemija, HbA1c, elekrolitni status, gasne analize i pH vrednost krvi, izračunata osmolarnost (osmolarnost (mosm/l)= 2 x (Na+K) + glukoza + ureja) i anjonska praznina (Na – (Cl+ HCO3)) na osnovu dobijenih laboratorijskih vrednosti. Traženo je prisustvo precipitirajućih faktora. Na osnovu fizikalnog nalaza, hematoloških parametara i drugih dijagnostičkih procedura intenzivno je tragano za prisustvom infektivnog žarišta.

Uvidom u medicinsku dokumentaciju analizirani su parametri: pol, godine života i tip dijabetesa. Beleženo je prisustvo precipitirajućih faktora. To su najčešće bili: akutni koronarni sindrom, CVI, infekcija i tip infekcije, akutni pankreatitis, uzimanje alkohola i neredovno uzimanje terapije koje dosta često pacijente vodi u akutne komplikacije dijabetesa. Praćena je predhodna glikoregulacija kroz HbA1c i prisustvo predhodnih akutnih i hroničnih komplikacija dijabetesa. Zabeležena je antidijabetesna i druga terapija pre hospitalizacije.

Zapisana je terapija sa liste intenzivne nege u prva tri dana hospitalizacije (dnevno određivanje količine tečnosti koja je primenjena u rehidrataciji, broj jedinica insulina aplikovan na dnevnom nivou, maksimalna količina datog K u danu, davanje bikarbonata). Zabeležena je i terapija sa kojom je pacijent otpušten iz bolnice i preporuka za dalje lečenje.

Praćen je razvoj komplikacija i ishod lečenja. Zabeleženi su: mortalitet, prisustvo neuroloških komplikacija, hipokalijemije, hipoglikemije, akutni respiratorni distres sindrom, edem pluća, akutna bubrežna insuficijencija, tromboza, rabdomioliza i gastrointestinalno krvarenje.

Podaci su analizirani metodama deskriptivne i analitičke statistike. Deskriptivni statistički metodi korišćeni za opisivanje uzorka su aritmetička sredina i standardna devijacija. Za poređenje grupa korišćeni su parametarski (jednofaktorska ANOVA, t test) i neparametarski testovi (hi kvadrat test). Za statističku obradu podataka korišćen je računarski softverski program SPSS. Podaci su prikazani tabelarno i grafički.

Rezultati

U naznačenom priodu, od 2007-2010. godine lečeno je 56 osoba. DKA je dijagnostikovana kod 54, a NHOK kod 2 pacijenta. Od 56 hospitalizovane osobe 29 osoba je ženskog, 27 muškog pola, prosečne starosti *51,8±18,8* godina (Tabela 2). Dijabetes mellitus tip 1 bio je prisutan u 26 (46,42%), a tip 2 u 30 (53,57%) pacijenata.

Tabelom 3 prikazana je distribucija hospitalizivanih u posmatranom periodu gde se primećuje opadajući trend

Tabela 2. Distribucija lečenih po polu i uzrastu; Table 2. Distribution treated by sex and age			
GODINE AGE	M M	Ž F	ukupno u uzrasnoj grupi total of the age group
18 – 39	8	7	15 (26.8%)
40 – 59	12	9	21 (37.5%)
60 – 79	7	10	17 (30.4%)
80 – 89	0	3	3 (5.3%)
Ukupno Total	27	29	56 (100%)

Tabela 3. Distribucija hospitalizovanih u posmatranom periodu Table 3. Distribution of hospitalized patients in the reporting period			
Godina Year	Ukupan broj hospitalizovanih TOTAL hospitalized	Hospitalizovani zbog ketoacidoze i hiperosmolarne kome Hospitalized for DKA and NHOK	Procentualni odnos Percentage ratio
2007. god 2007. year	462	17	3.68%
2008. god 2008. year	528	14	2.65%
2009. god 2009. year	632	15	2.37%
2010. god 2010. year	781	12	1.53%
Ukupno Total	2403	56	

u broju hospitalizacija zbog akutnih komplikacija dijabetesa kroz godine posmatranja.

U pacijenata sa DKA prosečne vrednosti na prijemu su bile: glikemija 32 mmol/l, HbA1c 11,6%, pH 6.89, HCO3 7,40 mEq/l. Kod pacijenata sa NHOK prosečna glikemija 60,35 mmol/l, HbA1c 11,2%, pH 7,43, a HCO3 19,8 mEq/l (Tabela 4).

Poredeći visinu glikemije, HbA1c, pH i bikarbonata na prijemu među pacijentima sa DKA i NHOK dobijena je visoko statistički značajna razlika u visini glikemije ($t(54)=6,03$, $p<0,01$) i vrednosti bikarbonata ($t(54)=3,72$, $p=0,01$). Za vrednosti HbA1c ($t(54)=0,25$, $p>0,05$) i pH krvi ($t(54)=0,81$, $p>0,05$) ne postoji statistički značajna razlika između ove dve grupe pacijenata.

Prosečna vrednost HbA1c bila je 11,6 %. Posmatrali smo u kojoj meri predhodna glikoregulacija utiče na ozbiljnost metaboličkog poremećaja u ketoacidozi. Jednofaktorskom parametarskom analizom varijanse (ANOVA) upoređene su vrednosti HbA1c u bolesnika sa blagim, umerenim i teškim oblikom ketoacidoze, podeljenih u date grupe na osnovu izmerenog nivoa bikarbonata (Tabela 5) i pH krvi na prijemu (Tabela 6). Ne postoji statistički značajna razlika u nivou HbA1c između ove tri grupe pacijenata.

Najčešći precipitirajući faktori bili su: infekcija i neadekvatna terapija. Znatno ređi uzroci nastanka DKA i NHOK bili su infarkt miokarda, cerebrovaskularni insult, akutni pankreatitis i akutno alkoholisano stanje (Grafikon1).

Kako je neadekvatna terapija važan precipitirajući faktor posmatrali smo terapiju na prijemu. Svi pacijenti sa NHOK i 16 osoba sa DKA bili su prethodno lečeni oralnim antihiperglikemijskim lekovima, dok je 29 njih sa DKA bilo prethodno na insulinskoj terapiji. Devet pacijenata (4 osoba- DM tip 1 i 5 osoba- DM tip2) nije imalo predhodnu terapiju. Od njih devet u 5 slučajeva u pitanju je bila prva manifestacija DM (de novo), a 4 bolesnika samoinicijativno su prekinula terapiju na duži period (Tabela 7).

Tabela 4. Vrednosti na prijemu;
Table 4. Values on admission

Prosečne vrednosti na prijemu Values on admission	DKA 54pacijenta 54 patients	NHOK 2 pacijenta 2 patients	P P
Glikemija mmol/l Glycemia mmol7l	32 ± 8.85	60.35 ± 15.14	<0.01
HbA1c % HbA1c %	11.6 ± 2.52	11.2% ± 1.7	>0.05
pH PH	6.89 ± 0.17	7.43 ± 0.1	>0.05
HCO3 mEq/l HCO3 mEq/l	7.40 ± 5.03	19.8 ± 5.23	=0.01
Anjonska praznina Anion gap	18	15	/
Osmolarnost mosm/l Osmolality mosm/L	348 ± 3.47	375 ± 2.56	/

Tabela 5. Prikaz vrednosti HbA1c u pacijenata sa različitim oblicima DKA na osnovu vrednosti bikarbonata
Table 5. HbA1C Value display in patients with various forms DKA based on the value of bcarbonates

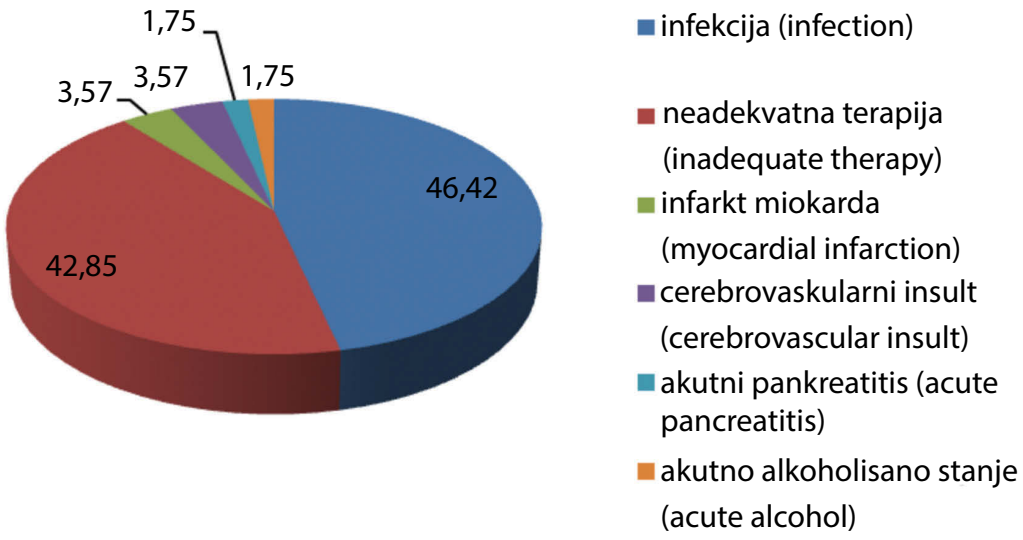
Nivo bikarbonata (mEq/l) Levels of bicarbonate	Vrednosti HbA1c HbA1c values	N N
< 10.00	11.57 ± 2.49	39
10.01 – 15.00	12.16 ± 2.23	10
>15.01	11.14 ± 3.60	5

F=0.315, p>0.05 (F2,51;0.05=3.23 i F2,51;0.01=5.18)

Tabela 6. Prikaz vrednosti HbA1c u pacijenata sa različitim oblicima DKA na osnovu nalaza pH krvi
Table 6. HbA1C Value display in patients with various forms DKA based on the value of blood pH

pH krvi	Vrednosti HbA1c	N
<7.00	11.64±3.36	14
7.01-7.24	11.90±1.93	28
>7.25	11.03±2.74	12

F=0.503, p>0.05 (F2,51;0.05=3.23 i F2,51;0.01=5.18)



Grafikon 1. Precipitirajući faktori za razvoj DKA i NHOK
Graph 1. Precipitating factors for the development of DKA and NHOK

Tabela 7. Prikaz predhodne terapije
Table 7. Previous therapy

ORA	Oralni antihiperglikemici Oral antidiabetics	Insulinska terapija Insulin	Bez terapije No therapy
Osobe sa DKA Patients diagnosed with DKA	16 (29.6%)	29 (53.7%)	9 (16.7%)
Osobe sa NHOK Patients diagnosed with NHOK	2 (100%)	/	/

Tabela 8. Glikemija po grupama u zavisnosti od predhodne terapije
Table 8. Glycemia in groups depending of previous treatment

Predhodna terapija Previous treatment	Glikemija na prijemu Glycemia on admission	N
Grupa 1 (oralna terapija) Group 1 (oral therapy)	29.77±4.80	16
Grupa 2 (insulinska terapija) Group 2 (insulin therapy)	31.99±7.26	29
Grupa 3 (bez predhodne terapije) Group 3 (without previous therapy)	27.68±5.75	9

Poređene su srednje vrednosti biohemijskih parametara (glikemije, bikarbinata i pH) po grupama koje su formirane na osnovu predhodne terapije. Ne postoji statistički značajna razlika u visini glikemije na prijemu između grupe 1 i grupe 2 ($t(43)=0,83$, $p>0,05$) niti između grupe 1 i grupe 3 ($t(23)=0,49$, $p>0,05$). Poredeći grupu pacijenata lečenih insulinom i one bez predhodne terapije t testom nije detektovana razlika u nivou glikemije na prijemu ($t(36)=1,34$, $p>0,05$) (Tabela 8).

Između grupe 1 i grupe 2 ($t(43)=2,16$, $p<0,05$), kao i između grupe 1 i 3 ($t(23)=2,02$, $p<0,05$) postoji statistički značajna razlika u nivou bikarbonata na prijemu. Između i grupe 2 i 3 ne postoji značajna razlika u nivou bikarbonata ($t(36)=1,62$, $p>0,05$) (Tabela 9). Predhodna terapija nije bitno uticala na nivo pH vrednosti krvi (Tabela 10). Izmedju grupa 1 i 2 ne postoji statistički značajna razlika u vrednosti pH krvi ($t(43)=1,61$, $p>0,05$). Takođe, ne postoji razlika između grupe 2 i 3 ($t(36)=0,28$, $p>0,05$) i grupe 1 i 3 ($t(23)=0,87$, $p>0,05$).

Lečenje hospitalizovanih pacijenata podrazumevalo je rehidrataciju, frakcionirano davanje bolusnog HM insulina, nadoknadu kalijuma i odgovarajuće lečenje precipitirajućeg faktora. Rehidratacija je ostvarena prvog dana sa prosečno 5,6 litara, drugog dana 4,08, a trećeg 3 litara infuzionih rastvora. Ukupna dnevna doza bolusnog HM insulina prvog dana bila je 81,04i.j, drugog 59,64 i.j, a trećeg 58,06 i.j. Maksimalna dnevna nadoknada kalijuma u obliku 7.4% rastvora KCL bila je 110mmol/l, a minimalna 20mmol/l. U tri bolesnika nije bilo potrebe za nadoknadom kalijuma (Tabela 11). Posmatrano je da li i u kojoj meri predhodna terapija kojom je pacijentima regulisan dijabetes melitus određuje intenzitet lečenja u

Tabela 9. Bikarbonati po grupama u zavisnosti od predhodne terapije
Table 9. Bicarbonates in groups depending of previous treatment

Predhodna terapija Previous treatment	Bikarbonati na prijemu Bicarbonates on admission	N
Grupa 1 (oralna terapija) Group 1 (oral therapy)	9.17±2.42	16
Grupa 2 (insulinska terapija) Group 2 (insulin therapy)	6.00±1.01	29
Grupa 3 (bez predhodne terapije) Group 3 (without previous therapy)	5.95±1.53	9

Tabela 10. Vrednost pH krvi po grupama u zavisnosti od predhodne terapije
Table 10. Value pH groups depending of previous treatment

Predhodna terapija Previous treatment	pH krvi na prijemu Blood pH on admission	N
Grupa 1 (oralna terapija) Group 1 (oral therapy)	7.15±0.15	16
Grupa 2 (insulinska terapija) Group 2 (insulin therapy)	7.07±0.16	29
Grupa 3 (bez predhodne terapije) Group 3 (without previous therapy)	7.09±0.19	9

Tabela 11. Prosečna terapija u prva tri dana intenzivnog lečenja
Table 11.Average treatment in the first three days of treatment

	Nadoknada tečnosti(L) Volume replacement	Insulinska Terapija (i.j.) Insulin therapy (i.j.)	Nadoknada kalijuma mmol/L(7.4%KCl) Compensation potassium mmol/L(7.4%KCl
Prvi dan First day	5.6±1.65	81.4±27.97	61±18.70
Drugi dan Second day	4.08±0.87	59.64±17.60	36±10.69
Treći dan Third day	3.1±1.01	58.06±19.70	45±7.90

prva tri dana u stanju dijabetesne ketoacidoze. Poredili smo prosečan broj jedinica insulina i prosečnu količinu date tečnosti u prva tri dana lečenja kod grupe pacijenta lečenih oralnom, insulinskom terapijom i u grupi bez terapije jednofaktorskom parametarskom analizom varijanse (ANOVA) (Tabela 12).

Empirijska vrednost Fisherovog količnika za jedinice insulina je F=1,07, p>0,05 (F2,51;0,05=3,23 i F2,51;0,01=5,18). Ne postoji statistički značajna razlika u broju jedinica insulina u prva tri dana lečenja između ove tri grupe pacijenata.

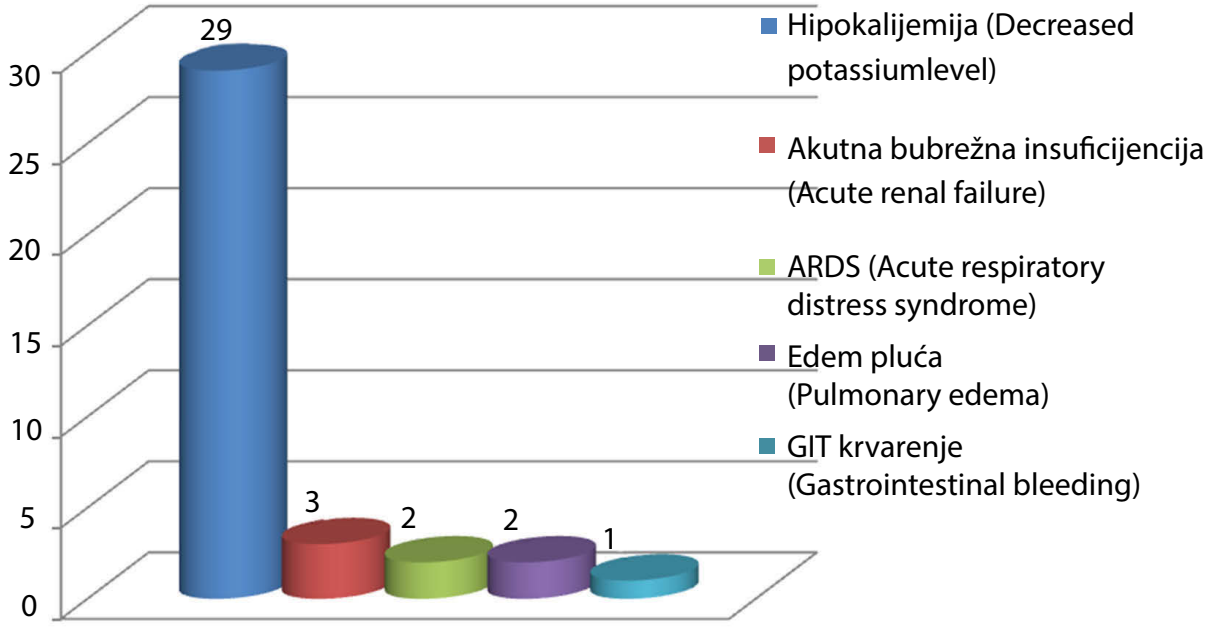
Empirijska vrednost Fisherovog količnika za količinu tečnosti je F=0,82, p>0,05 (F2,51;0,05=3,23 i F2,51;0,01=5,18).

Najčešća komplikacija bila je hipokalijemija, prisutna u 29 (51,78%) lečenih. Najniža vrednost kalijuma bila je 2,7mmol/l. Ostale komplikacije bile su: akutna bubrežna insuficijencija (3 osobe), akutni respiratorni distres sindrom (2 osobe), edem pluća (2 osobe) i gastrointestinalno krvavljenje (1 osoba) (Grafikon 2).

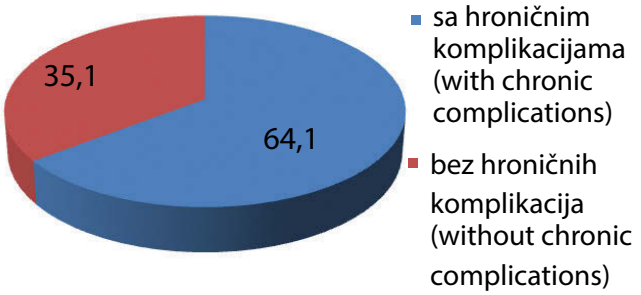
U toku lečenja svi pacijenti su pregledani od strane oftalmologa, neurologa, nefrologa u cilju ispitivanja i praćenja prisustnih hroničnih komplikacija. Hronične komplika-

Tabela 12. Uticaj predhodne terapije na lečenje ketoacidoze
Table 12. The impact of previous therapy for treatment of ketoacidosis

Predhodna terapija Previous therapy	prosečan br. jed insulina	Prosečn kol. Tečnosti	N
Grupa 1 (oralna terapija) Group 1 (oral therapy)	84.06±31.13	5.36±1.41	16
Grupa 2 (insulinska terapija) Group 2 (insulin therapy)	80.31±27.82	5.79±1.81	29
Grupa 3 (bez predhodne terapije) Group 3 (without previous therapy)	92.88±22.78	6.00±1.65	9



Grafik 2. Komplikacije lečenja
Graph 2. Complications of treatment

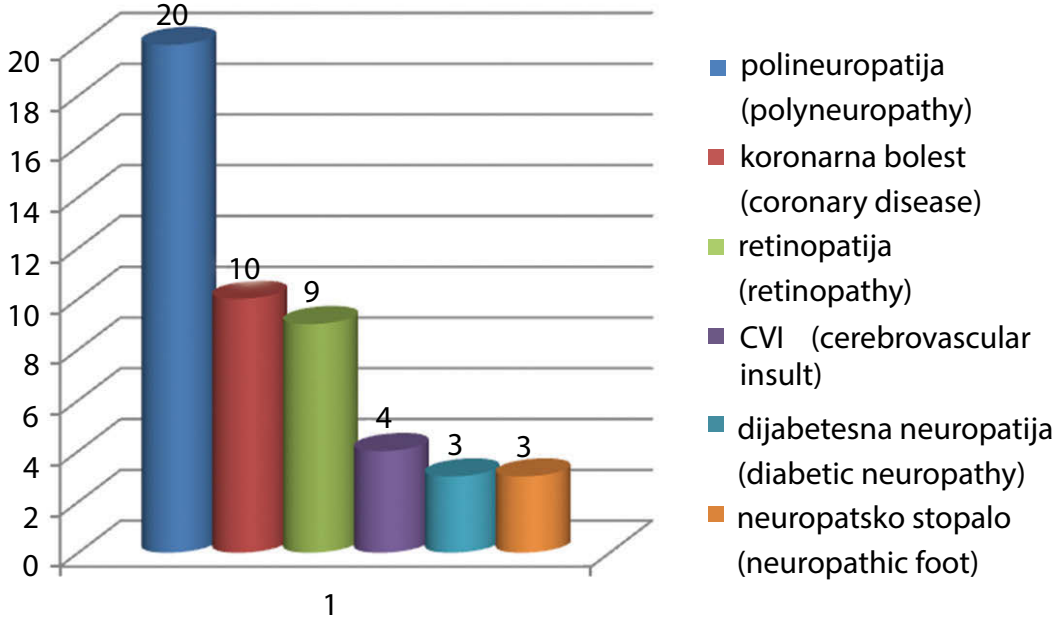


Grafik 3. Prisustvo hroničnih komplikacija
Graph 3. Presence of chronic complications

cije DM su bile prisutne kod više od polovine pacijenata, u 36 (64, 90%) osoba, dok 20 (35,10%) pacijenata nije imalo hronične komplikacije (Grafikon 3).

Polineuropatija je bila prisutna kod 20 osoba, retinopatija u 9 osoba, neuropatsko stopalo kod 3 pacijenta, koronarna bolest u 10 osoba, CVI u 4 osobe, dijabetesna nefropatija kod 3 pacijenta (Grafikon 4).

Po završetku lečenja, ukupno je 26 (46,42%) osoba otpušteno na bazal-bolusnoj terapiji humanim insulinom, 18 (32,14%) na bazal-bolusnoj terapiji analozima,



Grafik 4. Zastupljenost pojedinih hroničnih komplikacija
Graph 4. Prevalence of chronic complications

Tabela 13. Prikaz terapije na otpustu
Table 13. Therapy at discharge

Terapija na otpustu Therapy at discharge	DKA	NHOK
Bazal bolusna terapija HM insulinima Basal bolus HM insulin therapy	25	1
Terapija humanim analozima Basal bolus analogues	18	/
Premiks insulina i metformina Premix insulin metformin	11	/
Oralna terapija Oral therapy	1	1

11 (19,64%) na kombinovanoj terapiji premiks insulina i metformina, dok je dvoje (3,57%) otpušteno na oralnim antihiperglikemijskim lekovima (Tabela 13).

