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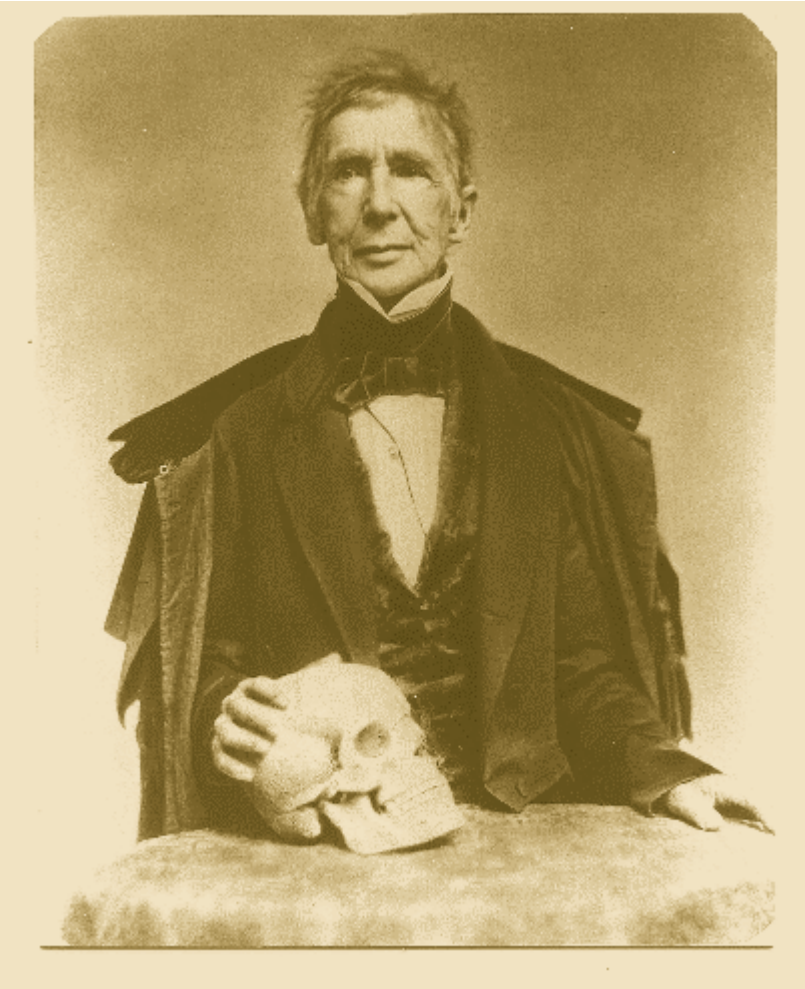

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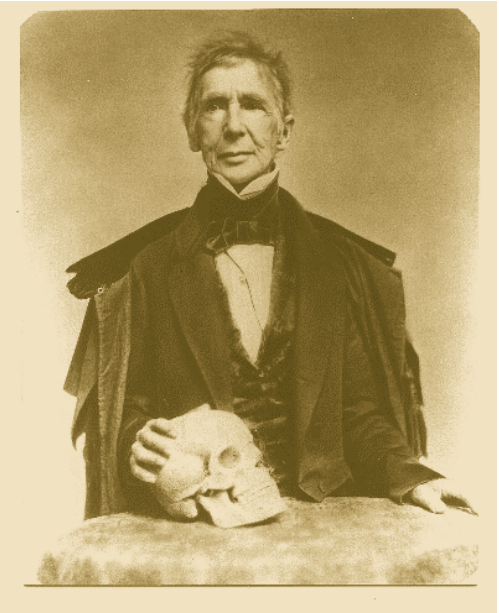


Dr John Collins Warren (1. avgust 1778 – 4. maj 1856), jedan od najčuvenijih američkih hirurga u 19. veku, prvi dekan *Harvard Medical School* (1816–1819) i jedan od osnivača *Massachusetts General Hospital* u Bostonu, najzaslužniji je za pokretanje časopisa *The New England Journal of Medicine and Surgery, and Collateral Branches of Science* (prvi broj izišao u januaru 1812), danas čuvenog *The New England Journal of Medicine* (NEJM). Dr Warren bio je autor prvog članka objavljenog u NEJM tačno pre 200 godina (vidi Uvodnik str. 139–40).

Dr. John Collins Waren (August 1, 1778 – May 4, 1856), one of the most famous American surgeons of the 19th century, was the first dean of Harvard Medical School and one of the founders of Massachusetts General Hospital. He played a leading role in establishing The New England Journal of Medicine and Surgery, and Collateral Branches of Science (first issue published in January 1812) which subsequently evolved into today`s The New England Journal of Medicine. He was the author of the first article published in the Journal exactly 200 years ago (see Editorial p. 139–40).

E D I T O R I A L

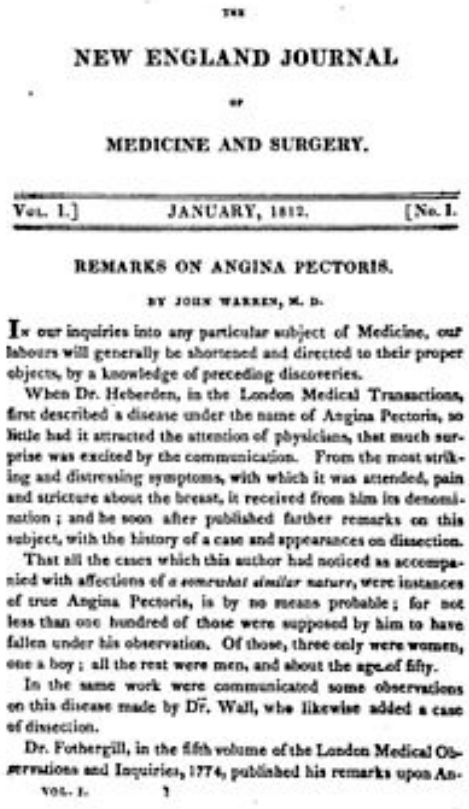
The Big Anniversary – 200 Years of The New England Journal of Medicine

Veliki jubilej – 200 godina *The New England Journal of Medicine*

Slobodan Obradović*, Dragana Obradović†

Military Medical Academy, *Clinic of Emergency Medicine, †Clinic of Neurology,
Belgrade, Serbia

In January 2012 The New England Journal of Medicine celebrates 200 years from the first issue so being the oldest medical journal which has been continually published. The Journal was founded by the Boston surgeons John Collins Warren, James Jackson, the founder of the Massachusetts General Hospital [1] and their distinguished colleagues. It was published under the name The New England Journal of Medicine and Surgery, and the Collateral Branches of Science. Since 1928 the Journal has had the today's name. The very first article was the paper "Remarks on Angina Pectoris" by John C. Warren [2] (Figure 1). In the last 200 years so many articles opening the new pages in the world of medi-



THE

NEW ENGLAND JOURNAL

OF

MEDICINE AND SURGERY.

VOL. I.] JANUARY, 1812. [No. I.

REMARKS ON ANGINA PECTORIS.

BY JOHN WARREN, M. D.

In our inquiries into any particular subject of Medicine, our labours will generally be shortened and directed to their proper objects, by a knowledge of preceding discoveries.

When Dr. Heberden, in the London Medical Transactions, first described a disease under the name of Angina Pectoris, so little had it attracted the attention of physicians, that much surprise was excited by the communication. From the most striking and distressing symptoms, with which it was attended, pain and stricture about the breast, it received from him its denomination; and he soon after published farther remarks on this subject, with the history of a case and appearances on dissection.

That all the cases which this author had noticed as accompanied with affections of *a somewhat similar nature*, were instances of true Angina Pectoris, is by no means probable; for not less than one hundred of those were supposed by him to have fallen under his observation. Of those, three only were women, one a boy; all the rest were men, and about the age of fifty.

In the same work were communicated some observations on this disease made by Dr. Wall, who likewise added a case of dissection.

Dr. Fothergill, in the fifth volume of the London Medical Observations and Inquiries, 1774, published his remarks upon An-

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Fig. 1 – The first article in the first issue of The New England Journal of Medicine (January 1812), at that time The New England Journal of Medicine and Surgery, and the Collateral Branches of Science

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cine have been published in the Journal. Today The New England Journal of Medicine is the leading medical journal in the world covering general and internal medicine with the impact factor of 53.486.

What is so special about the Journal? It is widely clinically orientated publishing the results of breakthrough clinical studies which become a part of the treatment guidelines for almost all branches of medicine. The meticulous and detailed presentation of the whole relevant data for each study are presented with full respect of ethical principles and understandable, practical end-points. Review articles correspond to important medical problems covering all relevant cutting-edge knowledge summarized into the useful information for the broad spectrum of different specialists with the state of art schemes, drawings, graphs and tables. Large epidemiologic studies reveal important data about trend of incidence, prevalence and treatment strategy for major diseases. Thankful for that we can realize what the results of medical efforts are in the struggle with major diseases of the mankind. Discoveries and detailed descriptions of new diseases, such as AIDS in the early eighties, are the Journal's specialty. However, the Journal never forgets rare diseases and the valuable attempts to solve the problems in these areas of medicine. Revolutionary therapeutic modalities and diagnostic tools such as percutaneous coronary interventions and the use of troponins for the diagnosis of myocardial infarction also entered the clinical practice through the gate of the Journal.

Case records of the Massachusetts General Hospital are the priceless treasure of the Journal. They are better than Sherlock Holmes stories, full of mystery, drama, different paths, and sophisticated details with a true solution at the end of the long labyrinth of medical knowledge. Images in Clinical Medicine are the parts of a never-ending Atlas written by the endless number of true medicine lovers.

The history of contemporary medicine is mirrored through the history of The New England Journal of Medicine. This is not the big anniversary only for the Journal; this is the Anniversary for all health care professionals around the world. Congratulations!!!

R E F E R E N C E S

1. *Brandt AM.* A reader's guide to 200 years of the New England Journal of Medicine. N Engl J Med 2012; 366: 1–7.
2. *Warren JC.* Remarks on angina pectoris. N Engl J Med 1812; 1: 1–11.

The Publisher, the Editorial Board and the Editorial Staff of the *Vojnosanitetski pregled* sincerely join to these congratulations, wishing The New England Journal of Medicine continues to be the leader in dissemination of medical knowledge worldwide.

ORIGINALNI ČLANCI

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Određivanje morfina, kodeina i 6-monoacetilmorfina metodom HPLC/MS u salivi heroinskih zavisnika

Determination of morphine, codeine and 6-monoacetylmorphine in saliva of substance-abuse patients using HPLC/MS methods

Vesna Milovanović*, Biljana Ćirić†, Jasna Milenković§, Vesna Kilibarda†, Marijana Ćurčić¶, Slavica Vučinić‡, Biljana Antonijević¶

***Agencija za hemikalije Republike Srbije, Beograd, Srbija; Vojnomedicinska akademija, Centar za kontrolu trovanja,** †Klinika za urgentnu i kliničku toksikologiju, ‡Odeljenje za toksikološku hemiju**, Beograd, Srbija;** §**Dom zdravlja „Dr Milutin Ivković“,** Služba laboratorijske dijagnostike, **Beograd, Srbija;** ¶**Univerzitet u Beogradu,** Farmaceutski fakultet, Katedra za toksikologiju „Akademik Danilo Soldatović“, **Beograd, Srbija**

Apstrakt

Uvod/Cilj. Saliva predstavlja alternativni matriks za identifikaciju sredstava zloupotrebe. Cilj ovog rada bio je optimizacija metode pripreme uzorka salive i određivanja metabolita heroina, morfina i 6-monoacetilmorfina (6-mam), i kodeina *liquid chromatography-mass spectrometry* (LC/MS) metodom i provera metode u realnim uslovima kod heroinomana. **Metode.** Priprema uzoraka vršena je tečno-tečnom ekstrakcijom uz smešu hloroforma i izopropil alkohola u odnosu 9 : 1. Ekstrakti su analizirani tehnikom HPLC/MS: razdvajanje na koloni Waters Spherisorb® 5 μm, ODS2, 4,6 × 100 mm, vršeno je primenom mobilne faze amonijum-acetat : acetonitril u odnosu 80 : 20 pri protoku od 0,3 mL/min. Masena detekcija je vršena u opsegu masa od 100 do 400 m/z. Primenjene su regresiona i korelaciona analiza za nivo verovatnoće 0,05. Određivanje prisustva morfina, kodeina i 6-mam vršeno je u uzorcima salive kod osoba kod kojih je test trakama utvrđeno prisustvo „opijata“ u urinu. **Rezultati.** Kalibracija je vršena u opsegu koncentracija 0,1–1 mg/L sa koeficijentom determinacije $R^2 > 0,99$. Dobijene su kalibracione krive: za morfin, y = 385531x + 14584; kodein, y = 398036x + 31542 i 6-monoacetilmorfin, y = 524162x – 27105. *Recovery* vrednosti za određivanje morfina i kodeina iznosile su 99%, a za 6-mam 94%. Limit detekcije predložene metode iznosio je 0,01 mg/L, a limit kvantifikacije 0,05 mg/L. U salivi uživalaca heroina koncentracija morfina kretala se u opsegu od 0,54 do 5,82 mg/L, kodeina od 0,05 do 5,33, a 6-mam od 0,01 do 0,68 mg/L i dobijena je statistički značajna korelacija između vrednosti za kodein i 6-mam. **Zaključak.** Predložena HPLC/MS metoda za određivanje sadržaja morfina, kodeina i 6-monoacetilmorfina u salivi je tačna, jednostavna, ekonomična i pogodna za rutinsku primenu, kao i za biomonitoring zloupotrebe heroina.

Ključne reči:
pljuvačka; morfin; kodein; hromatografija, tečna, pod vp; spektrometrija mase; heroin.

Abstract

Background/Aim. Saliva represents an alternative specimen for substances abuse determination in toxicology. Hence, the aim of this study was to optimize a method for saliva specimen preparation for heroin metabolites, morphine and 6-monoacetylmorphine (6-mam), and codeine determination by liquid chromatography-mass spectrometry (LC/MS), and to apply this method on saliva samples taken from the patients. **Methods.** Saliva specimen was prepared using liqiud/liquid extraction of morphine, codeine and 6-mam by mixture of chloroform and isopropanol (9 : 1; v/v). Extracts were analysed by HPLC/MS technique: separation column Waters Spherisorb® 5 μm, ODS2, 4.6 × 100 mm; mobile phase: ammonium acetate : acetonitile (80 : 20; v/v), mobile phase flow rate 0.3 mL/min; mass detection range: 100–400 m/z. Regression and correlation analyses were performed with the probalility level of 0.05. Concentrations of morphine, codeine and 6-mam were determined in saliva samples of the patients with “opiates” in urine identified by the test strips. **Results.** Calibration for each analysed substance was done in the concentration range from 0.1 to 1 mg/L and the coefficient of correlation was $R^2 > 0.99$. We obtained following calibration curves: y = 385531x + 14584; y = 398036x + 31542; and y = 524162x – 27105, for morphine, codeine and 6-mam, respectively. Recovery for morphine and codeine determination was 99%, while for 6-mam it was 94%. Limits of detection and quantification of a

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proposed method were 0.01 mg/L and 0.05 mg/L, respectively. Concentration of morphine in the saliva of the heroin users ranged between 0.54 and 5.82 mg/L, concentration of codeine between 0.05 and 5.33, and 6-mam between 0.01 and 0.68 mg/L. A statistically significant correlation between codeine and 6-mam concentrations was obtained. **Conclusion**. A proposed HPLC/MS method for morphine, codeine and 6-mam determination in saliva is accurate, simple, cheap and suitable for routine analysis and monitoring of heroin abuse.

Key words:
saliva; morphine; codeine; chromatography, high pressure liquid; mass spectrometry; heroin.

Uvod

Zloupotrebu psihoaktivnih supstanci u Srbiji karakteriše porast korišćenja svih vrsta droge, naročito sintetičke, kao i veliki broj slučajeva istovremenog korišćenja različitih vrsta droge. Prema podacima zdravstvenih centara u Srbiji, među registrovanim zavisnicima sredstava zloupotrebe dominantni su oni koji ova sredstva primenjuju intravenskim putem (65%) [1]. U periodu od januara 2008. do avgusta 2009. godine u Odeljenje za toksikološku hemiju Centra za kontrolu trovanja Vojnomedicinske akademije (VMA) u Beogradu primljena su 804 zahteva za analizu psihoaktivnih supstanci u urinu, među njima najveći broj iz Toksikološke ambulante i Klinike za toksikologiju VMA. Od tog broja, primenom komercijalnih test traka za detekciju psihoaktivnih supstancija, pozitivna reakcija na „opijate" ustanovljena je kod 275 uzoraka urina (neobjavljeni podaci).

U cilju biomonitoringa zloupotrebe heroina uobičajena je identifikacija kodeina, morfina i 6-monoacetilmorfina kao njegovih metabolita. Heroin se u potpunosti eliminiše iz organizma za oko 24 časa jer se u organizmu veoma brzo hidrolizuje do 6-monoacetilmorfina (6-mam), koji se dalje nešto sporije hidrolizuje do morfina, kao glavnog metabolita. U plazmi, takođe, dokazani su i acetilkodein i kodein, kao nečistoće ilegalnog heroina [2]. Metabolit 6-mam, 6-O-acetilmorfin, specifičan je za heroin, i njegovo određivanje u biološkom materijalu od velikog je značaja kada se sumnja na trovanje heroinom, jer se sam heroin teško može detektovati zbog brzog metabolizma [3].

Urin i krv su uobičajeni biološki materijali koji se koriste za detekciju i određivanje sredstava zloupotrebe. Upotreba salive kao alternativnog matriksa za detekciju ovih supstancija je sve veća u poslednjih nekoliko godina, zahvaljujući, pre svega, farmakokinetičkim istraživanjima koja su potvrdila zadovoljavajuću korelaciju između njihovog sadržaja u salivi i krvi [4].

Uzorkovanje salive je jednostavno i neinvazivno, a činjenica da sakupljanje salive može direktno da se nadgleda, bez narušavanja privatnosti ispitanika, smanjuje mogućnost falsifikovanja uzoraka, što je pogodnost u okolnostima testiranja vozača, radnika, ali i opšte populacije [5]. Pored toga, sa analitičkog stanovišta značajno je što je saliva kao matriks relativno neopterećena endogenim materijama koje bi mogle da interferiraju sa određivanjem same supstancije [6], a koncentracija ukupnih proteina u salivi čini svega 1% ukupnih proteina plazme, što je čini praktično deproteinizovanom tečnošću [7]. Zbog toga se saliva smatra dobrom alternativom za krv i za urin za dokazivanje skorašnje upotrebe sredstava zloupotrebe [8]. Za određivanje koncentracije sredstava zloupotrebe u salivi, najčešće se koriste tehnike gasne i tečne hromatografije sa masenom detekcijomposle pripreme materijala tečno-tečnom ili tečno-čvrstom ekstrakcijom [4].

Cilj ovog rada bio je optimizacija metoda pripreme uzorka salive i određivanje kodeina, i glavnih metabolita heroina, morfina i 6-monoacetilmorfina, *high-performance liquid chomatography mass spectrometry* (HPLC/MS) metodom.

Metode

Aparati

Za analizu su korišćeni tečni hromatograf Waters Alliance® (Waters Corporation, Milford, MA, USA), kolona Waters Spherisorb® 5 μm, ODS2, 4,6 × 100 mm (Waters Corporation, Milford, MA, USA), petlja 50 μL i maseni spektrometar Waters Micromass® ZQ™ (Waters Corporation, Milford, MA, USA).

Hemikalije i reagensi

U radu su korišćene komercijalno dostupne hemikalije i reagensi: azot čistoće 99,9999%; metanol, HPLC čistoće, i amonijum-acetat (Merck, Darmstat, Germany); hloroform, izopropil alkohol, amonijum-hidroksid i glacijalna sirćetna kiselina (Zorka Pharma p.a., Šabac, Srbija); acetonitril HPLC čistoće (J.T. Backer, Deventer, Netherlands); analitički standardi morfina, kodeina, 6-monoacetilmorfina (Sigma-Aldrich Corporation, St. Louis, MO, USA).

Osnovni rastvori morfina, kodeina i 6-monoacetilmorfina pripremljeni su u metanolu, u koncentraciji 1 g/L, svaki pojedinačno. Radni rastvori standarda koncentracije 0,10, 0,20, 0,30, 0,50 i 1,00 mg/L dobijeni su razblaživanjem osnovnih standarda u mobilnoj fazi.

Uzorkovanje i priprema uzoraka

Radom su obuhvaćeni rezultati dobijeni na uzorcima od 11 heroinskih zavisnika, muškog pola, starosti između 22 i 36 godina, od kojih je devet primenilo heroin *i.v.* putem, a dva ušmrkavanjem. Pet ispitanika nalazilo se u stanju kome, dva su bila somnolentna, a preostala četiri u svesnom stanju. Uzorkovanje salive je vršeno pomoću stimulatora lučenja salive – štapića sa sunđerčićem (Doa Multidiagnost S6 test®, Biognost, Zagreb, Hrvatska) natopljenim limunskom kiselinom. Uzorci su čuvani u hemijski čistim PVC posudama na temperaturi -20°C do određivanja.

Za pripremu uzoraka salive korišćena je tečno-tečna ekstrakcija. U uzorke dobijene rekonstitucijom uparene smeše standarda opijata (0,2 mL) u 2 mL *pool*-a salive, do-

dato je 3 mL smeše rastvarača hloroforma i izopropil alkohola u odnosu 9 : 1, i 100 µL amonijum-hidroksida da bi se postigla optimalna sredina za ekstrahovanje opijata (pH 9). Ekstrakcija iz 1 mL uzorka salive bolesnika vršena je dodavanjem 6 mL smeše rastvarača hloroforma i izopropil alkohola u odnosu 9 : 1, uz 200 µL amonijum-hidroksida. Posle 20 min mućkanja na horizontalnoj mućkalici, uzorci su centrifugirani 10 min na 4 000 obrtaja/min. Organski sloj je zatim uparen i izvršena je rekonstitucija u 1 mL mobilne faze. Ovako pripremljen ekstrakt analiziran je metodom HPLC/MS.

Metoda HPLC/MS

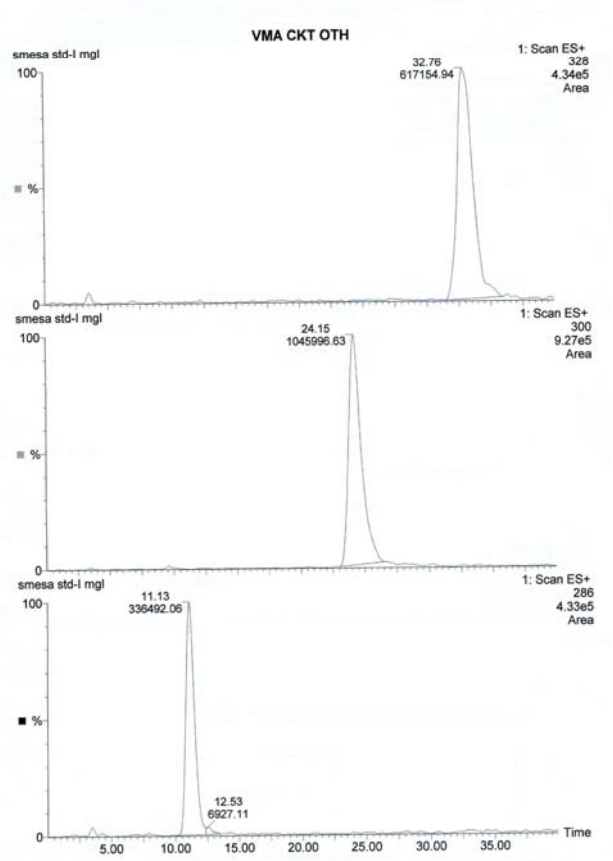
Hromatografski uslovi za HPLC-ESI-MS bili su sledeći: razdvajanje na koloni Waters Spherisorb® 5µm, ODS2, 4,6 × 100 mm vršeno je primenom mobilne faze koja se sastoji iz komponente A (5 mM amonijum-acetata + 0,1% sirćetne kiseline, pH = 3,5) i B (acetonitril + 0,1% sirćetne kiseline) u odnosu 80 : 20, pri protoku od 0,3 mL/min. Temperatura u autosempleru iznosila je 20°C, a injekciona zapremina 50 µL. Masena detekcija vršena je u opsegu masa od 100 do 400 m/z, centroidni mod, *interscan delay* 0,1 s, *scan time* 0,5 s, bez splitovanja, četiri načina snimanja (sa naponom na konusu 70, 60, 50 i 38 V i elektrosprej mod ES+); temperatura izvora bila je 150°C, temperatura desolvatacije 430°C; protok gasa: desolvatacioni 362 L/h, konusni 135 L/h; napon na kapilari 3 kV. Kalibracija i optimizacija aparata vršena je u odnosu na standard morfina (jon 286) koncentracije 10 mg/L, pri protoku 10 µL/min.

Za statističku obradu podataka korišćeni su programi Microsoft Office Excel i Statistica. Primenjena je regresiona i korelaciona analiza za nivo verovatnoće 0,05. Maseni spektri su obrađeni kompjuterskim programom Waters MassLynx™ (Waters Corporation, Milford, MA, USA).

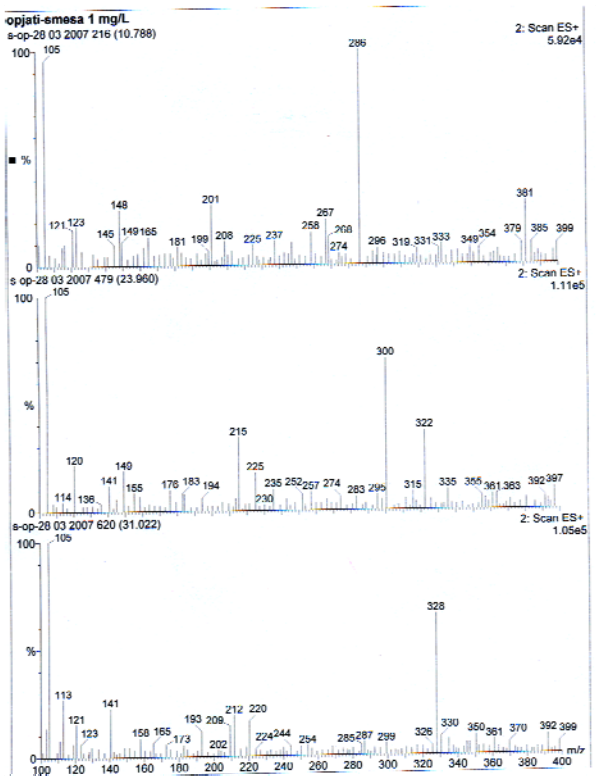
Rezultati

Pod zadatim hromatografskim uslovima retenciona vremena standarda pripremljenih u *pool*-u salive odgovarala su retencionim vremenima standarda u mobilnoj fazi i to: za morfin 11,1 min, za kodein 24,1 min i za 6-monoacetilmorfin 32,8 min, sa odstupanjem do 10%. Na slici 1 prikazani su hromatogrami morfina, kodeina i 6-monoacetilmorfina iz smeše standarda u mobilnoj fazi, a na slici 2 maseni spektri ispitivanih supstancija u kojima su identifikovane karakteristične jonske mase morfina (286 m/z), kodeina (300 m/z) i 6-monoacetilmorfina (328 m/z). Poređenja radi, na slikama 3 i 4 dati su hromatogram i maseni spektar ovih supstancija iz uzorka salive osobe (J.T.) kod koje je prethodno utvrđeno prisustvo „opijata“ u urinu.

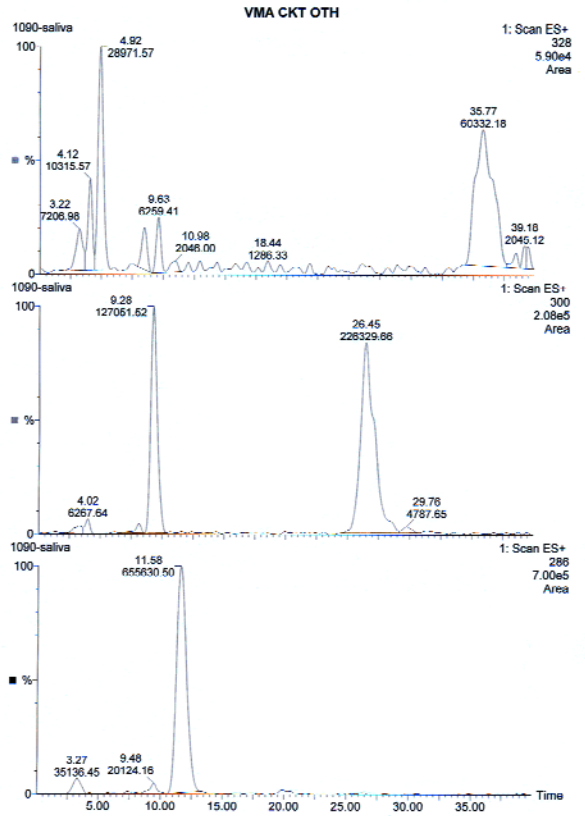
Zavisnost površine hromatografskog pika i koncentracije ispitivanih supstancija u salivi ispitana je regresionom analizom i dobijene su linearne kalibracione krive za analite od interesa sa koeficijentom determinacije $R^2 > 0,99$. Kalibracija je vršena u opsegu koncentracija 0,1–1 mg/L (0,1; 0,2; 0,3; 0,5; 1 mg/L) i dobijene su sledeće jednačine: za morfin, y = 385531x + 14584; za kodein, y = 398036x + 31542; za 6-mam, y = 524162x – 27105.



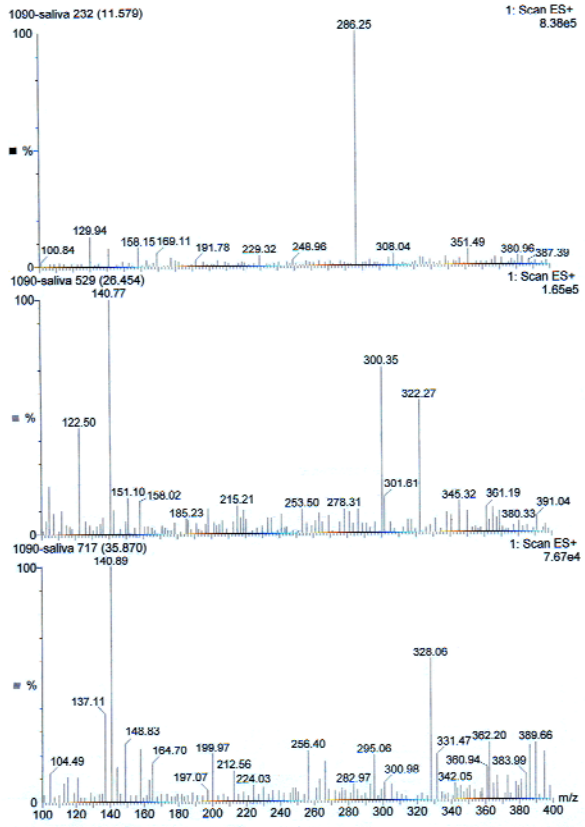
Sl. 1 – Hromatogram smeše standarda morfina, kodeina i 6-mam u mobilnoj fazi



Sl. 2 – Maseni spektar smeše standarda morfina, kodeina i 6- monoacetilmorfin (6-mam) u mobilnoj fazi
Morfin – 286 m/z; kodein – 300 m/z; 6-mam – 328 m/z



Sl. 3 – Hromatogram uzorka salive bolesnika koji je imao pozitivnu reakciju na opijate u urinu



Sl. 4 – Maseni spektar uzorka salive bolesnika koji je imao pozitivnu reakciju na opijate u urinu
Morfin – 286,25 m/z; kodein – 300,35 m/z; 6-monoacetilmorfin (6-mam) – 328,06 m/z

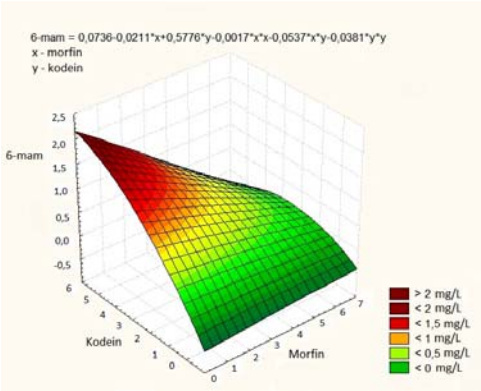
Korelacionom analizom potvrđena je tačnost metode sa koeficijentima korelacije: 0,9970 za morfin, 0,9982 za kodein i 0,9981 za 6-monoacetilmorfin. Dobijene su sledeće *recovery* vrednosti: za morfin 99%, za kodein 99% i za 6-monoacetilmorfin 94%. Računat na bazi pojedinačnih koncentracija analita *recovery* je varirao u rasponu 74–123% za morfin, 97–106% za kodein i 28–91% za 6-mam. Množenjem šuma aparata sa 3 i 5, izračunati su limit detekcije (LOD) od 0,01 mg/L, i limit kvantifikacije (LOQ) od 0,05 mg/L za sve ispitivane supstance.

Posle validacije metode, analizirano je 11 uzoraka salive bolesnika primljenih u Toksikološku ambulantu Centra hitne pomoći VMA i Kliniku za toksikologiju Centra za kontrolu trovanja VMA za koje je prethodno test trakama potvrđeno prisustvo opijata u urinu. Sadržaj ispitivanih supstancija u salivi bolesnika prikazan je u tabeli 1. Vrednosti

Tabela 1
Koncentracije morfina, kodeina i 6 monoacetilmorfina (6-mam) u salivi korisnika opijatne droge

Bolesnik	Koncentracija u salivi (mg/L)		
	morfin	kodein	6-mam
M.N.	5,42	0,33	0,01
T.N.	3,31	0,05	0,02
M.M.	1,80	0,29	0,08
Ž.M.	0,54	–	0,03
B.I.	1,06	–	0,27
P.B.	5,82	0,85	0,08
K.N.	4,50	5,33	0,65
M.A.	3,16	1,98	0,68
L.M.	1,34	–	–
I.D.	0,77	–	–
J.T.	1,87	0,22	0,09

koncentracija su se kretale u opsegu 0,54–5,82 mg/L za morfin, 0,05–5,33 za kodein, a za 6-monoacetilmorfin 0,01–0,68 mg/L. Koncentracije niže od LOQ dobijene su izračunavanjem posle merenja u koncentrisanim uzorcima. U cilju ispitivanja korelacije između koncentracija morfina, kodeina i 6-monoacetilmorfina u salivi ispitanika, primenili smo dva scenarija. U prvom scenariju uključili smo samo ispitanike kod kojih su izmerene vrednosti sve tri supstancije, a u drugom sve ispitanike, pri čemu smo u slučajevima kod kojih supstancije nisu detektovane koristili vrednost 0. Bez obzira na primenjeni scenario, statistički značajna korelacija dobijena je između vrednosti za kodein i 6-mam sa koeficijentom korelacije 0,84 u prvom, i 0,82 u drugom slučaju, uz nivo značajnosti $p = 0,02$ za oba scenarija. Jednačina koja opisuje korelaciju između koncentracija kodeina i 6-mam za prvi scenario iznosila je $y = 0,1312x + 0,0596$, a za drugi $y = 0,1293x + 0,0665$ sa koeficijentima determinacije (R^2) 0,70 i 0,67. Na slici 5 dat je trodimenzionalni prikaz korelisanih parametara.



Sl. 5 – Trodimenzionalni prikaz zavisnosti koncentracija morfina, kodeina i 6-monoacetilmorfina (6-mam) u salivi grupe bolesnika sa pozitivnom reakcijom na opijate u urinu

Diskusija

Saliva pripada kategoriji tzv. alternativnih uzoraka sa sve većim značajem za primenu u kliničko-toksikološkoj analizi [4]. Priprema uzoraka salive u svrhu određivnja lekova i sredstava zloupotrebe podrazumeva upotrebu tečno-čvrste [9], [10–13], ili tečno-tečne ekstrakcije [14]. Zapremina prikupljenog uzorka salive je često mala, pa se za detekciju supstanci primenjuju tehnike gasne [10] i tečne [9, 11, 12, 15–17] hromatografije sa masenom detekcijom [4], i tako se obezbeđuje visoka specifičnost i osetljivost. Metoda LC-MS primenjuje se kao potvrdni test za drogu u salivi [11]. U ovom radu, uzorci salive pripremani su tečno-tečnom ekstrakcijom, i dobijene su zadovoljavajuće *recovery* vrednosti. Morfin, kodein i 6-mam identifikovani su na osnovu retencionih vremena i masenih spektara čime je postignuta neophodna specifičnost analize, a linearnost je dobijena u opsegu 0,1–1 mg/L. Pri hromatografskoj analizi uzoraka salive pripremljenih ekstrakcionom procedurom navedenom u ovom radu nije primećeno prisustvo dodatnih pikova koji bi mogli da potiču od interferirajućih supstancija, što ukazuje na selektivnost primenjenog postupka. Osnovne prednosti predložene metode, u odnosu na metode opisane u literaturi, su jednostavnost pripreme uzorka i izvođenja hromatografske procedure i ekonomičnost. Predložena metoda, takođe, ima zadovoljavajuće karakteristike u pogledu osetljivosti, tačnosti i specifičnosti.

Saliva je lako dostupna za neinvazivno uzorkovanje koje može da se nadgleda, i relativno je neopterećena materijama koje bi mogle da interferiraju sa određivanjem analita. Pored navedenih prednosti upotrebe salive kao biološkog materijala, postoje i određena ograničenja. Naime, saliva se teško sakuplja kod dehidriranih, predoziranih zavisnika kod kojih se kao jedan od simptoma javlja suvoća usta, posebno ako uzimaju i stimulanse kao što je amfetamin. U poređenju sa urinom, u salivi je kraći period detekcije aktivnih supstanci, a i različiti načini sakupljanja salive (stimulisano i nestimulisano uzorkovanje) utiču na prinos analita od interesa. Savremeni analitički pristup problematici identifikacije i određivanja izdvaja primenu H PLC/MS metode zbog toga što se ovom tehnikom obezbeđuje visoka specifičnost i osetljivost, naročito ako se ima u vidu da je koncentracija metabolita u salivi često niža od koncentracije u tradicionalnim uzorcima [4, 15].

Difuzija jedinjenja iz krvi u salivu zavisi od unete doze i klirensa, osobina jedinjenja (pKa, liposolubilnosti, stepena vezivanja za proteine plazme, naelektrisanja, molekulske mase i prostorne konformacije), ali i od osobina salive (pH, brzine protoka, sastava) [18]. Vremenski period tokom koga supstance mogu da se detektuju u salivi zavisi i od puta unosa. Posle *iv* primene heroin se detektuje u prvom satu, a posle pušenja i ušmrkavanja tokom 24 h. Za morfin vreme detekcije je 0,5–24 h posle *im* primene, dok se kodein može naći u salivi 2–12 h nakon peroralnog uzimanja [6, 19, 20]. U ovom radu, vrednosti dobijene određivanjem morfina, kodeina i za 6-mam u salivi ispitanika nalazile su se u širokom opsegu koncentracija (tabela 1). Iako nismo imali podatke o dozama i vremenu proteklom od primene droge za ispitanike čiji su uzorci uzimani u rad, poznato je da koncentracije u salivi zavise i od kontaminacije usne duplje samim sredstvom zloupotrebe, naročito u prvom satu od primene [6, 21]. Pored doze i vremena, kontaminacija usne duplje mogla bi značajno da doprinese rasponu u dobijenim koncentracijama. Pujadas i sar. [10] u salivi bolesnika primljenih u jedinicu hitne pomoći zbog različitih povreda, posle tečno-čvrste ekstrakcije, metodom GC/MS odredili su vrednosti za morfin 34,6 ng/mL (0,0346 mg/L), za 6-mam 83,9 ng/mL (0,0839 mg/L) i kodein 372,0 ng/mL (0,372 mg/L). Metodom LC/MS-MS prilikom testiranja vozača, Wood i sar. [9] izmerili su koncentracije 6-mam od 238 i 291 µg/L (0,238 i 0,291 mg/L), morfina 725 i 1063 µg/L (0,725 i 1,063 mg/L) i kodeina 107 i 132 µg/L (0,107 i 0,132 mg/L), a Oiestad i sar. [20] 0,04–11 µmol/L (0,011–3,139 mg/L) i 0,024–20 µmol/L (0,007– 5,987 mg/L) za morfin odnosno kodein. Kod trudnica zavisnih od heroina, na metadonskoj terapiji, istom metodom dobijene su nešto niže vrednosti: 1,1–51,3 µg/L (0,0011 i 0,0513 mg/L) za morfin, 1,3–6,4 µg/L (0,0013 i 0,0064 mg/L) za kodein i 1,4–434,0 µg/L (0,0014 i 0,434 mg/L) za 6-mam [17].

U ovom radu koncentracije dobijene kod svih ispitanika opadale su po sledećem redosledu: morfin > kodein > 6-mam, osim kod jednog pacijenta kod kojeg je koncentracija kodeina bila veća od koncentracije morfina. Korelisanjem izmerenih vrednosti dobijena je statistički značajna korelacija između koncentracija kodeina i 6-mam, a na bazi prikazane jednačine (slika 5), moguće je predvideti koncentraciju jednog od metabolita što ima praktični značaj pri proceni ekspozicije heroinu, uključujući dinamiku i obim izloženosti.

Zaključak

Predložena metoda HPLC/MS za određivanje sadržaja morfina, kodeina i 6-mam u salivi je tačna, jednostavna i ekonomična, pa je zbog toga pogodnu za rutinsku primenu. Imajući u vidu činjenicu da ova metoda daje metabolički profil heroina, možemo reći da poseduje relativno visok informativni potencijal o vremenu i načinu njegove zloupotrebe.

Zahvalnica

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Glycosaminoglycans in the urinary bladder mucosa, tumor tissue and mucosal tissue around tumor

Glukozaminoglikani u mukozi mokraćne bešike, tkivu tumora i mukoznom tkivu oko tumora

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Abstract

Introduction/Aim. Glycosaminoglycans (GAG) are one of the main constituents of the connective tissue and cellular membrane. Their presence has been evidenced in mucosa and muscular tissue of the urinary bladder of both healthy individuals and those affected by carcinoma. This suggest their potential role in the onset of bladder carcinoma and follow-up of those patients. The aim of the study was to determine GAG levels in tumor tissue and the surrounding bladder mucosa in patients with bladder tumor, as well as in the bladder mucosa in patients with bladder carcinoma, and to compare the results according to the grade and stage of tumor and relapse. **Methods.** Tissue samples were taken in 61 patients (48 males and 13 females), mean age 61.5 years, range 40–92 years, obtained by transurethral resection (TUR) of bladder tumor, and 8 healthy persons. Determination of a total GAG content in the tissue samples was done by the Whiteman's method and then compared regarding the tumor grade and stage. **Results.** Tumor grade and stage directly correlated with the levels of GAG. The GAG levels were significantly higher in tumor samples as compared to healthy mucosa. **Conclusion.** Higher GAG levels were recorded in all the patients with bladder tumors comparing to smples obtained from healthy individuals. GAG levels do not predict tumor relapse.

Key words:
urinary bladder neoplasms; recurrence; glycosaminoglycans; sensitivity and specificity.

Apstrakt

Uvod/Cilj. Glikozaminoglikani (GAG) su glavni sastojci osnovne supstance vezivnog tkiva i ćelijske membrane. Njihovo prisustvo dokazano je u mukozi i mišićnom tkivu mokraćne bešike kako zdravih, tako i obolelih od karcinoma mokraćne bešike. Ta činjenica ukazuje na potencijalnu ulogu GAG u pojavi i praćenju obolelih od karcinoma mokraćne bešike. Cilj ovog rada bio je da se ispita značaj određivanja GAG u tkivu tumora i mukozi oko tumora mokraćne bešike prema stadijumu i gradusu tumora za predviđanje pojave recidiva superficijelnog tumora mokraćne bešike. **Metode.** Uzorci tkiva uzeti su od 61 bolesnika (48 muškaraca i 13 žena) prosečne starosti 61,5 godina, opseg 40–92 godine, tokom transuretralne resekcije (TUR) tumora mokraćne bešike. Određivanje ukupnog sadržaja GAG u uzorcima tkiva učinjeno je primenom metode Whiteman, a dobijene vrednosti potom su upoređene prema gradusu i stadijumu tumora. **Rezultati.** Dobijene vrednosti odnosa GAG pokazale su rast shodno rastu gradusa i stadijuma tumora i taj rast bio je značajan u odnosu na vrednosti GAG u mukozi zdravih osoba. Nije dobijena značajna razlika u vrednosti GAG kod osoba sa recidivom tumora u odnosu na sve ispitanike, kao grupu u celosti. **Zaključak.** Kod ispitanika u svim stadijumima i gradusima tumora dobijene su povišene vrednosti GAG u odnosu na vrednosti GAG kod ispitanika sa normalnom mukozom mokraćne bešike, ali se dobijeni rezultati ne mogu koristiti za predviđanje pojave recidiva bolesti.

Ključne reči:
mokraćna bešika, neoplazme; recidiv; glikozaminoglikani; osetljivost i specifičnost.

Introduction

Urinary bladder carcinoma is quite often asymptomatic and at the moment of detection invasive carcinoma is already present in 25% of cases. Out of the remaining 75% of superficial forms in about 15% of cases progression into invasive carcinoma occurs.