Ishod lečenja je bio upešan u 50 osoba (89,3%), dok je smrtni ishod nastupio u njih 6 (10.7%). Prosečna starost osoba koje su lečenje završile letalnim ishodom bila je 75±6,2 godina. U pitanju su bili pacijenti sa brojnim komorbiditetima i razvijenim makrovaskularnim komplikacijama dijabetes melitusa. Svi pacijenti su imali DKA, dok je u oba pacijenta sa NHOK uspešno saniran metabolički dizbalans i otpušteni su sa korigovanom terapijom. Četiri osobe sa DKA su prebačene na druga odeljenja nakon saniranja akutnog metaboličkog poremećaja.

Diskusija

Naše ispitivanje je pokazalo da akutne komplikacije dijabetes melitusa nisu česta pojava u hospitalizivanih pacijenata. Javljaju se pretežno kod pacijenata sa komorbiditetima i predhodno lošom glikoregulacijom.

Glavni precipitirajući faktor koji je pacijente dovodio u stanje DKA i NHOK je infekcija. Respiratorne infekcije, akutizacija hroničnog bronhitisa i pneumonija, kao i urinarne infekcije su i u drugim radovima zabeležene kao vodeći precipitirajući faktor (16). Infekcija kod obolelih od dijabetesa je ozbiljna pretnja za potencijalno veoma opasne kompilkacije. Potrebno je lociranje infektivnog žarišta i primena blagovremene terapije. Drugi razlog je neadekvatna antidijabetesna terapija. Tu spadaju neblagovremena i neadekvatna primena insulinske terapije, nedovoljna komlijansa i nepridržavanje predloženim merama higijensko-dijetetskog režima. Neželjeni kardiovaskularni događaji, akutni infarkt miokarda i cerebrovaskularni insult su treći precipitirajući faktor. Dobijeni podaci se slažu sa autorima drugih radova (17).

Prosečna starost pacijenata u našem radu bila je 51 godina. Najmlađi pacijent koji je hospitalizovan zbog akuntih komplikacija imao je 18 godina. Od svih hospitalizovanih zbok stanja DKA, blizu trećine bolesnika bilo je mlađe od 40 godina. To se slaže sa literaturnim podacima gde se dijabetesna ketoacidoza javlja kod mlađih, veoma često kao prva manifestacija dijabetesa. Prema podacima iz literature DKA se češće javlja kod DM tip 1, što nije slučaj sa prikazanom grupom u kojoj su brojniji bili pacijenti sa DM tip 2 (18).

Za razliku od dijabetesne ketoacidoze NHOK se prema podacima iz literature tipično javlja kod starijih ljudi na oralnim antihiperglikemijskim sredstvima. Prosečna starost pacijenta hospitalizovanih zbog NHOK je 79,5 godina. Razlog za pojevu hiperosmolarne neketonske kome kod starijih je loša kontrola dijabetesa, nedovoljno uzimanje tečnosti, upotreba diuretika i prisutna bubrežna slabost (19). Kod oba pacijenta sa NHOK u pitanju je bio DM tip 2 predhodno lečen oralnim antidijabeticima.

Prikazana je distribucija obolelih od DKA i NHOK u posmatranom četvorogodišnjem periodu. Pad u broji hospitalizovanih zbog akutnih komplikacija dijabetesa sa smanjenim procentualnim češćem u odnosu na ukupan broj hospitalizovanih se može objasniti poboljšanjem zdravstveno vaspitnog rada. Edukacijom lekara opšte medicine moguće je doprineti boljoj kontroli dijabetes melitusa i ranijem i blagovremenom prevođenju na insulin.

Naše ispitivanje nije pokazalo da postoji korelacija izeđu visine HbA1c i stepena metaboličkog poremećaja u akutnim komplikacijama dijabetesa, što možda ne bi bilo potvrđeno drugim ispitivanjem sa većim uzorkom. Takođe, prvobitna terapija ne određuje stepen metaboličkog poremećaja. Međutim, pacijenti lečeni oralnim antihiperglikemicima su imali niži stepen acidoze u odnosu na one lečene insulinom i one bez terapije. Ovo je moguće objasniti preostalom funkcijom β ćelija pankreasa u DM tip 2. U ovom radu nakon stabilizacije akutnog stanja nije određivana sekretorna funkcija β ćelija niti rezerva pankreasa. Prema našem saznanju ne postoje radovi koji su uporedili pojavu akutnih komplikacija dijabetesa sa sekretornom funkcijom β ćelija pankreasa. Predhodno lečenje nije pokazalo značajan uticaj na lečenje u prva tri dana intenzivnog tretmana.

Svi pacijenti su lečeni klasičnom terapijom za akutna stanja ove vrste. Cilj lečenja bio je da se postigne brza rehidratacija, normalizacija acido-baznog statusa, adekvatna regulacija glikemije, nadoknada K+. Pacijenti su na intenzivnom lečenju provodili u proseku 3 dana. Korigovana im je terapija i otpušteni su u stabilnom stanju. I pored intenzivnog tretmana razvijale su se komplikacije lečenja.

Najčešća komplikacija kod lečenih od DKA bila je hipokalijemija. Javljala se zbog loše nadoknade kalijuma u prvim i urgentnim terapijskim postupcima u ketoacidozi. Odluku o nadoknadi kalijuma treba uskladiti sa preporukama da se nadoknada K započinje kada je se-

rumski K<5,5 mmol/l. U 500 ml rastvora stavlja se10 ml 7,4% KCl. Akutna bubrežna insuficijencija kao druga po učestalosti komplikacija ketoacidoze, javljala se kod visoko febrilnih pacijenata, sa prisutnim teškim urinarnim infekcijama. Akutni respiratorni distres sindrom razvio se na terenu egzacerbacije hroničnog bronhitisa sa lošom respiratornom rezervom. Gastroinestinalno krvarenje nastalo je kod jednog pacijenta na terenu predhodne ulkusne bolesti. Učestalost pojedinih komplikacija u srodnim ispitivanjim je slična (20).

Mortalitet u našem istraživanju (10,7%) je bio viši u odnosu na podatke iz literature (2%), a vezan je za precipitirajući kardiovaskularni dogadjaj i starost pacijenata sa brojnim komorbiditetima (21). Prosečna starost pacijenata kod kojih je nastupio letalni ishod bila je 75 godina. Neophodno je napomenuti da su pacijenti koji su egzitirali uspešno vođeni i lečeni i toku trajanja DKA (u toku intenzivnog lečenja). U pitanju su stari pacijenti sa brojnim komorbiditetima (dugotrajnom hipertenzijom, ishemijskom bolešću srca, kardiomiopatijom, stanjem posle cerebrovaskularnog insulta) koji su nakon stabilizacije akutnog poremećaja najčešće dobijali CVI ili infarkt miokarda, što je i potvrđeno obdukcionim nalazima.

Kada je u pitanju razvoj komplikacija i ishod lečenja pacijenata sa NHOK, ograničenje je to što su u navedenom periodu hospitalizovana samo dva pacijenta sa ovim stanjem. Oba pacijenta su otpuštena u stabilnom stanju uz korekciju terapije sto implicira da je uspešnost lečenja u ovom slučaju bila 100%. U literaturni se nalazi podatak da je mortalitet u lečenih usled stanja NHOK od 15-50%. U drugim radovima se pominju tromboebolija i rabdomioliza kao česte komplikacije ovog stanja što se nije javljalo u naša dva pacijenta (22).

Hronične komplikacije dijabetes melitusa bile su prisutne kod više od polovine naših pacijenata sa DKA i NHOK. Prosečne vrednosti HbA1c su u obe grupe bile preko 11%. Prema Nathan et al. preračunavajući vrednost HbA1c u nivo glikemije dobija se podatak da su pacijenti u predhodnom tromesečnom periodu imali prosečan nivo glikemije 12-17,5 mmol/l (23), što jasno pokazuje dugoročno lošu glikoregulaciju. Loša komlijansa i neadekvatna terapija vode do nezadovoljavajuće glikoregulacije koja predhodi akutnim i hroničnim komplikacijama dijabetesa.

Za hronične komplikacije dijabetesa nema efikasne terapije. Lečenje se svodi na zaustavljanje progresije a ostalo je simptomatska terapija. Jako je važno rano prepoznavanje bolesti i dobra metabolička kontrola. Hronične komplikacije dijabetesa su takođe i vodeći uzrok umiranja. Prema Balkau B et al. mortalitet u obolelih od dijabetesa je dva puta veći u odnosu na nedijabetičare (24). Takođe, utvrđeno je da mortalitet usled kardiovaskularnih bolesti u opštoj populaciji opada a mortalitet od šećerne bolesti raste (25). U prikazanim podacima nije postojala statistički značajna razlika u visini glikoziliranog hemoglobina kod pacijenta sa i bez hroničnih komplikacija. Prisustvo hroničnih komplikacija se nije razlikovalo u pacijenata lečenih insulinom od onih lečenih oralnim antihiperglikemicima. Statistički ne postoji značajna razlika između prisustva hroničnih komplikacija u pacijenata lečenih insulinskom terapijom u odnosu na pacijente lečene oralnim antihiperglikemicima i onih koji su predhodno bili bez terapije. Ovi podaci imaju ograničenje jer su svi pacijenti predhodno imali visoke vrednosi HbA1c i ne postoji referentna kontrolna grupa.

Zaključak

I pored edukacije i potpune dostupnosti lekova za šećernu bolest, dijabetesna ketoacidoza i hiperosmolarna koma se javljaju, pretežno kod starijih osoba sa brojnim komorbiditetima.

Izuzetno je važan oprez kod obolelih od dijabetes melitusa u stanjima infekcije, koja nesumljivo predstavlja najznačajniji precipitirajući faktor za akutne komplikacije osnovne bolesti. Naročito je važan oprez kod starijih pacijenata koji imaju brojne komorbiditete i kod kojih se akutne komplikacije često završavaju letalnim ishodom. Neophodno je obratiti posebnu pažnju na njih u stanjima infekcije, stresogenih situacija i dehidratacije.

Redovnim praćenjem pacijenata sa dijabetesom, kontrolom glikemije i HbA1c mogu se sprečiti akutne komplikacije ove bolesti. Unapređenje edukacije pacijenata intenzivnim zdravstveno-prosvetnim radom neophodan je korak ka poboljšanju stava pacijenta prema bolesti i novom načinu života koji ona zahteva.

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ANTIBIOTIC OINTMENT IN THE MANAGEMENT OF RECURRENT, COMMUNITY-ACQUIRED BACTERIAL CYSTITIS IN WOMEN – OBSERVATIONAL STUDY

UPOTREBA ANTIBIOTSKIH MASTI U LEČENJU PONOVNIH BAKTERIJSKIH CISTITISA U ŽENA – OPSERVACIONA STUDIJA

Aleksić Predrag[1], Bančević Vladimir[1], Aleksić Aleksandra[2], Bančević Maja[3]

Summary

Acute bacterial cystitis is the most common form of community – acquired urinary tract infections in women. Urinary tract infections are the second most common reason for antibiotic prescription in the USA. There is a need for alternative treatments that would help to avoid the use of systematic antimicrobials thus reducing the incidence of microbial resistance and side effects of the drugs. The aim of this study is to evaluate the role of antibiotic ointment treatment in recurrent UTIs in women. We followed up 34 non – pregnant women with a previous history of recurrent UTIs who were instructed to apply antibiotic ointment Bacitracin/Neomycin, locally, on the external urethral meathus area, once a day, for 30 days. 88% of patients responded to the treatment, with no further recurrences in 67.6% of cases and there was a reduced (< 3 cases per year) recurrence in 20.5% of patients. Bacitracin/Neomycin ointment could be a safe, cheap and successful tool for recurrent UTIs in women. To our best knowledge, this is the first published pilot – study of topical antibiotic ointment usage in management and prevention of recurrent urinary tract infections. We need a double blinded, randomized study with a larger number of patients in order to collect more evidence to support this conclusion.

Key words: *urinary tract infections; antibiotic ointment.*

Sažetak

Akutno bakterisjki cistitis je najčešće zapaljenje urinarnog trakta u žena. Urinarne infekije su drugi najčešći uzrok propisivanja antibiotika u SAD. Upravo zbog toga, kao i rezistencije bakterija na sistemsku antibiotsku terapiju ali i neželjenih efekata terapije, danas se sve više ispituju i alternativni načini lečenja urinarnih infekcija. Cilj ovog rada je da ispita potencijanu ulogu antibiotskih masti u lečenju rekurentnih infekcija urinarnog trakta u žena. Pratili smo 34 žene, koje nisu bile gravidne, sa prethodnom istorijom rekurentnih urinarnih infekcija i koje su bile instruisane da aplikuju antibiotsku mast Bacitracin/Neomicin lokalno, na spoljašnji otvor mokraćnog kanala, jednom dnevno, u trajanju od 30 dana. U ispitivanoj seriji 88% pacijentkinja je imalo benefit od ove terapije, bez rekurencije infekcije u 67.7% slučajeva, dok se kod 20.5% pacijentkinja broj rekurencija urinarne infekcije smanjio na manje od tri za godinu dana. Na osnovu ovih rezultata, lokalna terapija Bacitracin/Neomicin antibiotskom mašću može biti jedan od potencijalnih jeftinih terapijskih izbora u lečenju rekurentnih urinarnih infekcija, bez značajnih neželjenih efekata. Po nšem saznanju, ovo je prva pilot studija u kojoj se ispituje potencijalna mogućnost lečenja i prevencije rekurentnih urinarnih infekcija lokalnom antibiotskim terapijom. Svakako, dalja randomizirana, duplo slepa ispitivanja, na većem broju ispitanica su neophodna za viši stepen pouzdanosti ovakve tvrdnje.

Ključne reči*: infekcije urinarnog trakta; antibiotske masti*

Introduction

Acute bacterial cystitis is the most common form of community – acquired urinary tract infections (UTIs) in women (1). Frequent recurrent UTIs might affect 10 – 20% of women after the initial UTI episode (2,3). Although uncomplicated recurrent UTIs are considered to be a benign condition, they can have a significant impact on the quality of life and healthcare costs (2). Recurrent UTIs with more than 3 episodes per year generally require antibiotic prophylaxis (3). There is a need for alternative treatments that would help to avoid the use of systematic antimicrobials thus reducing the incidence of microbial resistance and side effects of the drugs (4,5).

Methods

34 nonpregnant women (>= 18 years old) with documented community – acquired recurrent cystitis (at least 3 or more episodes per year) were successively recruited for the study at our outpatient service. The study was approved by the local institutional review board and informed consent was obtained from the patients. History and medical files were used to confirm the frequency of recurrent cystitis over the preceding 12 months, the absence of underlying urological abnormalities, and mode treatment. Acute bacterial cystitis was confirmed by

Table 1. - Patients data and treatment outcome

Patient code	Age (years)	Disease duration (years)	No of episodes (over the preceding 12 months)	Isolated pathogen	Follow up (months)	No of recurrences during follow up
1	24	2	6	E.coli	24	0
2	56	8	5	E.coli	24	1
3	20	2	7	Enterococcus	23	0
4	62	6	4	Pseudomonas	Lost to F/U	NA
5	19	2	7	E.coli	23	0
6	58	5	6	Proteus	23	0
7	34	10	3	Enterococcus	22	1
8	28	7	6	E.coli	22	1
9	23	4	5	E.coli	22	0
10	42	10	6	E.coli	21	0
11	52	6	5	Klebsiella	Lost to F/U	NA
12	22	3	6	Enterococcus	20	2
13	28	7	5	E.coli	20	0
14	55	5	6	E.coli	19	0
15	63	10	6	Proteus	18	0
16	22	4	5	Enterococcus	17	0
17	43	3	4	E.coli	16	0
18	25	7	6	E.coli	16	0
19	20	3	7	Proteus	15	0
20	18	1	7	E.coli	15	1
21	26	4	3	E.coli	14	0
22	24	2	5	E.coli	14	0
23	50	4	3	Proteus	13	1
24	51	2	4	Enterococcs	13	0
25	29	2	6	E.coli	Lost to F/U	NA
26	24	3	4	E.coli	12	0
27	30	5	5	Klebsiella	12	3
28	29	3	5	Enterococcus	11	1
29	55	4	4	E.coli	11	0
30	21	2	4	E.coli	10	0
31	25	2	3	E.coli	10	0
32	32	3	5	Enterococcs	10	0
33	22	3	4	E.coli	9	0
34	26	4	6	E.coli	9	2

urinalysis and positive culture defined as the isolation of uropathogen at 10 ^5 CFU/ml. Gynaecological examination, ultrasonography of the urinary tract and post-voided residual urine measurement were done to exclude the presence of intrauterine devices and obstructions, respectively. Acute cystitis was first treated with antimicrobials according to EAU guidelines. Thereafter, the women were instructed to liberally apply antibiotic ointment, 1 g of which contains 62.5 IU of Bacithracin and 825 IU of Neomycin (B/N ointment), over their external genitalia around external urethral orifice and perineum, once a day, for 30 days. The patients were seen on a monthly basis or any time in case of infection recurrence.

Results

At the end of the follow up period (range 24 to 9 months), 30 patients were evaluable. If women lost to follow up and those having 3 or more recurrences per year are considered to be treatment failures, then 30 (88%) patients responded to the treatment, with no further recurrences in 23 (67.6%) cases and a reduced number (< 3 per year) of recurrences in 7 (20.5%) patients. Side effects were not observed and the patients were highly satisfied with this treatment modality. It should be noted that prophylactic application of B/N ointment was effective in both pre- and postmenopausal women. (Summarized in Table 1.)

Discussion

In most cases the infection is caused by the patient's own intestinal bacteria which, having colonized the periurethral area, invade the bladder mucosa via the urethra (6). One possible way to prevent re – infection, might be to block the spread of microorganisms from the anal area to the external genitals in females. We thus tested the effect of a widely available antimicrobial ointment applied over the external genital area on the recurrence rate of community – acquired recurrent cystitis in women.

The described management of frequent recurrent community – acquired female cystitis may be worth considering, having in mind its safety and convenience. As most young women are concerned about the effect of antibiotics on their possible pregnancy and are unwilling to take drugs over a long period of time, due to recurrent UTIs, this method might offer high compliance and reasonable efficacy. Despite the obvious weakness of an uncontrolled, blinded and randomized observational study, the results obtained are very encouraging, approaching the efficacy rates of most antibiotic prophylaxis regimens to date (6,7,8). To test the possibility of the ointment exerting its effect simply as a physical barrier to the spread of bacteria, a randomized study should be designed to compare the effect of antibiotic ointment and its vehicle.

Conclusion

Bacitracin/Neomycin ointment could be a safe, cheap and successful tool for recurrent UTIs in women. To the best of our knowledge, this is the first published pilot – study with topical antibiotic ointment usage in management and prevention of recurrent urinary tract infections in women. We need a double blinded, randomised study with a larger number of patients for gathering more evidence for this conclusion.

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DIAGNOSTIC CAPABILITY OF PULSAR PERIMETRY IN EARLY PRIMARY OPEN ANGLE GLAUCOMA

ZNAČAJ PULSAR PERIMETRIJE U DIJAGNOZI RANOG GLAUKOMA

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Summary

Aim: to assess the ability of Pulsar perimetry in detecting an early glaucomatous visual field damage in comparison with Optic coherent tomography (OCT), in early glaucoma.

Material: Cross – sectional case study included 1 eye from 55 consecutive subjects containing: 27 healthy subjects and 28 patients with early Primary open angle glaucoma (POAG). Only 1 eye per subject was randomly selected, if both eyes met the inclusion criteria.

Methods: All the patients underwent OCT and visual field examination in addition to full

ophthalmic examinations. Receiver operating characteristic curves (ROC) were studied for all parameters. The sensitivity and specificity of the differences between normal and early glaucomatous eyes, the areas under the receiver operating characteristic curves (AROC) and positive, negative likelihood ratios were evaluated for all the single parameters and selected combined parameters.

Results: Individuals with early POAG had significantly higher values of FLV at GCC, MD and sLV at Pulsar perimetry compared to healthy individuals. There were 1.84% of cases with early glaucoma FLV, whereas there were 0.5% of cases with healthy eyes. Considering Pulsar perimetry, patients with early glaucoma had higher values of MD (0,23dB) and sLV (1,97dB), compared to MD (0,23dB) and sLV (1,62dB) in healthy individuals ($p<0.05$). The highest values of AROC had the following parametres: FLV AROC (0,648), MD AROC (0,691), sLV AROC (0,696).

According to AROC we combined the best three single parameters, FLV at GCC, MD and sLV at Pulsar perimetry, using a logistical diagnostic model. The largest AROC obtained was at the value of 0.790.

Conclusion: In conclusion, parameters obtained by OCT (FLV at GCC), and Pulsar perimetry (MD and sLV) were able to show the significant differences between early POAG patients and normal subjects. Combining parameters of FLV at GCC, MD and sLV at Pulsar perimetry showed higher diagnostic capability with those top three single parameters.

Key words: *Pulsar perimetry, early glaucoma, ganglion cell complex.*

Sažetak

Poređenje dijagnostičkog značaja pulsar perimetrije u odnosu na stanjenje sloja nervnih vlakana retine oko optičkog diska (RNFL), stanjenje ganglijskog kompleksa makule (GCC) i promene parametara papile (ONH) merenih optičkom koherentnom tomografijom (OCT) kod početnog glaukoma.

Studijom preseka obuhvaćeno je 55 ispitanika, pregledanih u Centru za vid „Oftalmika“, od 15.01 do 1.06. 2014 god. Ukupno 28 ispitanika imalo je postavljenu dijagnozu ranog glaukoma otvorenog ugla (MD 6 dB), a preostalih 27 ispitanika bilo je bez dijagnoze glaukoma. Za svakog ispitanika, nasumičnim odabirom izabrano je samo jedno oko.

Svim ispitanicima izvršen je kompletan oftalmološki pregled u midrijazi, pulsar perimetrija i OCT pregled RNFL-a, GCC-a i ONH. Za sve parametre izračunate su mere dijagnostičke valjanosti: senzitivnost (Sn), specifičnost (Sp), verodostojnost pozitivnog (LR+) i negativnog (LR-) testa i površina ispod ROC krive (AROC).

Ispitanici sa ranim glaukomom se statistički značajno razlikuju od zdravih osoba po vrednostima fokalnog gubitka volumena u GCC-u (FLV%), indeksu prosečnog gubitka (MD) i indeksu varijanse gubitka (sLV) u pulsar perimetriji. Kod njih je signifikantno veći FLV (1,84%) nasuprot zdravim očima (0,5%), kao i signifikantno veća vrednost MD (0,23dB) i sLV (1,97dB), nasuprot vrednostima MD (0,23dB) i sLV (1,62dB) u zdravim očima ($p<0.05$). Kod ovih parametara i vrednosti AROC su bile najviše: FLV AROC (0,648), MD AROC (0,691), sLV AROC (0,696). Međusobnom kombinacijom navedenih strukturnih i funkcionalnih parametara dobija se viša AROC vrednost (0,790), odnosno bolja diskriminantnost u odnosu na njihovu samostalnu primenu.

Fokalni gubitak volumena u GCC-u, kao i vrednosti MD i sLV u pulsar perimetriji su veće kod ispitanika obolelih od glaukoma. Kombinacija ovih parametara ima veći dijagnostički značaj u odnosu na njihovu samostalnu primenu. Stoga se Pulsar perimetrija u kombinaciji sa OCT pregledom može se smatrati senzitivnim parametrom u dijagnostici ranog glaukoma.

Ključne reči: *Pulsar perimetrija, rani glaukom, ganglijski kompleks makule*

Uvod

Primarni glaukom otvorenog ugla je optička neuropatija koja nastaje kao posledica disfunkcije i/ili apoptoze ganglijskih ćelija retine. Prve strukturne promene nastaju u ganglijskom kompleksu makule (GCC) i sloju retinalnih nervnih vlakana (RNFL), a zatim dolazi do promena na papili vidnog živca (ONH). Ove promene obično nastaju pre funkcionalnih ispada u vidnom polju. Skorije kliničke studije pokazuju da kod početnog glaukoma neki pacijenti ipak imaju rane funkcionalne ispade, pre nego što detektujemo strukturna oštećenja (1). Novije randomizirane studije pokazuju da početne promene kod ranog glaukoma mogu značajno da variraju, tako da je neophodno uraditi i strukturna i funkcionalna ispitivanja prilikom postavljanja dijagnoze ranog glaukoma (2).

Nove imidžing tehnike kao što su Hajdelberg retina tomografija (HRT) i optička koherentna tomografija (OCT) omogućavaju kvantitativnu objektivnu analizu neuroretinalnog oboda (NRO), ganglijskog kompleksa makule (GCC), sloja retinalnih nervnih vlakana (RNFL) i parametara papile vidnog živca (ONH). OCT je dijagnostička metoda koja je već pokazala dobre rezultate u diskriminaciji zdravih očiju od onih sa ranim glaukomom (3).

Što se tiče funkcionalnih ispitivanja standardna automatizovana perimetrija (SAP) je i dalje klinički standard u dijagnozi i praćenju glaukoma (4). Međutim SAP ima ograničenu senzitivnost u detekciji ranih glaukomnih ispada. Novi nekonvencionalni perimetrijski testovi kao što su kratkotalasna automatizovana perimetrija (SWAP), tehnologija dvostrukih frekvencija (FDT) i Pulsar perimetrija, razvijeni su u poslednjih 20 godina. Dizajnirani su da selektivno ispituju sub- populacije ganglijskih ćelija retine i njihove senzitivne puteve i detektuju rane funkcionalne ispade (5).

Cilj našeg ispitivanja bio je da uporedimo dijagnostički značaj Pulsar perimetrije i OCT- a, kod ranog glaukoma.

Materijal i metode

Studijom preseka obuhvaćeno je 55 ispitanika, pregledanih u Centru za vid „Oftalmika“, od 15.01 do 1.06. 2014 god. Dijagnozu ranog glaukoma otvorenog ugla imalo je 28 ispitanika, a preostalih 27 bilo je bez dijagnoze glaukoma. U ispitivanje je uključeno jedno oko po osobi, slučajnim odabirom. Svim ispitanicima izvršen je kompletan oftalmološki pregled u midrijazi, Pulsar perimetrija test P32 i OCT pregled ONH, RNFL-a (na 3.45 mm) i GCC-a. Kriterijumi za uključivanje u studiju: najbolja korigovana vidna oštrina veća ili jednaka 0.8, otvoren komorni ugao, odsustvo drugih očnih bolesti, zadovoljavajuća saradnja, dobar kavlitet snimaka na Pulsar perimetriji i OCT-u, kao i indeks prosečnog gubitka (MD) u vidnom polju manji od 6dB. Ispitanici su podeljeni u dve grupe. U grupi sa ranim glaukomom otvorenog ugla ispitanici su imali intraokularni pritisak (IOP) veći od 22 mmHg, normalan nalaz u SAP, bez udružene oftalmološke patologije. Grupu zdravih osoba činili su ispitanici sa visinom IOP ispod 21 mmHg, normalnim nalazom SAP, negativnom porodičnom anamnezom i odsustvom drugih očnih oboljenja.

Za Pulsar perimetriju koristili smo aparat Octopus 600, program P32. Pragovna senzitivnost je bazirana na TOP pragovnoj strategiji i izražena u novim mernim jedinicama koje mere prostornu rezoluciju kontrasta (src). OCT pregled izvršen je na Optovue aparatu, uključujući analizu GCC, RNFL i ONH. Kod Pulsar perimetrije testirali smo indekse vidnog polja: indeks prosečnog gubitka (MD) i indeks varijanse gubitka (sLV). Za OCT, analizirali smo sve dostupne parametre za GCC, RNFL I ONH.

Za sve parametre izračunate su mere dijagnostičke valjanosti testa: senzitivnost (Sn), specifičnost (Sp), verodostojnost pozitivnog (LR+) i negativnog (LR-) testa i AROC (površina ispod ROC – Receiver operating characteristic krive). Evaluirana je dijagnostička valjanost FD-OCT a i Pulsar perimetrije, u dijagnozi ranog glaukoma, komparacijom senzitivnosti i verodostojnosti testa za izabranu specifičnost (95% i 85%), AROC vrednost za pojedinačne parametre i njihovu međusobnu kombinaciju.

Rezultati

Raspodela prema polu pokazala je da je u grupi sa ranim glaukomom bilo statistički značajno više osoba muškog pola (61% muškaraca u grupi sa ranim glaukomom, u odnosu na 30% u grupi zdravih osoba, p=0.031). Što se tiče godina života, nije bilo statistički značajne razlike.

U grupi obolelih od glaucoma statistički signifikantno je veći volumen fokalnog gubitka (FLV%) u GCC-u (p< od 0.05). (Tabela 1.)

U pulsar perimetriji signifikantno su veći indeksi prosečnog gubitka (MD) i varijanse gubitka(sLV) (p< 0.05). (Tabela 2.)

Pojedinačni parametri kod kojih je bila najveća vrednost za površinu ispod ROC krive (AROC), za diskriminaciju glaukomatoznih očiju od zdravih, bili su: fokalni gubitak volumena (FLV) u ganglijskom kompleksu makule (GCC) kod OCT-a i indeks prosečnog gubitka (MD) i in-

Tabela 1. GCC kod glaukoma i zdravih

FLV%	Status	Broj	Srednja vrednost	St Dev	p
	Zdravi	27	0.5033	0.44356	<0.05
	Glaukom	28	1.8404	2.61663	

Tabela 2. Indeksi PULSAR perimetrije kod glaukoma					
	Status	Broj	Srednja vred-nost	St Dev	p
MD	Zdravi	27	0.233	1.4645	<0.05
	Glaukom	28	1.296	1.7451	
sLV	Zdravi	27	1.622	0.4594	<0.05
	Glaukom	28	1.975	0.4766	

Tabela 3. OCT/GCC: mere dijagnostičke valjanosti kod ranog glaukoma		
FLV%		P
Senzitivnost	60.71	
Specifičnost	74.05	
AROK	0.648	<0.05

Tabela 4. PULSAR: mere dijagnostičke valjanosti kod ranog gluakoma za MD indeks		
MD		P
Senzitivnost	60.71	
Specifičnost	74.07	
AROK	0.691	<0.05

Tabela 5. PULSAR:mere dijagnostičke valjanosti kod ranog glaukoma za sLV indeks		
sLV		P
Senzitivnost	75.0	
Specifičnost	62.96	
AROK	0.696	<0.05

deks varijanse gubitka (sLV) u Pulsar perimetriji (p< od 0.05). (Tabele 3-5.)

Međusobnom kombinacijom navedenih strukturnih (FLV) i funkcionalnih parametara (MD i sLV) dobija se najviša AROC vrednost i statistički značajno visoka povezanost (p < od 0.001), odnosno bolja diskriminantnost u odnosu na njihovu samostalnu primenu. (Tabela 6.)

Tabela 6. Mere dijagnostičke valjanosti kombinacija OCT i PULSAR		
FLV%, MD, sLV		P
Senzitivnost	82.14	
Specifičnost	70.37	
AROK	0.790	<0.001

Diskusija

Prevalenca glaukoma je u populaciji starijoj od 50 godina 3%, a kod starijih od 70 godina 5%. Najveći broj lečenih bolesnika je u starosnoj grupi od 65 do 75 godina (6). Prema našim rezultatima statistički značajno je bio veći broj muškaraca u grupi sa ranim glaukomom, mada druge studije pokazuju da približno isto obolevaju pripadnici oba pola (7).

Fokalni gubitak volumena u GCC-u, kao i vrednosti MD i sLV u pulsar perimetriji su veće kod ispitanika obolelih od glaukoma.

U grupi obolelih od glaucoma dobili smo statistički signifikantno veći volumen fokalnog gubitka (FLV%) u GCC-u. Optička koherentna tomografija je ranije kod glaucoma uglavnom korišćena za merenje debljine sloja retinalnih nervnih vlakana oko papile (RNFL). Primena nove tehnologije Fourier domain OCT, zahvaljujući velikoj brzini i visokoj rezoluciji pruža nove mogućnosti u otkrivanju ranih strukturnih oštećenja.

Sa pojavom aparata nove generacije postalo je moguće izdvojiti ganglijski kompleks makule, izmeriti njegovu debljinu i analizirati ga odvojeno od ostalih slojeva retine.

Ganglijske ćelije na zadnjem polu, čine oko 35 % debljine retine s tim da je njihova najveća gustina na 7,2 stepena oko foveje, što odgovara površini od centralnih 9 stepeni vidnog polja. Prilikom pregleda vidnog polja standardnom perimetrijom (SAP), samo 1/10 test tačaka ispituje ovu lokaciju. Ganglijske ćelije prve stradaju kod glaukoma i potrebno je da bude uništeno najmanje 40% ovih ćelija, da bi se pojavili prvi defekti u vidnom polju, kada je u pitanju SAP. Fokalni gubitak volumena (FLV) u GCC u je jedan od značajnih parametara strukturnog očtećenja, u dijagnozi ranog glaukoma. Skorašnja studija Tana i autora sa Kalifornijskog univerziteta, je to pokazala (8).

Kada smo analizirali parametre pulsar perimetrije, signifikantno su bili veći indeksi prosečnog gubitka (MD) i varijanse gubitka(sLV), u grupi ispitanika sa ranim glaukomom. Pulsar perimetrija razvijena je pre 20 godina od strane Gonzalesa i saradnika (9). Ova novija perimetrijska metoda ispituje senzitivne puteve magnocelularnih ćelija ganglijskog kompleksa retine. Ove ćelije ćine 10% retinalnih ganglijskih ćelija i deo su magnocelularnog senzitivnog puta koji ima veliku brzinu sprovođenja i osetljiv je na stimuluse malog kontrasta (10). Razvijena je je 2000g (8). Test P32koristi cirkularnu sinusoidnu rešetkastu sliku koja ispituje 66 tačaka u centralnih 30 stepeni vidnog polja. Trepćući stimulus je veličine 5 stepeni, ima centrifugalno kretanje (kao talas koji se širi kada kamenčić ubacite u vodu) u dve alternirajuće faze, sa promenom kontrasta koji se smanjuje od centra ka periferiji. Pulsar perimetrija između ostalog testira funkciju kontrastne senzitivnosti, kombinuje promenu kontrasta stimulu-

sa (svetlo-tamno), sa promenom stimulusa u prostoru i ograničenom vremenskom intervalu. Dosadašnji rezultati dali su pozitivne rezultate u ranoj detekciji glaukomatoznog oštećenja (11). Kombinacija ovih parametara ima veći dijagnostički značaj u odnosu na njihovu samostalnu primenu. Izračunavanjem mera dijagnostičke valjanosti, pojedinačni parametri kod kojih je bila najveća vrednost za površinu ispod ROC krive (AROC), za diskriminaciju glaukomatoznih očiju od zdravih, bili su: fokalni gubitak volumena (FLV) u ganglijskom kompleksu makule (GCC) kod OCT- a i indeks prosečnog gubitka (MD) i indeks varijanse gubitka (sLV) u Pulsar perimetriji. Međusobnom kombinacijom navedenih strukturnih (FLV) i funkcionalnih parametara (MD i sLV) dobija se najviša AROC vrednost, odnosno bolja diskriminantnost u odnosu na njihovu samostalnu primenu.

Početne promene kod ranog glaukoma mogu značajno da variraju. Obično se prvo javljaju rana strukturna oštećenja u ganglijskom kompleksu makule i sloju retinalnih nervnih vlakana (12). Međutim, u nekim slučajevima funkcionalni ispadi prethode strukturnim promenama. Zahvaljujući novim perimetrijskim metodama, moguće je ispitivati funkciju sub-populacija ganglijskih ćelija retine i njihovih senzitivnih puteva. Ovo nam omogućava da kod nekih pacijenata detektujemo rane funkcionalne ispade, koji nastaju pre nego što dođe do strukturnih oštećenja (13). Dijagnoza ranog glaukoma je kompleksna i uvek je neophodno uraditi i strukturna i funkcionalna ispitivanja.

Zaključak

Na kraju možemo zaključiti da je Pulsar perimetrija u kombinaciji sa OCT pregledom senzitivna metoda za detekciju ranih ispada kod početnog glaukoma. Zajednička analiza funkcionalnih i strukturnih parametara ipak ima veći dijagnostički značaj u dijagnozi ranog glaukoma, nego njihova pojedinačna primena.

Lista skraćenica

SAP – Standardna automatizovana perimetrija
OCT – Optička koherentna tomografija
GCC – Ganglijski kompleks makule
ROC – Receiver operating characteristic kriva
AROK – Površina ispod ROC krive
FLV – Fokalni gubitak volumena u GCC- u
MD – Indeks prosečnog gubitka u vidnom polju
sLV – Indeks varijanse gubitka u vidnom polju
RNFL – Sloj nervnih vlakana retine
ONH – Papila vidnog živca

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THE ASSESSMENT OF RISK FACTORS FOR RETINOPATHY OF PREMATURITY

PROCENA RIZIKO FAKTORA KOD RETINOPATIJE PREMATURITETA

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Summary

Retinopathy of prematurity (ROP) is a potentially blinding eye disorder that primarily affects premature infants. Our study was conducted in order to determine which risk factors lead to the development of retinopathy of prematurity.

This retrospective study included 108 newborns with birth weight (BW) < 1500 g and gestation age (GA) < 33 weeks, over the period of two years, who were treated at the Clinic of Pediatric, University Hospital, Clinic Centre Banja Luka. In all preterm children, the impact of risk factors conditioned preterm birth (gestational age and birth weight), parameters of general health status (respiratory distress syndrome, apnea, perinatal asphyxia, frequent use of blood derivatives, sepsis, hyperbilirubinemia) and parameters of the treatment with oxygen therapy.

Out of 108 infants who fit the screening criteria, ROP was detected in 64 (59.2%) infants, 21(19.4%) of which had severe ROP requiring surgical intervention. Severe ROP was expressed in only 7.8% (5/64) of infants with GA > 30 weeks and in 12.5% (8/64) of infants with BW > 1250 g, compared to 25% (16/64) of infants with GA < 30 weeks and 20.3% (13/64) of infants with BW < 1250 g. The incidence of severe ROP was statistically significantly more frequent with progressively smaller birth weight BW < 1250 g (p <0.01) and the lower GA (gestational age) < 30 weeks (p <0.01). Using multiple logistic regression analysis for ROP, a long-term oxygen therapy (OR,15:54CI, 1.99-120.79) and a long duration of mechanical ventilation (OR,9.97; CI,3.06-32.51), there were obtained factors with a strong connection to the development of severe ROP. The following factors have a slightly lower correlation to the development of severe ROP: birth weight < 1250 g, gestation age < 30 weeks, respiratory distress syndrome, apnea, frequent use of blood derivatives and early sepsis.

Prematurity and low birth weight are significant risk factors for the development of ROP. Compromised pulmonary function with long-term oxygen therapy and frequent use of blood derivatives are important factors in the development of severe ROP.

Keywords: *retinopathy of prematurity, risk factors, preterm children*

Sažetak

Retinopatija prematuriteta (ROP) je oboljenje oka koje može da vodi slepilu kod prevremeno rođene dece. Naša studija je sprovedena sa ciljem utvrđivanja koji faktori rizika dovode do razvoja retinopatije prematuriteta.

Retrospektivna studija obuhvata 108 novorođenčadi porođajne težine < 1500 g i gestacije starosti < 33 nedelje, lečenih tokom dvogodišnjeg perioda na Klinici za dječije bolesti, Univerzitetske bolnice Kliničkog centra Banja Luka. Kod sve prevremeno rođene dece je ispitivan uticaj faktora rizika uslovljenih prevremenim rođenjem (gestacijska starost i porođajna težina), parametrima opšteg zdravstvenog stanja (respiratorni distres sindrom, apnea, perinatalna asfiksija, politransfuzije, sepsa, hiperbilirubinemija) i parametrima lečenja sa oksigeno terapijom.

Od 108 novorođenčadi koji su imali kriterijume za praćenje, ROP je prisutan kod 64 (59.2%) novorođenčeta od kojih je 21 (19.4%) imalo teški ROP koji zahteva hiruršku intervenciju. Teški ROP se ispoljava kod samo 7.8% (5/64) novorođenčadi gestacijske starosti veće od 30 gestacijskih nedelje i kod 12.5% (8/64), PT > 1250 g u odnosu na 25% (16/64) koji su gestacijske starosti < 30 nedelja i 20.3% (13/64), PT < 1250 g. Učestalost pojave teškog ROP-a je statistički visoko značajna što je porođajna težina manja PT < 1250 g (p<0,01) i što je niža gestacija < 30 nedelja (p<0,01).

Koristeći multiplu logističku regresionu analizu dobili smo prediktivne faktore sa jakom povezanošću za pojavu teškog ROP-a, dugotrajnu oksigeno terapiju (OR, 15.54; CI, 1.99-120.79) i dugu primenu mehaničke ventilacije (OR, 9.97; CI, 3.06-32.51). Nešto nižu povezanost za razvoj teškog ROP-a imaju porođajna težina < 1250 g, gestacija starost < 30 nedelja, respiratorni distres sindrom, apnee, politransfuzije i rana sepsa.

Prematuritet i niska porođajna težina su veoma značajni faktori rizika za razvoj ROP-a., a kompromitovana plućna funkcija uz dugotrajnu oksigeno terapiju i politransfuzije su važni faktori u razvoju teškog ROP-a.

Ključne reči: *retinopatija prematuriteta, faktori rizika, prevremeno rođena deca*

Introduction

Retinopathy of prematurity (ROP) is a disease that affects the retinal blood vessels during their development and it manifests itself by cessation of normal blood vessel development and by the occurrence of proliferative retinopathy. It occurs in several development phases and retrolental fibroplasia represents a completely destroyed visual function which is manifested by blindness.

Numerous risk factors which obstruct normal development of vascularization of the retina are responsible for the occurrence of retinopathy of prematurity. The most significant among them are: short gestation, low birth weight, long – term use of supplemental oxygen and many other factors which are mutually combined and complemented.

By improving neonatal care, survival rates are higher in children born too small for their gestational age and in children with low birth weight which increases the incidence of retinopathy of prematurity and leads to the development of more severe forms of diseases.[1, 2] The prevention of retinopathy of prematurity is focused on the elimination of risk factors and ophthalmologic screening.[3] Detecting and recognizing the early stages of this disease and timely and adequate treatment mean developing good visual function.

On the other hand, studies show that complex and expensive surgical procedures in treating severe stages of ROP followed by ablation and fibrovascular organization of vitreous body achieve good anatomical results. However, they are not accompanied by improved visual function. [4]

Material and methods

Patients

A two – year retrospective study was conducted in the Department of Pathological Neonatology with Prematurity and in the Department of Intensive Care of the Paediatric Clinic of the University Hospital Clinical Centre Banja Luka. The study included infants born in the Maternity Home of the Clinic of Gynaecology and Obstetrics of the University Hospital Clinical Centre Banja Luka and infants referred from regional centres in the Republic of Srpska. We conducted an analysis of 108 preterm infants on the basis of criteria for ROP screening programmes. Infants with severe congenital malformations and chromosome disorders and infants that had died before ophthalmological examination were excluded from the study. Examinees were divided into three groups: infants without ROP; infants with ROP, but with no need of surgical intervention; infants with "severe" disease that had to be treated with laser surgery.

Methodology of ophthalmologic screening

Criteria of ophthalmologic screening for retinopathy of prematurity are accepted according to the protocols of the American Academy of Paediatrics.[5,6] The selection for screening included preterm infants born earlier than 33 weeks of gestational age and with birth weight below 1500 g, as well as infants that were at additional risk for ROP. Preterm infants at risk were selected for screening by paediatricians – neonatologists from the Department of Intensive Care. Maximum mydriasis was achieved by administration of Cyclopentolate 0.5%, eye drops and anaesthetic Novesine 0.4% was also instilled immediately prior to examination. The exam was made with the use of binocular indirect ophthalmoscope and 20 D magnifier. In order to examine peripheral areas of retina better, indentator was used [7]. During the screening process, the development of retinal blood vessels was monitored and preterm infants with ROP were selected.[8] By adhering to the criteria of the International Classification of Retinopathy of Prematurity– ICROP, data from each examination were entered into documentation on the localization of the endings of retinal blood vessels – (zone 1 – 3), expansion and stage 1 – 5.[9] Age when ROP was detected, maximum stage of ROP, localization and ROP treatment were determined.

Identification of risk factors

Demographic data that were analysed included: gestational age in weeks, birth weight and sex. Clinical data included perinatal asphyxia, respiratory distress syndrome (RDS), apnoea, use of surfactant, pneumonia, persistent ductus arteriosus, sepsis, intraventricular haemorrhage, hyperbilirubinemia that required phototherapy.

With regard to the treatment, the oxygen was used in the form of controlled higher concentration of inspired oxygen (diffusion via atmosphere of the incubator or "hood"), by non – invasive positive – pressure ventilation and/or conventional mechanical ventilation. The condition of retinal blood vessel network was analysed with respect to the duration of the use of oxygen therapy and the form of respiratory support. All preterm infants treated with oxygen were continuously monitored by the use of pulse oximetry technique. Percutaneous value of hemoglobin oxygen saturation in arterial blood (SaO2) was measured.

Using the SPSS Statistics 20.0 software package, a statistical analysis was made. Methods of descriptive and analytical statistics were used in the analysis. In univariable comparison of risk factors between groups without ROP, infants with ROP that were not in need of a surgical treatment and ROP that required surgical treatment, Student's t – test and Chi – square test with an adequate significance level of $p<0.05$ were used. Predictive factors for the development of ROP were estimated by using multivariable logistic regression. Odds ratio was calculated and the 95% confidence interval for each risk factor was estimated.

Table 1. Anthropometric characteristics of preterm infants

	Without ROP	With ROP that does not require surgery	Severe ROP
Gestational age, weeks	31.6±1.3	30.8±1.4	28.8±1.5
Birth weight, g	1772.7±253.9	1469.6±282.2	1154.3±224.9
Sex			
Male	30	21	9
Female	14	22	12
Total	44	43	21

Results

The paper presents results of the two – year retrospective study conducted with the aim of diagnosing retinopathy of prematurity and determining the risk factors that lead to the development of the disease.

Of the total number of infants (1186) treated in our departments, 9. 1 % of them (108) met the criteria to be monitored due to ROP. Sixty preterm infants were males and forty – eight were females. The incidence of severe ROP that required to be surgically treated was 21/108 (19.4%).

The first ophthalmologic screening examination was made in all infants starting from 31st week up to 37th week of gestation. The mean value was 34. 8±1.63 GW. ROP was present in 64 out of 108 (59.2%), out of which 21/108 (19.4%) had severe ROP that required to be surgically treated. No abnormalities were detected in 44 out of 108 (40. 7%).

The mean value of gestational age in all examined infants was 30.8±1.7 weeks (range 26 – 33), while the mean value of birth body weight was 1533.2±346.4 g (range 740 – 2370).

The mean value of birth body weight in infants with ROP that required to be surgically treated was 1154.3±224.9 g (range 740 – 1460) and gestational age was 28.8±1.5 weeks (range 26 – 31.4). Birth body weight in infants without ROP was 1772.7±597, 3g and gestational age was 31.6±1.3 weeks (Table 1).

Table 2 shows the characteristics of preterm infants without ROP and with ROP that do not require surgical intervention in comparison with infants with severe ROP that require surgical treatment, which was confirmed with ophthalmological screening examination. The incidence of severe ROP is significantly higher if birth weight is lower i.e. < 1250 g ($p<0.01$) and if gestation is shorter i.e. < 30 weeks ($p<0.01$). Prolonged oxygen therapy is highly statistically significant in infants that will develop severe ROP ($p=0.000$). Development of severe retinopathy of prematurity in comparison with the presence or absence of systemic diseases was also analysed and tested. The presence of a severe stage of ROP is statistically more frequent in a more severe stage of respiratory distress due to hyaline membrane disease, ($p<0.05$). The incidence of severe ROP is highly statistically significant if perinatal asphyxia, frequent apnoea, early sepsis are present (Table 2).