The risk of tumor progression and relapse is paralelly increased with higher clinical stage and grade as well as the size and number of primary tumors. In our country, according to the latest statistical report for the year 2004 [1] the incidence of bladder carcinoma was 6.7% for male (the fourth most common malignancy) and 2.1% for female population (the ninth most common malignancy), while the

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mortality rate was 4% and 1.6% for male and female population, respectivly.

The change in glucosaminoglycons (GAG) level is interesting in the diagnosis and follow-up of bladder carcinoma. It has been established that GAG is the main constituent of the basic layer of the connective tissue and cellular membrane. Their presence has been evidenced in mucosa and muscular tissue of the urinary bladder [2–4] in both healthy bladders and those affected by carcinoma. Their role in antibacterial adherence and formation of urinary stones [5–8] is already known. All this is suggestive for the potential role that GAG can play in the occurrence of urinary bladder tumors. Changes occurring in the epithelium and deeper structures cases of bladder tumors, suggest that the whole bladder mucosa is preparing for the occurrence of malignancy (primary and recurrent tumor).

The aim of our study was to determine GAG levels in tumor tissue and bladder mucosa of patients with tumor, as well as in healthy mucosa of the bladder, and to compare the results regarding tumor grade and stage as well as in cases of tumor relapse.

Methods

Our study comprised 61 patients with bladder tumor (48 males and 13 females) aged 40–92 years (average age 61.5 years) and 8 controls with healthy bladder mucosa of the comparable age (6 males and 2 females).

The material was sampled by transurethral resection (TUR) in all subjects. Sixty-one tumor biopsies were taken as well as 46 samples of bladder mucosa adjacent to the tumor and no macroscopic signs of abnormalities. Control samples were taken by TUR in 8 patients with no signs of malignancy, but the presence of urinary stones or infections.

The samples were subjected for biochemical analysis as well as for histological analysis. Hematoxylin eosin was used for histological staining. The material taken for biochemical studies was stored at -20°C, and subsequently homogenized at a Potter-Elvehiem's homogenizer at 3000 rpm. The material prepared in this way, both tumorous and mucosal tissues, were studied using the Whitman's method for determination of GAG. The values of GAG were presented in grams of tissue proteins, since proteins are a constant parameter in the tissue.

Tissue GAG levels were determined by the Whitman's method [9], from a complex with Alcian Blue 8 GX stain (Alcian Blue 8 GX 0.5 g/L in 0.05 mol/L Na-acetate buffer with addition of 0.05 mol/L $MgCl_2$, pH 5.8), subsequently dissolved in Na-lauril sulfate. The intensity of color was measured by a spectrophotometer at 620 nm.

Tissue proteins were determined with Commasi Brilliant Blue (CBB) stain by spectrophotometry at 595 nm.

The results were processed by nonparametric statistic tests: Mann-Whitney *U*-test, Kruskall-Wallis test and Wilcoxon test.

Results

Distribution of the patients regarding tumor grade and gender is given is Table 1.

Table 1

Distribution of patients according to gender and tumor grade

Grade	Gender		Total	
	male	female	n	%
Papilloma	1	1	2	3.3
Grade I	13	3	16	26.2
Grade II	33	8	41	67.2
Grade III	1	1	2	3.3

Two patients with grade III tumors entered the study. No signs of invasion were found and they were not particularly analyzed in relation to tumor grade.

According to histopathologic findings and clinical picture, using TNM (Tumor Nodus Metastasis) and World Health Organization (WHO) clasification systems, tumors were classified into three stages (Figure 1).

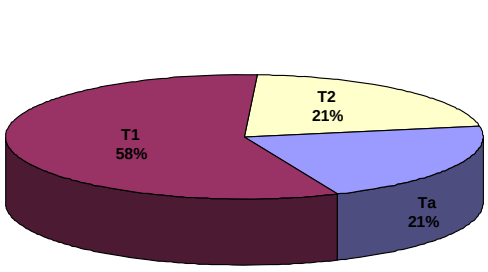

Fig. 1 – Distribution of patients according to the tumor stage

The biochemical evaluation was done in 57 out of 61 tumor samples (in 4 cases due to techical reasons evaluation was not possible), 46 samples of heatlhy appearing mucosa from urinary bladder with tumor, and 8 samples of healthy bladder mucosa (Table 2).

The values of GAG were expressed in mg GAG/g tissue proteins, in relation to tumor grade in tumor tissue as well as bladder mucosa of patients with tumor (Table 3).

In Table 4 the relationship between GAG values and clinical stages of the tumor is given.

According to individual values of GAG in tumor and mucosal tissue of patients with tumor relapse, no differences in GAG values were noted which might suggest the occurrence of relapse although the values remained within the range obtained elsewhere.

Two year follow-up of tumor relapses according the tumor grade and stage is shown in Table 5.

Table 2

Distribution of glycosaminoglucans (GAG) in different tissue samples

Samples	Number of specimens	GAG (mg/g tissue proteins) mean (min–max)
Bladder tumor	57	105.52 (19.1–1024.59)
Bladder mucosa	46	142.94 (4.6–1000.0)
Control bladder	8	39.23(18.87–88.0)

Table 3

Glycosaminoglucans (GAG) values in tissue samples of bladder tumor and mucosa according to the tumor grade

Samples / tumor grade	Number of specimens	GAG (mg/g tissue proteins) mean (min–max)
Tumor samples		
Papilloma	2	37.11 (35.65–38.57)
Grade I	16	75.55 (19.1–137.91)
Grade II	37	120 (24.47–1024.59)
Grade III	2	138.92 (130.84–146.99)
Bladder samples		
Papilloma	2	13.54 (10.69–16.39)
Grade I	13	176.5 (18.7–608.7)
Grade II	31	137.21 (4.6–1000.0)
Grade III	0	0
Control bladder samples	8	39.23 (18.87–88.0)

Table 4

Glycosaminoglucans (GAG) values in tissue samples of bladder tumor and mucosa according to the tumor stage

Samples / tumor stage	Number of specifiens	GAG (mg/g tissue proteins) mean (min–max)
Tumor samples		
Ta	13	52.86 (19.1–114.79)
T1	33	135.37 (24.47–1024.59)
T2	11	78.2 (30.16–146.99)
Bladder samples		
Ta	12	98.19 (10.69–25.0)
T1	24	177.29 (16.14–1000.0)
T2	10	114.18 (4.6–250.0)
Control bladder samples	8	39.23 (18.87–88.0)

Table 5

Distribution of relapses according to the tumor grade and stage

Tumor stage	Tumor grade				Relapses
	P	I	II	III	n
Ta	1	–	–	–	1
T1	–	1	6	–	7
T2	–	–	4	1	5

P – Papilloma

Discussion

According to the available information on the behavior of superficial bladder carcinoma, it is diffucult to predict further course and occurrence of relapses and outcome of the disease. Not a single tumor marker has been found to have specificity and sensitivity which would make it useful for clinical practice. Therefore, efforts have been focused on detecting a substrate which would satisfy all relevant criteria for a good tumor marker. In addition to the above, we believe that the whole bladder mucosa is influenced by carcinogens, and that one or more mucosal fields are the vulnerable sites where the absence of protective factors permit neoplasm proliferation. It is difficult to say whether the removed primary, solitary neoplastic lesion still presents a local predisposing factor for the relapse on the same site, or, some other sites, relieved from the protective noxa become new sites of *de novo* tumor growth since the whole mucosa suffers the influence of the noxious factor.

A decision to evaluate GAG has been made since the available referential data suggested their insufficiently studied role in bladder tumors [10, 11]. We focused on qualitative

study of GAG intending to identify and possibly, show a correlation of GAG in tumor and mucosa of patients with superficial bladder tumors which might suggest tumor recurrence. The results of de Klerk [2] point out that total GAG levels are mildly reduced in 13 (100%) cases of bladder transitional cell carcinoma with large individual variations. He believes that, due to the variability, total GAG level cannot indicate different grades and stages of tumors. However, our results indicate the opposite: elevation of total GAG values correlates with the increase in tumor stage and grade. All tumors had more elevated GAG values in comparison with healthy mucosa. Moreover, the noted variability in GAG values, in each individual case, remains within the grade and stage of the tumor. The content of particular GAG fractions within the total GAG content cannot be compared since we did not include a qualitative analysis in our study.

The results of our study concerning total GAG content, suggest increased GAG levels in tumorous and mucosal tissue in patients with tumors, as compared with the controls. The difference was statistically significant ($p < 0.05$). It remains to be answered whether the recorded GAG values result from the discharge of GAG from tumorous tissue, *ie* preparation of the mucosa for the occurrence of tumor, since the control mucosal tissue does not contain high GAG levels. We recorded elevation of GAG values with higher tumor grade. The lowest values are found in papilloma while the highest were found in cases with tumor grade III. This applies to GAG values in tumorous tissue, while in the same group of patients GAG values in mucosa not affected with tumor is somewhat different. Thus, papillomatous mucosa is characterized with GAG levels below normal, which may result from mucosal stress. Tumor increase GAG levels increase in the surrounding tissue with increase of tumor grade, but not strictly following the tumor grade. It is, nevertheless always increased, and the difference from the controls is statistically significant ($p < 0.05$). Clinically, GAG levels in tumor tissue rises with the incerase in tumor stage. The lowest increase was noted in Ta stage and it is no statistically significant ($p > 0.05$) when compared with the controls, while in stages T1 and T2 showed a significant increase.

Mucosa of patients with tumor also shows increased GAG levels in all stages of the disease, and the difference from the controls was statistically significant ($p < 0.05$).

Out of the 61 patients, relapse was noted in 13 in a 24-month period.

Relapse most commonly occurred in patients with grade II tumor, as well as in those with T1 and T2 stages. In none of the analyzed cases with relapse, GAG values in tumor tissue and mucosa deviated from other analyzed cases of the group as a whole, so that the obtained results cannot be used to predict relapse of the disease.

Conclusion

Our study shows that GAG levels rise with the rise in tumor grade and stage in the tumor tissue as well as in surrounding mucosa. Elevation of GAG levels in mucosa of patients with tumor was higher than in tumor itself, suggesting a very important question to be answered.

Whether tumor is an only manifestation of a process occurring in the bladder mucosa with future behavior what is probably already determined remains unclear.

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Tumor necrosis factor-alfa and interleukin-4 in cerbrospinal fluid and plasma in different clinical forms of multiple sclerosis

Vrednosti faktora nekroze tumora alfa i interleukina 4 u likvoru i plazmi bolesnika sa različitim formama multiple skleroze

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Abstract

Background/Aim. Multiple sclerosis (MS) is an immune-mediated central nervous system disease characterized by inflammation, demyelination and axonal degeneration. Cytokines are proven mediators of immunological process in MS. The aim of this study was to investigate whether there is a difference in the production of the tumor necrosis factor alpha (TNF-alpha) and interleukin-4 (IL-4) in cerebrospinal fluid (CSF) and plasma in the MS patients and the controls (other neurological non-inflammatory diseases) and to determine a possible difference in these cytokines in plasma and CSF in different clinical forms of MS. **Methods.** This study involved 60 consecutive MS patients – 48 patients with relapsing-remitting MS (RRMS) and 12 patients with secondary progressive MS (SPMS). The control group consisted of 20, age and sex matched, non-immunological, neurological patients. According to the clinical presentation of MS at the time of this investigation, 34 (56.7%) patients had relapse (RRMS), 14 (23.3%) were in remission (RRMS), while the rest of the patients, 12 (20.0%), were SPMS. TNF-alpha and IL-4 concentrations were measured in the same time in CSF and plasma in the MS patients and the controls. Extended disability status score (EDSS), albumin ratio and IgG index were determined in all MS patients. **Results.** The MS patients had significantly higher CSF and plasma levels of TNF-alpha than the controls ($p < 0.001$ for both samples). IL-4 CSF levels were significantly lower in the MS patients than in the controls ($p < 0.001$), however plasma levels were similar. The patients in relapse (RRMS) and with progressive disease (SPMS) had higher concentrations of CSF TNF-alpha levels than the patients in remission ($p < 0.001$). IL-4 CSF levels in relapse (RRMS) and SPMS groups were lower than in the patients in remission. The patients in remission had an unmeasurable plasma TNF-alpha level and the patients with SPMS had significantly lower IL-4 levels in plasma than the patients in relapse and remission ($p < 0.001$). The only significant correlation between cytokine level with either EDSS, or albumin ratio, or IgG index, was found between CSF TNF-alpha levels and albumin ratio in the patients with relapse (R square = 0.431, $p < 0.001$). **Conclusion.** According to the obtained data MS relapse was characterized by high concentrations of TNF-alpha in CSF and plasma and low concentrations of IL-4 in CSF. Remission was characterized by high concentrations of IL-4 and low concentrations of TNF-alpha both in CSF and plasma. SPMS was characterized with lower concentrations of TNF-alpha and IL-4 compared to relapse, both in CSF and plasma.

Key words:
multiple sclerosis; tumor necrosis factor-alpha; interleukin-4; plasma; cerebrospinal fluid; disease progression; treatment outcome.

Apstrakt

Uvod/Cilj. Multipla skleroza (MS) je imunološki posredovana bolest centralnog nervnog sistema koju karakterišu inflamacija, demijelinizacija, degeneracija aksona i glioza. Citokini su važni medijatori imunoloških procesa kod MS. Cilj ove studije bio je da se ispita postojanje razlike u produkciji inflamacijskog citokina faktora nekroze tumora alfa (TNF-alfa) i antiinflamacijskog citokina interleukina 4 (IL-4) u likvoru i plazmi bolesnika sa različitim kliničkim faktorima MS i kod bolesnika sa drugim neurološkim neinflamacijskim oboljenjima (kontrolna grupa). **Metode.** U studiju je bilo uključeno 60 bolesnika sa MS, 48 sa relapsno-remitentnom MS (RRMS) i 12 bolesnika sa sekundarno progresivnom MS (SPMS). Kontrolnu grupu je sačinjavalo 20 bolesnika sa neurološkim, neimunološkim bolestima. U vreme ispitivanja, 34 (56,7%) bolesnika bilo je u fazi pogoršanja, 14 (23,3%) u fazi remisije, dok je 12 (20%) bolesnika imalo SPMS. Citokini TNF-alpha i IL-4 određivani su istovremeno u plazmi i likvoru bolesnika sa MS i kontrolne grupe. Nivo neurološkog poremećaja, izmeren korišćenjem *The Expanded*

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Disability Status Scale (EDSS), albuminski koeficijent i IgG indeks određivani su kod svih bolesnika sa MS. **Rezultati.** Bolesnici sa MS imali su značajno više koncentracije TNF-alfa i u likvoru i u plazmi, u poređenju sa bolesnicima kontrolne grupe ($p < 0,01$). Koncentracija IL-4 u likvoru bila je značajno niža kod bolesnika sa MS, nego kod bolesnika kontrolne grupe ($p < 0,01$), dok su koncentracije plazmatskih vrednosti bile slične. Bolesnici u fazi pogoršanja (RRMS) i sa SPMS imali su više koncentracije TNF-alfa u likvoru, nego bolesnici u fazi remisije bolesti (RRMS), ($p < 0,01$). Koncentracija IL-4 u likvoru bila je niža kod bolesnika sa RRMS u fazi pogoršanja i SPMS, nego kod bolesnika koji su bili u remisji bolesti ($p < 0,01$). Kod bolesnika u remisiji nije nađen TNF-alfa u plazmi, dok su bolesnici sa SPMS imali značajno niže koncentracije IL-4 u plazmi u poređenju sa bolesnicima u fazi pogoršanja i u remisiji ($p < 0.01$). Jedina statistički značajna korelacija nađena je između koncentracije TNF-alfa u likvoru i albuminskog koeficijenta i to kod bolesnika u fazi pogoršanja bolesti (R *square* = 0,431, $p < 0,01$). **Zaključak**. Prema rezultatima našeg istraživanja, pogoršanje MS karakterišu visoke koncentracije TNF-alfa u likvoru i plazmi i niske koncentracije IL-4 u likvoru. Remisiju bolesti karakterišu visoke koncentracije IL-4 i niske koncentraije TNF-alfa i u plazmi i u likvoru. Progresivnu formu (SPMS) karakterišu niže koncentracije TNF-alfa i IL-4 u poređenju sa relapsom, i to i u plazmi i u likvoru.

Ključne reči:
multipla skleroza; faktor nekroze tumora; interleukin-4; plazma; cerebrospinalna tečnost; bolest, progresija; lečenje, ishod.

Introduction

Multiple sclerosis (MS) is a central nervous system (CNS) disease characterized by inflammation, demyelination and axonal degeneration. The etiology of MS is still unknown. The results of numerous experimental and clinical studies support the thesis of MS being immunologically mediated disease [1–4]. According to currently accepted theory, autoreactive Th1 cells (CD4+) directed at myelin or oligodendrocyte antigens, initiate aberrant immune response in peripheral blood, away from immunologically protected and privileged CNS. Through interaction with adhesive molecules Th1 cells cross the damaged blood-brain barrier, inside the CNS they have to be reactivated and in further interaction between Th1 cells, macrophages, residential CNS cells and B cells, numerous inflammatory, anti-inflammatory cytokines, auto-antibodies, oxidative species and enzymes are produced [1]. Recently, the presence of Th17 cells has been acknowledged, characterized by interleukin (IL)-17A production, another potent proinflamatory cytokine, which causes upregulation of several inflammatory cytokines such as the tumor necrosis factor-alpha (TNF-alpha), IL-1beta, IL-6, IL-8 [3]. They have a potential to damage myelin, directly or indirectly with consequent axonal damage. So, cytokines are important mediators of immune response in MS [5], and possible shifting toward Th1 and Th17, with the down regulation of Th2 cytokine response, might be one of the important causes of ongoing inflammatory damage in MS. However, an interplay between immune mediated inflammation and neurodegeneration seems to play crucial role in MS development and progression [6].

Clinically, MS is characterized by the phases of remissions and relapses in the majority of patients, relapsing-remitting MS (RRMS). In so calledthe time course, most of these patients develop secondary progressive form of MS – secondary progressive MS (SPMS), while the smallest proportion of patients is characterized by the progressive course from the onset – primary progressive MS (PPMS). Some studies [7, 8] suggest that different immunopathological mechanisms might be involved in the development of different MS types. That might explain different course and prognosis of the disease, necessity of different therapeutic approach in different MS types and unresponsiveness of SPMS and PPMS to current immunomodulatory treatment [9].

There are few studies that compare concentrations of cytokines both in cerebrospinal fluid (CSF) and plasma in MS patients [7, 10] and to the best of our knowledge none that compares concentrations of cytokines in both compartments, in clinically different stages of MS. TNF-alpha is one of the most potent inflammatory cytokines with the confirmed role in direct myelin damage [11]. It enhances expression of adhesion molecules on endothelial cells and lymphocytes [12], induces secretion of interferon gamma and other inflammatory cytokines and chemokines [13], and indirectly through activation of macrophages and microgial cells, stimulates the production of reactive oxidative species, nitric oxide and lytic enzymes [14]. On the other hand, IL-4 is an anti-inflammatory Th2 cytokine with the proposed protective role in MS [15–18].

Hence, the aim of our study was to investigate whether there is a difference in the production of TNF-alfa and IL-4 in CSF and plasma in MS patients and patients with other non-inflammatory neurological diseases, to determine a possible difference in the production of these cytokines both in plasma and CSF in different stages of MS – relapse, remission, progressive phase, and to evaluate a correlation between cytokine concentrations in CSF and plasma and clinical impairment, albumin ratio, intrathecal IgG synthesis.

Methods

Sixty consecutive patients from the Department of Neurology, Military Medical Academy, Belgrade were enrolled in the study after obtaining a permission of the Ethical Committee of the Military Medical Academy in Belgrade. All of them had clinically definite diagnosis of MS according to Poser criteria [19] and had either exacerbation (relapse), or SPMS or clinically stable disease (remission). None of them were on immunosuppressive treatment at least 3 months prior the study. Clinical assessment was performed by using an expanded disability status scale (EDSS) score [20]. The control group consisted of 20 age and gender matched patients with non-inflammatory neurological dis-

eases (epilepsy, spasmodic torticolis and hereditary neuropathy).

In all the patients, CSF and blood samples were obtained and after spinning stored at -70ºC for cytokine analysis. Cytokine analysis was done by the ELISA method (Genzyme, Predicta), according to the written instructions. Apart from cytokine analysis, blood and CSF samples were used for evaluation of albumin ratio and IgG index. Albumin ratio was calculated according to the formula: CSF albumin/serum albumin, while IgG index was calculated by using the formula – IgG CSF / IgG serum : albumin CSF / albumin serum (normal values: < 5.7 and < 0.7 respectively) [21].

Discrete variables are shown as counts and percentages. Continuous variables are presented as median with the interquartile range (iqr) (25-75 percentile). TNF-alpha and IL-4 CSF and plasma levels were compared between the MS patients and the control group by the Mann-Whitney test. Comparison of TNF-alpha and IL-4 CSF and plasma levels within the MS subgroups were done by using Kruskal-Wallis test. A correlation between TNF-alpha CSF levels and albumin ratio was presented as a linear regression curve. A *p*-value of less than 0.05 was accepted as statistically significant.

Results

The majority of the patients were classified as RRMS – 48 (80.0%) and 12 patients (20.0%) had secondary progressive disease (SPMS). According to the clinical presentation at the time of this investigation, 34 (56.7%) patients had relapse, 14 (23.3%) were in remission, while the rest of the patients were in secondary progressive phase of MS 12 (20.0%). In our study group female predominance was present (60.0%), average age was 43.5 ± 3.2 the disease duration was 5.6 ± 2. Average EDSS in our group was 3.8 ± 0.7.

Concentrations of TNF-alpha and IL-4 in CSF and plasma for MS patients and controls analyzed in this study are shown in Table 1.

TNF-alpha was detected in CSF of all MS patients and in none of the patients of the control group. In plasma, it was detected in 38 (63.3%) of MS patients, and in 7 patients (35.0%) of the control group, with significantly higher values in the MS group ($p < 0.001$). There was no significant difference in TNF-alpha concentrations in CSF and plasma in the MS group.

In relation to clinical presentation of MS, higher concentration of TNF-alpha in CSF was found in the relapse group than in SPMS (without a statistical significance between these two groups), and the lowest in the remission phase of the disease ($p < 0.001$). TNF-alpha was not detected in plasma of the patients in remission. However, TNF-alpha was detected in plasma in the majority of relapse patients, 30 (88.2%), and in all the patients with SPMS, without a significant difference between these two groups.

IL-4 was detected in plasma of 37 (61.7%) and in CSF of 54 (90%) of MS patients and in all the patients in the control group, in both CSF and plasma (Table 1). IL-4 was significantly lower in CSF of the MS group compared with the controls ($p < 0.001$), while there was no difference between its' plasma concentrations in the two groups ($p = 0.154$). CSF concentration of IL-4 was significantly lower as compared with plasma concentration in MS group ($p < 0.05$).