With the aim of demonstrating the significance of immaturity as risk factor, a multiple logistic regression model was designed. It includes all risk factors, i.e. gestational age, birth weight and various diseases such as respiratory distress syndrome, presence of apnoea, perinatal asphyxia, sepsis and hyperbilirubinemia. When it comes to most of the examined risk factors, there is a strong connection between severe ROP that requires surgery and exposure to the risk factors in infants with lower birth weight (OR 9.25; CI 3.20-26.69), shorter gestation (OR 10.06; CI 3.29-30.76), presence of apnoea (OR 14.24; CI 4.30-47.19), diseases such as respiratory distress syndrome (OR 3.76; CI 1.26-11.17), perinatal asphyxia (OR 6.50; CI 2.15-19.64), early sepsis and poly – transfusions (Table 3).

There is an exceptionally strong connection between severe ROP that requires to be surgically treated and exposure to oxygen therapy, especially when received for more than 10 days (OR 15.54; CI 1.99-120.79), and long – term mechanical ventilation (OR 9.97; CI 3.06-32.51). Pneumonia, late – onset sepsis, intraventricular haemorrhage, hyperbilirubinemia and presence of persistent ductus arteriosus are not statistically significant for manifestation of severe ROP.

Discussion

In our study, higher ROP incidence is related to lower birth weight, shorter gestation, long – term oxygen therapy, mechanical ventilation, early sepsis, and blood poly – transfusions. Risk factors shown in literature vary because of the differences in methodology and in indications for the treatment of ROP [10].

During the two – year period of our activities, the general incidence of severe ROP was 1.8%. 21 out of 108 (19.4%) preterm infants had severe ROP requiring surgical intervention. Developing countries tend to demonstrate the presence of ROP requiring surgical intervention in

Table 2. Relationship between retinopathy of prematurity and risk factors			
	Without ROP and with ROP that does not require surgery	**Severe ROP**	**p**
Gestational age, weeks			
GA<30	21	16	<0.01
GA>30	66	5	
Birth weight			
BW<1250 g	13	13	<0.01
BW>1250 g	74	8	
Perinatal asphyxia			
Yes	9	9	0.001
No	78	12	
RDS			
Yes	40	16	0.013
No	47	5	
Apnoea			
Yes	20	17	0.000
No	67	4	
Early sepsis			
Yes	12	12	<0.01
No	75	9	
Hyperbilirubinemia			
Yes	71	17	>0.05
No	16	4	
Oxygen therapy			
Yes	79	21	0.000
No	8	0	
Mechanical ventilation			
Yes	26	17	<0.05
No	61	4	

RDS – respiratory distress syndrome

infants born too large for their gestational age and infants born with greater birth weight, while the incidence is very diverse.[10, 11] Studies conducted worldwide indicate the differences in ROP incidence: Mathew and associates [12] from Scotland 4.8%, Pishava and associates [13] from Iran 9.5%, Yang and associates [14] from China 25%, Karna and associates [15] from the USA 7.8%, Ahmed and associates [16] from Bangladesh 4.4%, Wani and associates [17] from Kuwait 7.8%. The study of Hussain and associates [18] indicates a significant reduction in incidence and severity of ROP due to the application of surfactant. General incidence of ROP in infants was 21.3%, and the incidence of severe ROP was 4.6%.

In our study, screening was averagely performed in 54 infants within one – year period. The average birth weight was 1533.2 g, the mean gestational age was 34.8±1.63 weeks. In Goble's study the average gestational age of in-

Table 3. Multiple logistic regression analysis of risk factors related to the occurrence of ROP

	OR	95% CI	p
Gestational age, < 30 weeks	10.06	3.29-30.76	0.000
Birth weight < 1250 g	9.25	3.20-26.69	0.000
RDS	3.76	1.26-11.17	0.013
Surfactant	6.50	2.15-19.64	0.000
Apnoea	14.24	4.30-47.19	0.000
Pneumonia	1.71	0.63-4.66	0.289
Perinatal asphyxia	6.50	2.15-19.64	0.000
Early sepsis	8.33	2.89-23.99	0.000
Late sepsis	0.35	0.09-1.29	0.104
IVH	2.73	0.60-12.49	0.180
Poly-transfusions	10.86	3.53-33.41	0.000
Hyperbilirubinemia	0.96	0.28-3.23	0.945
PDA	3.18	0.81-12.49	0.085
Mechanical ventilation	9.97	3.06-32.51	0.000
Long-term oxygen therapy >10 days	15.54	1.99-120.79	0.001

OR: Odds ratio; CI-95% confidence interval, RDS – respiratory distress syndrome, IVH – intraventricular haemorrhage PDA – persistent (patent) ductus arteriosus

fants who had undergone screening due to ROP was 29.1 weeks.[19]

In our study, shorter gestational age was established as a statistically highly significant risk factor ($p<0.01$) for the occurrence of ROP. The average gestational age among examinees was 30.8 ± 1.7 weeks and it ranged between 26 and 33 weeks. The incidence of ROP in infants with birth weight below 1250 g was 20.3% (13/64), whereas in infants with BW >1250 g, the incidence was 12.5%. All the studies stress preterm birth is a significant risk factor and that the incidence of ROP increases with immaturity. [20-23] Darlow and associates agree with such an interpretation and they state that children born before 25th gestational week are twenty times more likely to have severe form of ROP compared to children born after 28th gestational week.[24]

In our study, in the examined group of infants with the birth weight > 1460 g and gestational age > 31.4 weeks, there is no severe ROP, but there is a smaller number of infants with birth weight below 1000 g, which can be explained by a lower survival rate of children with low body weight, in our conditions. Similar results were obtained in other developing countries such as ours.[25-27]

As for the occurrence of ROP, Chen and associates have concluded that the exposure to oxygen is of greater importance for infants born at 23 – 25 weeks, while the infection is associated with ROP among infants born at 28 – 29 weeks.[19] In the study of Alpay and associates, the influence of numerous risk factors for development of ROP was explored. They emphasized apnoea, respiratory distress syndrome (RDS) and oxygen therapy as significant independent risk factors for the development of ROP.[28] In our study the presence of more severe respiratory distress due to hyaline membrane disease is significantly higher ($p<0.05$) in infants in whom ROP requires surgical treatment. Other author's studies also confirmed that respiratory distress syndrome affected the development of ROP.[21, 25, 29]

In our study, in infants who received long – term oxygen therapy, there was a significantly frequent occurrence of ROP that required to be surgically treated ($p<0.01$). Oxygen therapy was used for longer periods of time in children with low birth weight and shorter gestation and those children at the same time had more frequent occurrence of ROP requiring surgical treatment.

An optimal level of oxygen in the treatment of preterm infants is a constant quest. [22] It is being explored within a wide range of oxygen concentration. Control of oxygenation is achieved by elimination of the application of high oxygen concentrations. Lower concentrations will significantly reduce the incidence of severe ROP, as this was shown in the study of Wright and associates.[30] In most of the studies, the value of haemoglobin oxygen saturation

in preterm infants born before 32nd week ranged between 89% and 94%; in others between 85% and 95% or 83% and 93%. The application of controlled and limited saturation of haemoglobin with oxygen induced a decrease of the incidence of ROP requiring surgical intervention, [31, 32, 33] but there has been an increase in mortality. [34, 35]

In our study, the length of the application of mechanical ventilation was significantly more frequent ($p<0.05$) in the group of infants with ROP requiring surgical treatment than in infants who did not have ROP or who had ROP which did not require surgical treatment. Many authors emphasize that longer application of mechanical ventilation is associated with the development of severe forms of ROP.[21, 36-38] Finer and associates published the study in which they pointed out a lower incidence of severe ROP and chronic lung disease in children with very low birth weight during a restrictive application of mechanical ventilation by using non – invasive ventilation with positive pressure, in the nine – year period. [39] Mechanical ventilation is applied in preterm infants with severe forms of respiratory distress syndrome and they depend on a higher concentration of oxygen for achieving adequate saturation. During the application of mechanical ventilation, fluctuations of oxygenation are more frequent and a higher risk of hyperoxia is present. Key guidelines for reducing the incidence of ROP are avoiding hyperoxia early in the life of an infant, constant maintenance of a certain saturation of haemoglobin with oxygen and achieving a satisfactory growth rate.[40]

By using multiple logistic regression analysis, we found that a predictive factor with a strong relation to the occurrence of severe ROP was a longer exposure to oxygen therapy (OR, 15.54; CI, 1.99-120.79) and longer application of mechanical ventilation (OR, 9.97; CI, 3.06-32.51). The duration of oxygen therapy and higher oxygen concentration are directly related to the duration of mechanical ventilation. Immaturity and compromised lung function due to hyaline membrane disease are significant etiological factors for the development of ROP. Our results are consistent with the results which Shah and associates obtained.[21] Lower birth weight, shorter gestation, respiratory distress syndrome, the use of surfactant, presence of apnoic crisis, perinatal asphyxia, early sepsis and larger number of blood transfusions have a slightly lower correlation to the development of severe ROP.

Fortes Filho and associates [41] divided the children according to the gestation and monitored the impact of risk factors on the development of ROP. The conclusion was that those infants born before 32nd gestational week developed ROP due to the general immaturity and that infants born after 32nd week developed ROP because of being more ill. Preterm infants who developed ROP had some other severe associated diseases which may lead to disability, later in life.[42]

Numerous risk factors influence the development of ROP. Since this is a complex issue, it is necessary to work constantly on the exchange of experiences between different institutions and to acquire new information in order to improve prevention and to improve the disease outcome.

Conclusion

By analysing the risk factors which affect the development of severe ROP in high – risk preterm infants, we have affirmed that significant predictive factors are immaturity, lower birth weight and shorter gestation with longer application of supplemental oxygen. Also, significant predictive risk factors include: the application of a larger number of blood transfusions, perinatal asphyxia, apnoea and early sepsis. Compromised pulmonary function due to respiratory distress syndrome which requires the application of a surfactant and a longer application of mechanical ventilation is associated and related with the development of severe ROP. Prevention of preterm birth, reasonable application of oxygen therapy and mechanical ventilation are necessary for reducing the incidence of retinopathy of prematurity. All of the above – mentioned can reduce the incidence and severity of ROP.

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PHARMACOGENETICS IN CLINICAL PRACTICE: CHALLENGES AND OPPORTUNITIES

FARMAKOGENETIKA U KLINIČKOJ PRAKSI: IZAZOVI I MOGUĆNOSTI

Gordana Dragović[1], Nada Dolić[2], Božana Dimitrijević[2], Milan Jovanović[3], Marko Barović[2], Đorđe Jevtović[4]

Summary

Pharmacogenetics studies the influence of genetic variation on drug response, while pharmacogenomics is a genome – wide and multifactorial extension of the term pharmacogenetics. Both pharmacogenetics and pharmacogenomics are the backbones of the concept called personalized medicine. Although a "personalized" approach has always been a goal of good medical practice, there is a new aspect of the extensive use of molecular data to tailor drug therapy to an individual patient, in order to maximize therapeutic benefit and minimize adverse events.

The main causes of inter – individual variability in a patient are genetic variations in genes responsible for enzymes synthesis, which participate in absorption, distribution, metabolism and excretion (ADME system) of drugs. So, all patients can be classified in four groups, according to their genotype: slow metabolizers, intermediate metabolizers, ultrafast metabolizers and extensive metabolizers who are most frequent and are considered to be a standard group of patients. The group of most essential metabolism enzymes is represented by a superfamily of cytochrome P450 (CYP2D6, CYP2C9, CYP2C19, etc.). Enzymes from this family contribute to inter – individual differences in drug concentration in the plasma. The effects of polymorphisms in the CYP P450, range from an ineffective drug therapy to severe toxicity it can cause (e.g. drugs with a narrow therapeutic range (e.g., Warfarin) in patients with a reduced activity of CYP P450).

Pharmacogenetic testing is carried out for a number of reasons: for selecting patients most likely to achieve therapeutic efficacy, in order to reduce side effects and determine the most appropriate dose, in order to achieve efficiency and a higher degree of therapy safety. The advantage of pharmacogenetic testing is reflected in the fact that it can be applied prior to drug application. Pharmacogenetics should satisfy patients' need for an effective therapy without side effects, physicians' need for assistance in selecting an adequate remedy and dosage for a particular patient, as well as other health care professionals, government agencies and pharmacists' needs.

Key words: *pharmacogenetics, genetic polymorphisms, CYP 450, personalized medicine.*

Sažetak

Farmakogenetika proučava uticaj genetskih polimorfizama na terapijski odgovor određenog leka, dok farmakogenomika pored uticaja gena ispituje i ostale multifaktorijalne uticaje interindividualnih, tj. intraindividualnih odgovora na propisanu standardnu terapiju. Individualni pristup, odnosno personalizovana terapija predstavlja osnovni cilj farmakogenetike, u kome je od ključne važnosti postići maksimalni terapijski uspeh, uz istovremeno postizanje minimalnog broja pratećih neželjenih efekata propisane terapije.

Osnovni uzrok interindividualne varijabilnosti pacijenata su genetičke varijacije (pojedinačni nukleotidni polimorfizmi) u genima zaduženim za sintezu enzima koji učestvuju u procesu apsorpcije, distribucije, metabolizma i eliminacije lekova. Svi pacijenti se mogu podeliti na osnovu svog genotipa na 4 klase: spori metabolizeri, intermedijarni metabolizeri, ultrabrzi metabolizeri i ekstenzivni metabolizeri koji su i najčešće zastupljeni i smatraju se standardnom grupom pacijenata. Najbitniju grupu ovih enzima predstavlja superfamilija citohroma P450 (CYP2D6, CYP2C9, CYP2C19, itd). Enzimi iz ove familije doprinose interindividualnim razlikama u koncentraciji lekova u plazmi. Tako se posledice polimorfizama CYP P450 kreću se od neefikasne medikamentozne terapije do ozbiljnih toksičnosti koje ona može prouzrokovati. Jedan takav primer predstavljaju lekovi uske terapijske širine (npr. varfarin, opšti anestetici, citostatitici, itd.) kod pacijenata sa smanjenom aktivnošću CYP P450.

Kada je reč o kliničkoj primeni, od farmakogenetike svi mnogo očekuju: pacijenti koji zahtevaju efikasnu terapiju bez neželjenih efekata, lekari kojima je potrebna pomoć u izboru adekvatnog leka i doze za određenog pacijenta, zdravstveni radnici koji moraju da pronađu način da unaprede kvalitet zdravstvene zaštite uz istovremeno smanjenje troškova, vladine agencije kojima su potrebna pouzdana ispitivanja prilikom izdavanja terapijskih vodiča i zakona, kao i farmaceutskoj industriji koja nastoji da skupe supstance, koje pretenduju da postanu lek, ne izgube usled nepredviđene toksičnosti u kasnim fazama razvoja leka.

Ključne reči: *farmakogenetika, genetski polimorfizmi, CYP P450, individualna terapija.*

Uvod

Farmakogenetika je disciplina koja istražuje uticaj genetskih polimorfizama na terapijski odgovor, odnosno na efikasnost i bezbednost leka. Cilj farmakogenetike je personalizovana terapija, u okviru koje bi se mogao prepisati najefikasniji lek u adekvatnoj dozi za pojedinačnog pacijenta. Iako je „personalizovani" pristup lečenju oduvek bio znak dobre medicinske prakse, detaljno korišćenje molekularnih podataka prilikom „krojenja" terapije po meri određenog pacijenta, u cilju poboljšanja terapeutskog ishoda i smanjenja neželjenih dejstava, pruža sasvim novi aspekt lečenja.

Farmakogenetika se napaja sa dva glavna istraživačka izvora, osnovnog koji nastoji da otkrije genetske polimorfizme i razume biološke genotipsko-fenotipske odnose, i klinički orijentisanog izvora koji se nadovezuje na osnovno znanje u istraživanju odnosa genetskih faktora i fenotipova terapijskog odgovora, i primene novih dijagnostičkih procedura u njegovom prenošenju u kliničku praksu.

Farmakogenetika u kliničkoj praksi

Svakodnevna praksa pokazuje da nemaju svi pacijenti isti farmakološki odgovor na primenjenu standardnu terapiju. Neki pacijenti imaju zanemarljivu korist od terapije, dok kod drugih ista terapijska doza dovodi do pojave neželjenih i toksičnih efekata [1, 2].

Farmakogenetika/farmakogenomika proučava ulogu genetskih faktora u dispoziciji i odgovoru na lek. Pored toga, faktori okruženja (navike u ishrani, pušenje, istovremena primena drugih lekova, izloženost toksičnim materijama), fiziološke razlike (uzrast, pol, pridružene bolesti, trudnoća) i komplijansa pacijenata, zajedno doprinose interindividualnim varijacijama u metabolizmu leka i posledičnom terapijskom odgovoru.

Terapeutski monitoring leka (*Therapeutic Drug Monitoring*; TDM), kao najranija personalizovana terapijska procedura kod primene određenog leka, se sprovodi tek nakon davanja leka, dok se farmakogenetička testiranja mogu sprovesti i pre nego što se započne s terapijom. Farmakogenetičko testiranje je univerzalno za mnoge lekove i dovoljno je sprovesti ga samo jednom. Na osnovu činjenice da nije uvek moguće razdvojiti faktore životne sredine od genetskih uticaja kao uzroke varijabilnosti u odgovoru na lek, kombinovanje klasičnog TDM (fenotipska strategija) i genotipizacije metaboličkih svojstava leka, trenutno se smatra najsofisticiranijim načinom individualizacije doziranja pojedinih lekova [3-6].

Farmakogenetička istraživanja

Jedan od mogućih uzročnika interindividualne varijabilnosti su genetičke varijacije. Od posebnog značaja su pojedinačni nukleotidni polimorfizmi (PNP) gena koji kodiraju sintezu enzima zaduženih za metabolizam i transport lekova [7].

Najveći stepen PNP-a nađen je u genima uključenim u metabolizam lekova; odnosno metabolizam približno 40% lekova koji se nalaze u svakodnevnoj kliničkoj praksi se odvija pod dejstvom enzima čiju sintezu kodiraju geni sa PNP-om [8]. Trenutno su najbitniji ovakvi enzimi superfamilije citohrom P450 (kao što su CYP2D6, CYP2C9, CYP2C19, CYP2C8, CYP3A4, CYP3A5), tiopurin metil transferaza (TPMT), uridin difosfat glukuronosil transferaza (naročito UGT1A1, UGT1A4, UGT2B7), N-acetil transferaza 2 (NAT2), dihidro pirimidin dehidrogenaza (DPD) i organski katjonski transporter 1 (OCT1) [5, 9-12].

Međutim, danas se zna da i geni koji nemaju veze sa farmakokinetičkim svojstvima lekova, utiču na terapijski učinak ili bezbednost lekova. Takvi geni mogu direktno da utiču na receptore, odnosno sisteme transdukcije lekova, ili mogu biti samo indirektno povezani sa farmakodinamičkim mehanizmima dejstva leka. Na primer, gen ADRB2 koji kodira beta-2 adrenergički receptor i njegova dva nesinonimna pojedinačna nukleotidna polimorfizma (Arg16Gly i Glu27Gln) intenzivno se proučavaju da bi se jasno odredila njihova uloga u terapiji teških oblika astme [13, 14].

CITOHROM P450

Subpopulacione podele pacijenata na osnovu genotipa/lokusa

Na osnovu svog genotipa/lokusa, svi pacijenti mogu se podeliti u četiri klase: spori metabolizeri (SM), intermedijarni metabolizeri (IM), ekstenzivni metabolizeri (EM) i ultrabrzi metabolizeri (UM). Ekstenzivni metabolizeri su najčešće zastupljeni i smatraju se standardnom grupom pacijenata. Opis ove klasifikacije zajedno sa posledicama dejstva prilikom postojanja razlike u parovima gena na efikasnost, odnosno toksičnost lekova prikazan je u tabeli 1. [3].

Klinički značaj polimorfizma CYP P450

Opšte je prihvaćeno da varijacije u genima koji kodiraju enzime iz familije citohrom P450 (P450) doprinose interindividualnim razlikama u koncetraciji lekova u plazmi, što dovodi do varijabilnosti u efikasnosti i bezbednosti primenjenog leka kod različitih pacijenata.

Moguće posledice polimorfizma CYP P450 kreću se u rasponu od ozbiljne toksičnosti do neefikasne medikamentozne terapije. Genetski određeno smanjenje enzimske aktivnosti CYP P450 može imati važne implikacije kod lekova uske terapijske širine kao što su varfarin, kod koga povećane koncetracije u plazmi dovode do toksičnosti. U slučaju prolekova, kao što su kodein ili klopidogrel, slaba

Tabela 1. Farmakogenetička klasifikacija pacijenata na osnovu genotipa/lokusa [3]

Klasa	Par gena Raspored alela	Očekivani uticaj na aktivni lek	Očekivani uticaj na prolek
Spori metabolizeri (SM)	Obe pozicije u genu koje čine par imaju varijantu koja rezultira nefunkcionalnim ili odsutnim proteinom.	Smanjena efikasnost u konverziji aktivnog leka u neaktivni metabolit. Povećan rizik od viših koncentracijskih nivoa aktivnog leka i posledične kliničke toksičnosti.	Nesposobnost da se prolek konvertuje u aktivni metabolit. Ukoliko prolek nema terapijska svojstva, efikasnost neće biti zadovoljavajuća uprkos povećanim dozama.
Intermedijarni metabolizeri (IM)	Jedan član u genskom paru poseduje varijantu koja rezultira nefunkcionalnim ili odsutnim proteinom, a drugi poseduje varijantu koja rezultira proteinom smanjene funkcionalnosti.	Smanjena efikasnost u konverziji aktivnog leka u neaktivni, povećava rizik od povišenih nivoa aktivnog leka i kliničke toksičnosti. Započinjanjem primene leka u malim dozama, postepenim povećanjem, efikasnost se postiže ranije nego kod EM.	Smanjena efikasnost konverzije neaktivnog proleka u aktivni metabolit. Očekivano smanjenje efikasnosti prilikom standardnih doza održavanja.
Ekstenzivni metabolizeri (EM)	Svaki član para ima sekvencu konzistentnu s potpuno funkcionalnim proteinom.	Aktivni lek primenjen u standardnoj dozi metabolisan u neaktivne komponente, postiže efikasnost bez ili uz minimalne neželjene efekte.	Prolek konvertovan u aktivni metabolit postiže efikasnost bez ili uz minimalne neželjene efekte.
Ultrabrzi metabolizeri (UM)	Lokus nasleđen od jednog roditelja ima sekvencu konzistentnu sa funkcionalnim proteinom. Lokus nasleđen od drugog roditelja ima dve ili više sekvence koje rezultiraju funkcionalnim proteinom, ili jedan član genskog para ima sekvencu konzistentnu sa funkcionalnim proteinom, a drugi ima varijantu koja uzrokuje proizvodnju povećane količine funkcionalnog proteina.	Povećana efikasnost konverzije aktivnog leka u neaktivni metabolit i povišen rizik of smanjene efikasnosti pri standardnim dozama.	Povećana efikasnost konvertovanja proleka u aktivne metabolite, i s tim povezan povišen rizik od toksičnosti usled viših nivoa aktivnih metabolita od očekivanih.

enzimska aktivnost može da spreči postizanje terapeutskih koncentracija u plazmi i da dovede do terapijskog neuspeha. Međutim, dupliranje gena CYP2D6 može izazvati toksične reakcije na kodein, usled akumulacije aktivnih metabolita. Za lekove široke terapijske širine, kao što su selektivni inhibitori preuzimanja serotonina i beta-blokatori, kliničke implikacije varijacija gena CYP P450 imaju nešto manji klinički značaj. Tako na primer, nasledni nedostatak CYP2C9 može ostati nedetektovan u toku života pojedinca, osim ukoliko mu nije propisan lek uske terapijske širine, kao što je varfarin, koga metaboliše CYP2C9. Slično tome, pojedinac sa neaktivnim CYP2D6 može proći bez ijednog neželjenog efekta, osim ako ne uzima lek kao što je tioridazin, koji može proizvesti štetne posledice na srčani rad kod sporih metabolizera CYP2D6.