All the patients with progressive MS (SPMS), those in remission and 70% of the relapse patients had detectable CSF levels of IL-4. The highest CSF concentration of IL-4 was found in the remission group and it was significantly higher than in the relapse and progressive MS groups.

We did not find a correlation between either of the cytokine and EDSS.

On the other hand, we found a significant linear correlation between albumin ratio and TNF-alpha concentration in CSF of the relapse MS group (Figure 1). No correlation was found between IgG index and cytokine concentrations in the CSF and plasma of the MS patients.

Table 1

Concentrations of TNF-alpha and IL-4 in cerebrospinal fluid (CSF) and plasma of the multiple sclerosis (MS) patients and the control group

Body fluid	TNF–alpha				IL–4			
	MS group		Control group		MS group		Control group	
	Median (IQR) (pg/mL)	Detectability (%)	Median (IQR) (pg/mL)	Detectability (%)	Median (IQR) (pg/mL)	Detectability (%)	Median (IQR) (pg/mL)	Detectability (%)
CSF								
All patients	198.8 (88.9–330.5)	100.0	Not detected	0.0	60.8 (23.1–106.1)	90.0	650.0 (543.1–769.3)	100.0
Relapse	197.8 (81.6–246.9)	100.0	/		77.5 (56.0–90.1)	70.0	/	
SPMS	165.2 (93.2–226.4)	100.0	/		64.6 (48.2–74.7)	100.0	/	
Remission	9.8 (4.3–15.1)	100.0	/		253.0 (202.1–294.6)	100.0	/	
p	ns[1] < 0.001[2]				ns[5] < 0.001[6]			
Plasma								
All patients	263.3 (133.2–444.2)	63.3	47.7 (28.5–63.4)	35.0	200.3 (64.3–421.2)	61.0	370.6 (268.2–490.6)	100.0
Relapse	300.4 (210.0–360.2)	88.2	/		244.5 (211.6–288.2)	62.0	/	
SPMS	231.1 (152.1–299.1)	100.0	/		26.0 (19.5–33.1)	50.0	/	
Remission	Not detected	0.0	/		144.8 (122.7–164.7)	50.0	/	
p	ns[3], 0.001[4]				*p* < 0.001[7]			

[1] **Non-significant difference in CSF TNF-alpha levels for the relapse and the SPMS group.**
[2] **Significant difference in CSF TNF-alpha levels for both the relapse and the SPMS group and the remission group.**
[3] **Non-significant difference in plasma TNF-alpha levels for the relapse and the SPMS group.**
[4] **Significant difference in plasma TNF-alpha levels for both the relapse and the SPMS group and the remission group.**
[5] **Non-significant difference in CSF IL-4 levels for the relapse and the SPMS group.**
[6] **Significant difference in CSF IL-4 levels for both the relapse and the SPMS group and remission group.**
[7] **Significant difference in plasma IL-4 levels for all three groups.**
SPMS – secondary progresive multiple sclerosis; IQR – the interquartile range.

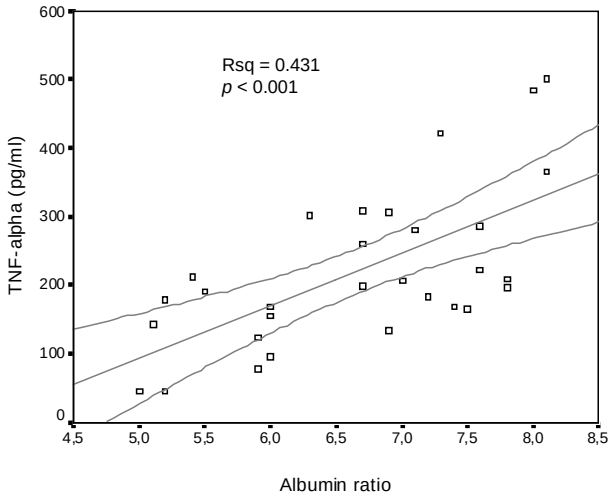

Fig. 1 – Correlation between cerebrospinal fluid TNF-alpha levels and albumin ratio in the relapse multiple sclerosis patients

Discussion

The role of TNF-alpha in MS pathogenesis is implicated by several studies – it has been identified in acute and chronic MS lesions [22], TNF-alpha secretion from monocyte has been shown to be higher before [23] and during MS relapse [24] and, recently, it has been reported that increased TNF-alpha concentrations is related to fatigue in MS [25].

We detected TNF-alpha in CSF of all MS patients, but it was not detected in CSF of the patients with other neurological non-inflammatory diseases. Our findings are in concordance with the results of Tsukada et al. [11] and Baraczka et al. [26]. Other authors [8] detected TNF-alpha in a smaller percentage of MS patients, though the studies were performed in a significantly smaller patient group. In relation to clinical stage of MS, TNF-alpha concentrations differ among the groups. The highest concentration was found during the relapse phase, as were noted in other studies [7, 27]. Rather high values of TNF-alpha were detected in CSF of SPMS in our study, though lower compared to relapse values. Other authors reported high concentration of TNF-alpha in progressive MS, form as well [27, 28]. In relation to cytokine production from peripheral mononuclear blood cells derived from MS patients and stimulated *in vitro*, no difference in TNF-alpha production was reported in progressive and stable MS [29]. However, there is no data related to TNF-alpha concentrations in progressive and relapse group of MS patients within the same study and using the same methodology. Increased TNF-alpha concentrations in the CSF potentate inflammatory response, by augmenting macrophage functions, production of other inflammatory cytokines, lytic enzymes and reactive oxidative species (ROS). The importance of ROS in MS is confirmed in our previously published data [30, 31]. We reported increased index of lipid peroxidation both in CSF and plasma, increased concentrations of superoxid anion radical in plasma and increased activity of two anti-oxidative enzymes, superoxide dismutase and glutation reductase [31].

On the other hand, in remission TNF-alpha level was significantly lower, though still present in CSF. Shaw et al. [32] did not find a significant difference in TNF-alpha concentrations in the stable MS patients and the control group both in CSF and sera, while Sharief et al. [27] did not detect TNF-alpha in CSF of the stable MS patients. Our findings support the thesis of continuous inflammatory response within the CNS during the MS course [6, 28, 32]. One could speculate that among other inflammatory factors, high concentrations of TNF-alpha in CSF is needed for clinical presentation of MS relapse and/or progression of the disease.

A very recent report emphasizes the role of TNF-alpha in relapse by finding that suppressive effect of glucocorticoids on TNF-alpha production is associated with its clinical effect in MS [33]. Similar pattern of TNF-alpha production was found in plasma of our MS patients group. High levels were found in the relapse and SPMS group, higher than in CSF though not significantly, while in the stable MS patients TNF-alpha was not detected in plasma. Detectability of TNF-alpha in plasma varies in different studies. It was detected in rather small percentage [10], or in one third of MS patients [7]. Only Sharief et al. [27] found significantly higher concentrations of TNF-alpha in CSF compared to plasma. In our group of MS patients detectebility of TNF-alpha in CSF was 100%, while in plasma it was detected in 63.3% of the MS patients and in the control group in only 35.0% of patients**.** Having in mind autocrine function of cytokine, it would not be expected to find such high levels of TNF-alpha in plasma. Although pathological process is within the CNS, immunologicaly privileged and protected compartment, systemic reaction is still present. High levels of TNF-alpha in plasma might be explained by systemic reaction to stress, MS relapse being powerful stressful event, especially since TNF-alpha was not detected in plasma of stable MS patients**.** In other words, during the MS course inflammatory reaction and immunological response are not limited within the CNS, but widespread. The results of recent studies [34, 35] are consistent with this hypothesis. Significant and sustained increase of serum TNF-alpha was found in healthy subjects a day after a psychologically stressful event [34]. We did not find a correlation between CSF values of TNF-alpha and EDSS as others did [23]**.** In our study, albumin ratio, a marker of the blood-brain barrier damage correlated with the CSF values of TNF-alpha in the patients with relapse**.** No relationship was found between the intrathecal IgG synthesis and TNF-alpha values in plasma and CSF in our group of patients.

However, it seems that immunoregulatory role of TNF-alpha is far more complex, since MS treatment with anti-TNF-alpha antibodies resulted in further demielynation and disease progression [36].

Some studies have shown that production of IL-4 and IL-10, both Th2 cytokines, is increased in stable [17] and interferon beta treated MS patients [37] and reduced in clinically active phase of MS [38]. That is in concordance with our results. In the patients with relapse and those with SPMS, IL-4 concentration in CSF was low and even undetectable in one third of the relapse patients. On the contrary, in CSF of stable patients IL-4 concentration was significantly higher and

detected in all. However, IL-4 showed a different secretion pattern in plasma. The highest concentration was detected in relapse patients, than in stable patients, and the lowest in SPMS. The secretion pattern of both cytokines, pro-inflammatory TNF-alpha and anti-inflammatory IL-4 appears to be the same as far as plasma concentrations are concerned. Relapse is characterized by high concentrations of TNF-alpha and other inflammatory cytokines in plasma, while high concentrations of IL-4 might be a consequence, an effort to dampen systemic inflammatory response. According to our results, systemic cytokine response and cytokine secretion within the CNS may not be in concordance with the cytokine dominant function in a case of relapse or remission, as some experimental data has shown [39]. IL-4 shows inhibitory effect on TNF-alpha secretion and augments TNF-alpha sR production *in vitro* [40], however in our study no negative correlation was found between IL-4 and TNF-alpha concentrations neither in CSF nor in plasma. Our data confirm previous reports of lack of correlation between CSF and plasma values of IL-4 and EDSS [15]. No correlation was found either for intrathecal IgG synthesis, nor for albumin ratio and IL-4 plasma and CSF values, as previously reported [38].

A misbalance found between inflammatory and anti-inflammatory cytokines, is one of many immune dysregulations in MS and one should be aware of oversimplified interpretation [41]. However, these results support the thesis that modification of cytokine profiles could be associated with prevention of disease relapse and maintaining remission of MS. Present therapeutic strategies in MS are shifted from immunomodulation to more aggressive immunosupressive treatments with monoclonal antibodies [42]. Clinical improvement in those studies has been shown to correlate with decrease of inflammatory cytokines and increase of anti-inflammatory cytokine production [43–45].

Conclusion

According to our data MS relapse is characterized by high concentration of TNF-alpha in CSF and plasma and low concentration of IL-4 in CSF and high in plasma. Remission is characterized by high concentration of IL-4 and low concentrations of TNF-alpha both in CSF and plasma. SPMS is characterized with the same cytokine pattern as relapse, though both cytokines, inflammatory TNF-alpha and anti-inflammatory IL-4, have been detected in lower concentrations.

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^{123}I-FP-CIT brain SPECT (DaTSCAN) imaging in the diagnosis of patients with movement disorders – First results

SPECT scintigrafija mozga korišćenjem ^{123}I-FP-CIT (DaTSCAN) u dijagnostici bolesnika sa poremećajem pokreta – prvi rezultati

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Abstract

Background/Aim. ^{123}I-FP-CIT brain single-photon emission computed tomography (SPECT), DaTSCAN imaging, offers a possibility to study structural and biochemical integrity of presinaptic dopaminergic neurotransmitter system. The aim of this study was to evaluate the usefulness of ^{123}I-FP-CIT brain SPECT scintigraphy in patients with extrapyramidal diseases. **Methods.** Fifteen patients (8 males and 7 females), aged 26–81 years, presenting with extrapyramidal symptoms entered the study. Out of them, 7 patients were diagnosed with definite clinical form of idiopathic Parkinson's disease (PD) or clinical probable for PD clinical stage 2–4 using the Hoehn&Yahr scale (H&Y); 6 patients were with atypical parkinsonism (AP), 1 patient with essential, and 1 with psychogenic tremor. SPECT was performed 180 min after injection of 185 MBq ^{123}I-FP-CIT using a dual head Gamma camera. Sixty four one minutes' frames were acquired using a noncircular rotation mode into a 128 × 128 image matrix. Transverse slices were reconstructed using a 0.6 order Butterworth filter. Visual interpretation was based on striatal uptake, left to right asymmetry and substructures most affected. The ratio of binding for the entire striatum, caudate and putamen to nonspecific binding in occipital cortex was calculated. SPECT findings were categorized as normal and abnormal (incipient, moderate and severe presinaptic deficit). **Results.** ^{123}I-FP-CIT uptake was reduced in the striatum of 6/7 patients with PD and 5/6 patients with AP. Two patients with PD and AP showed a negative finding. The remaining 2 negative results were obtained in the patients diagnosed with essential tremor and psychogenic tremor. The mean striato-occipital ratio (SDR) of the most affected side was lower in the patients with PD. **Conclusion.** Our first results confirm the usefulness of ^{123}I-FP-CIT brain SPECT in differential diagnosis of extrapyramidal diseases.

Key words:
parkinsonian disorders; parkinson disease; diagnosis, differential; tomography, emission-computed, single photon; radionuclide imaging.

Apstrakt

Uvod/Cilj. Scintigrafija mozga pomoću ^{123}I-FP-CIT (DaTSCAN) tehnikom jednofotonske emisione komjuterizovane tomografije (SPECT), omogućava ispitivanje strukturnog i biohemijskog integriteta presinaptičkog dopaminergičkog neurotransmiterskog sistema. Cilj ovog rada bio je procena doprinosa ^{123}I-FP-CIT SPECT scintigrafije mozga kod bolesnika sa ekstrapiramidnim poremećajima. **Metode.** Ispitivanjem je bilo obuhvaćeno 15 bolesnika (osam muškaraca i sedam žena), starosti 26–81 godine, sa kliničkim manifestacijama ekstrapiramidnog sindroma. Od ukupnog broja bolesnika, kod sedam inicijalno je postavljena dijagnoza Parkinsonove bolesti (PD) ili sumnja na PD, kod šest bolesnika dijagnoza atipičnog parkinsonizma (AP), a kod dvoje dijagnoza esencijalnog, odnosno psihogenog tremora. Snimanje mozga rađeno je SPECT tehnikom, dvoglavom gama kamerom, 180 min posle davanja obeležavačke doze ^{123}I-FP-CIT-a aktivnosti 185 MBq. Akvizicija 64 jednominutna frema vršena je po necirkularnoj orbiti u matrici 128 × 128. Transverzalni preseci su rekonstruisani korišćenjem 0,6-rednog Butterworth filtera. Vizuelna interpretacija nalaza bazirana je na nakupljanju radiofarmaka u strijatumu, asimetriji vezivanja levo/desno i najzahvaćenijim supstrukturama. Indeks vezivanja je računat na osnovu odnosa nakupljanja u strijatumu, kaudatumu i putamenu i nespecifičnog nakupljanja u okcipitalnom korteksu. Nalazi SPECT-a su kategorizovani kao normalni i patološki (početni, umereni i izraženi presinaptički deficit). **Rezultati.** Nakupljanje ^{123}I-FP-CIT bilo je oslabljeno u regiji strijatuma kod šest od sedam bolesnika sa

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pouzdanom dijagnozom ili sumnjom na PD, kao i kod pet od šest bolesnika sa AP. Dva negativna nalaza dobijena su kod dve bolesnice sa PD i AP. Preostala dva negativna nalaza dobijena su kod dva bolesnika sa esencijalnim, odnosno psihogenim tremorom. Srednja vrednost strijato-okcipitalnog indeksa vezivanja na zahvaćenoj strani mozga bila je niža kod bolesnika sa PD. **Zaključak.** Naši prvi rezultati potvrđuju doprinos ^{123}I-FP-CIT SPECT scintigrafije mozga u diferencijalnoj dijagnostici bolesnika sa ekstrapiramidnim poremećajima.

Ključne reči:
parkinsonov sindrom; parkinsonova bolest; dijagnoza, diferencijalna; tomografija, komjuterizovana, emisiona, jednofotonska; scintigrafija.

Introduction

The term Parkinson's disease refers to a group of neurodegenerative conditions considered to primarily come from abnormalities of basal ganglia function. PD is one of the most common neurodegenerative diseases affecting about 329 per 100 000 people (age-adjusted prevalence rate) [1]. Although the etiology of PD is not completely understood, the condition probably results from a combination of polygenic inheritance, environmental exposures, and gene–environment interactions. Data has implicated mitochondrial dysfunction, oxidative damage, aberrant protein aggregation, and deficits in ubiquitin-mediated protein degradation as playing key roles in the etiopathogenesis of PD [2–4]. In PD degeneration of dopaminergic neurons and their projection to the striatum is a slowly evolving process that may take decades to develop. In most cases the diagnosis of PD is straightforward when cardinal clinical signs and symptoms as bradykinesia, rigidity, and resting tremor are present [5]. However, these main features of PD are shared, at least in part by essential tremor, multisystem atrophy, progressive supranuclear palsy, vascular parkinsonism, dementia with Lewy bodies, corticobasal degeneration, Alzheimer's disease, and drug-induced parkinsonism. Besides, delineating PD from the above mentioned parkinsonian disorders distinguishing PD from normality can also be difficult, especially in early stage of the disease [6].

A reliable test to diagnose PD is important for at least two reasons. Prognosis and management of PD and other parkinsonian disorders differ considerably [7], and an objective disease marker would facilitate the development of neuroprotective therapies [8]. Several procedures have been proposed to diagnose PD: functional imaging with positron emission tomography (PET) or single photon emission computed tomography (SPECT), transcranial sonography, olfactory and neuropsychological tests, biomarkers and DNA tests [9–12].

Imaging of the dopaminergic system with SPECT is used since radiopharmaceuticals for imaging the presynaptic dopamine transporter, as well as the dopamine D2 receptors became commercially available.

Dopamine transporters are localized on dopaminergic nerve endings and are lost in the process of degeneration in PD. They can be used as markers for the integrity or for the degree of loss of dopaminergic nerve endings. The cocaine derivative ^{123}I-labeled N-(3-fluoropropyl)-2ß-carbomethoxy-3ß-(4-iodophenyl)nortropane (FP-CIT) (DaTSCAN®, GE Healthcare) binds with high affinity to dopamine reuptake sites in the striatum and can be used to visualize dopaminergic nerve terminals *in vivo* in the human brain. The nuclear medicine method – brain SPECT with ^{123}I-FP-CIT is a valuable tool for discriminating neurodegenerative parkinsonian syndromes with an associated presynaptic dopaminergic deficit from diseases without presynaptic neurodegeneration [13]. SPECT with ^{123}I-FP-CIT is a sensitive marker of dopaminergic degeneration, and the degree of striatal binding reduction in PD correlates with disease severity [14, 15]. Dopamine transporter imaging offers the prospect of a quick, objective method to confirm or exclude presynaptic parkinsonism (PD) in inconclusive cases [16].

The aim of the study was to evaluate our first results with ^{123}I-FP-CIT SPECT in patients with extrapyramidal diseases.

Methods

Since January 2009, 15 patients (age range 26–81, median 67 years) with clinical findings suggestive of extrapyramidal disorders have been referred to our institution for ^{123}I-FP-CIT brain SPECT. All parkinsonian patients fulfilled clinical diagnostic criteria for PD [5]. The disease staging (modified Hoehn &Yahr score [17]) was given at the time of SPECT examination all but one patient with PD and atypical parkinsonism (AP) (range 1.00–4.00, mean 2.70 ± 1.07 standard deviation).

Thyroid uptake was blocked before the scan by administration of 400 mg of perhlorate at least 30 min prior to the injection. All subjects received 150–185 MBq (4–5 mCi) of ^{123}I-FP-CIT in slow intravenous injection. During the acquisition, the patient's head was fixed in with elastic band to minimize motion artifacts.

Acquisition started between 3 and 4 hours after intravenous injection of commercially available radiopharmaceutical ^{123}I-FP CIT (DaTSCAN®, GE Healthcare). Data were acquired with a double-head camera (Vertex, Adac) using low-energy-general purpose collimators, in 64 × 64 matrix size. Sixty four projections were acquired at 60 s per view with the camera heads following a non-circular orbit, resulting in a total scan time of 32 min. Total brain count of more than 1.5 million was achieved in all examinations. SPECT data were reconstructed by back projection filtered with a Butterworth filter (0.6 cut of value, order 8).

SPECT images were interpreted visually and semiquantitatively using region of interest techniques to assess specific ^{123}I-FP-CIT binding in striatum and striatal subregiones.

The findings were classified as normal if symmetric intense tracer uptake in striatum and striatal subregiones presented. Abnormal findings were categorized as incipient, moderate and severe presinaptic deficit. Incipient presinaptic deficit was defined as asymmetrical one-sided slightly re-

duced putamen uptake. Moderate presinaptic deficit was defined as reduction in specific/nonspecific tracer binding and poor visualization of putamen in both sides, and, finally, severe presinaptic deficit as significant reduction of specific binding resulting in vizualisation of background activity through the brain hemispheres.

For analysis of striatal ^{123}I-FP-CIT binding, 3 transaxial slices representing the most intense striatal binding were summed. Irregular regions of interest were constructed manually in areas corresponding to the right and left striatum, caudate, and putamen. Irregular regions of interest were also drawn in area corresponding to the occipital cortex (Figure 1).

The mean specific basal ganglia binding was calculated from the mean counts per pixel in the whole striatum, caudate nucleus, and putamen (specific binding), dividing the results by the mean counts per pixel in the occipital cortex (nonspecific binding). The ratios expressed as mean ± standard deviation, were compared between the patients.

Results

Subject demographic data are given in Table 1. Four patients had normal striatal ^{123}I-FP-CIT binding (Figure 2) with no significant differences in striatal or subregional binding ratios. Eleven patients had abnormal ^{123}I-FP-CIT SPECT findings – assigned as incipient, moderate and severe presinaptic deficit was found in 2, 5 and 4 patients, respectively (Figures 3 and 4). Subanalyses showed diminished

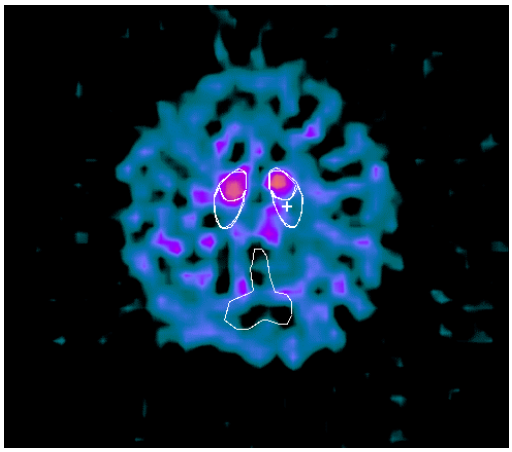

Fig. 1 – Irregular regions of interest were constructed manually in areas corresponding to the right and left striatum, caudatum, putamen, and occipital cortex

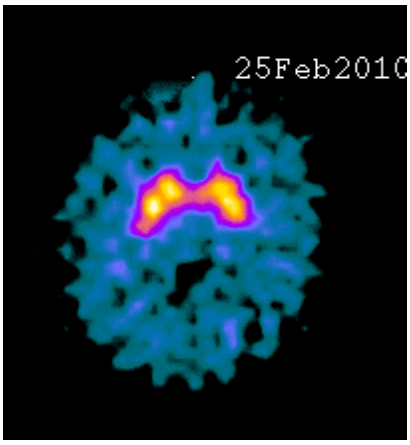

Fig. 2 – Normal ^{123}I-FP-CIT SPECT in patient with essential tremor

Table 1

Demographic and clinical characteristics of patients with Parkinson`s disease (PD)

Parameter	PD	AP	ET/PT
Patients number	7	6	2
Age (year) ($\bar{x}$ ± SD)	66.7 ± 8.8	59.8 ± 20.3	65.0 ± 21.2
Sex (M/F)	3/4	4/2	1/1
Modified H&Y score	2.42 ± 0.93	3.10 ± 1.24*	–

***1 patient missing; AP – atypical parkinsonism, ET – essential tremor, PT – psyhogenic tremor, H&Y score – Hoehn&Yahr score**

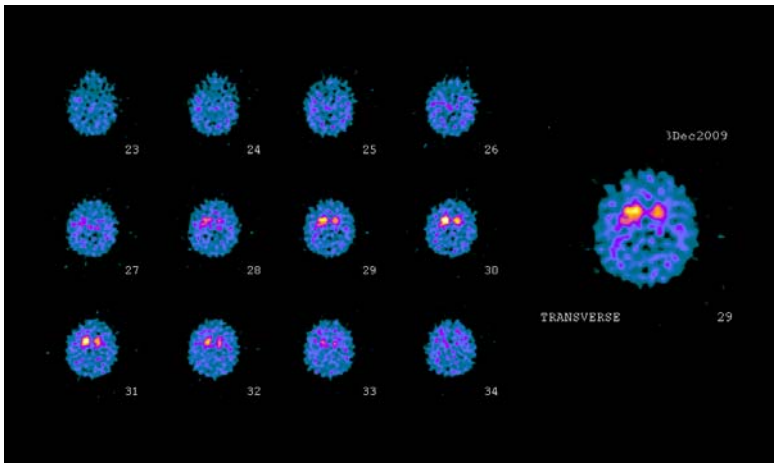

Fig. 3 – ^{123}I-FP-CIT SPECT interpreted as moderate presinaptic dopaminergic deficit in patient with Parkinson`s disease (clinical stage 2.0 H&Y)

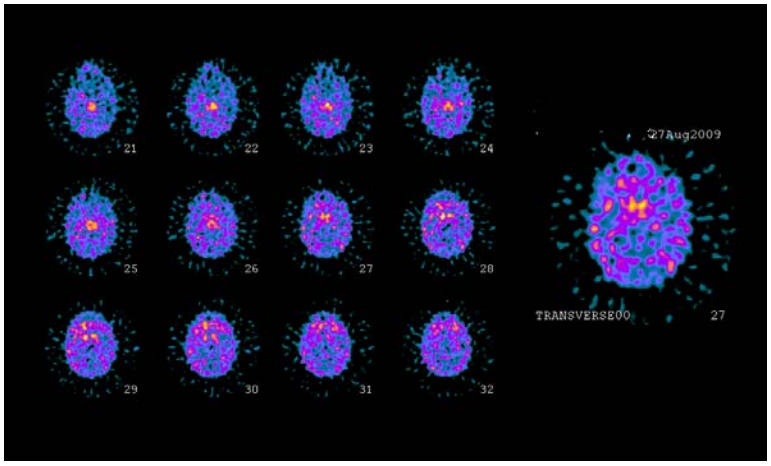

Fig. 4 – ^{123}I-FP-CIT SPECT interpreted as severe presinaptic dopaminergic deficit in patient initially diagnosed with atypical parkinsonism (clinical stage 4.0 H&Y)

binding in the caudate (2.15 ± 0.56 and 2.16 ± 0.56 for the right and left caudate, respectively), diminished binding in the putamen (1.82 ± 0.32 and 1.86 ± 0.47 for the right and left putamen, respectively).

^{123}I-FP-CIT binding ratios in the whole striatum, the caudate, and the putamen are presented in Table 2.

pathic PD either clinical probable for PD. All but one patient had a pathological ^{123}I-FP-CIT SPECT finding graded as moderate to severe presinaptic dopaminergic deficit. Normal ^{123}I-FP-CIT SPECT findings was obtained in a 72-year old woman diagnosed with PD in early stage (H&Y = 2). Rather than the false negative result, the pausible explanation may

Table 2

Specific ^{123}I-FP-CIT uptake ratio observed in striatum, caudatum, and putamen of patients with Parkinson`s disease (PD)

Patient N°	Clinical diagnosis	SPECT visually estimated*	Striatal region					
			Striatum		Caudatum		Putamen	
			R	L	R	L	R	L
1	PD	3	1.09	1.15	1.26	1.36	1.3	1.0
2	PD	1	1.77	1.72	2.04	2.01	1.77	1.49
3	ET	1	3.72	3.72	4.24	4.8	4.45	3.56
4	AP	5	1.65	1.52	1.79	1.71	1.85	1.48
5	AP	3	1.87	2.04	2.54	2.58	2.07	2.17
6	PD	2	1.89	2.16	2.93	3.04	1.89	2.28
7	PD	3	1.97	1.71	2.37	1.94	1.58	1.52
8	AP	3	2.5	2.15	2.5	2.54	2.46	1.91
9	PD	5	1.97	1.73	1.97	2.05	1.6	1.5
10	PT	1	2.37	2.4	2.42	2.82	2.42	2.47
11	PD	5	1.38	1.6	1.3	1.52	1.47	2.72
12	PD	3	2.12	2.24	2.47	2.47	1.92	2.05
13	AP	1	2.62	2.39	3.31	3.33	2.88	2.07
14	AP	2	2.27	2.24	2.77	2.85	2.01	2.05
15	AP	5	1.72	1.81	1.83	1.75	1.94	1.88
$\bar{x}$ ± SD			2.06 ± 0.62	2.03 ± 0.58	2.38 ± 0.76	2.45 ± 0.87	2.10 ± 0.71	2.01 ± 0.61

AP – atypical parkinsonism, ET – essential tremor, PT – psyhogenic tremor, R – right, L – left,

***1 – normal DaTSCAN, 2 – incipient presynaptic deficit, 3 – moderate presynaptic deficit, 5 – severe presynaptic deficit**

Discussion

There is no confirmatory test for PD except the hystopathological one. Although idiopathic PD represents the most prevalent form of Parkinson′s syndrome, at early stages of disease it could be difficult to differentiate idiopathic PD from other forms of parkinsonism solely on clinical grounds, taking in considerations that share, at least partialy, similar symptoms such as tremor, bradykinesia, rigidity, gait disturbance, speech or swallowing difficulties and autonomic dysfunction [18].

Out of 15 patients enroled in our study, seven were already diagnosed patients with clinical definite form of idiobe that the patient initially diagnosed as having PD actually have another disorder; moreover, taking into account that it occurs in 10%–25% of PD cases when reviewed pathologically [19]. In addition, approximately 10% of patients diagnosed clinically with early PD have normal dopaminergic functional imaging (Scans Without Evidence of Dopaminergic Deficit [SWEDDs]) [20, 21] .The diagnosis in these patients has been debated: is it early PD, some previously unrecognized form of PD, or not PD at all? A recent study analysing clinical details including non-motor symptoms in 25 tremulous SWEDDs patients in comparison with 25 tremor-dominant PD patients showed that underlying pathophysio-

logy of SWEDDs differs from PD but has similarities with primary dystonia [22]. Distinguishing these patients from PD is important, cause the correct diagnosis will help avoid inappropriate PD drug treatments. Study comparing DaT-SPECT results in autopsy findings in 25 patients with parkinsonian disorders suggests that DaT-SPECT can reliably document dopaminergic degeneration in Lewy body disorders [23].

The mean striatal FP-CIT binding was decreased in our patients already diagnosed as having PD (1.79 ± 0.36, 1.81 ± 0.35 for the right and left striatum, respectively), concordantly with literature data. Striatal ^{123}I-FP-CIT binding is reduced in PD in proportion to disease severity [14, 15]. Studies with 6-18F-fluoro-L-dopa PET suggest that the disease process in PD first affects the posterior putamen, followed by the anterior putamen and the caudate nucleus [24]. Our results showed that the putamen ^{123}I-FP-CIT binding was markedly reduced in subgroup of PD patients (1.73 ± 0.40, 1.76 ± 0.39 for the right and left putamen, respectively) *vs* the mean putamen binding (2.10 ± 0.71, 2.01 ± 0.61 for the right and left putamen, respectively) (Table 2).

Clinical rating scales, H&Y and the Unified Parkinson`s Disease Rating Scale (UPDRS) scores, have a high sensitivity and specificity for the clinical diagnosis of PD [25]. These rating scales, however, are more difficult to apply in patients with atypical parkinsonism. The clinical diagnosis is most difficult early in the disease when the signs and symptoms are quite subtle. Moreover, symptoms in PD become apparent only after a critical level of cell loss – the "symptom threshold" [26] – requiring a loss of approximately 80% of dopamine innervation.

We found interesting a case of 50-year-old woman with atypical parkinsonian syndrome and a ^{123}I-FP-CIT SPECT finding suggestive for PD performed in another institution 9 months ago. For the reason she clinically was not likely to be diagnosed with PD, we obtained a control scan. The typical abnormal finding consisting for the severe presinaptic deficit in PD was shown. Our finding contributed to clinical characterization of patient, which is of importance in therapy selection and prognosis.

Several studies on patients with known PD in early stage showed the ability to differentiate between PD and normally with 100% specificity. In distinguishing Alzheimer disease from Dementia Lewy body, *in vivo* findings of presynaptic dopaminergic imaging correlated well with neuropathological findings at autopsy, suggesting a remarkable sensitivity of 77%–88% and a specificity of 87.9%–100.0% [27–29].

According to the results of a meta-analysis of the literature data on diagnostic accuracy of SPECT in parkinsonian syndromes [30], SPECT with presynaptic radiotracers are highly accurate in differentiation between patients with PD and essential tremor. We had only two patients with clinical diagnosis suggestive to essential or psychogenic tremor and we obtained normal results in both of them. Normal SPECT FP-CIT finding enabled clinicians to exclude a neurodegenerative parkinsonian syndrome. The use of ^{123}I-FP-CIT SPECT can prove or exclude high sensitivity nigrostriatal dysfunction in cases of monosymptomatic tremor (dystonic tremor, essential tremor, Parkinson tremor) and facilitates early and accurate diagnosis. Furthermore, a normal ^{123}I-FP-CIT SPECT is helpful in supporting a diagnosis of drug-induced, psychogenic and vascular parkinsonism by excluding underlying true nigrostriatal dysfunction [31, 32].

Recent study of Antonioni et al. [33] reported that ^{123}I-FP-SPECT is likely to be regarded as economically advantageous to differentiate essential tremor from PD, increasing time on potentially beneficial therapy at a lower cost to healthcare system.

In addition, the study comparing the scanning techniques ^{123}I-FP-CIT and F-DOPA (positron emmision tomography tehnique) reported the sensitivity of 91% for both and specificity of 100% and 90% for ^{123}I-FP-CIT and F-DOPA, respectively, in the diagnosis of presynaptic dopaminergic deficits in early phases of PD [34].

A limitation of our study is that we did not include the healthy volunteer control group because of the absence of validated control population for ^{123}I-FP-CIT. In addition, manually constructed regions of interest may be the source of errors, as well. Using voxel-based analysis with anatomic standardization or validated an automated technique combining perfusion and ^{123}I-FP-CIT SPECT may contribute in the differential diagnosis of parkinsonism under clinical circumstances [35].

Conclusion

Imaging presynaptic dopamine transporter as an important diagnostic tool for patients with parkinsonian syndromes has become a routine clinical procedure in nuclear medicine departments in Europe. The technique is relatively accurate to differentiate patients with PD in an early phase from healthy one, patients with PD from those with essential tremor, and PD from vascular parkinsonism.

Based on the first 15 ^{123}I-FP-CIT SPECT studies performed in our Institute, we showed that ^{123}I-FP-CIT SPECT studies provide useful information used to confirm or exclude PD leading to change the diagnosis and patient management. ^{123}I-FP-CIT SPECT can therefore be considered useful tool in the diagnosis of patients with atypical clinical presentation of PD.

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ORIGINAL ARTICLE

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Frequency and changes in trends of leading risk factors of coronary heart disease in women in the city of Novi Sad during a 20-year period

Promene učestalosti i tendencije kretanja vodećih faktora rizika od koronarne bolesti srca kod žena Novog Sada tokom 20-godišnjeg perioda

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Abstract

Backround/Aim. From 1984 to 2004 the city of Novi Sad participated through its Health Center "Novi Sad" in the international Multinational MONItoring of Trends and Determinants in CArdiovascular Disease (MONICA) project, as one of the 38 research centers in 21 countries around the world. The aim of this study was to determine frequency and changes of trends in leading risk factors of coronary heart disease (CHD) and to analyze the previous trend of movement of coronary event in women in Novi Sad during a 20-year period. **Methods.** In 2004, the fourth survey within MONICA project was conducted in the city of Novi Sad. The representative sample included 1,041 women between the age of 25 and 74. The prevalence of risk factors in CHD such as smoking, high blood pressure, elevated blood cholesterol, elevated blood glucose and obesity was determined. Also, indicators of risk factors and rates of coronary events in women were compared with the results from MONICA project obtained in previous three screens, as well as with the results from other research centres. χ^2-test, linear trend and correlartion coefficient were used in statistical analysis of results obtained. **Results.** It was observed that during a 20-year period covered by the study, the prevalence of the leading risk factors for the development of CHD in the surveyed women was significantly increasing and in positive correlation with the values of linear trend. Also, the increase of morbidity rates and mortality rates of coronary event were in positive correlation. The decrease was only recorded in the period from 1985–1989 (the implementation of the intervention programme). **Conclusion.** Upon analysing the increase in prevalence of leading risk factors of CHD and significant increase in the rates of coronary event, we can conclude that health status of women in Novi Sad during a 20-year period was deteriorating.

Key words:
coronary disease; risk factors; women.

Apstrakt

Uvod/Cilj. Od 1984. do 2004. godine Novi Sad je preko Doma zdravlja „Novi Sad", kao istraživačkog centra, učestvovao u međunarodnom projektu *Multinational MONItoring of Trends and Determinants in CArdiovascular Disease* (MONICA), kao jedan od 38 istraživačkih centara u 21 zemlji sveta. Cilj rada bio je da se kod žena Novog Sada utvrdi promena učestalosti i vodećih faktora rizika od koronarne bolesti srca (KBS) i da se sagledaju dosadašnje tendencije koronarne bolesti srca tokom 20-godišnjeg perioda. **Metode.** U Novom Sadu 2004. godine izvršeno je četvrto istraživanje u okviru projekta MONICA, na uzorku od 1,041 žene, starosti 25–74 godine. Utvrđena je prevalencija vodećih rizičnih faktora koronarne bolesti srca: pušenja, povišenog krvnog pritiska, povišenog holesterola i glukoze u krvi, i gojaznosti. Izvršena je komparacija prevalencije rizičnih faktora, i stope koronarnih događaja sa rezultatima dobijenim u prethodna tri istraživanja u Novom Sadu, kao i rezultatima dobijenim u ostalim istraživačkim centrima koji su učestvovali u projektu MONICA. Statistička značajnost analiziranih podataka određivana je pomoću χ^2-testa, linearnog trenda i koeficijenta korelacije. **Rezultati.** Tokom 20-godišnjeg praćenja, prevalencija vodećih faktora rizika od razvoja KBS kod ispitivanih žena je u značajnom porastu i pokazuje pozitivnu korelaciju sa vrednostima linearnog trenda. Pozitivnu korelaciju, takođe, pokazuje i porast stope incidencije i mortaliteta od koronarnih događaja. Pad se beleži samo 1987. godine (sprovođenje interventnog programa). **Zaključak.** Analizirajući povećanje prevalencije faktora rizika od KBS i posledično značajno povećanje stope mortaliteta, može se zaključiti da se situacija kod žena u Novom Sadu znatno pogoršala tokom dvadesetogodišnjeg praćenja.

Ključne reči:
koronarna bolest; faktori rizika; žene.

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Introduction

Cardiovascular diseases (CVDs) are the leading cause of death among women in the modern world [1–3]. Coronary heart disease (CHD) is among the major CVDs. Basically, CHD as a consequence of an accelerated process of atherosclerosis is caused by a number of risk factors. Some of them cannot be changed (age, gender, heredity), while the majority of risk factors can be modified by changing the life style. Among the factors mentioned in the first place are: smoking, obesity, high blood pressure, elevated blood cholesterol, elevated blood sugar level [2–4]. Coronary risk is known to be multi-factorial, so that the effect of one risk factor will be combined and modified by the effect of the others [5, 6].

The intensive implementation of an integrated program of prevention and control of CVDs in Finland in the period from 1975 to 2000 significantly reduced CHD morbidity and mortality due to a significant reduction of leading risk factors [7–9].

The project Monitoring of Trends and Determinats in Cardiovascular Disease (MONICA) was the largest World Health Organisation (WHO) research study which included 21 countries and 38 research centers [10–12]. Health Center in Novi Sad was nominated as a research centre and participated in this project from 1984 until 2003 [10–12].

The aim of this study was to determine frequency and changes of trends in leading risk factors of CHD and to analyze the previous trend of coronary event in women in Novi Sad during a 20-year period.

Methods

The study of the project MONICA included the entire population of Novi Sad aged between 25 and 74 years, but a separate analysis was done only for women after the fourth screening in 2004. The fourth screening was conducted on a representative sample consisting of women aged between 25 and 74 years [10, 11]. The number of women in the sample was 1,222 divided into five age groups. The response rate was 85.2%. A total number of responding women was 1,041 [13, 14].

Risk faktors included in the screening were: smoking, high blood pressure, elevated blood cholesterol, high blood sugar and obesity. All indicators of risk factors, measurements and laboratory tests were expressed in accordance with the recommendation European Health Risk Monitoring (EHRM) [15].

High systolic blood pressure is a systolic blood pressure 140 mmHg and higher, or diastolic 90 mmHg and higher (WHO-ISH, 1999) or blood pressure of a person already been under treatment for hypertension [15].

Obesity is estimated on the basis of the values of body mass index (BMI), taken as the value ≥ 30 kg/m^2 [15].

Hypercholesterolemia is considered to exist for the values ≥ 5.0 mmol/L recommended by EHRM [15].

Elevated blood glucosa values are considered to be values ≥ 6.1 mmol/L, while a value of 7.0 mmol/L or more are considered as a preliminary diagnosis of diabetes [15].

Smoking indicators are daily smokers and average number of cigarettes smoked per day [15].

Risk factors found in women in 2004 (smoking, high blood pressure, elevated cholesterol levels and obesity) were compared with the results obtained in earlier studies in the project MONICA (1984/85, 1988/89, 1994/95) [16].

Fatal and nonfatal coronary events were monitored through population-based Registers. The data source was the Register that operated from 1983 until 2004 with the break between 2000 and 2002. Coronary event represents confirmed myocardial infarction and confirmed death from CHD [12, 13, 15].

The results of other MONICA centers were also compared [12–14].

In the statistical data processing the descriptive statistical method was used: mean value ($\bar{x}$) and the standard deviation (SD). The statistical significance of the analyzed data was determined by using χ^2-test (for the level of significance either $p < 0.05$ or $p < 0.01$ was taken), linear trend and correlation coefficient.

Results

The prevalence of smoking among women was 41.7% (35.7% smoked daily and 6.0% occasionally). On average, women started to smoke at the age of 21. The highest percentage of women smokers was in the age group between 25 and 34 years (48.7%) and the lowest in the age group between 65 and 74 years (15.7%). The difference in age was statistically significant ($\chi^2 = 20.06$, $p < 0.001$). There was a high level of correlation between smoking and age of the women ($R^2 = 0.87$). The average number of cigarettes smoked per day per woman was 15.8 (Table 1). A total of 34.1% of the women stopped smoking during the last five years before 2004 and 47.8% more than five years before 2004.

Table 1

The average value of risk factors for coronary heart disease (CHD) in women in Novi Sad

Risk factors for CHD	$\bar{x}$	SD	Min.	Max.
Number of cigarettes smoked per day	15.8	8,5	1	60
Blood pressure (mmHg)				
systolic	129	24	85	231
diastolic	81	12	56	132
Total cholesterol (mmol/L)	6.06	1.26	2.6	16
Glucose (mmol/L)	5.76	1.93	2.6	19

$\bar{x}$ – mean value; SD – standard deviation; Min. – minimum value; Max. – maximum value

The prevalence of hypertension (140/90 mmHg or more) in women was 34.0%. The average value of systolic pressure was 129 mmHg systolic and 81 mmHg diastolic (Table 1).

The prevalence of high blood cholesterol (5.0 mmol/L or more) in women was 81.8%. The average value of cholesterol was 6.06 mmol/L (Table 1).

The prevalence of obesity in women (BMI ≥ 30 kg/m^2 and over) was 24.1%. The percentage of those with BMI between 25 kg/m^2 and 30 kg/m^2 was 34.5%. The average BMI of women was 26.7 kg/m^2.

The prevalence of women with elevated blood glucose (value ≥ 6.1 mmol/L) was 19.8%, of which the prevalence of women with blood sugar level of ≥ 7 mmol/L was 9.5%. and they were recognised as potential diabetics.

The average number of risk factors in women ranged from 0 to 5. The percentage of women with 2 risk factors was 35.1% and without risk factors was 7.7% of the surveyed women (Figure 1). The average number of risk factors in women was 1.92.

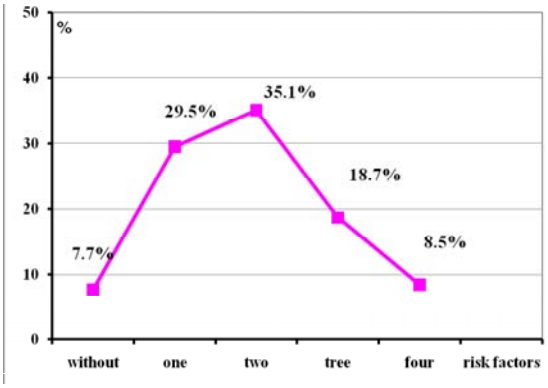

Fig. 1 – The frequency of risk factors in women in Novi Sad

In comparison with the earlier screenings in 1984/85, 1988/89 and 1994/95 the prevalence of smoking among women of Novi Sad had significantly increased (from 30.8% to max 41.7%). The prevalence of hypertension had also increased from 27.9% to 34%. The prevalence of hypercholesterolemia had increased from 29.6% to 81.8%. The prevalence of obesity had increased from 46.7% to 58.6%. During a 20-year follow-up, the prevalence of risk factors was in a significant increase. The prevalence of the studied risk factors showed a positive correlation with the values of the linear trend (Figure 2).