Genotipski testovi kojima se detektuju varijacije mnogih P450 gena danas su dostupni u komercijalne svrhe. Imajući u vidu raspon lekova čijeg metabolizma nema bez aktivnog učešća CYP P450, upotreba takvih testova mogla bi omogućiti pristup širokoj implementaciji individualizacije medikamentozne terapije. Do sada je funkcionalni polimorfizam otkriven za gene CYP2A6, CYP1A2, CYP2C9, CYP2C19, CYP2D6 i CYP3A4/5. Danas najmanje 25 lekova u svojim uputstvima, koje je odobrila Uprava za hranu i lekove (*US Food and Drug Administration*; FDA) sadrži

Tabela 2. Primeri lekova čije uputstvo sadrži informacije o genotipu [15-30]

Lek	Genotip	Klinička implikacija
Antidepresivi, anksiolitici i antipsihotici		
Aripiprazol	CYP2D6	Povećana izloženost leku kod SM CYP2D6; preporučuje se smanjenje doze.
Klozapin	CYP2D6	Moguće povećanje koncentracije u plazmi kod SM CYP2D6; nejasan klinički značaj.
Diazepam	CYP2C19	Povećana enzimska aktivnost može dovesti do pojačane sedacije.
Doksepin	CYP2D6	Povećana koncentracija u plazmi kod SM CYP2D6; potrebno smanjiti dozu.
Fluoksetin	CYP2D6	Povećana koncentracija u plazmi S-fluoksetina kod SM; nejasan klinički značaj.
Protriptilin	CYP2D6	Povećana koncentracija u plazmi kod SM CYP2D6; potrebno smanjiti dozu.
Risperidon	CYP2D6	Povećana koncentracija u plazmi kod SM CYP2D6; klinički značaj nejasan.
Tioridazin	CYP2D6	Povećana koncentracija u plazmi kod SM CYP2D6; što povećava rizik od lekom izazvane prolongacije QT-intervala i aritmija [16]. Tioridazin je kontraindikovan kod SM CYP2D6.
Venlafaksin	CYP2D6	Povećana koncetracija u plazmi kod SM CYP2D6; može povećati rizik od neželjenih efekata.
Analgetici		
Celekoksib	CYP2C9	Povećana izloženost leku kod onih sa alelima smanjene funkcionalnosti CYP2C9;. Kod pacijenata sa **3/*3* genotipom neophodno smanjiti dozu.
Kodein	CYP2D6	Smanjen nivo morfina u plazmi i analgetski efekat kod SM CYP2D6 [17-20].
Tramadol	CYP2D6	Smanjen nivo *O*-dezmetiltramadola u plazmi ismanjen analgetski efekt kod SM CYP2D6 [17-20].
Kardiovaskularni lekovi		
Karvedilol	CYP2D6	Povećan nivo *R*-karvedilola u plazmi kod SM CYP2D6; potencijalno povećan rizik od neželjenih efekata, kao što je vrtoglavica.
Klopidogrel	CYP2C19	Samnjen antiagregacioni efekat kod SM CYP2C19; moguć povećan rizik od neželjenih kardiovaskularnih poremećaja, uključujući i trombozu stenta [21, 22].
Metoprolol	CYP2D6	Povećan nivo u plazmi kod SM CYP2D6; nejasan klinički značaj [23, 24].
Propafenon	CYP2D6	Povećan nivo u plazmi kod SM CYP2D6; nejasan klinički značaj.
Propranolol	CYP2D6	Povećan nivo u plazmi kod SM CYP2D6; nejasan klinički značaj [23, 24].
Varfarin	CYP2C9	Smanjen klirens S-varfarina kod nosilaca alela CYP2C9 smanjene metaboličke funkcije; potrebno smanjiti dozu; povećan rizik od krvarenja [25-28].
Inhibitori protonske pumpe		
Omeprazol	CYP2C19	Potencijalno smanjena efikasnost kod EM CYP2C19 u poređenju sa SM [29].
Rabeprazol	CYP2C19	Potencijalno smanjena efikasnost kod EM CYP2C19 u poređenju sa SM [30].

Skraćenice: SM – spori metabolizeri; EM – ekstenzivni metabolizeri; IM – intermedijarni metabolizeri

farmakogenetičke informacije koje se odnose na P450 enzime [15]. Primeri ovakvih lekova dati su u tabeli 2. Na uputstvima se od P450 gena pominju CYP2C9, CYP2C19 i CYP2D6. Upravo zato je ovaj pregledni rad fokusiran na genetske varijacije bitne za enzime CYP2C9, CYP2C19 i CYP2D6 kao i lekove koje metabolišu navedeni enzimi.

CYP2C9 genotip

Enzim CYP2C9 metaboliše približno 15% lekova sa kliničkom primenom, uključujući i neke antikoagulante (npr. *S*-varfarin), hipoglikemike (npr. tolbutamid), blokatore receptora angiotenzina II (npr. losartan), antiepileptike (npr. fe-

nitoin) i nesteroidne antiinflamatorike (npr. diklofenak) [31]. Do danas je identifikovano 35 varijanti gena CYP2C9 [32].

Uticaj CYP2C9 genotipa na terapijsku efikasnost varfarina

Varafin je najčešće propisivan lek za prevenciju tromboembolije. Ima usku terapijsku širinu i dozira se prema internacionalnom normalizovanom odnosu (*International Normalised Ratio*, INR), pa se za većinu indikacija preporučuje INR ospeg 2 ili 3 [33]. Rizik od tromboze raste kod primene antikoagulacijskih doza ispod terapijskog nivoa [34, 35], dok INR opseg veći od 4 povećava rizik od krvarenja. Varfarin je lek koji je izazovno kontrolisati, velikim delom zbog toga što doza neophodna da bi se postigao terapijski efekat varira i dvadesetostruko kod različitih pacijenata [36].

Kao pomoć lekarima u određivanju doze varfarina, kada je poznat genotip CYP2C9, publikovano je više algoritama [37-40]. Mnogi lekari i zdravstveni radnici očekuju rezultate kliničkih istraživanja, koja su u toku, pre nego što u potpunosti usvoje protokole za doziranje varfarina na osnovu genotipa.

CYP2C19 genotip

Funkcionalnost varijanti CYP2C19

Enzim CYP2C19 metaboliše pribižno 10% lekova u kliničkoj praksi, uključujući *S*-mefenitoin, inhibitore protonske pumpe i nelfinavir. Enzim CYP2C19 takođe je odgovoran za biotransformaciju klopidogrela u farmakološki aktivan oblik. Genetski nedostatak u CYP2C19 posredovanoj eliminaciji *S*-mefenitoina prvi put je zabeležen 1979.godine [41]. Do danas je identifikovano 28 varijanti CYP2C19 gena [42].

Uticaj genotipa CYP2C19 na terapijsku efikasnost klopidogrela

Klopidogrel je antiagregacioni lek koji se nalazi u širokoj upotrebi kod pacijenata sa kardiovaskularnim oboljenjima. Pokazalo se da klopidogrel, u kombinaciji sa aspirinom, smanjuje morbiditet i mortalitet pacijenata sa akutnim koronarnim sindromom (ACS), koji se leče medikamentoznom terapijom ili koronarnom revaskularizacijom [43-45]. Dualna antiagregaciona terapija koja se sastoji od klopidogrela i aspirina, takođe smanjuje rizik od koronarne tromboze stenta, nakon perkutane koronarne intervencije (PCI) [46]. Kod različitih pacijenata efikasnost klopidogrela znatno varira, pa se kod 25% pacijenata koji su primili terapiju javlja rezidualna *ex vivo* agregacija trombocita [47]. Kod ovakvih pacijenata postoji povećan rizik od ozbiljnih neželjenih srčanih stanja, kao što su infarkt miokarda i tromboza stenta.

Uputstvo za primenu klopidogrela ažurirano je u martu 2010.godine kao odgovor na izveštaje o smanjenoj efikasnosti u prisustvu alela CYP2C19 smanjene metaboličke funkcionalnosti [48]. Uputstvo upozorava na smanjenu efikasnost kod sporih metabolizera i navodi da je dostupno genetsko testiranje na alele CYP2C19 smanjene funkcionalnosti. Uputstvo dalje savetuje zdravstvene radnike da razmotre alternativne opcije za pacijente označene kao SM. Međutim, uputstvo ne precizira u kojim slučajevima bi trebalo raditi genotipizaciju, kao ni koje korake preduzeti kod pacijenata kojima se testom utvrdi prisustvo alela smanjene metaboličke funkcionalnosti. Osim toga, uputstvo ne pominje IM, koji su jasno u povećanom riziku od kardiovaskularnih poremećaja u poređenju sa EM, mada manjem od SM.

Nedavno je konzorcijum za kliničku implementaciju farmakogenetike, podržan od strane američkog Nacionalnog instituta za zdravlje (*Nacional Institutes of Health,* NIH), publikovao vodiče za genotipom navođenu antiagregacionu terapiju kardiovaskularnih bolesti [49]. Ovi vodiči ne daju čvrste preporuke o tome koje pacijente treba genotipizovati, već nude dva potencijalna pristupa. Prema prvom, svi pacijenti koji imaju ACS, ili im je izvršena PCI, treba da budu genotipizirani, dok su prema drugom, u fokusu pacijenti sa visokim i umerenim rizikom, kao što su oni sa istorijom tromboze stenta, dijabetesa, insuficijencije bubrega, ili onih sa visokorizičnim koronarnim angiografskim karakteristikama. Kod pacijenata za koje postoje podaci o CYP2C19 genotipu, standardne doze klopidogrela se preporučuju za EM i UM. Alternativna terapija prasugrelom, tikagrelorom ili cilostazolom preporučujuje se za IM ili SM [49].

Uticaj genotipa CYP2C19 na terapijsku efikasnost inhibitora protonske pumpe

Inihibitori protonske pumpe (IPP), kao što su omeprazol, esomeprazol, pantoprazol, lansoprazol i rabeprazol su prolekovi i delimično se metabolišu zahvaljujući CYP2C19. Aleli CYP2C19 smanjene metaboličke funkcionalnosti povećavaju koncentraciju inhibitora protonske pumpe u plazmi i stvaraju veću supresiju želudačne kiseline [50-52]. U skladu s ovim podacima stoji činjenica da se viša stopa eradikacije *Helicobacter pylori* postiže ili uz pomoć dualne (IPP i amoksicilin) ili triple (IPP, amoksicilin i klaritromicin) terapije kod pacijenata sa defektnim alelom CYP2C19. To naročito važi u pogledu prijavljenih stopa izlečenja standardnim dozama omeprazola (20 mg/dan) i amoksicilina od 100%, 60% i 29% za EM [29]. Genotip CYP2C19 takođe utiče na efikasnost inhibitora protonske pumpe u lečenju gastroezofagealnog refluksa.

Slično CYP2C9, postoje podaci da se ekspresija gena CYP2C19 menja tokom odrastanja, pri čemu se puna ekspresija dostiže tek sa 10 godina starosti [53]. Kao posledica toga, odnos između genotipa CYP2C19 i odgovora na inhibitor protonske pumpe može da se razlikuje između odraslih i pedijatrijskih pacijenata [53].

Genotip CYP2D6

Enzim CYP2D6 metaboliše oko 25% lekova sa kliničkom primenom iz više različitih klasa kao što su antidepresivi, antipsihotici, antihipertenzivi i analgetici.

Uticaj genotipa CYP2D6 na terapijsku efikasnost opioidnih analgetika

Kodein i tramadol su prolekovi koji se uz pomoć CYP2D6 konvertuju u svoje aktivne metabolite morfin, odnosno *O*-desmetiltramadol. Spori metabolizeri CYP2D6 postižu niže koncentracije metabolita, pa je analgetski efekat pri uobičajenim dozama ovih lekova minimalan [17-20]. Za razliku od toga, pacijenti sa dupliranim genom CYP2D6 i fenotipom ultrabrzog metabolizma nakon uzimanja kodeina ili tramadola, u riziku su od toksičnih koncentracija morfina i *O*-desmetiltramadola u plazmi [54-56]. Postoje izveštaji o ozbiljnim respiratornim depresijama i bolovima u abdomenu kod pacijenata koji su primali analgetike na bazi kodeina, a za koje je naknadno utvrđeno da imaju fenotip ultrabrzog metabolizma [55, 56]. Pored toga, prijavljeni su i slučajevi teške opioidne toksičnosti, pa čak i smrtni slučajevi, kod odojčadi koje su dojile majke sa fenotipom ultrabrzog metabolizma (UM) [57]. Ovi podaci ukazuju na to da analgetike koji sadrže kodein i tramadol treba davati samo pacijentima koji pripadaju fenotipu EM, da bi se izbegli analgetski neuspeh kod SM, odnosno toksičnost kod UM.

Farmakogenetsko testiranje u kliničkoj praksi

Farmakogenetičko testiranje se vrši prevashodno zbog nekoliko razloga: u selekciji pacijenata sa najvećim izgledima za postizanje terapijske efikasnosti, u cilju smanjenja neželjenih efekata i određivanju adekvatne doze leka kako bi se postigle efikasnost i što veći stepen bezbednosti primenjene terapije. Noviji podaci ukazuju na to da je većina propisanih lekova efikasna kod ne više od 60% osoba koje ih uzimaju, a znatan broj pacijenata razvije neki od ozbiljnih neželjenih efekata, koji često zahtevaju hospitalizaciju. Klinička i regulatorna zajednica prepoznale su u širokom spektru farmakogenetičkih testova ozbiljan potencijal kako bi se izmenila postojeća standardna medicinska praksa. Tako su danas informacije o genetičkom testiranju sastavni deo uputstava za upotrebu nekih od lekova, kao što su abakavir, varfarin, klopidogrel, irinotekan, maravirok, cetuksimab itd. [5].

Izazovi u kliničkim istraživanjima

Više od 200 registrovanih lekova u svetu, što čini oko 10% lekova odobrenih od strane FDA, sadrži farmakogenetičke informacije, što je još uvek mali broj lekova. Ipak, navedeni podatak pokazuje potencijalni značaj odnosa između genetskih polimorfizama i farmakokinetike odnosno terapijskog odgovora.

Kada je reč o kliničkoj primeni, od farmakogenetike svi mnogo očekuju: pacijenti koji zahtevaju efikasnu terapiju bez neželjenih efekata, lekari kojima je potrebna pomoć u izboru adekvatnog leka i doze za određenog pacijenta, zdravstveni radnici koji moraju da pronađu način da unaprede kvalitet zdravstvene zaštite uz istovremeno smanjenje troškova, vladine agencije kojima su potrebna pouzdana ispitivanja prilikom izdavanja terapijskih vodiča i zakona, kao i farmaceutskoj industriji koja nastoji da skupe supstance, koje pretenduju da postanu lek, ne izgube usled nepredviđene toksičnosti u kasnim fazama razvoja leka.

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PHARMACEUTICAL THERAPY OF CHRONIC VENOUS DISEASE

MEDIKAMENTNA TERAPIJA HRONIČNE VENSKE BOLESTI

Đorđe Radak[1, 2, 3], Vuk Sotirovic[1]

Summary

Chronic venous disease (CVD) is a common condition and a global phenomenon that affects a significant part of the population worldwide. The majority of patients with CVD have symptoms that significantly affect their daily activities and deteriorate the quality of their life. Treatment modalities vary from medical and other types of conservative therapies, less invasive endovenous intervention to radical surgical procedures. The purpose of this article is to underline the importance of venoactive drugs in the treatment of patients with CVD.

Key words: *CVD, pharmaceutical therapy, venoactive drugs*

Apstrakt

H*ronična venska bolest je često stanje i globalni fenomen koji utiče na značajni deo svetske pupulacije. Najveći broj pacijenata koji boluje od hronične venske bolesti, ima simptome koji u značajnoj meri utiču na njihove dnevne aktivnosti i narušavaju njihov kvalitet života. Modaliteti lečenja variraju od medikamentne i ostalih tipova konzervativne terapije, preko minimalno invazivnih endovenoznih procedura do radikalnih hirurških procedura. Cilj ovog rada je da se podvuče značaj venoaktivnih lekova u tretmanu pacijenata sa hroničnom venskom bolešću.*

Ključne reči: *hronična venska bolest, medikamentna terapija, venoaktivni lekovi*

Introduction

Chronic venous disease (CVD) is a common condition and global phenomenon that affects a significant part of the population worldwide (1). CVD includes the full spectrum of morphological and functional abnormalities of the venous system irrespective of whether they produce any symptoms.

The grading of chronic venous disorders (CVD) was simplified and standardized by the introduction of the Clinical, Etiological, Anatomical, and Pathophysiological (CEAP) classification system (2). The CEAP classification categorizes limbs into seven classes from C0 to C6. Each clinical class is further characterized by the subscript (S) if the categorized limb is symptomatic or the subscript (A) if the limb is asymptomatic. The international character of CEAP classification allows precise comparisons between countries and continents.

It has to be noted that the majority of patients with CVD have symptoms that significantly affect their daily activities and deteriorate the quality of their life. However, it is not easy to confirm a positive correlation between signs and symptoms of CVD. CVD could be associated with a whole range of symptoms such as: pain, heaviness, restless legs, tingling, aching, burning, night muscle cramps, swelling, sensations of throbbing or itching skin, leg tiredness and/or fatigue (3). In addition, these symptoms could be a part of some other non – venous chronic and acute diseases and conditions: obesity, neurological reasons, standing or sitting professions, or arterial occlusive disease (4).

In addition to others, a very specific group of patients has been isolated in recent years. These patients have no visible signs of disease, but they are constantly reporting venous – like symptoms and are thus a real "nightmare" for both GPs and vascular surgeons (2, 5, 6). There are approximately 20% of patients with CVD in C0s category and identification of these patients could be crucial from diagnostic and therapeutic point of view.

Along with CVD pandemic, a different treatment modality has been developed in order to deal with and control the disease in the early stages.

Treatment modalities vary from radical surgical procedures, over less invasive endovenous interventions, to medical and other types of conservative therapies.

The purpose of this article is to underline the importance of venoactive drugs in the treatment of patients with CVD.

Pharmaceutical therapy of CVD – Venoactive drugs

Veno-active drugs (VADs) constitute a diverse group of medications, which are synthetic but mostly have herbal origin. Five main types of VADs have been identified (7):

1. Alpha – benzopyrones, notably coumarin;
2. Gamma – benzopyrones, also known as flavonoids,

which include simple diosmins, micronized purified flavonoid fraction (MPFF), and the rutosides, including rutin, troxerutin, and hydroxyethylrutosides (Hr);
3. Saponins, including horse chestnut seed extract (HCSE) and ruscus aculateus extract;
4. other herbal extracts, including anthocyans, proanthocyanidins (grape seed extract, red-vine – leaf extract), *Ginko biloba* extract, and *Centella asiatica* extract;
5. synthetic products (chemical family of quinons) which include naftazone and calcium dobesilate.

Due to diversity of VADs, there are multiple mechanisms of their action (7):

- The most important mechanism of action is their impact on inflammatory processes in venous valves and the vein wall: scavenging of free radicals, blocking the propagation of oxidative reactions and reinforcing inherent cellular antioxidant capacity. Notably MPFF has a significant anti – inflammatory effect in the early stage of inflammatory cascades: by inhibiting leukocyte – endothelial interactions;
- Actions on venous tone – most of them act by modulating noradrenergic signaling, by reducing norepinephrine metabolism in the cases of MPFF and hydroxyethyl-rutosides or by agonism of venous α1-adrenergic receptors in the case of *Ruscus* extracts;
- Actions on capillary permeability (edema) – with their antioxidant and anti – inflammatory effects, it is not surprising that many of the major VADs have been shown to reduce capillary hyperpermeability, MPFF treatment significantly reduces plasma VEGF in patients with skin changes, and plasma VEGF has been proposed as a marker of MPFF therapy;
- The positive effect on lymphatic circulation and lymph flow;
- Reduction of blood viscosity and improvement in blood flow – several VADs have been shown to reduce blood viscosity and/or erythrocyte aggregation, including MPFF, troxerutin and calcium dobesilate.

The place of Venoactive drugs in daily clinical practice

The concept of venoactive drugs is more than attractive. According to a perfect scenario, VADs could reduce progression of CVD, symptoms related to CVD and even development of severe stages and the occurence of venous ulcers and all the accompanying complications.

In recent guidelines, only some of VADs have found their place in the management of CVD (7,8). (Table 1)

Recommendations for the use of venoactive drugs in guidelines are based on the 'Grading of Recommendations Assessment, Development and Evaluation' (GRADE) system (9, 10). The GRADE system differs from other schemes described in the guidelines in the fact that separate levels are assigned for the recommendation of treatment and for the quality of evidence on which the recommendation is based. Recommendations are classified as either strong (grade 1) or weak (grade 2), and quality of evidence as high (grade A), moderate (grade B) or low (grade C). Importantly, the GRADE system recognizes that large observational studies may provide evidence of moderate or even high quality, particularly if the estimation of the magnitude of the treatment effect is very large. In current clinical practice, the major point of interest concerning these drugs is to reduce symptoms related to CDV.

Table 1. Recommendations for venoactive drugs from the international consensus meeting in Cyprus, November 2012.

Indication	Venoactive drug	Recommendation	Quality of evidence	Grade
Relief of venous symptoms ($C0_s$ to $C6_s$) and edema (C3)	**MPFF**	**Strong**	**Moderate**	**1B**
	Simple diosmins	Weak	Poor	2C
	Rutosides (O-betahydroxyethyl)	Weak	Moderate	2B
	Calcium dobesilate	Weak	Moderate	2B
	HCSE	Weak	Moderate	2B
	Ruscus extracts	Weak	Moderate	2B
	Gingko biloba	Weak	Poor	2C
	Other VADs	Weak	Poor	2C
Adjunctive treatment of primary venous ulcer (C6)	**MPFF**	**Strong**	**Moderate**	**1B**

Micronized purified flavonoid fraction (MPFF) treatment strategy

Micronized purified flavonoid fraction has a number of vein – specific anti – inflammatory effects that relieve symptoms at all stages of CVD. In several placebo – controlled trials, MPFF was associated with a significantly greater improvement in many of the symptoms of CVD after 2 months compared to placebo ($P< 0.001$ MPFF versus placebo) or simple diosmin ($P< 0.05$ MPFF versus simple diosmin). Moreover, symptom relief with MPFF was achieved rapidly and maintained in the long term (11).

In a meta-analysis of 459 patients, MPFF significantly reduced the symptoms associated with venous ulcers after 4 and 6 months of the treatment (12) MPFF is also beneficial for post – surgery pain, (13, 14, 15) and the pain associated with pelvic congestion syndrome (16). Patients receiving MPFF 2 weeks before and continuing for 14 days after varicose vein surgery required significantly less analgesic use than the control group (13, 14). In a cross – over study, women were randomized to receive either MPFF or placebo. After 6 months, mean pain scores were significantly lower in the MPFF group compared to placebo ($P<0.05$) (16).

In recent guidelines for the management of CVD, MPFF has been assigned a high level of recommendation as a first – line treatment for venous symptoms in any stage of CVD (7). It should be noted (Table 1) that the recommendation for MPFF is strong, based on benefits that clearly outweigh the risks and evidence of moderate quality (grade 1B) for the indication of relief of venous symptoms in $C0_s$ to $C6_s$ patients, including those with CVD – related edema. MPFF retains its strong recommendation for use as adjuvant therapy in treating venous ulcers (7).