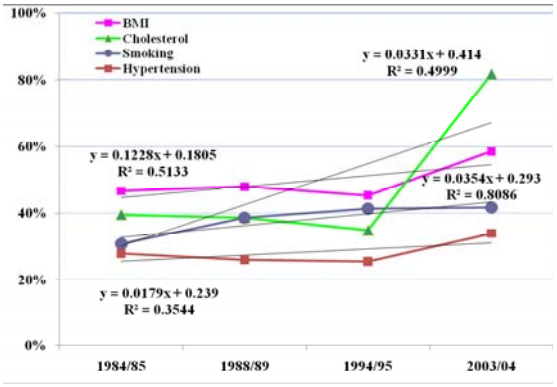

Fig. 2 – Chages in trends of risk factors in women in Novi Sad

Smoking ($\chi^2 = 10.98$, $p < 0.05$) Hypertension ($\chi^2 = 18.89$, $p < 0.001$)
Cholesterol ($\chi^2 = 257.68$, $p < 0.001$) Body mass index – BMI ($\chi^2 = 72.89$, $p < 0.001$)

Coronary events in the women showed a statistically significant increase from values close to the linear trend line (R^2 = 0.80). At the beginning of the study, in 1983, there were 154 coronary events per year and at the end, in 2004, there were 340 coronary events per year. The fall was recorded in 1987 and reduced by 15%. In the subsequent period, the coronary events among women were increased by 120% (Figure 3).

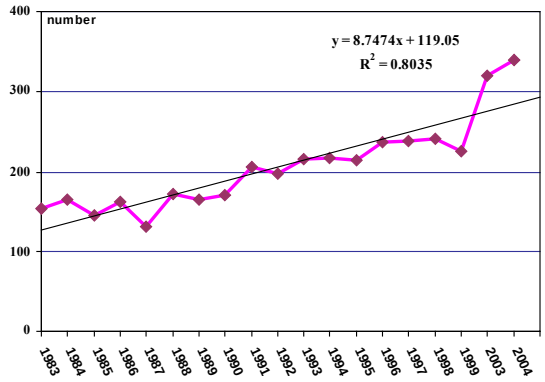

Fig. 3 – Coronary events in female population in Novi Sad

Mortality from CHD in women also showed a statistically significant increase from the values close to the linear trend line (R^2 = 0.74). The fall was recorded in 1987 (Figure 4).

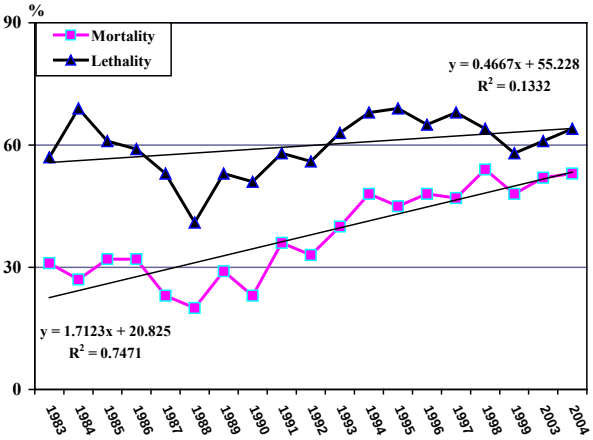

Fig. 4 – Mortality from coronary events in women in Novi Sad

Lethality from coronary ischemic events declined until 1987, and then increased until 1999, and then declined again until 2003, and then increased again until 2004 (Figure 4).

The authors also made comparison of indicators of risk factors in women, with results from the project MONICA obtained in other research centers [12].

The prevalence of female daily smokers increased in Novi Sad. It was in the 11th place in the beginning of the study, and in the 6th place at the end the study in comparison with other centers [12, 17, 18]. In terms of the average systolic blood pressure Novi Sad was in the 15th place in the begin-

ning, and in the 4th place at the end of the study. By the amount of total cholesterol in women Novi Sad was in the 31st place in the beginning and in the 3rd place at the end of the study. This makes Novi Sad a center of the highest increase in cholesterol levels in comparison with other centers [12]. The average total cholesterol in the blood of women was in the range of 4.5 mmol/L (Beijing, China) and 6.3 mmol/L (Kaunas, Lithuania) [12].

With a total number of risk factors in women Novi Sad was in the 17th place in the beginning of the study, but in the first place in the end. Thus, Novi Sad was among 8% of the centers with the largest increase of number of risk factors in women (Novi Sad, China, Switzerland and Poland). In 60% of research centers there was a statistically significant decrease in the total average number of risk factors (Russia, Poland, United Kingdom, Germany, Italy, Finland, France, Australia), while in 20% there were no significant changes [12, 17, 18] (Figure 5).

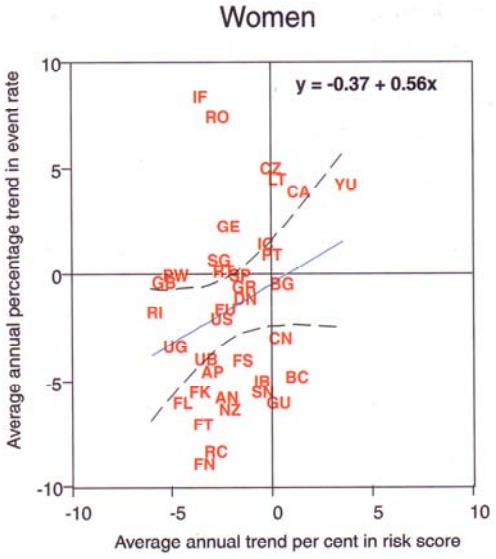

Fig. 5 – Chages in trends of coronary events in women included in the MONICA study compared with the number of risk factors [12]

By analysing the average annual change rates of coronary events it can be observed that in 42% of the 38 research centers there was a reduction in coronary events during the decade, and in 16% of the centers there was a significant increase in the rate of coronary events. Novi Sad belongs to a group of centers with a significant increase. In 50% of research centers, there was a significant decrease in mortality rates from coronary heart disease. In 25% of the centres there was no change, and Novi Sad is one of the 25% of centers with a significant increase in mortality from coronary heart disease in women [12, 17, 18] (Figure 5).

In the proportion of CVD in the crude death rate for women, Novi Sad was in the 9th place in the beginning and in the 4th place in the end of the study. In terms of coronary events there has also been a deterioration in Novi Sad, increasing from 20th place in the beginning to the 11th in the end of the study [12, 17, 18]. Compared with the results in 38 research centers in the 21 country of the world, women in Novi Sad could be classified among most vulnerable in relation to CVD [12].

Discussion

Cardiovascular diseases in women in modern world are characterized, in the first place, by incidence and mortality in relation to other diseases. Coronary heart disease is among the leading CVDs and prevention of this disease is necessary in the shortest possible time [1–3, 6, 8, 19]. Due to reduced production of estrogen after menopause in women, there is an increase in risk of developing CVDs [20], so preventive measures for women in the period after menopause are especially important, since the incidence of diseases of CVD increases with age [4, 20–22].

The results of our screening show that prevalence of risk factors significantly increased during a 20-year period. Novi Sad is among a few centres in which the average number of risk factors in women increased (China, Switzerland, Poland) [12, 17, 18].

In most of research centres there was a significant reduction in risk factors, and a significant reduction of daily smokers in 50% of research centers (USA, Great Britain, Iceland, China, Australia). In 45% of research centers, there was a significant decrease in the average systolic blood pressure (Poland, Germany, Russia, France), and in 50% of them, there was a statistically significant decrease in the average cholesterol in blood (Sweden, Russia, France, Finland, Italy) [12, 17, 18].

There was a significant reduction in morbidity from CHD in 63% centers (Russia, France, Australia, Finland, Sweden, Iceland), and a significant decrease of mortality in 60% centres (Australia, France Italy, Sweden, USA) [12, 17, 18].

At the beginning of the study, Novi Sad was among the best research centres up to 1991, when a significant decrease in morbidity and mortality from CHD was registered. The mortality from CHD was reduced by 15%. It was the time of intensive implementation of the intervention programme. In the subsequent period, after 1992, risk factors, morbidity and mortality from CHD among women significantly increased. This situation can be explained by the presence of new risk factors, stress, due to the difficult economic situation, state of war, sanctions placed by the United Nations, the deficiency in food (fewer vitamins, especially antioxidants) and deficiency of medication, especially for treatment of hypertension.

The situation did not change even after the end of the war and lifting sanctions placed by the United Nations. In this period Serbia was in a difficult economic situation, and began to reform the health care system.

Conclusion

Upon analysing the increase in prevalence of leading risk factors of CHD and significant increase in rates of coronary event, we can conclude that health status of women in Novi Sad during a 20-year period was deteriorating.

We hope that a recently adopted National Programme for Prevention and Control of CVDs will open new perspectives for more efficient work in this field. A special place and importance would be given to the specific prevention measures related to women.

The results of the research also show that female population is increasingly affected by leading risk factors of CHD in Novi Sad. Most effective measure is the reduction of risk factors which could be achieved at the primary health care level using available cost-effective measure. Experience from the period of intensive implementation of the intervention programme is a very strong evidence that even in the present conditions it is possible to improve the situation.

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Efikasnost TNF-α antagoniste i drugih imunomodulatora u terapiji bolesnika sa oftalmološkim manifestacijama Behčetove bolesti i HLA B51 pozitivnih vaskulitisa

Efficacy of TNF-α antagonist and other immunomodulators in the treatment of patients with ophthalmologic manifestations of Behcet's disease and HLA B51 positive vasculitis

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Apstrakt

Uvod/Cilj. Behčetova bolest je genetski uslovljen, imunološki posredovan multisistemski okluzivni vaskulitis malih krvnih sudova, posebno venula, nepoznate etiologije. Cilj rada bio je analiza elemenata kliničke slike, aktivnosti oboljenja kao i terapije bolesnika sa oftalmološkom manifestacijom Behčetove bolesti. **Metode.** Ovom prospektivno-retrospektivnom studijom obuhvaćeno je 11 bolesnika iz dva oftalmološka klinička centra, kojima je dijagnoza Behčetove bolesti postavljenja na osnovu Internacionalnih kriterijuma za Behčetovu bolest iz 2006 godine. Simptomi i znaci oboljenja ispitivani su prospektivno i retrospektivno u vreme aktivne manifestacije bolesti. Efekat terapije procenjivan je praćenjem najbolje korigovane vidne oštrine i inflamacije u staklastom telu pre i posle primene terapijskog modaliteta. Primenjena terapija sastojala se od primarne kortikosteroidne terapije u aktivnoj fazi bolesti koja je dopunjavana drugim lekom izbora iz grupe imunosupresiva. U našem slučaju to je bio ciklosporin ili metotreksat. Refrakterni slučajevi sa lošom vidnom prognozom lečeni su i trećim lekom, biološkim preparatom infliksimabom, TNF-α antagonistom. **Rezultati.** Prosečna starost 11 bolesnika sa Behčetovom bolesti bila je 50,6 godina. HLA B-5(51) tip bio je pozitivan kod 81% bolesnika, a *pathergy* test kod 36% bolesnika. Promene na oku bile su u vidu vitritsa (100%), zadnjeg uveitisa (45%), panuveitisa (54%), vaskulitisa retine (54%), cistoidnog edema makule (54%), cistoidne degeneracije makule (18%). Povišen intraokularni pritisak imalo je 27% bolesnika. Parametri aktivnosti bolesti nisu pokazali statistički značajno odstupanje kod ispitivanih bolesnika ($p > 0,05$). Uočeno je statistički značajno poboljšanje vidne oštrine ($p < 0,05$) lošijeg oka nakon terapije. Takođe, utvrđeno je statistički visokoznačajno smanjenje inflamacije u lošijem oku u ispitivanoj grupi bolesnika ($p = 0,001$). **Zaključak.** Utvrđeno je statistički značajno poboljšanje vidne oštrine i smanjenje inflamacije kod ispitivanih bolesnika primenom terapijskog protokola na bazi kortikosteroida, ciklosporina (metotreksata) ili TNF-α antagoniste. Visoka cena terapije i potencijalne komplikacije predstavljaju ograničavajuće faktore za propisivanje TNF-α antagoniste.

Ključne reči:
behčetov sindrom; mrežnjača, bolesti; uveitis; dijagnoza; ciklosporin; metotreksat; lečenje, ishod.

Abstract

Bacground/Aim. Behcet's disease is genetically conditioned, immune-mediated multisystem occlusive vasculitis of small blood vessels, espesially venules, of unknown etiology. The aim of this study was to analyze the clinical features, disease activity and therapy of the patients with ophthalmologic manifestation of Behcet's disease. **Methods.** In this study symptoms and signs of the disease were analyzed both prospectively and retrospectively during the active manifestation of the disease. The diagnosis was reached according to the International Criteria for Behcet's Disease (2006). The treatment effects were evaluated based on the presence of the best corrected visual acuity and the inflammation of the vitreous humour before and after the application of our therapeutic method. The applied therapeutic modality consisted of the primary application of corticosteroid therapy in the active stage of the disease complemented with the choice of drugs from the immunosuppressive group. In this study there drugs were cyclosporine or methotrexate. A treatment refractory patients with poor vision prognosis were treated with a third

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drug, the biological preparation infliximab, a tumor necrosis factor-alpha (TNF-α) antagonist. **Results.** The mean age of 11 patients with ophthalmologic manifestation of Behcet's disease was 50.6 years. HLA B-5(51) was positive in 81% of the patients while 36% of the patients had positive pathergy test. Changes in affected eyes included vitritis (100%), posterior uveitis (45%), panuveitis (54%), retinal vasculitis (54%), cystoid macular edema (54%), and cystoid degeneration (18%). Increased intraocular pressure was observed in 27% of the patients. There was no statistically significant variation in disease activity parameters in any of the patients ($p > 0.05$). A statistically significant improvement in visual acuity ($p < 0.05$) and a high statistically significant decrease of inflammation of the worst affected eyes ($p = 0.001$) were detected. **Conclusion.** Our therapeutic method is useful for producing the optimal therapeutic plan for the acute – chronic stage of the difficult ophtamological manifestation of Behcet's desease as well as the prevention of relapse. However the high cost of the therapy and the potential complications should be taken into consideration when prescribing this therapy, especially a TNF-α antagonist.

Key words:
behcet syndrome; retinal diseases; uveitis; diagnosis; cyclosporine; methotrexate; treatment outcome.

Uvod

Behčetova bolest (BB) je genetski uslovljen, imunološki posredovan multisistemski okluzivni vaskulitis malih krvnih sudova, posebno venula, nepoznate etiologije. Oboljenje je pretežno obostrano i u kratkom vremenskom periodu dovodi do slepila ako se ne dijagnostikuje na vreme.

Behčetova bolest je nazvana po Hulusi Behçetu, turskom dermatologu koji je 1937. godine prvi opisao trijas simptoma – recidivantne aftozne ulceracije bukalne sluznice, genitalne ulceracije i uveitis [1].

Najprihvaćenije objašnjenje patogeneze BB obuhvata genetsku, infektivnu i autoimunsku komponentu. Smatra se da spoljašnji stimulus pokreće imunsku reakciju kod genetski osetljivih – predisponiranih HLA B-51 osoba. Infekcija ili inflamacijsko stanje mogu dovesti do prezentacije antigena. Navodi se lista mogućih infektivnih i/ili auto antigena [2]. Smatra se da HLA-B51 povezuje pul polipeptida niskog afiniteta, koji mogu dovesti do neefikasne indukcije tolerancije [3]. Posledični imunski odgovor je produkcija citokina, uključujući i faktor nekroze tumora alfa (TNF-α) i aktivacija T-ćelija [4]. Endotel se aktivira povišenim solubilnim ICAM-1 u serumu bolesnika sa oftalmološkim manifestacijama BB [5]. Iako su opisana raznovrsna antitela, malo njih igra ulogu u samoj patogenezi ove bolesti. Neke studije koriste *proteomic* pristup i trijažu endotelne ekspresije novih autoantitela [6, 7], koja indukuju dalje funkcionalne efekte. Veruje se da takav pristup može dovesti do novih dijagnostičkih testova i do boljeg razumevanja ovog oboljenja.

Bitna imunološka i histopatološka obeležja BB su CD4 pozitivne T-ćelije, ćelije „prirodne ubice“ (CD8+CD56+ NKT) [8] i hiperaktivni neutrofili indukovani citokinima TNF-α, IL-8, GM-CSF, hiperprodukcijom oksigena i povećanom endotelnom citotoksičnošću. Takođe, uočeno je da γδ-T ćelije reaguju na mikobakterijski 65-kDa *heat shock* protein i njihove homologe humane peptide 60-kDa 9 (HSP60). Ovo ukazuje na to da su oni mogući specifični epitopi kao i da su neki infektivni agensi (streptokokni antigen, stafilokoknini endotoksin, peptidi *Escherichia coli*) mogući triger. Ćelije γδ-T dalje stvaraju interleukin (IL)-2, TNF-α, INF-γ i IL-8 [10, 11].

Kod bolesnika sa trombozom u kliničkoj slici uočeni su disfunkcija i oštećenje endotela, koagulacija i poremećaj fibrinolitičkog puta (defekt proteina C, proteina S, faktor V).

Dijagnoza ovog oboljenja zasnovana je na Internacionalnim kriterijumima za BB (ICBD) [12] iz 2006 godine. Iako nema dijagnostičkih laboratorijskih testova za BB, uočen je povišen nivo imunoglobulina, posebno imunoglobulina A u akutnoj fazi, dok su C-reaktivni protein (CRP) i sedimentacija eritrocita retko povišeni kod bolesnika sa nodoznim eritemom, tromboflebitisom i artritisom, a nisu povišeni kod manifestacija centralnog nervnog sistema i okularnih ili mukokutanih bolesti. U akutnoj fazi bolesti povišeni su serumski amiloid A [13], beta-2 mikroglobulin [14] i neopterin [15]. Autoantitela, uključujući antineutrofilna citoplazmatska antitela, povremeno su pozitivna, ali nemaju dijagnostički značaj. Ovde je potrebno da se isključe oboljenja kao što su psorijaza, idiopatska plućna fibroza, ulcerozni kolitis, reumatoidni artritis koja, takođe, mogu imati povišen nivo IL-8. Takeuchi i sar. [15] ispitivali su nivo serumske alkalne fosfataze, i CRP kod bolesnika sa aktivnom BB. Došli su do zaključka da se nivo alkalne fosfataze može koristiti kao marker koji određuje aktivnost bolesti, kao i da postoji pozitvna korelacija između nivoa alkalne fosfataze i CRP kod bolesnika sa aktivnom BB.

Terapija BB obuhvata primenu kortikosteroida, imunomodulatora, pre svega ciklosporina [16], potom antimetabolite i alkilirajuće preparate. Smatra se da nove imunomodulatorne supstance, kao što su interferon (IFN)-α i TNF-α antagonisti poboljšavaju inače lošu prognozu u odnosu na očuvanje vida kodove bolesti jer su efikasni i kada su drugi imunosupresivni modulatori neuspešni. Ciklosporin i azatioprin su pokazali pozitivan efekat u lečenju uveitisa, a zbog neurotoksičnosti ciklosporin se izbegava kod bolesnika sa neurološkim manifestacijama, dok su izveštaji za IFN i TNF-α antagoniste sa manjim brojem opisanih slučajeva. Kolhicin i azatioprin su efikasni kod BB gde, pored očnih manifestacija u kliničkoj slici, dominira artritis, što važi i za vaskulitise, neurološke i gastrointestinalne forme BB [17]. Evropska liga za reumatizam predložila je protokol za lečenje BB [18].

Cilj ovog rada je analiza elemenata kliničke slike i terapije bolesnika sa oftalmološkim manifestacijama BB i obuhvatio je tip i način okularnih manifestacija BB i analizu aktivnosti bolesti, dijagnostički efekat fluoresceinske angiografije, učestalost HLA-B51 antigena i procenu efikasnosti primenjenog terapijskog modaliteta.

Metode

Studijom je bilo obuhvaćeno 11 bolesnika sa uveitisom kao oftalmološkom manifestacijom BB, lečenih u dva oftalmološka klinička centra. Simptomi i znaci BB analizirani su prospektivno i retrospektivno u vreme aktivne manifestacije ove bolesti. Kao mera aktivnosti oboljenja kontrolisani su parametri SE, IgA, alkalna fosfataza. Rukovodili smo se protokolom za lečenje BB Evropske lige za reumatizam, kao i našim iskustvima. U dijagnostici i praćenju očnih manifestacija bolesti korišćeni su biomikroskop, direktna i indirektna oftalmoskopija i fluoresceinska angiografija.

Kontinuirano su praćeni klinički tok bolesti, najbolje korigovana vidna oštrina boljeg i goreg oka, i promene u makuli, a stepen inflamacije staklastog tela registrovan je kao retke ćelije, od 1+ do 4+ [20]. Takođe, praćena je aktivnost bolesti merenjem nivoa CRP, SE, nivoa alkalne fosfataze i, IgA antitela. Ima autora koji u tu svrhu mere nivo pojedinih citokina posebno IL-8 u serumu ovih bolesnika jer je uočeno da se mononuklearne ćelije, fibroblasti i endotelne ćelije boje IL-8 antitelima. Merenje IL-8 nije rađeno zbog cene ove analize i nemogućnosti da se uradi u svakom centru. *Pathergy* test je, takođe, korišćen kao deo dijagnostičkog protokola.

Rezultati

Behčetova bolest, bilateralno zastupljena, dijagnostikovana je kod svih 11 bolesnika (7 muškaraca i 4 žene) (tabele 1 i 2).