In conclusion, CVD is a global phenomenon that has almost pandemic proportions. In order to deal with this massive phenomenon, several therapeutic options have been developed. Apart from very popular surgical and less invasive procedures, venoactive drugs have been trying to find their place on the global medical scene for many years. Today, latest guidelines have started to recommend venoactive drugs, especially MPFF, as a standard symptom relief therapy, in every stage of CVD. However, promising beneficial effects and expansion of their use are yet to be explored in further multicenter trials.

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THE ROLE OF L-ARGININE IN CARDIOVASCULAR SYSTEM

ULOGA L-ARGININA U KARDIOVASKULARNOM SISTEMU

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Summary

The essential amino acid, L-Arginine (L-Arg) has an important role in the cardiovascular system. Literature data show that L-Arg is the only substrate for the production of nitric oxide (NO), from which L-Arg develops its effects on the cardiovascular system. As a free radical, NO is synthesized in all mammal cells by L-Arg with the activity of NO synthase (NOS). In the states of hypertension, diabetes, hypercholesterolemia and vascular inflammation, a disorder occurs in the metabolic pathway of the synthesis of NO from L-Arg which all together bring alterations to blood vessels. Clinical studies show that L-Arg has an effect on thrombocytes, the process of coagulation and the fibrolytic system. All the new data summarized in this review suggest that L-Arg could be one of important therapeutic molecules for improving cardiovascular disorders.

Keywords: *L-Arg, NO, NOS, arginase, cardiovascular system*

Sažetak

Esencijalna aminokiselina, L-Arginin (L-Arg) ima veoma važnu ulogu u kardiovaskularnom sistemu. Podaci iz literature pokazuju da je L-Arg jedini supstrat za produkciju azot-monoksida (NO), preko koga L-Arg i ostvaruje svoje efekte na kardiovaskularni sistem. Kao slobodni radikal, NO se sintetiše u svim ćelijama sisara od L-Arg uz aktivnost enzima NO sintaze (NOS). U stanjima hipertenzije, dijabetesa, hiperholesterolemije i vaskularne inflamacije dolazi do poremećaja metaboličkog puta sinteze NO od L-Arg, što sve zajedno dovodi do oštećenja krvnih sudova. Kliničke studije ukazuju da L-Arg može imati efekte na trombocite, proces koagulacije kao i na fibrinolitički sistem. U okviru ovog preglednog članka sumirani su najnoviji podaci iz literature koji sugerišu da bi L-Arg mogao biti jedan od bitnih terapeutskih molekula u poboljšanju lečenja kardiovaskularnih poremećaja.

Ključne reči: *L-Arg, NO, NOS, arginaza, kardiovaskularni sistem*

Opste osobine L-Arginina

Do danas je poznato najmanje pet enzima koji koriste esencijalnu aminokiselinu L-Arginin (L-Arg) kao supstrat za svoju aktivnost. Osim arginil-tRNA sintetaza, četiri grupe enzima kod sisara koriste slobodni L-Arg kako supstrat i to su: azot-monoksid (NO) sintaza (NOS) (najmanje tri izoforme), arginaza (2 izoforme), L-Arg -glicin amidinotransferaza i L-Arg dekarboksilaze [35]. Iako postoji više puteva katabolizma L-Arg, postoji samo jedan put sinteze L-Arg, i to je put koji vodi od citrulina [35]. Kod sisara, citrulin se sintetiše u crevima od glutamina i prolina, a glavno mesto za endogenu biosintezu L-Arg od citrulina je u bubrezima [16; 49]. Osim u bubrezima, citrulin će metabolisati u L-Arg u svim tkivima koja eksprimiraju enzime argino-sukcinat sintetazu (ASS) i argino-sukcinat liazu (ASL) u ciklusu poznatom kao "citrulin-NO ciklus" [35; 49; 64]. Velike količine L-Arg se sintetišu u ciklusu uree u hepatocitima, i na ovaj način sintetisani L-Arg se odmah hidrolizuje u ornitin i ureu, što ima za posledicu da urea ciklus ne pruža dovoljno L-Arg za celokupan organizam. Promene u dostupnosti L-Arg kao i krajnjih produkata različitih metaboličkih puteva L-Arg u organizmu mogu imati značajne fiziološke posledice [35]. U slučaju nedovoljne sinteze endogenog L-Arg, neophodno je obezbediti dodatni unos L-Arg ishranom za optimalan rast, [54] i za regeneraciju tkiva [49; 51]. U zavisnosti od stanja uhranjenosti i faze razvoja, normalne koncentracije L-Arg u plazmi ljudi su u rasponu od 40 to 100 μmol/l [4].

Pokazano je da metabolizam L-Arg ima veoma važnu ulogu u kardiovaskularnom sistemu [35], upravo zbog njegove uloge prekursora za sintezu molekula NO, slobodnog radikala koji se sintetiše u svim ćelijama sisara od L-Arg uz aktivnost enzima NOS [42; 46; 49; 61]. Podaci iz literature pokazuje da je L-Arg jedini supstrat za produkciju NO, preko kaga i ostvaruje svoje efekte na kardiovaskularni sistem. Kliničke studije u koje su bili uključeni hipertenzivni i dijabetični pacijenti kao i zdrave osobe, ukazuju da L-Arg može regulisati vaskularnu hemostazu [14; 18; 19; 44]. Eksperimentalni rezultati dobijeni na životinjama kao i *in vitro* podaci sugerišu da L-Arg može imati efekte na trombocite, process koagulacije kao i na

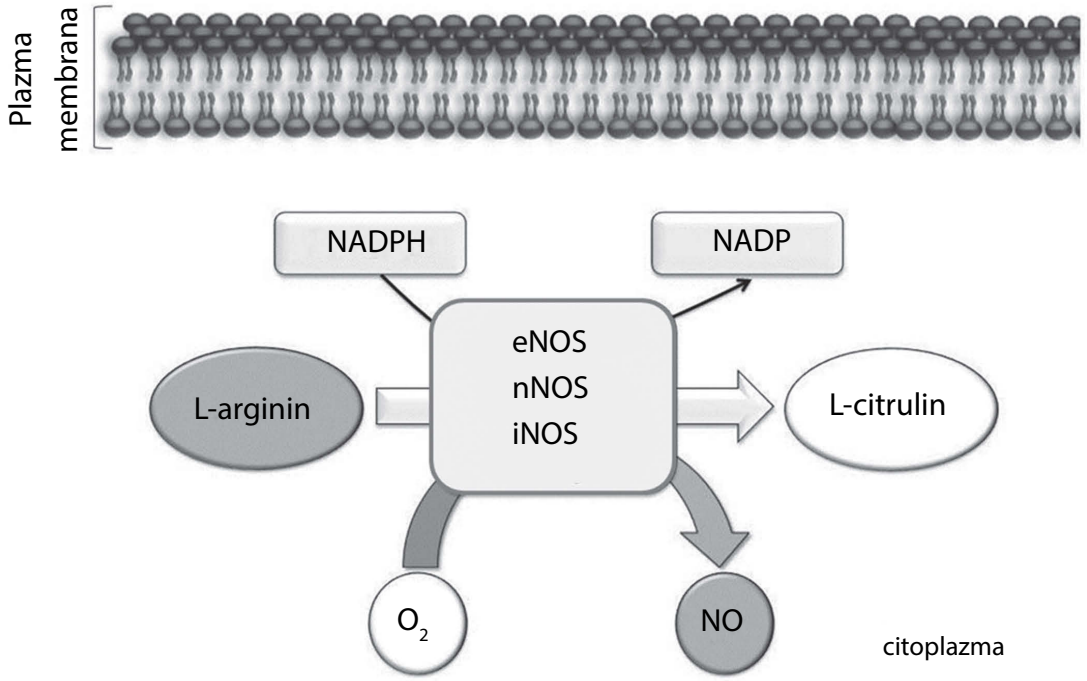
fibrinolitički sistem [2; 19; 28; 44; 53], što ukazuje na novi terapeutski potencijal aminokiseline L-Arg [14]. U okviru ovog preglednog članka sumirani su najnoviji podaci iz literature koji ukazuju na značaj i ulogu L-Arg u fiziologiji i patofiziologiji kardiovaskularnog sistema.

Uloga L-Arginina u kardiovaskularnom sistemu

U kardiovaskularnoj fiziologiji i patofiziologiji ključnu ulogu ima efekat NO na vaskularni endotelium [35]. Poremećaj metaboličkog puta L-Arg/NO u endotelu je jedan od najčešćih mehanizama kojima faktori rizika za kardiovaskularna oboljenja, kao što su hiperholesterolemija [5], hipertenzija [38], pušenje [8], dijabetes [27], homocisteinemia [50], i vaskularna inflamacija [23], ostvaruju svoje negativne efekte na zidove krvnih sudova [25]. Aminokiselina L-Arg je jedini prekursor za sintezu NO. Katabolički enzimi L-Arg, koji svojim delovanjem najviše utiču na kardiovaskularni sistem jesu NOS i arginaza [31; 35; 45; 47].

Tri izoforme NOS su klonirane i okarakterisane do sada: neuronalna NOS (nNOS), inducibilna NOS (iNOS) i endotelna NOS (eNOS) [7; 30; 66], i sve tri izoforme NOS su prisutne u kardiovaskularnom tkivu [24; 25; 41; 43]. L-Arg je substrat enzima NOS, koji ga konvertuje u aminokiselinu L-citrulin uz oslobađanje NO **(Slika 1.).** Iz ovako nastalog citrulina, L-Arg, takođe, može biti i recikliran pomoću enzima ASS i ASL [35; 64], ali i razložen arginaznom.

L-Arg se pod delovanjem enzima arginaze pretvara u L-ornitin, koji je prekursor u sintezi poliamina i uree, molekula bitnih za ciklus uree [49]. Takođe, L-Arg je i prekursor za kreatin, jedinjenje koje ima značajnu ulogu u energetskom metabolizmu mišića, nerava i testisa. Kreatin takođe, bitno doprinosi katabolizmu L-Arg i sintezi agmatina i proteina [54; 55; 59]. Indukcija enzima arginaze dovodi do pojačanog katabolizma L-Arg do ornitina [60]. Pošto je L-Arg limitirajući faktor za sintezu NO [26], može se očekivati da je i aktivnost arginaze uključena u indukovanu sintezu NO. Na osnovu zapažanja da inhibicija aktivnosti arginze dovodi do povećane proizvodnje NO u endotelu, [1; 10; 21; 67; 69; 70], arginaza u endotelnim ćelijam najverovatnije ima ulogu u regulaciji dostupnosti substrata za sintezu NO [35]. U patofiziološkim stanjima kao što su hipertenzija i ishemijska reperfuzija, aktivnost endotelne arginaze je povećana što doprinosi disfunkciji endotela, daljim smanjenjem koncentracije L-Arg, što sve zajedno dovodi do disfunkcije NOS i poremećene produkcije NO [1; 21; 35; 43; 67; 68; 70]. Pokazano je da su aktivnosti oba enzima, i NOS i arginaze značajno smanjeni u endotelnim ćelijama diajbetičnih pacova u poređenju sa kontrolama [35; 63]. Aktivnost arginaze može biti smanjena ili inhibirana *N*-hidroksiargininom, posrednikom u signalnom putu NOS [52]. Tako, usled niske aktivnosti arginaze, dostupno je više L-Arg kao supstrata za enzime eNOS i iNOS čija aktivnost je stimulisana, što za rezultat ima povećanu produkciju NO [47]. Međutim, ekspresija arginaze kao i metabolizam L-Arg u različitim patofiziološkim stanjima nije u potpunosti okarakterisan, pa u skladu sa tim, nivo ekspresije ili aktivnosti arginaze može predstavljati terapijsku metu za neka kardiovaskularna oboljenja [35; 52].



Slika 1. ***Shematski prikaz mehanizma nastanka azot-monoksida (NO) od L-Arginina u endotelnim ćelijama***

NO*- azot-monoksid;* ***eNOS****- endotelna NO-sintaza;* ***iNOS****- inducibilna NOS;* ***nNOS****- neuronalna NOS;* ***NADP****- nikotinamid adenin dinukleotid fosfat.*

Akutna i hronična primena L-Arg poboljšava funkciju endotela u animalnim modelima hiperholesterolemije i ateroskleroze [3]. Pokazano je da povećanje koncentracije L-Arg u endotelnim ćelijama dovodi do povećanja produkcije NO od strane eNOS na dozno zavisan način [20]. Povećanje koncentracije L-Arg u plazmi dovodi do povećane produkcije kako vaskularnog tako i sistemskog NO [34; 36; 52; 56; 58; 64]. Studije u kojima su korišćene glatke mišićne ćelije krvnih sudova (VSMC) pokazale su da je limitirajući faktor za povećanje mogućnosti sinteze NO uz pomoć iNOS upravo mogućnost recikliranja L-Arg [35; 65]. Schott i sar. pokazali su da aktivnost iNOS zavisi od koncentracije ekstracelularnog L-Arg [40]. Pojava u kojoj dodatno povećanje koncentracije L-Arg u ćelijama u kojima je koncentracija L-Arg u fiziološkom opsegu i pri kojoj bi trebalo da je enzim iNOS saturisan, dovodi do povećanja koncentracije NO, nazvana je "L-argininski paradoks" [17; 32]. U prilog ovom paradoksu ide i mogućnost postojanja intracelularnog pula L-Arg koji nije dostupan enzimu iNOS i neophodan je upravo ekstracelularni L-Arg za aktivnost iNOS i sledstvenu produkciju NO [6; 11; 37]. Dakle, termin "L-argininski paradoks" odnosi se na specifičnu situaciju u kojoj suplementacija L-Arg stimuliše aktivnost NOS i produkciju NO, iako je nivo L-Arg u plazmi u fiziološkim granicama [9].

Pokazano je da sistemsko ili oralno davanje L-Arg poboljšava kardiovaskularnu funkciju i smanjuje ishemiju srca kod pacijenata sa bolešću koronarnih arterija [34; 49; 62]., kao i da dovodi do smanjenja krvnog pritiska i vaskularnog otpora u bubrezima kod hipertenzivnih bolesnika s normalnom ili nedovoljnom bubrežnom funkcijom [22; 49]. Iako je koncentracije L-Arg u plazmi nepromijenjena kod pacijenata sa hiperholesterolemijom, oralno ili intravenozno, davanje L-Arg može povratiti funkcije endotela kod ovih pacijenata [33]. Osim toga, pošto se većina endogenog L-Arg sintetiše u bubrezima, disfunkcija bubrega može takođe pridoneti redukciji nivoa L-Arg u plazmi [35]. Kod pacijenata sa sindromom kratkog creva, zapaženo je da je stvaranje citrulina smanjeno, što dovodi i do smanjenja nivoa L-Arg u plazmi [13; 35; 39; 57], pa se stoga, L-Arg smatra esencijalnom aminokiselinom u isharni u pacijenata s oslabljenom funkcijom bubrega ili creva [35]. Postoji mišljenje da je ateroskleroza uzrokovana nedostatkom L-Arg, najverovatnije usled poremećenog odnosa u koncentraciji lizina i L-Arg [29] kao i interakcije između estrogena i metabolizma L-Arg [12; 49].

Podaci iz literature, kao i naši kako objavljeni [15; 45; 47; 48] tako i naši preliminarni rezultati koji se odnose na uloge L-Arg u kardiovaskularnom sistemu, sugeriše se da bi L-Arg mogao biti jedan od bitnih terapeutskih molekula u poboljšanju lečenja kardiovaskularnih poremećaja.

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THE ROLE OF THE NITRIC OXIDE SYNTHASES IN BRAIN ISCHEMIA DURING CAROTID ENDARTERECTOMY

ULOGA AZOT-MONOKSID SINTAZA U STANJIMA ISHEMIJE MOZGA TOKOM KAROTIDNE ENDARTEREKTOMIJE

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Summary

According to the World Health Organization, 15 million people per year are affected by stroke. The most common cause of stroke is brain ischemia, which occurs in almost 85% of cases. Ischemia caused by thromboembolism is defined as permanently or temporarily decreased blood flow which prevents an adequate delivery of oxygen, glucose and other important nutrients, leading progressively to metabolic changes and cell apoptosis. Carotid endarterectomy (CEA) can cause hypoxic – ischemic states of the brain or acute brain ischemia (ABI) leading eventually to stroke. The main cause of ABI as a result of CEA is cerebral hypoperfusion caused by clamping of carotid arteries, when hypoxia occurs.. Hypoxia per se is one of the triggers of complex physiological responses in the body, including the release of various mediators of inflammation. One of these inflammatory mediators is nitric oxide (NO), a free radical which has numerous physiological effects and also plays an important role in the immune response of the organism. However, NO may be very harmful and cause cell and tissue damage. The lack of literature data on the role of endothelial NOS (eNOS) and inducible NOS (iNOS) during CEA, as well as the mechanisms of their regulation in ischemic conditions, suggest that intensifying future research in this field is very important. An insight into molecular mechanisms of iNOS activity and expression regulation will certainly help to develop new therapeutic strategies for treating harmful effects of free radicals, especially uncontrolled production of NO.

Key words: *carotid endarterectomy, acute brain ischemia, nitric oxide, endothelial nitric oxide synthase, inducible nitric oxide synthase.*

Sažetak

Prema podacima Svetske zdravstvene organizacije, 15 miliona ljudi godišnje doživi moždani udar. Najčešći uzročnik moždanog udara je ishemija mozga, koja se dešava u skoro 85% slučajeva. Moždana ishemija izazvana tromboembolijskim događajima definiše se kao trajno ili prolazno smanjenje cirkulacije krvi, što za posledicu ima nedostatak kiseonika, glukoze i ostalih važnih nutritijenata, dovodeći postepeno do metaboličkih promena i apoptoze ćelija. Tokom operativnih zahvata kao što je karotidna endarterektomija (CEA) može doći do hipoksično-ishemičnog stanja mozga ili akutne ishemije mozga (ABI), kao i do samog moždanog udara. Glavni uzrok ABI u toku CEA je cerebralna hipoperfuzija koja je uzrokovana klemovanjem karotidne arterije, pri čemu dolazi do hipoksije, što može predstavljati jedan od okidača za niz fizioloških odgovora organizma, među kojima je oslobađanje različitih medijatora inflamacije. Jedan od medijatora inflamacije je i azot monoksid (NO), slobodni radikal koji pored mnogobrojnih fizioloških efekata ima važnu ulogu i u samom imunom odgovoru organizma. Međutim, NO može biti veoma štetan i svojim delovanjem dovesti do oštećenja ćelija i tkiva. Nedostatak podataka u literaturi o ulozi endotelne NOS (eNOS) i inducibilne NOS (iNOS) tokom CEA, kao i mehanizama njihove regulacije u stanjima ishemije, ukazuju na pravac kojim treba da se usmere buduća istraživanja. Poznavanje molekularnih mehanizama regulacije aktivnosti i ekspresije iNOS, svakako će pomoći razvoju novih terapijskih strategija u tretmanu štetnih efekata produkcije slobodnih radikala, pre svega nekontrolisane produkcije NO.

Ključne reči: *karotidna endarterektomija; akutna moždana ishemija; azot monoksid; endotelna azot monoksid sintaza, inducibilna azot monoksid sintaza.*

Uvod

Moždana ishemija izazvana tromboembolijskim događajima definiše se kao trajno ili prolazno smanjenje cirkulacije krvi, što za posledicu ima nedostatak kiseonika (O_2), glukoze i ostalih važnih nutrijenata, dovodeći postepeno do metaboličkih promena i apoptoze ćelija [1, 2]. Stvaranje plaka na zidovima krvnih sudova prvo dovodi do pojave privremenih simptoma, poznatih kao prolazni ishemijski napad (TIA; *engl. Transient Ischemic Attack*), pri kome se privremena ishemija javlja u mozgu, kičmenoj moždini ili mrežnjači, bez izazivanja infarktnog stanja [3]. Za razliku od trajne ishemije, TIA ne uzrokuje trajno oštećenja mozga [4]. TIA je kratkotrajna epizoda vaskularne disfunkcije, često označena kao „mini šlog", sa simptomima moždanog udara i trajanja od nekoliko minuta do 24 časa [5, 6].

Iako je iznenadna okluzija krvnog suda izazvana tromboembolijskim događajima najčešći uzrok ishemičnog oštećenja mozga [2], primećeno je da tokom operativnih zahvata kao što je karotidna endarterektomija (CEA; *engl. Carotid Endarterectomy*) može doći do hipoksično-ishemičnog stanja mozga ili akutne ishemije mozga (ABI; *engl. Acute Brain Ischemia*), kao i do samog moždanog udara [2, 7]. Glavni uzrok moždanog udara u toku CEA je cerebralna hipoperfuzija koja je uzrokovana klemovanjem karotidnih arterija, dok su u intraoperativnom, kao i postoperativnom periodu tromb, embolizam i cerebralna hiperperfuzija glavni uzročnici lošeg kliničkog ishoda [6, 8-11].

Narušena ravnoteža između oksidativnih i antioksidativnih procesa ima važnu ulogu u patologiji ABI [12, 13]. Naime, povećana produkcija slobodnih radikala, kao što su superoksid anjon radikal (O_2^-), azot monoksid (NO), vodonik peroksid, peroksinitrit i visoko reaktivni hidroksil radikal može imati ključnu ulogu u patogenezi ABI [14]. Danas, jedan od glavnih ciljeva istraživanja ABI nastale tokom CEA, je razvijanje terapijskih strategija usmerenih ka smanjenju oštećenja mozga nastalog oksidativnim stresom, kroz razumevanje molekularnih mehanizama nastanka ABI [11]. Jedan od terapijskih pristupa mogao bi biti stimulisanje antioksidativne aktivnosti u krvi koja obezbeđuje zaštitu protiv neuroloških oštećenja u stanju ishemije mozga [6, 12].

NO je slobodni radikal koji je uključen u različite fiziološke i patofiziološke procese, a nastaje aktivnošću enzima azot-monoksid sintaza (NOS; *engl. Nitric Oxide Synthase*) [15]. Jedna od glavnih uloga NO je vazodilatatorni efekat koji ostvaruje u krvnim sudovima. NO je molekul koji je prisutan u celom organizmu, ali se u fiziološkim uslovima primarno sintetiše u endotelnim ćelijama krvnih sudova i od velike je važnosti za homeostazu kardiovaskularnog sistema (KVS) zbog svoje kardioprotektivne uloge [6, 15].

Nedostatak podataka u literaturi o ulozi NOS, endotelne (eNOS) i inducibilne (iNOS), tokom CEA kao i mehanizama njihove regulacije u stanjima ishemije, ukazuju na značaj istraživanja u ovoj oblasti. Poznavanje molekularnih mehanizama regulacije aktivnosti i ekspresije NOS, svakako će pomoći razvoju novih terapijskih strategija u tretmanu štetnih efekata produkcije slobodnih radikala, pre svega nekontrolisane produkcije NO u stanjima ishemije mozga.

Akutna ishemija mozga (ABI)

Moždano tkivo je izuzetno osetljivo na ishemiju, tako da i kratki periodi ishemije mogu izazvati niz kompleksnih događaja u neuronima, koji mogu dovesti do apoptoze ćelija [16, 17]. ABI je neuropatološko stanje koje karakteriše apoptoza neuronskih ćelija koja je izazvana nizom patofizioloških događaja, među kojima je jedan od bitnijih činilaca oksidativni stres [12]. Veliki broj novonastalih slobodnih radikala u toku ishemije mozga mogu dovesti do funkcionalnih i strukturnih oštećenja neurona, usled oštećenja lipida, proteina i nukleinskih kiselina [12, 14, 18]. Različiti regioni mozga imaju različit stepen tolerancije na ishemiju, pri čemu je bela masa tolerantnija na ishemiju od sive mase mozga [16, 17]. Takođe, određene populacije neurona, kao što su CA1 piramidalni neuroni hipokampusa, mnogo su osetljivije na ishemiju od drugih, poput dentatne granule neurona [16, 17]. Moždana ishemija rezultuje naglim smanjenjem protoka krvi najčešće izazvanim naglom okluzijom krvnog suda, što dovodi do gubitka neuroloških funkcija [1, 2].