Tabela 1

Prikaz dijagnostičkih karakteristika bolesnika sa Behčetovom bolešću i HLA B51 pozitivnim vaskulitisom

Bolesnici	Vidna oštrina				Opacitati				Kriterijumi ISG	*Patergy* test
	pre terapije		posle terapije		pre terapije		posle terapije			
	b.o.	g.o.	b.o.	g.o.	b.o.	g.o.	b.o.	g.o.		
1.	0,5	0,01	0,7	0,01	++	++++	+	+	3	+
2.	0,1	0,03	0,3	0,1	++	+++	+	++	3	+
3.	0,5	0,08	0,9	0,8	++++	+++	+	+	3	+
4.	1	0,5	1	0,9	+	++	+	++	2	
5.	1	0	1		-	+-	-	-	2	
6.	0,9	0,1	0,9	0,2	+	++	+	+	2	
7.	1	0,1	1	0,2	-	++	-	+	3	
8.	1	0,3	1	0,5	-	+	-	-	3	
9.	0,2	0,01	1	1	-	+	-	-	3	+
10.	1	1	1	1	+	++	+	+	2	
11.	1	0,1	1	0,3	+	+++	-	+	3	

ISG – *International Study Group for Behcet's Disease*
b.o. – bolje oko; g.o. – gore oko

Tabela 2

Prikaz kliničkih karakteristika bolesnika sa Behčetovom bolešću i HLA B51 pozitivnim vaskulitisom

Bolesnici	Dijagnoza				Terapija	
	Životno doba (godine) / pol (muški – M, ženski –Ž)	prednji uveitis	zadnji uveitis	CME OD,OS	lekovi	doza
1.	21/M	Ø	vaskulitis infiltrati	+	prednizolon ciklosporin infliksimab	20 mg 5 mg/kg 5 mg/kg
2.	15/Ž	Ø	periflebitis	- +	prednizolon metoteksat infliksimab	10 mg 12,5 mg 5 mg/kg
3.	45/M	granulomatozni hipertenzivni iridiciklitis	flebitis	+ +	prednizolon metotreksat ciklosporin	0,4 mg/kg ↓ 7,5 mg 5 mg/kg
4.	24/M	serofibrinozni icidociklitis	flebitis	+ -	prednizolon	1 mg/kg
5.	58/M	Ø	vazokluzivni sindrom	CDM	prednizolon	1 mg/kg
6.	71/Ž	serofibrinozni iridociklitis	flebitis, NVS, hemoftalmus	+ -	prednizolon	0,5 mg/kg
7.	45/M	serofibrinozni hipertenzivni iridociklitis	flebitis	- -	prednizolon	1mg/kg
8.	52/Ž	serofibrinozni iridociklitis	retinitis, hemoragije retine eksudati retine	+ -	prednizolon	0,5 mg/ kg
9.	35/M	serofibrinozni hipertenzivni iridociklitis	retinitis -hemoragije retine, eksudati	- -	prednizolon metotreksat	1 mg/kg 0,1 mg/kg
10.	71/Ž	Ø	vitritis	- -	prednizolon	0,3 mg/kg
11.	46/M	serofibrinozni iridociklitis	flebitis	CDM	ciklosporin	1–3 mg/kg

CDM – cistoidna degeneracija makule, CME – cistoidni edem makule; NVS – neovaskularizacija

Prosečna starost bolesnika sa okularnim manifestacijama BB iznosila je 50,6 ± 15,8 godina, a HLA B51 antigen je dijagnostikovan kod 9 (81,2%) od 11 bolesnika. U vreme dijagnostikovanja bolesti prosečna starost bolesnika bila je 44,8 ±15,7 godina.

Oftalmološke manifestcije bolesti (tabela 3), bile su u formi panuveitisa kod šest bolesnika (54,5%) i zadnjeg uveitsa kod pet bolesnika (45,4%). Prednji uveitis je bio u formi granulomatoznog iridociklitisa kod jednog bolesnika (9,1%), hipertenzivna forma kod 3 bolesnika (27,3%). Znaci vaskulitisa uočeni su kod sedam bolesnika (63,3%), a znaci vazookluzivnog sindroma bili su prisutni kod jednog bolesnika (9,1%). Cistoidni edem makule (CEM) je dijagnostikovan kod šest bolesnika (54,5%), kod jednog bolesnika obostrano; cistoidna degeneracija makule je postojala kod dva bolesnika. Vitritis je bio prisutan kod svih jedanaest bolesnika sa okularnim manifestacijama BB.

Odstupanja vrednosti IgA i alkalne fosfataze nisu bila statistički značajna ($p > 0,05$). *Pathergy* test je bio pozitivan kod četiri bolesnika. HLA tipizacija urađena je kod 9 (81%) od ukupno 11 bolesnika i kod svih devet HLA-B51 bila je pozitivna.

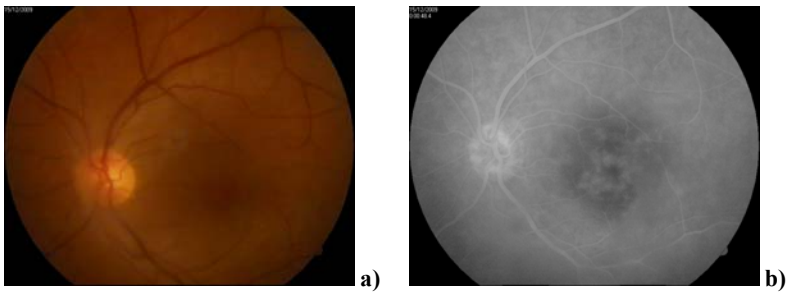
Fluoresceinska angiografija urađena je kod četiri (36,3%) bolesnika (slike 1–4) sa namerom da nam jasnije prikaže stanje makule, to jest CEM, kao i znake ishemije i neovaskularizacije. Kod jedne bolesnice neovaskularizacija je dovela do totalnog hemoftalmusa na jednom oku i parcijalnog hemoftalmusa na drugom oku. Promena je kasnije tretirana laser fotokoagulacijom u smislu dalje prevencije komplikacija.

Tabela 3

Prikaz oftalmoloških manifestacija kod 11 bolesnika sa Behčetovom bolešću i HLA B51 pozitivnim vaskulitisom

Dijagnoza	Bolesnici	
	n	%
Panuvetis	6	54,5
Zadnji uveitis	5	45,4
Povišen intraokularni pritisak	3	27,3
Hipopion	3	27,3
Vitritis	11	100
Retinitis (okluzije vene, hemoragije, eksudati)	4	36,4
Vaskulitis	7	63,7
Cistoidni edem makule	6	54,5
Citoidna degeneracija makule	2	18,2

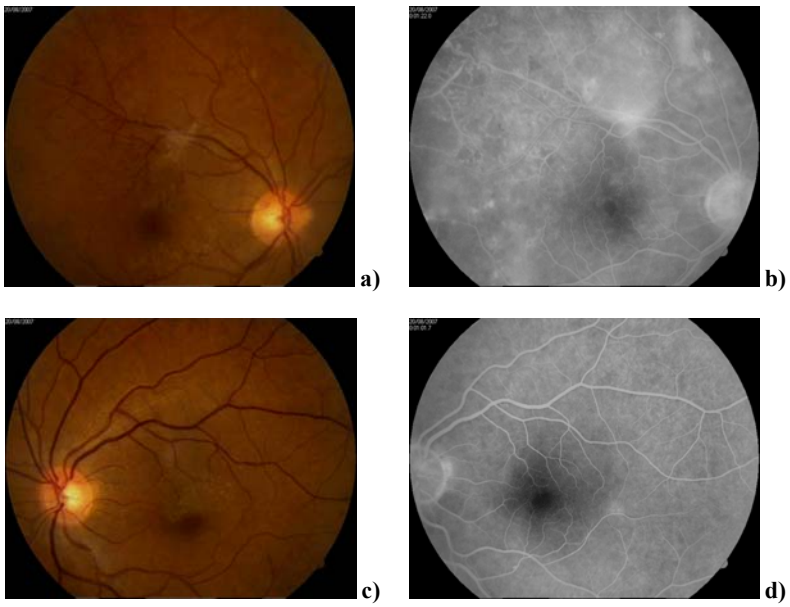
Prema analizi uporednog *t*-testa (SPSS 16,0) između varijabli, vidna oštrina pre terapije – bolje oko i vidna oštrina



a) **b)**

Sl. 1 – Oftalmološke manifestacije Behčetove bolesti kod bolesnika starog 45 godina

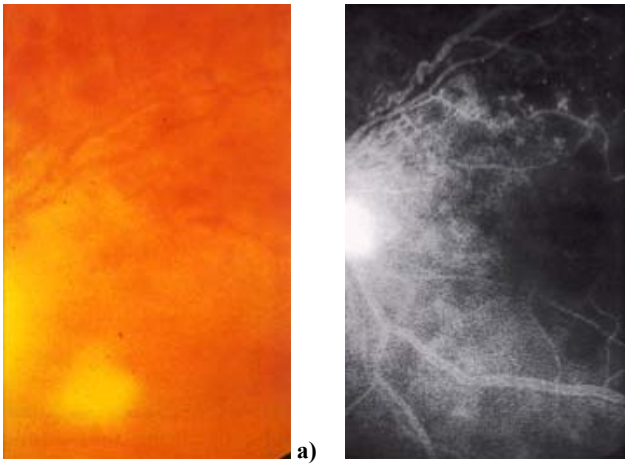
a) fotofundus cistoidnog edema makule boljeg oka; b) fluoresceinska angiografija boljeg oka (nakon lečenja ciklosporinom i niskim dozama kortikosteroida posle izvesnog poboljšanja recidiv i pad vidne oštrine)



a) **b)** **c)** **d)**

Sl. 2 – Prikaz očiju bolesnice sa Behčetovom bolesti

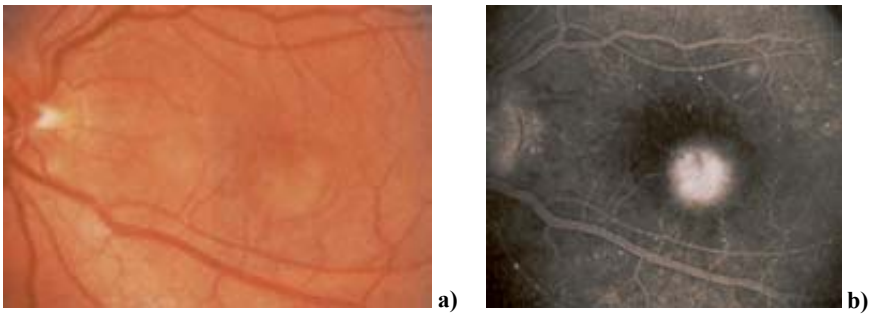
a) fotofundus goreg oka (retinalni vaskulitis, nevaskularizacija i, kasnije, hemoftalmus kao komplikacija); b) fluoresceinska angiografija goreg oka; c) fotofundus boljeg oka (retinalni vaskulitise gde je nakon kortikosteroide terapije rađena laser foto koagulacija); d) fluoresceinska angiografija boljeg oka



a) b)

Sl. 3 – Prikaz oka bolesnice sa Behčetovom bolesti (retinalni vaskulitis sa neovaskularizacijom; započeto lečenje infliksimabom)

a) fotofundus oka; b) fluoresceinska angiografija istog oka



a) b)

Sl. 4 – Prikaz oka bolesnice sa Behčetovom bolesti na terapiji infliksimabom

a) fotofundus oka; b) fluoresceinska angiografija istog oka

posle terapije – bolje oko nije nađena statistički značajne razlike ($p > 0,05$) što je i očekivano za bolje oko (četiri bolesnika sa vidnom oštrinom 1,0). Primenom istog testa na vidnu oštrinu lošijeg oka ovih jedanaest bolesnika nađeno je da između varijabli vidna oštrina pre terapije – gore oko i vidna oštrina posle terapije – gore oko postoji statistički značajna razlika ($p < 0,05$).

Prema analizi uporednog *t*-testa (SPSS 16,0) u prvoj testiranoj grupi između varijable opacitati pre terapije – bolje oko i varijable opacitati posle terapije – bolje oko nije bilo statistički značajne razlike ($p = 0,195$). U drugoj testiranoj grupi između varijable opacitati pre terapije – gore oko i varijable opacitati posle terapije – gore oko, postojala je statistički značajna razlika ($p = 0,001$).

Diskusija

Smatra se da je uveitis autoimunsko-autoinflamatorno oboljenje oka sa predominacijom CD4+ T-ćelija. Terapija zadnjih uveitisa i panuveitisa zasniva se na ovoj činjenici. Terapija inflamacije oka i komplikacija do kojih dolazi u sklopu BB podrazumeva lečenje u akutnoj fazi bolesti, u hroničnoj fazi i terapiju recidiva.

Behčetova bolest je apsolutna indikacija za imunomodulatornu terapiju. Bolesnici sa prednjim uveitisom ne moraju se lečiti sistemskom terapijom. Inflamacija u staklastom telu i fenomen curenja na fluoresceinskoj angiografiji indikacije su za uvođenje imunomodulatorne terapije čak i u odsustvu drugih kliničkih manifestacija bolesti na zadnjem segmentu.

Terapija akutne faze bolesti podrazumeva primarnu terapiju kortikosteroidima, dozirano miligram na kilogram telesne težine po šemi. Nekada je potrebno uključiti jedan ili dva imunosupresiva iz grupe kao što su ciklosporin A, alkilirajući preparat, kao što su ciklofosfamid ili hlorambucil ili antimetabolite metotreksat, azatioprin, mikofenolat mofetil. U većini slučajeva ove kombinacije su efikasne i uspevaju da kontrolišu inflamaciju i dovode do poboljšanja vidne oštrine i smanjenje inflamacije u staklastom telu. U teškim slučajevima (funkcionalni monokulusi) indikovana je primena anti-TNF-α preparata infliksimaba.

U našem radu za procenu efikasnosti terapijskog protokola svih jedanaest bolesnika koristili smo dva parametra – vidnu oštrinu bolesnika, kao i inflamaciju u staklastom telu, pre i posle primene terapije.

U studiji smo koristili ciklosporin kao drugi lek izbora, početna doza 3–5 mg/kg, primenjen kod tri bolesnika. Cilj terapije ciklosporinom bio je da se ubrza resorpcija CEM i popravi vidna funkcija, a dugoročno da se spreči preteća ishemija i sledstvena neovaskularizacija retine i, takođe, da se kod dva bolesnika kortikosteroidi isključe iz terapije zbog njihovih lokalnih i sistemskih neželjenih dejstava. Primenje-

na je kombinacija ciklosporina 3 mg/kg sa niskim dozama kortikosteroida 0,4 mg/kg, sa pozitivnim uspehom kod dva bolesnika. Kod jednog bolesnika došlo je do potpune resorpcije CEM i smirivanja inflamacije u zadnjem segmentu oka, a kod drugog bolesnika do stabilizacije vidne oštrine na jednom oku i potpune kontrole inflamacije. Kod jednog bolesnika terapija kortikosteroidima i ciklosporinom 5 mg/kg nije dovela do zadovoljavajućeg efekta, te je terapija zamenjena anti-TNF-α preparatom, infliksimabom.

Terapija anti-TNF-α lekom sprovedena je kod ukupno dva bolesnika kada je sva prethodna terapija postala nedovoljna za kontrolu inflamacije. Terapija anti-TNF-α agensima bazira se na uočenom povišenom TNF-α u serumu i očnoj vodici bolesnika sa uveitisom [19–21] kao i kod eksperimntalnih autoimunskih uveitisa. Komercijalno dostupni anti-TNF-α preparati su etanercept (Enbrel, Wyeth) koji je rekombinantni humani TNFRp75-Fc fuzioprotein. Sastoji iz dva humana TNF receptora (TNFRs) od 75 kDa (p75) povezana sa Fc delom humanog IgG1. Adalimumab (Humira, Abbott) je noviji preparat „punih" humanizovanih anti-TNF-α antitela koja se daju u dvonedeljnim intervalima supkutano.

Nama je bio dostupan infliksimab (*Remicade, Schering Pluogh*), himerik humano/mišjih monoklonskih anitela na TNF-α. Daje se u dozi od 3–5 mg/kg u intravenskoj infuziji u 2–4 nedeljnim intervalima. Kontrola intraokularne inflamacije uočava se već od prvog dana terapije, vidna oštrina popravila se u jednom slučaju za jedan red već nakon prve infuzije, dok resorpcija vitritisa i retinitisa počinje nešto kasnije, posle sedmog dana. Cistoidni edem makule se, takođe, resorbuje. Marcomichelakis i sar. [21] ističu efikasnost infliksimaba kod dugotrajnog CEM u uveitisu. Prateća imunosupresivna terapija kod dva bolesnika na infliksimabu bila je ukinuta. Vidna oštrina se popravila se kod jedne bolesnice za dve linije na boljem oku i za četiri linije na gorem oku, a kod drugog bolesnika za jednu liniju.

Infliksimab su naši bolesnici dobro podnosili i nije bilo neželjenih sporednih dejstava u smislu tuberkuloze. Naše iskustvo je da je infliksimab efikasan u postizanju i održavanju remisije BB. Posebno je indikovan kada su drugi terapijski modaliteti iscrpljeni i vidna oštrina kao posledica CEM u pogoršanju. Ponovljene infuzije su neophodne za održavanje remisije. Jedna doza infliksimaba od 5mg/kg efikasna je za intraokularnu inflamaciju, ali ponovljene infuzije su neophodne za prevenciju recidiva. Teške forme uvitisa kod bolesnika sa BB preporučljive su za lečenje infuzijama i u periodima kraćim od osam nedelja (2–4 nedelje). Jedan od ograničavajućih faktora za primenu ovog leka je cena. Postoji i mogućnost razvijanja antitela na mišju komponentu infliksimaba.

Zaključak

Ovaj rad doprinosi boljem prepoznavanju simptoma aktivnosti BB, uspešnoj terapiji njenih težih oftalmoloških manifestacija, kao i stvaranju optimalnog terapijskog plana za akutno-hroničnu fazu bolesti i prevenciju recidiva. Pozitivna iskustva sa infliksimabom u lečenju težih formi oftalmoloških manifestacija BB ohrabrujuća su i daju nadu za smanjenje loše vidne prognoze ovog teškog oboljenja, uprkos činjenici da su biološki preparati još uvek skupi.

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Primena verzije upitnika *Oral Impacts on Daily Performance* na srpskom jeziku za procenu kvaliteta života vezanog za oralno zdravlje

Applicability of a Serbian version of the "Oral Impacts on Daily Performance (OIDP)" index – assessment of oral health-related quality of life

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Apstrakt

Uvod/Cilj. Upitnik *Oral Impacts on Daily Performance* (OIDP) dobro je poznat psihometrijski test za procenu kvaliteta života u zavisnosti od oralnog zdravlja koji se koristi širom sveta. Primenom ovog upitnika procenjuje se stepen u kojem oralnozdravstveni problemi utiču na svakodnevne aktivnosti ispitanika u poslednjih šest meseci. Cilj ove studije bio je da se OIDP indeks prevede na srpski jezik, proveri njegova pouzdanost u praksi i time verifikuje na našem području. **Metode.** Prateći internacionalno ustanovljeni metod, OIDP upitnik preveden je korišćenjem standardizovane metodologije koja se sastojala od prevoda upitnika sa engleskog na srpski jezik, povratnog prevoda i pilot studije. **Rezultati.** U studiju su bila uključena 44 ispitanika (24 muškog i 20 ženskog pola), starosti preko 65 godina, pri čemu je korišćena preliminarna srpska verzija OIDP upitnika. Tegobe u domenu oralnog funkcionisanja najviše su bile izražene tokom jela (47,7%) i govora (36,4%), dok je postojao mali uticaj teškoća u domenu psihosocijalne sfere. Prilikom utvrđivanja interne konzistencije testa uočili smo da je korigovani koeficijent korelacije za sva pitanja bio unutar zadovoljavajućih kriterijuma (> 0,20). *Cronbach's alpha* koeficijent, koji se izračunavao za pouzdanost testa bio je 0,75. **Zaključak.** Na osnovu rezultata ovog istraživanja zaključeno je da je ovaj upitnik pogodan za korišćenje u svakodnevnoj praksi na srpskom govornom području i da može pružiti korisne informacije pri proceni kvaliteta života.

Ključne reči:
usta, higijena; usta, zdravlje; upitnici; psihometrija; kvalitet života; osetljivost i specifičnost.

Abstract

Background/Aim. The Oral Impacts on Daily Performance (OIDP) is a well-known psychometric test used internationally to assess the oral health-related quality of life. The interview and self-administrated questionnaire both assess the degree to which oral health problems have affected the life of the participants over the previous 6 months. The aim of this study was to translate the OIDP index into Serbian and to assess its reliability in practice as its initial verification in the Serbian speaking area. **Methods.** Following an internationally established methods, the OIDP scale was translated using standardized methodology that consisted of forward translation, pilot study and backward translation. **Results.** A pilot study was carried out with 44 respondents (24 males i 20 females) using a preliminar Serbian version of the OIDP index. All patients were aged over 65 years. A total of 68.2% of the participants replied that they had at least one OIDP impact on daily life in the past 6 months. These troubles were most prominent during eating (47.7%) and speaking (36.4%), but there is a little impact of troubles in the domain of psychosocial sphere. The corrected item-total correlation coefficients for all items were above the minimum recommended level of 0.20 for including an item in a scale. The standardized Cronbach's alpha coefficient was 0.75. **Conclusion.** Based on these results, we can conclude that this index is suitable for use in everyday practice in Serbian speaking area providing useful information required to assess oral health-related quality of life.

Key words:
oral hygiene; oral health; questionnaires; psychometry; quality of life; sensitivity and specificity.

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Uvod

Kvalitet života je već dugi niz godina u središtu zanimanja brojnih istraživača kao jedna od tema važnih u životu svakog čoveka. Istorijski gledano, definicije i merenja kvaliteta života mnogo su se razlikovale i menjale [1]. Kvalitet života postao je predmet interesa u psihologiji, filozofiji, socijalnim naukama, kliničkoj medicini i stomatologiji i u sistemu zdravstvene zaštite [2].

U literaturi postoje brojna istraživanja kvaliteta života i pojedinih aspekata koji taj kvalitet čine. Sa aspekta stomatologije, naročito je značajan koncept koji se odnosi na kvalitet života u zavisnosti od oralnog zdravlja (*Oral Health-Related Quality of Life* – OHRQoL). Ovaj koncept razvio se tek u poslednjih nekoliko decenija, dok mu se ranije nije pridavala značajna pažnja [3]. Oralne bolesti pripisivale su se vlastitom neugodnom iskustvu ispitanika bez nekih znatnih posledica na stanje opšteg zdravlja i dobrobiti pojedinca. Gerson [4] je u svojoj studiji o percepciji oralnih bolesti izneo opšte mišljenje da oralni problemi nisu opravdanje za izostanak sa posla, jer ih ne doživljava kao deo klasične percepcije bolesti. Dunnell i Cartwright [5] podupiru takvo razmišljanje ističući da su glavobolja, osip, opekotine i zubni problemi sporedni problemi, a ne bolest.

Znatan doprinos današnjem poimanju OHRQoL dale su zemlje Zapadne Evrope i SAD tražeći opravdanje za sve veće troškove zdravstvenog sistema koji bi trebalo da unapredi zdravlje populacije, a koji se više nije mogao pravdati samo statističkim podacima mortaliteta i morbiditeta [6]. Takođe, u pogledu oralnih bolesti bilo je jasno da klinički pokazatelji kao što su zubni karijes i periodontalne bolesti nisu dovoljni za procenu oralnozdravstvenog statusa i njegovog uticaja na kvalitet života ispitanika. Iz tog razloga, korišćenje isključivo kliničkih merenja u oceni opšteg i oralnog zdravlja kod ispitanika svih uzrasta široko je kritikovano jer nije uspevalo da uključi funkcijske i psihosocijalne aspekte zdravlja i nije adekvatno prezentovalo zdravstveni status, funkciju i potrebe individue [7, 8]. Stoga, osim potrebe za kliničkim istraživanjima bolesti, rasle su i potrebe za merenjima zdravstvenog stanja društva, što je pratio razvoj standardizovanih upitnika koji su se mogli primeniti u istraživanjima na velikim populacijama.