Ishemično oštećenje mozga čine patološki događaji koji mogu biti izazvani moždanim udarom, teškim povredama glave, kardiorespiratornim zastojem, kao i nekim operativnim procedurama poput CEA, vantelesnog krvotoka i indukovane hipotenzije [7]. Ovi patološki putevi ishemične kaskade nastali tokom nekoliko minuta mogu da izazovu nepovratna oštećenja neurona [1, 19], dovodeći do hipoksično-ishemičnog stanja [2]. Regioni mozga kao što su hipokampus, amigdaloidna jedra i prefrontalni korteks odgovaraju na akutni i hronični stres i pokazuju promene u morfologiji i biohemiji, koje su u velikoj meri reverzibilne [20]. Moždana ishemija deluje kao faktor stresa tj. stresor i na taj način stimuliše hipotalamusnu-hipofiznu-adrenalnu osovinu (HPA; *engl. hypothalamic-pituitary-adrenal axis*) [11, 21]. Brojne studije pokazuju da endokrine promene HPA osovine mogu takođe dovesti do moždane ishemije [11, 21]. Kao posledica CEA nastaje složena biološka reakcija, poznata kao hipermetabolički odgovor na stres, koja je posredovana HPA osovinom, a karakteriše je patološka aktivacija autonomnog nervnog sistema i podizanje nivoa kateholamina [11, 22, 23]. Pored toga, ova reakcija dalje implicira hemodinamičke, metaboličke, inflamatorne i imunološke promene usmerene na uspostavljanje homeostaze i oporavak [8]. Metaboličke i endokrine promene koje na-

staju tokom operacije, uključujući povećano snabdevanje O_2, povećan katabolizam i oštećenje funkcija imunog sistema su povezane sa lošim postoperativnim i kliničkim ishodom [8]. Pored oštećenja tkiva koja nastaju tokom prolazne ishemije, dodatna oštećenja tkiva i mikrocirkulacije nastaju i tokom reperfuzije tkiva. [9, 10].

Karotidna endarterektomija (CEA) i akutna ishemija mozga

Karotidna endarterektomija je hirurška procedura u karotidnoj arteriji, koja se koristi za smanjenje rizika od moždanog udara [11]. Asimptomatska karotidna stenoza podrazumeva prisustvo stenotičnih lezija na karotidnimarterijama, u bolesnika koji namaju i nisu imali neurološke simptome moždane ishemije [11]. Simptomatska stenoza podrazumeva istovremeno prisustvo stenoze karotidnih arterija i simptoma cerebralne ishemije i ima visok rizik od moždanog udara, ali za razliku od asimptomatske stenoze, nastupa 2 dana nakon pojave simptoma [24]. Prema preporuci Nacionalnog instituta za zdravlje i kliničku veštinu SAD-a (NICE; *engl. National Institute for Health and Care Excellence,* USA) pacijenti sa umerenim do ozbiljnim (50-99 % začepljenja) stenozom unutrašnje karotidne arterije i simptomima, moraju se operisati najbolje u roku od 2 nedelje od nastanka simptoma [24]. Pacijenti sa asimptomatskom stenozom unutrašnje karotidne arterije imaju veći rizik od nastanka moždanog udara u poredjenju sa opštom populacijom, ali manji rizik od pacijenata sa simptomatskom stenozom. Učestalost moždanog udara u svetu, uključujući fatalni moždani udar je 1-2 % na godišnjem nivou [24], dok je smrtnost pacijenata od endarterektomije tokom hirurške intervecije 1-2 % [24]. Svake godine preko 25 000 ljudi u Srbiji doživi moždani udar a čak 10% ih je mlađe od 30 godina [25]. Dve velike kliničke studije su pokazale da operacija karotida 30 dana od moždanog udara smanjuje rizik od smrtnosti za 3%, dok kod asimptomatskih pacijenata sa stenozom za čak 60 % i produžava životni vek za najmanje 5 godina nakon operacije [26].

Postoje podeljena mišljenja među hirurzima oko načina tretiranja asimptomatskih pacijenata, tj. da li je dovoljno tretirati pacijente lekovima ili je neophodna operacija [27]. Tradicionalni način endarterektomije podrazumeva otvaranje arterije i uklanjanje plaka, dok noviji pristup uključuje endovaskularnu angioplastiku, koja je endoskopska metoda i postavljanje katetera oko luka aorte i do karotidne arterije (stenta) [11]. Kateter sadrži balon za proširenje arterije i ubacuje stent koji drži arteriju otvorenom. U nekoliko kliničkih ispitivanja 30 dana nakon srčanog ili moždanog udara smrtnost je bila znatno veća nego kod stenta sa CEA (9,6 % bez endovaskularne angioplastike u odnosu na 3,9 % sa) [28]. CEA je zlatni standard za tretiranje simptomatskih pacijenata sa karotidnom aterosklerozom [11, 29]. Međutim, uspešnost ove operativne procedure zavisi od stope pre i postoperativnih neželjenih neuroloških događaja [30, 31]. ABI izazvana TIA inicira kompleksni niz događaja u centralnom nervnom sistemu i HPA osovini, koji na kraju mogu dovesti do nervnog i ćelijskog oštećenja. Mozak je izuzetno osetljiv na ishemiju i kao odgovor na stres pokazuje promene u morfologiji i biohemijskim procesima, koje su u velikoj meri reverzibilne [31]. Za ove promene se zna da modifikuju funkciju HPA osovine, ali njihovi mehanizmi još nisu razjašnjeni. Patogeneza ABI je složena i obuhvata više mehanizama, uključujući proizvodnju slobodnih radikala, dovodeći do oksidativnog stresa koji doprinosi neurološkim oštećenjima. Kroz interakciju sa velikim brojem molekula, reaktivne vrste kiseonika (ROS; *engl. reactive oxygen species*) mogu uništavati nepovratno ili menjati funkciju ćelijskih lipida, proteina i nukleinskih kiselina, kao i prekinuti ćelijske signalne puteve nakon cerebralne ishemije. Cilj CEA je da se spreče negativne posledice stenoze karotidnih arterija, sekundarne ateroskleroze, tj. ishemičnog moždanog udara [11].

Kao i kod bilo koje operacije, pažljiva procena relativne koristi i rizika od postupka se traži na individualnoj osnovi pacijenta. Perioperativni CEA rizik za smrtnost u narednih 30 dana treba da bude manji od 3 % za asimptomatske pacijente, odnosno za 6 % u pacijenta sa simptomima. Pacijenti sa simptomima obično imaju TIA bez većih posledica ili blaži moždani udar, koji može uticati na disfunkciju jedne strane tela, uključujući poremećaj govora ili vida [4].

Uloga azot monoksida (NO) u stanjima ishemije mozga

U fiziološkim uslovima NO se primarno sintetiše u endotelnim ćelijama krvnih sudova i uključen je u različite procese važne za homeostazu KVS [15]. Disfunkcija endotela tokom različitih patoloških stanja u KVS često je inicirana smanjenom sintezom NO. Međutim, hiperprodukcija NO može dovesti do oštećenja ćelija direktnom promenom strukture proteina ili indirektno kroz formiranje visoko reaktivnog peroksinitrita [32, 33] što se najčešće dešava u različitim patofiziološkim stanjima, poput ishemije mozga [32]. Povećan nivo NO dovodi do inhibicije enzima uključenih u regulaciju metabolizma i DNK sinteze [12, 34-36]. Pokazano je da tokom ABI dolazi do povećane produkcije NO [37-39].

Uloga NO tokom ishemije mozga je složena [39]. Neuroprotektivna uloga NO se ogleda u poboljšanju protoka krvi posle ishemijskog događaja, pri čemu dolazi do vazodilatacije, inhibicije agregacije trombocita i adhezije leukocita [40, 41]. Međutim, pri velikim koncentracijama NO dolazi do povećanog afiniteta NO prema gvožđu i tiolnim grupama u proteinima, što dovodi do neurotoksičnosti [12, 42, 43]. Naime, NO reaguje sa superoksid anjonima pri čemu nastaju peroksinitriti, koji predstavljaju jake oksidante i tako narušavaju metabolizam

gvožđa [40, 44, 45]. Podaci iz literature ukazuju da štetni efekti NO u moždanom tkivu mogu biti povezani sa povećanim postishemijskim oslobađanjem ekscitatornih neurotransmitera [40, 46, 47]. Regulacija koncentracije NO može se ostvariti pomoću antagonista glutamatnog receptora, pošto povećan nivo NO inicira proizvodnju glutamata. Takođe, reperfuzija tkiva nakon ishemije povećava nivo oksigenacije tkiva, ali i znatnu produkciju NO i superoksida, što može dovesti do brzog porasta peroksinitrita [48].

Uloga azot monoksid sintaze (NOS) u stanjima ishemije mozga

NOS je enzim koji konvertuje aminokiselinu L-arginin (L-Arg) u L-citrulin, pri čemu NO nastaje kao krajnji produkt enzimske reakcije. Do sada su opisane tri izoforme NOS: neuronalna NOS (nNOS; tip-I; *engl. neuronal NOS*), inducibilna NOS (iNOS; tip-II; *engl. inducible NOS*) i endotelna NOS (eNOS; tip-III; *engl. endothelial NOS*) [49-51]. Tokom ishemije mozga kao i nakon reperfuzije tkiva dolazi do aktivacije sve tri izoforme NOS enzima, iNOS, eNOS i nNOS. [12, 47, 52]. Smatra se da tokom ishemije mozga dolazi do aktivacije nNOS u neuronima i glija ćelijama i do ponovnog preuzimanja glutamata u sinapsama. Pored toga, aktivacija NMDA receptora (*engl. N-methyl-D-aspartate receptors*) rezultuje povećanjem intracelularnog kalcijuma (Ca^{2+}) [52], što dodatno povećava aktivnost nNOS. Povećana aktivnost iNOS i eNOS u vaskularnom endotelu tokom ishemije mozga, verovatno potiče usled aktivacije u makrofagima i infiltriranim neutrofilima [53] tako da razvijanje novih strategija u cilju inhibicije ili inaktivacije NOS može predstavljati novi terapijski pristup pošto se zna da povećana aktivnost nNOS i iNOS prouzrokuje neurotoksičnost [39].

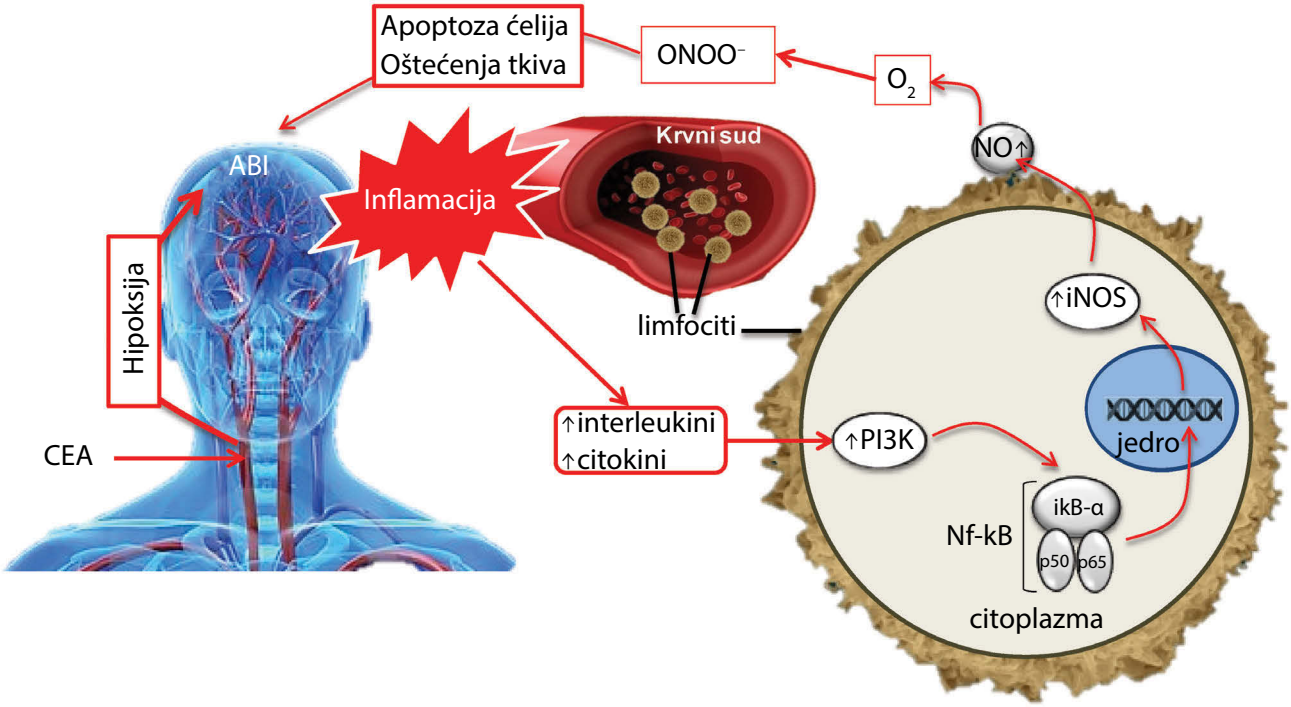
Enzim eNOS je pretežno vezan za ćelijsku membranu [54]. Kada je neaktivan, vezan je za kaveolin, dok povećanje intracelularne koncentracije jona Ca^{2+} dovodi do vezivanja kalmodulina za eNOS i aktiviranja ovog enzima. Aktiviranje eNOS zahteva dimerizaciju enzima, prisustvo supstrata L-arginina, i kofaktor BH4 (engl. (6R)-5,6,7,8-tetrahydro-L-biopterin). Stimulusi koji dovode do povećanja intracelularne koncentracije Ca^{2+} dovode i do sinteze NO. eNOS zahteva veću koncentraciju Ca^{2+} za svoju aktivnost od iNOS [55]. NO stvoren u niskim dozama od strane eNOS izoforme, funkcioniše kao signalni molekul u nekoliko bioloških procesa, uključujući i regulaciju vaskularnog tonusa, remodelovanje vaskulature (dilataciju i stanjivanje zida) i angiogenezu. Povećana ekspresija eNOS može imati važnu ulogu u regulisanju angiogeneze nakon moždanog udara [56]. Međutim, pokazano je da veoma visoke doze eNOS mogu uticati nepovoljno po organizam [57]. Naime, rizik od infarkta kod miševa sa injeciranim eNOS je značajno povećan 24 časa nakon okluzije srednje cerebralne arterije (MCA) u poređenju sa normalnim mišem [57].

Enzim iNOS je predominantno lokalizovan u citosolu ćelije [54]. iNOS je enzim sa velikom mogućnošću sinteze NO i može da sintetiše i do 1000 puta više NO od eNOS [15]. Ovako nastali NO može imati štetne efekte, jer u velikim koncentracijama reaguje sa superoksidnim anjonima, što dovodi do nastanka visoko reaktivnih vrsta O_2 [58]. Do ekspresije iNOS dolazi nakon indukcije, usled inflamacije posredovane citokinima-indukovanim faktorima transkripcije kao što je npr. nukleusni faktor-κB (NFκB; *engl. Nuclear Factor-κB*) koji se vezuje za elemente u okviru promotora gena za iNOS ili nekim drugim patofiziološkim stimulusima [59, 60]. Kalmodulin ostaje nekovalentno vezan za kompleks iNOS i stoga predstavlja suštinsku podjedinicu ove izoforme [61]. Zajedno sa drugim izoformama, postoje vezivna mesta za NADPH, FMN i FAD [62]. Aktivnost iNOS je pokazana u širokom spektru ćelija i tkiva [63-65], kao što su makrofazi [66], hondrociti [67], Kupferove ćelije, hepatociti [68, 69], neutrofili [70], zatim u pulmonarnom epitelu [71], limfocitima [72] i vaskulaturi [73]. Regulacija proizvodnje NO preko iNOS nužno se javlja tokom transkripcije i translacije [73]. Ekspresija iNOS gena i naknadno prevođenje iRNK je kontrolisana velikim brojem agonista, posebno proinflamatornim medijatorima. Citokini uključeni u regulaciju ekspresije iNOS su faktor nekroze tumora (TNF-α), interleukin-1β (IL–1β) i interferon-γ (IFN-γ), slobodne masne kiseline (SMK) koje učestvuju u imunskom odgovoru. Takođe je pokazano da u stanju hipoksije dolazi do povećane ekspresije iNOS [74].

Jedna od osnovnih razlika između eNOS i iNOS je u mehanizmu regulacije njihove aktivnosti. Enzim eNOS je konstitutivna izoforma NOS koja je Ca^{2+} i kalmodulin zavisna, odnosno njena aktivnost je regulisana nivoom intraćelijskog Ca^{2+}, dok je aktivnost iNOS, koji takođe sadrži kalmodulin, nezavisna od koncentracije Ca^{2+} [75]. Dakle, za aktivaciju eNOS je potrebna veća koncentracija Ca^{2+} u odnosu na iNOS [55]. Takođe, za razliku od eNOS koji je aktivan u fiziološkim uslovima, aktivacija iNOS nastaje kao odgovor na različite faktore poput citokina [76], endotoksina ili oksidativnog stresa [77] u različitim patofiziološkim uslovima [78].

Zaključak

Hipoksično-ishemična stanja mozga nastala tokom CEA, usled hipoperfuzije, predstavljaju glavni uzrok lošeg kliničkog ishoda [6, 8-11]. Razumevanje molekularnih mehanizama u regulaciji iNOS u patološkim procesima ABI nastale tokom CEA, je od izuzetne važnosti. Razvoj inflamacije, kao i narušena ravnoteža između oksidativnih i antioksidativnih procesa imaju važnu ulogu u patologiji ABI [12-14]. Važni medijatori inflamatornih i oksidativnih procesa su iNOS i NO, stoga bi jedan od potencijalnih terapijskih pristupa mogao biti i smanjenje aktivnosti i ekspresije iNOS i prekomerne produkcije NO, odnosno stimulisanje antioksidativne aktivnosti u krvi (**Slika 1.**).



Slika 1. Predloženi mehanizam regulacije aktivnosti i ekspresije iNOS tokom CEA. CEA- karotidna endarterektomija; **ABI–** akutna moždana ishemija; **PI3K–** fosfatidilinozitol-3 kinaza; **O_2-** kiseonik; **NO-** azot oksid; **iNOS-** inducibilna NOS; **NFκB-** nukleusni faktor kapa B; **IκBα-** I kapa B alfa; **$ONOO^-$** peroksinitrit; **↑–** fiziološko povećanje; (figura čoveka je preuzeta i modifikovana sa sajta http://www.dreamstime.com/royalty-free-stock-image-cardiovascular-system-image5564156).

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DIAGNOSTIC AND THERAPEUTIC MANAGEMENT OF PATIENTS WITH ADRENAL INCIDENTALOMA

DIJAGNOSTIČKE I TERAPIJSKE MERE LEČENJA PACIJENATA SA ADRENALNIM INCIDENTALOMOM

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Summary

Routine and frequent use of computerized tomography (CT) angiography in vascular disease detection has led to frequent suprarenal gland abnormal findings that could, if undiagnosed, significantly complicate the course of the future treatment. The term adrenal incidentaloma refers to adrenal lesion discovered serendipitously during an imaging investigation. Adrenal incidentalomas do not constitute a single pathological entity. Major concerns are risks of malignancy and autonomous hormone secretion. The majority of all adrenal incidentalomas (approximately 79%) are nonfunctioning benign lesions. Among functioning lesions subclinical cortisol excess is most frequently found. All patients with adrenal incidentaloma should undergo clinical, biochemical, and imaging evaluation for malignancy and hormone production. In this review, we discuss the current issues in diagnostic and therapeutic management of patients with adrenal incidentaloma. Follow-up of patients with adrenal incidentalomas involves the assessment of growth and development of hormonal function. After this review analysis several useful protocols could be designed to help vascular surgeons to adequately treat patients with concomitant vascular disease and adrenal incidentaloma.

***Key words**: adrenal gland, incidentaloma, tumor, autonomous hormone production, functional testing, imaging.*

Sažetak

Rutinska i učestala upotreba kompjuterizovane tomografije (CT) angiografije pri dijagnostici vaskularnih oboljenja dovela je do čestog otkrivanja promena na nadbubrežnim žlezdama koje u značajnoj meri mogu kompikovati ishod lečenja ovih pacijenata. Termin adrenalni incidentalomi se odnosi na adrenalni leziju slučajno otkrivenu tokom imidžing procedura. Adrenalni incidentalomi ne predstavljaju pojedinačni patološki entitet. Najveća briga predstavlja rizik od maligniteta i hormonske aktivnosti. Većina od svih adrenalnih incidentaloma (približno 79%) su nefunkcionalne benigne lezije. Među funkcionalnim lezijama najčešće se susreću pacijenti sa subkliničkim kortizolskim ekscesom. Svi pacijenti sa adrenalnim incidentalomima trebaju biti podvrgnuti kliničkoj,biohemijskoj i imidžing evaluaciji za malignitet i hormonsku aktivnost. U ovom radu mi razmatramo aktuele dijagnostičke i terapijske probleme vezane za pacijentesa adrenalnim incidenralomima. Praćenje ovih pacijenata obuhvata praćenje rasta i hormonske funkcije adrenalnih incidentaloma. Nakon analiziranja ovog pregleda, moguće je napraviti nekoliko korisnih protokola koji bi pomogli vaskularnim hirurzima u adekvatnom lečenju pacijenata sa udruženim vaskularnim oboljenjima i adrenalnim incidentalomima.

***Ključne reči:** nadbubrežna žlezda, incidentalom, tumor, nezavisna hormonska produkcija, funkcionalno testiranje, imidžing.*

Introduction

Adrenal incidentaloma (AI) is an adrenal mass, generally 1 cm in diameter or larger, discovered through imaging study performed for indications other than evaluation of adrenal disorder. (1) Adrenal incidentalomas are found in the adrenal cortex or medulla and may be hormonally active or nonfunctional, malignant or benign. (2) The prevalence of AI at autopsy was found to be less than 1% in patients younger than 30 years old and up to 7% in patients aged 70 or older. (3) Imaging studies yielded similar findings: AI were found in approximately 2–4% of the middle aged, increasing up to more than 10% in the elderly, peaking between the fifth and the seventh decade. (4) Because of its increasing prevalence, AI is now recognized as a common clinical problem and has even been proclaimed as “endocrine epidemic of A-I-D-S” – Adrenal Incidentaloma Discovered Serendipitously. (5)

The prospectively validated management of a patient with AI has not been established although a “state of the science statement” and Medical guidelines for the management of adrenal incidentalomas by have been published by the National Institute of Health and American

Table 1. Etiology of incidentally found adrenal mass (adrenal incidentaloma)

1. ***Adrenal cortical lesions***
   - Adenoma
   - Adenocortical carcinoma
   - Nodular hyperplasia
2. ***Adrenal medullary tumors***
   - Pheochromocytoma
   - Ganglioneuroma/neuroblastoma
3. ***Other adrenal tumors***
   - Myelolipoma, lipoma, lymphoma, haemangioma, hamartoma, teratoma
4. ***Metastasis***
   - Lung, breast, kidney, gastrointestinal tract tumors, lymphoma, melanoma
5. ***Infection***
   - Abscess, tuberculosis, fungi, cytomegalovirus
6. ***Infiltration***
   - Amyloidosis, sarcoidosis
7. ***Cysts and pseudocysts***
   - Parasitic, endothelial, degenerative adenoma
8. ***Haemorrhage***
9. ***Pseudoadrenal masses***
   - Stomach, pancreas, kidney, spleen, liver, vascular lesions (e.g. aneurysms and tortuous splenic veins)

Association of Clinical Endocrinologists and American Association of Endocrine Surgeons (AACE/AAES), respectively. (6,7) The challenge is to recognize and treat an infrequent AI that involves a significant risk, either because of hormonal activity or because of its malignancy. (8) Adrenal incidentalomas do not constitute a single pathological entity. Etiology underlying incidentally discovered adrenal mass is presented in Table 1.

We will discuss the diagnostic and therapeutic approach in the following cases of AI: nonfunctional benign adrenal mass, hormone – secreting adenomas, adrenal nodular hyperplasia, pheochromocytoma and adrenal malignancy.

Clinical approach in a case of AI

Nonfunctioning benign adrenal mass

Approximately 79% of all AIs are nonfunctioning benign lesions including adrenocortical adenoma, myelolipoma, ganglioneuroma and adrenal cyst. If functional screening is negative, the diagnosis is most likely nonfunctioning adenoma, the most frequently found adrenal tumor of all. (9)

Adrenocortical adenomas usually appear as non – highly vascular, homogenous lesions with smooth, encapsulated margins. In some isolated cases, these lesions progress to functioning tumors. (14)

Imaging studies cannot reliably distinguish between functioning and nonfunctioning adrenal adenomas. The second most commonly found benign adrenal mass is myelolipoma, a tumor consisted of fat and bone marrow elements. Due to its structure myelolipoma presents with characteristic imaging appearance. (9)

Cortisol producing-adenoma

Autonomous cortisol secretion was found in 5.3% of patients with AI. The autonomous cortisol production is referred to as subclinical hypercortisolemia because these patients lack full phenotypic manifestations of Cushing syndrome. However, these patients are more likely to suffer from obesity, hypertension, diabetes mellitus, and osteoporosis. (10) Patients with subclinical hypercortisolemia usually have normal values of morning cortisol level. However, disturbances in the circadian rhythm of cortisol excretion may occur in the form of slightly increased nocturnal cortisol concentrations. (11) Adrenal autonomy is best assessed by dexamethasone suppression test. The test consists of the administration of 1 mg of dexamethasone (3 mg of dexamethasone may be also applied) at 11pm followed by the measurement of morning serum cortisol concentration at 8am. The result of cortisol level greater than 138 nmol/L (5 μg/dL) is standardly used to define autonomous cortisol production. (12) The use of a much lower cut – off level of 50 nmol/L increases the sensitivity, but it also increases the rate of false positive results. (13) The result of <1.8 μg/dL has been proposed as the best negative predictive value.

The following tests can be performed either to confirm or rule out autonomous cortisol production: 2 – day high – dose dexamethasone suppression test, late – night salivary cortisol measurement and 24 – hour urinary free cortisol test. Cortisol value of 70 nmol/L measured after 2 mg daily dexamethasone (for 48h) is used by some as a cut off value for identifying patients with autonomous cortisol secretion. (13) Low adrenocorticotropic hormone (ACTH) concentration can be found in patients with autonomous cortisol production due to suppression. On the other hand, low dehydroepiandrosterone – sulphate level may also be found as a result of insufficient ACTH production. Adrenal scintigraphy may be used for assessing lesion functionality.

A reasonable strategy is to consider adrenalectomy in younger patients (<40 of age), those with low ACTH levels, and in cases of autonomous cortisol production – associated obesity, hypertension, diabetes mellitus and osteopenia. A patient with subclinical hypercortisolemia should receive glucocorticoid therapy peri- and postoperatively because of the risk of acute adrenal insufficiency. (1,14,15) Glucocorticoid therapy may last 6 to 18 months after unilateral adrenalectomy.(7)

Primary hyperaldosteronism: aldosterone – producing adenoma and adrenal nodular hyperplasia

Primary hyperaldosteronism is caused by aldosterone – producing adrenal masses, mostly by adenoma or nodular hyperplasia. Adrenal nodular (micronodular or macronodular) hyperplasia may involve one or both adrenal glands and is more frequent than aldosterone – producing adenoma. Primary hyperaldosteronism is the most common cause of secondary hypertension while approximately 1% of adrenal incidentalomas have proved to be aldosterone – producing adenomas. (1,9) Primary hyperaldosteronism should be suspected in the cases with early onset of hypertension, usually refractory to medication, and hypokalemia. However, these patients may have normal levels of potassium in the blood. The measurements of plasma aldosterone concentration (PAC) and plasma renin activity (PRA) are used to calculate PAC/PRA ratio (APR) which is the most commonly used test for screening of primary hyperaldosteronism. (16) The APR is most sensitive when blood is obtained 2h after waking up in the morning, in upright position, after a brief period of rest. An elevated PAC (>20 ng/dL) and a high APR (>30) are highly indicative for hyperaldosteronism. (9) However, the cut-off for a positive result may be laboratory dependent. Thus, PAC≥15 ng/dL and APR ≥20 have also been used as indicators for aldosterone – producing adrenal masses. (1) The interpretation of test results should be noted in caution in patients receiving some medications including antihypertensives, and whenever possible, such therapy should be discontinued prior to testing. (1,9) The negative suppression of aldosterone level after a salt challenge may be used as additional confirmatory test for primary hyperaldosteronism. (17)

When primary hyperaldosteronism is diagnosed it is important to determine the subtype of the disease. Patients with aldosterone – producing adenoma tend to be younger (<40 years old), have more severe hypertension and hypokalemia, and have higher PAC than those with adrenal nodular hyperplasia. However, there is no specific biochemical evaluation that reliably differentiates the subtype of primary hyperaldosteronism. (9) The proposed therapy for aldosterone – producing adenoma is surgical (in patients with unilateral source of aldosterone excess). However, preoperative adrenal vein sampling is mandatory for surgical decision if CT scan shows bilateral or no lesions (especially in patients over 40 years old) to exclude bilateral adrenal hyperplasia that is treated with selective and nonselective mineralocorticoid receptor blockers. (7,18)

Sex hormones-producing adenoma

Sex hormone – producing adrenocortical tumors are rare and typically occur in the presence of clinical manifestations of autonomous hormone secretion. Routine screening for excess androgens or estrogens in patients with adrenal incidentalomas is therefore not recommended. (1)

Clinically silent pheochromocytoma

Approximately 4 – 7% of AIs have proved to be clinically silent pheochromocytomas. (19) These tumors may be benign or malignant. Even when clinically silent this tumor can be lethal. (20) In approximately 25% of patients, pheochromocytoma is associated with familial syndromes (multiple endocrine neoplasia type 2, von Hippel-Lindau disease), thus genetic testing should be performed, especially in young patient. (7)

Radiological findings indicative for pheochromocytoma consist of increased attenuation on unenhanced CT, prominent vascularity of the mass, delayed washout of contrast medium, and high signal intensity on T2 – weighted MRI. (21) Biochemical assessment for pheochromocytoma is necessary in all patients with AI.

Measuring fractionated metanephrines and catecholamines in a 24 – hour urine are most widely used biochemical tests for diagnosis of pheochromocytoma. The additional measurement of fractionated catecholamines in the 24-hour urinary specimen is helpful in diagnosing dopamine – secreting pheochromocytoma. The measurement of plasma free metanephrines can also be used, but the value of this test is limited because of its low specificity. (22) The use of tricyclic antidepressants, decongestants, amphetamines, reserpine, and phenoxybenzamine should be discontinued to eliminate false – positive results. Surgical resection should be performed in all patients with pheochromocytoma and α-adrenergic blocking agent (phenoxybenzamine, doxazosin, or phenoxybenzamine/metyrosine) should be administered 1 to 3 weeks preoperatively. Long – term follow – up is advised because 10% to 15% of pheochromocytomas were found to be recurrent. (7)

Primary adrenocortical carcinoma

Primary adrenocortical carcinoma is a rare tumor with poor prognosis. The estimated prevalence of adrenal carcinoma in the general population is approximately 12 cases per 1 000 000. (23) Among the patients with adrenal incidentalomas, adrenocortical carcinoma was found in 4.7% of cases. (10) Although adrenocortical carcinoma can develop at any age, a bimodal age distribution was found, with the disease peaking before the age of 5 and in the 4th to 5th decade of life. (2) This tumor is functional in two thirds of cases: hypercortisolemia either alone or in association with virilisation is the most frequent presentation. Estrogen- and aldosterone- secreting adrenal carcinomas are rarely found. (8,24) The tumor size determines the risk of malignancy: adrenal cortical carcinoma accounts for 2% of AI up to 4 cm in size, 6% of tumors between 4.1 cm and 6 cm and 25% of tumors larger than 6 cm. (25) Imaging methods are helpful in differing between adrenocortical malignancy and benign lesions. Using CT, higher pre-contrast attenuation values (>10 Hounsfield Units) are usually obtained for malignant lesions because they generally contain less lipids in comparison to adrenal adenomas. After contrast administration, adrenocortical carcinomas present as inhomogeneous lesions with irregular borders and low percentage of contrast washout (<40%). MRI and CT have comparable accuracy in differentiation between benign and malignant adrenal lesions.

Adrenocortical carcinomas usually present as hypointense lesions on both MRI T1- and T2-weighted images with strong enhancement after contrast injection and delayed washout. (26, 27) Adrenocortical carcinoma can also be visualized by positron emission tomography (PET). Open adrenalectomy is the procedure of choice in the treatment of primary adrenocortical carcinoma.

Adrenal metastasis

Adrenal metastases account for 50 – 75% of all AI in patients with history of malignant disease. Adrenal metastases are bilateral in 10 – 15% of cases, they are usually larger than 3 cm and most frequently originate from primary tumors of the lung, breast, kidney, gastrointestinal tract, and melanoma or lymphoma. Adrenal hypofunction may occur due to tumor growth. In addition to imaging techniques, CT – guided fine – needle aspiration biopsy may be applied. Pheochromocytoma should always be excluded before fine – needle aspiration biopsy in order to avoid the potential hypertensive crisis. (8) If PET is performed, most malignant lesions will show avidity for [^{18}F]-fluorodeoxyglucose. However, it is difficult to separate primary adrenocortical carcinoma from adrenal metastasis by using PET scan.

Follow-up of patients with AI

Any adrenal mass with concerning imaging characteristics and/or lesions ≥4 cm should be surgically removed because of an increased risk of adrenal carcinoma. Follow – up of patients with adrenal incidentaloms involves assessment for growth and development of hormonal function.

The increase in size of a benign – appearing adrenal mass is used to screen for malignancy. Approximately 15% of AI increase in size during follow – up or may even shrink over time. There is no adrenal mass growth cut-off that can reliably confirm or exclude a malignant lesion. Patients with AI <4 cm and with radiologic characteristics consistent with benign lesions need to have imaging re-evaluation at 3 to 6 months and then annually for 1 to 2 years.(7) However, some recommend first scan within 6 months followed by annual CT for up to 4 – 5 years. Importantly, repeated CT scans increase the chance of causing fatal malignancy due to ionizing radiation. (8) If adrenal tumor grows (≥0.8 cm or ≥1 cm) and/or become hormonally active during follow – up, surgical removal should be considered. (28,7)

Hormonal evaluation should be performed at the time of diagnosis and then annually for up to 5 years. (7) The risk of hormonal progression was found to increase in the first 3 years and then remain stable. There is an opinion that hormonal follow – up in patients with non – functional AI ≤ 2 cm is probably of limited value because these lesions rarely progress in size or become functional. (8)

The impact of adrenal incidentaloma on cardiovascular pathology

Patients with adrenal incidentalomas have been reported to have an increased risk for cardiovascular diseases. (29) Accordingly, subtle cortisol production was found to be an independent risk factor for hypertension in patients with AI. (30) Moreover, patients with subclinical hypercortisolemia were found to have increased prevalence of adverse metabolic and cardiovascular outcomes. (31) Increased prevalence of insulin resistance, a major risk factor for cardiovascular events, has been reported in patients with functional AI. (32,33) Decreased insulin sensitivity was also reported in patients with nonfunctional AI. (34) Notably, patients with nonfunctional AI were found to have increased carotid intima media thickness. (35) and impaired cardiac morphology and function. (36) It has been suggested that some degree of adrenal autonomy that is not recognized by current methods, is responsible for increased cardiometabolic risk in patients with nonfunctional AI. (29) Accordingly, the level of *in vitro* steroid production was found to be similar in hormonally inactive adrenocortical tumors and subclinical hypercortisolism/overt Cushing syndrome suggesting cortisol autonomy in silent adrenal lesions. (37) Thus, increased prevalence of AI in patients with cardiovascular diseases is not only due to frequent use of imaging methods, but it is partly influenced by impact of AI on cardiovascular system.

Concluding remarks

According to the AACE/AAES guidelines, all patients with adrenal incidentaloma should undergo clinical, biochemical, and imaging evaluation for hypercortisolism, aldosteronism (if hypertensive), the presence of a pheochromocytoma, or a malignant tumor. (7) Surgical removal should be applied in lesions ≥4 cm, pheochromocytoma, aldosterone producing – adenomas and unilateral nodular hyperplasia, cortisol – producing lesions with adverse clinical manifestations of hypersecretion, and in those with proved/suspected primary adrenocortical carcinoma. There is no definite recommendation for AI follow – up. Radiological reevaluation is advised in first 6 months, and then annually for 1 – 5 years. Hormonal screening should be done annually for 5 years. However, hormonal follow – up may be questioned for non – functioning AI which is ≤ 2 cm in size. Surgical removal should be considered in patients with tumor growth (≥0.8 cm or ≥1 cm) and/or *de novo* hormonal activity. After this review analysis several useful protocols could be made to help vascular surgeons to adequately treat patients with concomitant vascular disease and adrenal incidentalomas.

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VASCULAR AGE AND CAROTID INTIMA – MEDIA THICKNESS

VASKULARNA STAROST I DEBLJINA INTIMO-MEDIJALNOG KOMPLEKSA KAROTIDNIH ARTERIJA

Brankica Tepavčević[1], Sandra Radak[2,3]

Summary

Measurement of carotid intima – media thickness with B – mode ultrasound is a noninvasive and highly reproducible technique for detecting and quantifying subclinical atherosclerosis. Several large, prospective, epidemiologic studies have shown that this method accurately identifies prevalence and incidence of cardiovascular disease, independently of traditional risk factors. The value of this method is that it can be used to determine patients' vascular age. Measurement of carotid intima – media thickness reveals current atherosclerotic burden, it is feasible in a clinical setting and it can be integrated into coronary heart disease risk assessment models.

Vascular age is substitution for chronological age and it is used in order to improve coronary heart disease risk prediction taking current carotid atherosclerotic burden into account.

Key words: *vascular age, atherosclerosis, cardiovascular diseases, prevention, risk factors.*

Sažetak

Merenje debljine intimo-medijalnog kompleksa karotidnih arterija B modom ultrazvuka, je neinvzivna tehnika i pouzdana metoda za detekciju i kvantifikaciju subkliničke ateroskleroze. Više velikih prospektivnih epidemioloških studija pokazuju da ova metoda precizno identifikuje prevalencu i incidencu kardiovaskularnih bolesti u odnosu na tradicionalne riziko faktore. Vrednost metode je u tome što se može koristi za odredjivanje vaskularne starosti bolesnika. Merenje debljine intimo-medijalnog kompleksa može nam kvantitativno pokazati aterosklerotska oštećenja, koja možemo koristiti u proceni postojećeg rizika za kardiovaskularne bolesti i proceniti vaskularnu starost svakog pacijenta. Vaskularna starost je zamena za hronološku starost i koristi se da bi se unapredilo davanje prognoze i izračunavanje rizika za oboljenje koronarnih arterija u zavisnosti od aterosklerotiskih oštećenja na karotidnim arterijama.

Ključne reči: *vaskularna starost, ateroskleroza, kardiovaskularne bolesti, prevencija, faktori rizika*

Introduction

Measurement of carotid intima – media thickness (CIMT) with B – mode ultrasound is a noninvasive and highly reproducible technique for quantifying atherosclerotic burden. It is a well – validated research tool, but it is not used widely as a clinical tool, even though the American Heart Association Prevention Conference V concluded that CIMT could be considered for further clarification of coronary heart disease (CHD) risk assessment (1). Several studies have demonstrated that CIMT predicts future cardiovascular events (1,2). For demonstration purposes, the Atherosclerosis Risk in Communities (ARIC) study will be focused on, because it has a well – defined scanning protocol and published data (2). In ARIC, increasing CIMT identified prevalent cardiovascular disease including angina, myocardial infarction, stroke, transient ischemic attack, and peripheral vascular disease. More importantly, the presence of increased CIMT predicted future CHD events, both for men and women (8).

Framingham CHD risk estimates are strongly influenced by chronological age; however, atherosclerotic burdens of individuals with the same chronological age and similar risk profiles can differ substantially (1,3). An imaging test that quantifies atherosclerotic burden and that can be integrated with existing risk stratification paradigms, could be a very useful clinical tool (3).

A rich database from several clinical and epidemiologic trials, that used CIMT, provides an opportunity to adjust a patient's chronological age to their atherosclerotic burden, a concept that is called "vascular age" (8). For example, a 45 – year – old white man who has a CIMT of 0.8 mm is actually a 60 – year – old male (vascular age) based on the median CIMT value, taking sex and race into account (2,3).

Vascular health screening program should use vascular age as a part of the clinical risk prediction program. The standard imaging protocol from the ARIC study, to scan 1 – cm segments in each carotid artery, can be used. For each patient, vascular age is estimated using a statistical model on the basis of published nomograms from ARIC study using their sex, race, chronological age, and CIMT value (7).

Carotid Ultrasound Imaging

The carotid arteries were imaged with an 8.0 MHz linear array ultrasound transducer. The common carotid artery segment was defined as the distal 1 cm of the common carotid artery, immediately proximal to the origin of the bulb.

Determination of Vascular Age

Vascular age was determined by linear regression modeling using published nomograms of CIMT percentiles (5th, 10th, 25th, 50th, 75th, 90th, and 95th) according to chronological age, race and gender (6). Linear and nonlinear regression models were constructed for each of the CIMT percentile functions for each carotid arterial segment. Composite CIMT values were used to determine vascular age, defined as the age at which the composite CIMT value for an individual of a given race and gender would represent the median value (50th percentile) in the ARIC study. Specifically, the linear 50th percentile function by chronological age, gender, and race was used to project the age of each subject based on their composite CIMT value. If each of a given subject's segmental CIMT values were at the 50th percentile for their chronological age, gender, and race, their composite CIMT would be at the 50th percentile and their vascular age would be equal to their chronological age. For example, a 45 –year – old black female with a composite CIMT of 0.593 mm would have a CIMT percentile of 50% and a vascular age of 45 years; however, a 45 – year – old black female with a composite CIMT of 0.678 mm would have a CIMT percentile

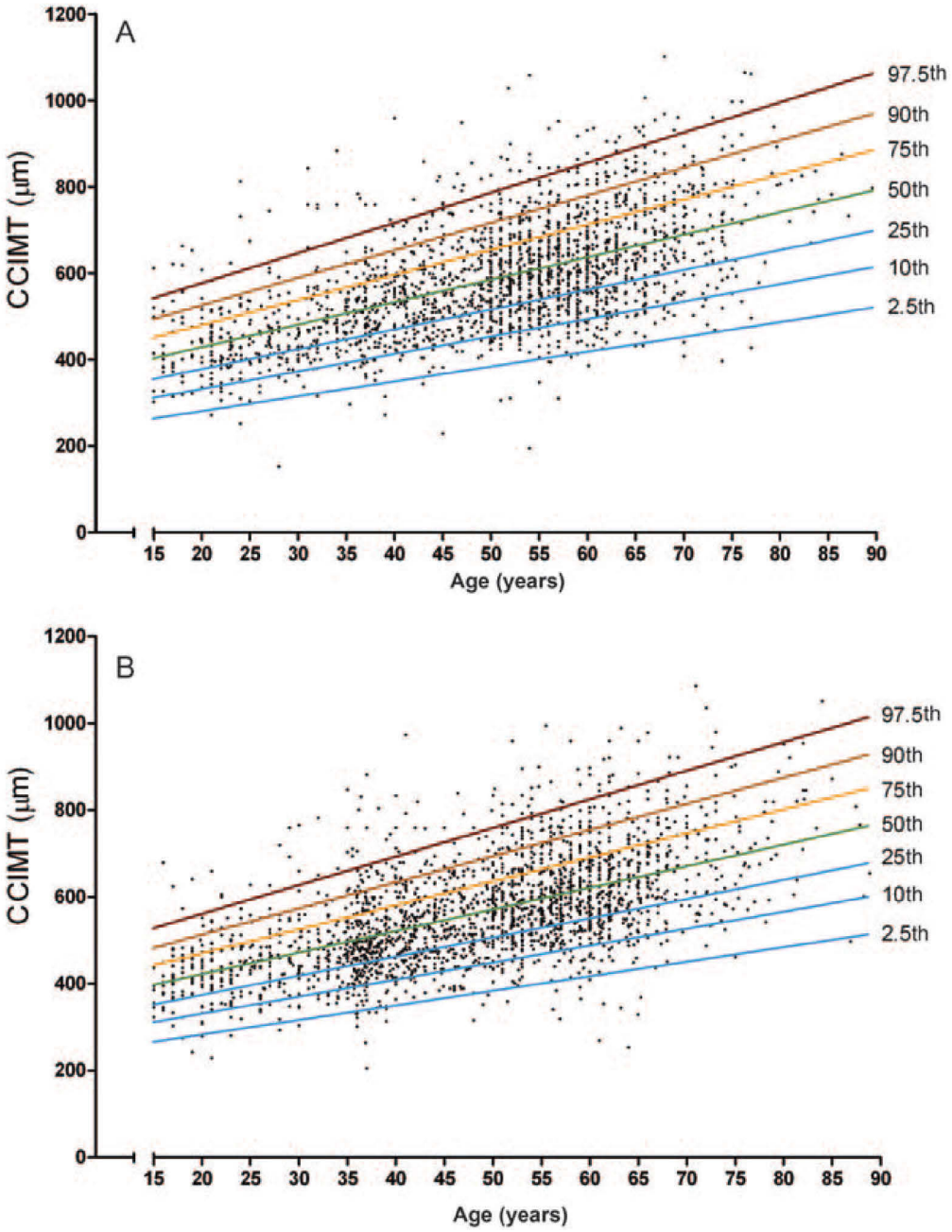

Figure 1. Age-specific percentiles of common carotid artery intima – media thickness (CCIMT) in healthy sub – population: (A) men; (B) women (9).

of 71% and a vascular age of 55 years. Finally, vascular age was substituted for chronological age in the Framingham CHD risk prediction model, resulting in modified CHD risk estimates.

Discussion

The CIMT measurement can be used in conjunction with well validated and previously published population norms to determine vascular age (4). Vascular age represents an atherosclerotic burden, which varies between individuals of the same chronological age despite similar CHD risk profiles. Thus, population – based risk estimates can be modified by this direct assessment of an individual's current atherosclerotic burden. When vascular age replaced chronological age in CHD risk prediction algorithms, an estimated CHD risk was altered substantially. Evaluating atherosclerotic burden using CIMT may help individualize therapy for the primary prevention of CHD events.

Like all ultrasound techniques, determining CIMT requires training of sonographers and readers, as well as strict attention to quality control. Training programs for determining CIMT in research and clinical settings have been established. The reproducibility of this test in our clinical laboratory is similar to that reported in the literature (3-5). Since high-resolution vascular ultrasound transducers, modern ultrasound machines, and sonographers are available in most active clinical environments, the assessment of CIMT appears to be ready for mainstream use (8).

Conclusion

Measurement of CIMT is feasible in a clinical setting, and its use to determine vascular age can alter CHD risk assessment. Determining patients' vascular age could potentially improve the applicability of population – based CHD risk estimates to the management of an individual patient by accounting for age – related variation in atherosclerotic burden. CIMT measurement might help to identify previously unrecognized high – risk individuals and could help clinicians with better primary prevention strategies for an individual patient.

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Prof. dr Đorđe Radak

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