Naime, danas postoji ukupno 20 identifikovanih i klinički verifikovanih indeksa koji mere uticaj stanja oralnog zdravlja na kvalitet života. Svi indeksi u originalnoj verziji su na engleskom jeziku, a samo je 10 prevedeno na druge jezike. Upitnici se međusobno razlikuju u pogledu dimenzija koje pojedini upitnik meri, ukupnog broja pitanja, formulacije pitanja i tipa ponuđenih odgovora [9]. Indeksi koji se preporučuju za ispitivanje kvaliteta života starijih ispitanika su: GOHAI, SOSHI, OHIP-49, DIDL, OHIP-14, OIDP I OHIP-G [7, 10, 11].

Struktura svih indeksa podrazumeva određeni broj pitanja na osnovu kojih se dobijaju informacije direktno od ispitanika. Pojedina pitanja se odnose na bol i neugodnost, druga na socijalne i psihološke posledice, što zajedno obuhvata sve aspekte OHRQoL. Pitanja su jednostavna, a najčešća formulacija glasi: „Kako biste procenili zdravlje svojih zuba, desni i usta?“ Ponuđeni odgovori su, takođe, jednostavni i rangiraju se na Likertovoj skali (raspon pet bodova) od „odlično“ do „nezadovoljavajuće“. Pojedinac rangira svoje oralno zdravlje u odnosu na elemente kvaliteta života za koje smatra da su najvažniji i izražava ga na spomenutoj skali. Dodatna pomoć u popunjavanju upitnika može se sastojati od instrukcija koje se daju ispitaniku i na taj način mu se objašnjava polje na koje se pitanje odnosi.

Ovo je prvo istraživanje koje koristi OIDP. To je višedimenzijski upitnik, orginalno sastavljen na engleskom jeziku, čiji su autori Tsakos i sar. [12]. Ovim indeksom meri se frekvencija i ozbiljnost oralnih uticaja na dnevne aktivnosti starijih ispitanika [12]. Za razliku od drugih mera, OIDP odnosi se samo na treći nivo merenja (ICIDH teorijski okvir) [13], pokazujući time jaku teorijsku koherentnost, i smanjuje mogućnost dvostrukog bodovanja istih oralnih uticaja na različitim nivoima [14]. Ovaj upitnik sastoji se od 10 aktivnosti koje pokrivaju fizičke, psihološke i socijalne dimenzije svakodnevnog života.

Od pojave originalne verzije, OIDP je korišćen u različitim studijama kod adultne populacije u Velikoj Britaniji i Grčkoj [12, 14], Tajlandu [15], Tanzaniji [16], Ugandi [17], Norveškoj [18] i Iranu [19]. Ovaj upitnik je usvojen za epidemiološka istraživanja populacija različitog doba i pokazalo se da je pouzdan i valjan. S obzirom na međunarodnu prihvaćenost upitnika, cilj ovog rada bio je da se OIDP indeks prevede na srpski jezik, proveri njegova pouzdanost u praksi i time, verifikuje na našem govornom području.

Metode

Prevođenje OIDP upitnika za odrasle osobe na srpski jezik uključivao je tri glavna koraka: prevod OIDP upitnika sa engleskog na srpski jezik, povratni prevod i pilot studiju.

Srpska verzija OIDP upitnika razvijena je u skladu sa preporučenim lingvističkim metodom [20] koji se koristi internacionalno i na čijim načelima su se bazirala uputstva autora. U prvom koraku, nakon dobijanja dozvole od autora orginala za razvoj srpske verzije, prevod sa engleskog na srpski jezik uradila su dva profesionalna prevodioca koji dobro poznaju stomatološku terminologiju. Svaki prevodilac radio je nezavisno i priredio prevod orginalnog upitnika samostalno bez konsultacije sa drugim prevodiocem ili istraživačkim timom. Dobijene su dve verzije originalnog upitnika koje su se veoma malo razlikovale, a zatim je uz konsultacije sa istraživačkim timom napravljena „konsenzus-verzija“ koja je testirana na malom uzorku od 10 ljudi. Ovo testiranje odnosilo se na ispitivanje razumevanja pitanja iz upitnika od strane ovih ispitanika. Dalje, postupak je podrazumevao da se ta konsenzus verzija upitnika na srpskom jeziku prevede na engleski jezik putem povratnog prevoda od strane osobe koja poseduje odlično znanje engleskog jezika, a koja se nalazi na usavršavanju iz oblasti stomatološke protetike u zemlji sa engleskim govornim područjem. Povratni prevod bio je urađen bez znanja orginalnog teksta na engleskom jeziku. Nakon dobijanja ove dve verzije prevoda (konsenzus verzije na srpskom jeziku i povratnog prevoda na engleskom jeziku) prevodioci su zajedno sa istraživačkim timom diskutovali o neslaganju i napravili preliminarnu srpsku verziju OIDP upitnika (tabela 1).

Tabela 1

Srpska verzija indeksa *Oral Impacts on Daily Performance* (OIDP)

1 : Da li ste u poslednjih 6 meseci imali ikakve poteškoće tokom .....
zbog problema sa Vašim ustima, zubima ili zubnim nadoknadama?
*Ako je odgovor „ne“ vi ćete označiti „0“ i oceniti prisustvo teškoće tokom sledeće aktivnosti/ponašanja!
*Ako je odgovor „da“, vi morate popuniti preostala pitanja (2–5)!

2: Da li ste imali ove poteškoće prilikom ..... redovno/povremeno ili kroz određeno razdoblje/kraće vreme?
*Ukoliko su se poteškoće javljale redovno/povremeno, vi morate zabeležiti učestalost teškoće u pitanju 3.
*Ukoliko su se poteškoće javljale kroz određeno razdoblje/kraće vreme, vi morate zabeležiti trajanje teškoće u pitanju 4!

3: Koliko često ste u poslednjih 6 meseci imali ovakve poteškoće?
5 Gotovo svaki ili svaki dan
4 3–4 puta nedeljno
3 Jednom do dvaput nedeljno
2 Jednom do dvaput mesečno
1 Ređe od jednom mesečno

4: Koliko dugo ste u poslednjih 6 meseci imali ovakve poteškoće?
5 Preko 3 meseca ukupno
4 Od 2–3 meseca
3 Od 1–2 meseca
2 Od 5 dana do 1 meseca
1 Do ukupno 5 dana

5: Koristeći skalu od 0 do 5, gdje 0 označava mali uticaj, a 5 označava vrlo ozbiljni uticaj, koji broj bi po Vašem mišljenju najbolje odražavao uticaj poteškoća na Vaš svakodnevni život?

Kada je u pitanju struktura ovog upitnika, on se može podeliti na deo koji se tiče oralnog funkcionisanja, odnosno tegoba koje ispitanik ima u obavljanju osnovnih oralnih funkcija (jelo, govor, čišćenje zuba ili proteza, spavanje i odmaranje) i na deo koji se odnosi na njegov psihički odgovor, odnosno na tegobe i socijalne posledice istih. Pomoću ovog upitnika procenjuje se da li oralni problemi postoje i prati se njihova frekvencija i ozbiljnost koje utiču na svakodnevni život ispitanika u periodu od 6 meseci. Upitnik se fokusira na 10 bazičnih dnevnih aktivnosti kao što su: ishrana, govor, čišćenje zuba ili proteza, spavanje, odmaranje, pokazivanje zuba bez osećaja nelagodnosti, održavanje uobičajnog emocionalnog stanja bez razdražljivosti, izvršavanje laganih fizičkih aktivnosti, izlaženje izvan stambenog prostora i uživanje u druženju sa drugim ljudima. Konstrukcija upitnika je takva da se svaki odgovor boduje od 0 do 5, u zavisnosti od toga u kojoj meri ispitanik oseća tegobu (tabela 2). Skor OIDP izražava se kao suma skorova koji su rezultat množenja frekvencije i ozbiljnosti oralnog uticaja (*performance score = severity score x frequency score*) za svaku aktivnost, a zatim podeljeni sa maksimalnim mogućim skorom. Veći OIDP skor pokazuje lošiji oralnozdravstveni status.

Sledeći korak u verifikaciji ovog indeksa predstavljalo je organizovanje pilot studije i ocenjivanje stepena razumevanja pitanja, kao i mogućnost primene upitnika u ovoj populaciji. Pilot studija uključivala je 44 osobe starijeg životnog doba koje su bile približnih godina i sa područja na kome se obavljalo ispitivanje, sa umerenim socioekonomskim varijacijama i podjednakim brojem ispitanika oba pola. Istraživanje je obavljeno u periodu od aprila do oktobra 2009. godine u Odseku za stomatologiju Medicinskog fakulteta u Foči. Pitanja su postavljali istraživači svakom ispitaniku ponaosob i bila su praćena detaljnom diskusijom o stepenu razumevanja svakog pitanja. U slučaju poteškoća u razumevanju, davana su alternativna objašnjenja. Pri tome, izbegavalo se sugerisanje odgovora ispitaniku.

Tabela 2

Način bodovanja uticaja oralnih tegoba na 10 uobičajnih dnevnih aktivnosti

Aktivnosti / ponašanja	Prisustvo teškoće	Period		Efekat
	Ne: 0 / Da: 1	učestalost	trajanje	
Jelo	0 / 1	1–5	1–5	0–5
Govor	0 / 1	1–5	1–5	0–5
Čišćenje usta	0 / 1	1–5	1–5	0–5
Spavanje	0 / 1	1–5	1–5	0–5
Odmaranje	0 / 1	1–5	1–5	0–5
Smeškanje, smejanje, pokazivanje zuba bez osjećaja nelagodnosti	0 / 1	1–5	1–5	0–5
Emocionalno stanje	0 / 1	1–5	1–5	0–5
Izvršavanje laganih fizičkih aktivnosti	0 / 1	1–5	1–5	0–5
Izlazak vani,u posetu nekome	0 / 1	1–5	1–5	0–5
Uživanje u druženju s drugim ljudima	0 / 1	1–5	1–5	0–5

U samom procesu prevođenja ovog upitnika naišlo se na neka neslaganja između orginalnog teksta i povratnog prevoda. Naime, postojali su komentari o značenju i interpretaciji fraze *find it difficult to attend to your oral care* (*brushing your teeth or taking care of your dentures*) u povratnom prevodu, iako je u orginalnom tekstu postojala fraza *cleening teeth or dentures*. Tumačenja data u zagradama smatrala su se neophodnim jer su se pitanja odnosila na oralnu higijenu i izraz „zaštita oralna" bi mogao izazvati nerazumevanje. Posle diskusije, ova rečenica je revidirana u *find it difficult to clean your mouth* (*for example brushing teeth*). Takođe, pošto su ispitanici bili stari preko 65 godina, pitanje koje se odnosilo na izvršavanje radnih obaveza je izbačeno, jer se smatralo neadekvatnim za ovu populaciju. Nakon ovih korekcija napravljena je preliminarna verzija OIDP upitnika na srpskom jeziku.

U svrhu kulturološke adaptacije i verifikacije OIDP upitnika na srpskom govornom području neophodno je bilo izvršiti ocenu pouzdanosti upitnika. Statistička obrada podataka je urađena u SPSS 11,5 programu za Windows. Pouzdanost upitnika procenjivana je na osnovu vrednosti korigovanog koeficijenta korelacije (*corrected-item-total correlation coefficient)* za sva pitanja i *Cronbach's alpha* koeficijenta.

Rezultati

U pilot studiji koja je uključivala 44 ispitanika (24 muškog i 20 ženskog pola), korišćena je preliminarna verzija upitnika OIDP na srpskom jeziku. Svi ispitanici bili su stari preko 65 godina, prosečno 71,2 godine. Najveći broj ispitanika koji je učestvovao u ovoj studiji živeo je u urbanom području na teritoriji grada Foče (68,2%) i bio srednjeg nivoa obrazovanja (50,0%). Takođe, 24 (54,5%) ispitanika bilo je bez zuba, dok je 20 (45,5%) ispitanika bilo sa određenim brojem zuba, ali bez prisutnih zubnih nadoknada.

Prilikom analiziranja podataka iz pilot studije uvidelo se da veliki broj ispitanika ukazuje na uticaj oralnozdravstvenih problema na njihove dnevne aktivnosti u poslednjih šest meseci. Prevalencija oralnog uticaja, merena indeksom OIDP, bila je vrlo visoka. Naime, 68,2% osoba doživelo je najmanje jedan oralni uticaj tokom poslednjih šest meseci. U pogledu oralnog funkcionisanja, tegobe su najviše bile izražene tokom jela (47,7%) i govora (36,4%), dok je mali uticaj teškoća postojao u psihosocijalnoj sferi (tabela 3).

Korigovani koeficijent korelacije (*corrected-item-total correlation coefficients)* i *Cronbach's alpha* koeficijent upućivali su na to da je ovaj indeks imao odličnu unutrašnju konzistentnost. Korigovani koeficijent korelacije za sva pitanja bio je iznad minimalnog preporučenog nivoa od 0,20. Dalje, koeficijent *Cronbach's alpha* bio je veći od preporučenog nivoa i iznosio je 0,75 (tabela 4).

Tabela 3
Uticaj oralnih tegoba na uobičajne dnevne aktivnosti ispitanika starije dobi

Dnevne aktivnosti	Ispitanici	
	n	%
Jelo	21	47,7
Govor	16	36,4
Čišćenje usta	9	20,5
Spavanje	2	4,5
Odmaranje	6	13,6
Smejanje bez osećaja nelagodnosti	12	27,3
Emocionalno stanje	11	25,0
Izvršavanje laganih fizičkih aktivnosti	7	15,9
Izlazak van stambenog prostora	5	11,4
Uživanje u druženju sa drugim ljudima	4	9

Tabela 4
Vrednosti korigovani koeficijent – ukupna korelacija (*corrected item-total correlation*) za OIDP indeks

Dnevne aktivnosti	*Corrected item-total correlation*
Jelo	0,30
Govor	0,26
Čišćenje usta	0,37
Spavanje	0,47
Odmaranje	0,44
Smejanje bez osećaja nelagodnosti	0,67
Emocionalno stanje	0,42
Izvršavanje laganih fizičkih aktivnosti	0,56
Izvršavanje laganih fizičkih aktivnosti	0,32
Uživanje u druženju sa drugim ljudima	0,32
Alpha	0,75

Diskusija

Za potrebe ovog istraživanja, prevod na srpski jezik urađen je bez većih problema i poređenje između originalnog OIDP i povratnog prevoda nije upućivalo na veće sadržajne

ili konceptualne razlike. Ekvivalentne reči lako su pronađene zahvaljujući jednostavnoj strukturi upitnika OIDP. S druge strane, kao veći izazov, pokazao se pokušaj da se shvate teorijske osnove OIDP i njegov pristup u merenju kvaliteta života [20].

Na osnovu ovog istraživanja može se zaključiti da upitnik OIDP poseduje tri karakteristične osobine. Prvo, upitnik ne procenjuje samo postojanje i stepen oralnozdravstvenih problema već i obim u kojem takvi problemi utiču na svakodnevni život. Drugo, ovaj upitnik je naročito koristan za procenu kvaliteta života osoba starijeg doba koji nisu smešteni u specijalizovane ustanove. Treća karakteristika upitnika OIDP jeste da uključuje praćenje učestalosti i trajanja oralnih nelagodnosti. Na primer, GOHAI sadrži pitanja o učestalosti nelagodnosti u poslednja tri meseca, pri čemu se nelagodnost javlja samo jednom i nastavlja tokom određenog vremenskog perioda. Međutim, upitnik OIDP je pažljivo planiran tako da može da se odnosi na oba parametra (ozbiljnost i trajanje oralnih nelagodnosti).

Tokom sprovođenja pilot studije, predviđena je mogućnost višestukog izbora odgovora za ispitanike, što se smatralo veoma važnim da bi se istraživanje moglo nesmetano nastaviti. Višestruki izbor odgovora korišćen je da olakša ispitaniku donošenje odluke. Promene nisu napravljene u sumiranju (zbrajanju) ili redosledu pitanja.

Tokom analiziranja rezultata ovog istraživanja uočeno je da je ukupna incidencija oralnog uticaja na dnevne aktivnosti bila 68,2%. Međutim, dosadašnje studije pokazivale su različite rezultate zavisno od populacije koja je bila ispitivana i od toga koji upitnik je korišćen. Tako, npr, rezultati studije koja je bila izvedena u Velikoj Britaniji ukazivali su na znatno nižu vrednost oralnog uticaja [21, 22]. Međutim, s obzirom na to da su u ovoj studiji bile korišćene različite mere OHRQoL (OHIP-14 i OHRQoL-UK), njihova direktna uporedivost sa ovom studijom je ograničena. Nuttall i sar. [21] pokazali su da 51% osoba sa zubima u Velikoj Britaniji ima na neki način izražen uticaj oralnozdravstvenih problema na njihov svakodnevni život. U Tajlandu [15], gdje je ispitivanje izvedeno na ispitanicima starosti od 35 do 44 godina, učestalost oralnog uticaja bila je čak veća (73,6%) nego u Velikoj Britaniji. Najčešće „napadnuta“ dnevna aktivnost bilo je „žvakanje“, na što upućuju i rezultati ove studije. U ovoj studiji, problemi vezani za govor i jasno izgovaranje pojedinih glasova bili su prisutni kod 36,4%, a problemi sa čišćenjem usta kod 20,5% ispitanika. Segment sa pitanjima iz psihosocijalne sfere ukazivao je da ispitanici nisu imali velike poteškoće u ovom domenu, kao što potvrđuju i rezultati studije koja je sprovedena u Srbiji, a u kojoj je korišćen OHIP kao indikator za procenu kvaliteta života [23]. Kada je u pitanju spavanje, samo 4,5% ispitanika istaklo je da zbog problema sa zubima ima takve poteškoće. Takođe, samo 11,4% ispitanika ukazalo je da su ovi problemi uticali na njihov svakodnevni život ometajući ih da izlaze izvan stambenog prostora, a 9,1% istaklo je da su ove tegobe uticale da uživaju u druženju sa drugim ljudima.

Kada je u pitanju pouzdanost ovog upitnika, može se istaći da OIDP upitnik ima jako dobru unutrašnju konzistentnost, pri čemu su sva pitanja imala korigovani koeficijent korelacije > 20 [24], tako da su se sva pitanja mogla uključiti u upitnik pokazujući time dobru homogenost upitnika. Takođe, koeficijent *Cronbach's alpha* je imao vrednost veću od 0,7 [20], što i potvrđuje ovaj zaključak. U daljem toku istraživanja, planira se potvrda validnosti i pouzdanosti upitnika na većem broju ispitanika, kao i primena ovog psihometrijskog testa za ispitivanje posledičnog uticaja oralnozdravstvenog statusa na kvalitet života starijih ispitanika na srpskom govornom području.

Zaključak

Na osnovu rezultata ovog istraživanja, zaključeno je da je upitnik OIDP pogodan za korišćenje u svakodnevnoj praksi i da može pružiti korisne informacije pri proceni kvaliteta života zavisnog od oralnog zdravlja ispitanika. Rezultati ove studije ukazali su da oralnozdravstveni problemi sve više utiču na svakodnevni život starijih ispitanika. Takođe, ovo istraživanje daje predlog verzije OIDP upitnika na srpskom jeziku, ali za definitivnu verifikaciju na našem govornom području, potrebno je uraditi kliničko istraživanje na većem broju ispitanika.

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Imedijatno opterećenje implantata fiksnim zubnim nadoknadama – studija na psima

Immediate implant loading with fixed dental restorations – an animal model study

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Apstrakt

Uvod/Cilj. Imedijatno opterećenje implantata smatra se jednim od najznačajnijih dostignuća savremene dentalne implantologije. Rezultati novijih kliničkih i eksperimentalnih studija pokazali su da samo implantati visoke primarne stabilnosti mogu biti izloženi protokolu imedijatnog opterećenja zubnom nadoknadom sa predvidivim ishodom. Cilj studije bio je da se ispita mogućnost uspešne primene protokola imedijatnog opterećenja implantata različitog mikrodizajna fiksnim zubnim nadoknadama. **Metode.** U eksperimentalno istraživanje uključena su dva psa, obezubljena obostrano u premolarnoj regiji gornje i donje vilice. Nakon tri meseca od ekstrakcije zuba, ugrađena su, po predviđenoj šemi, četiri različita implantata po kvadrantu (n = 32): Mk III TiUnite (Nobel Biocare, Sweden), ITI TPS (Straumann, Switzerland), 3I-Osseotite (Implant Innovation, USA) i XiVE Cell-Plus (Friadent, Germany). Implantati su imedijatno opterećeni fiksnim zubnim nadoknadama, četvoročlanim mostovima od plemenite legure zlato-platina, dva dana posle implantacije. Stabilnost implantata i mogućnost imedijatnog opterećenja ocenjivana je na osnovu analize rezonantnih frekvencija (RFA). **Rezultati.** Šest meseci nakon implantacije i imedijatnog opterećenja fiksnom zubnom nadoknadom, svi mostovi bili su u funkciji i svi implantati uspešno integrisani, ne pokazujući znake mobilnosti. Sumiranjem dobijenih vrednosti koeficijenta stabilnosti implantata (ISQ) ustanovljeno je da su rezonantne frekvencije bile značajno veće kod implantata u donjoj vilici. Rezultati eksperimentalnog istraživanja pokazali su da su sve analizirane površine ostvarile dobru implantatnu stabilnost. Utvrđen je porast ISQ vrednosti kod svih implantata u mandibuli i delimičan pad ISQ vrednosti za implantate u maksili, nakon šest meseci funkcionalnog opterećenja fiksnim zubnim nadoknadama. **Zaključak.** Ispitivani endoosealni implantati nisu pokazali različit stepen oseointegracije jer se rezultati merenih parametara hirurških (ISQh) i protetkih (ISQp), nisu statistički značajno razlikovali između implantatnih sistema.

Ključne reči:
implantati, stomatološki; stomatološka enosalna implantacija; oseointegracija; zubna proteza, parcijalna, fiksna; psi; zubna proteza, retencija.

Abstract

Background/Aim. Immediate loading is considered to be the most innovative technique in contemporary implant dentistry. Recent clinical and experimental findings have demonstrated that only implants with high primary stability can be subjected to immediate loading protocol with predictable results. It is generally accepted that the most important prerequsite for successful osseointegration is achievement and maintenance of implant stability. The aim of this *in vivo* study was to investigate the possibility for successful application of immediate loading protocol in implant systems with different surface properties. **Methods.** In the experimental study 2 mongrel dogs were edentulated bilaterally in the mandibular and maxillary premolar areas. After 3 months implants were placed in a pattern 4 different commercially available implants per quadrant (n = 32): Mk III TiUnite (Nobel Biocare, Sweden), ITI TPS (Straumann, Switzerland), 3I-Osseotite (Implant Innovation, USA) and XiVE Cell-Plus (Friadent, Germany). Implants were subjected to immediate loading with 4 unit gold cast bridges, 2 days post implantation. The assessment of implant stability and immediate loading possibilities were done by performing Resonance frequency analysis (RFA). **Results.** After a 6-month loading period all bridges were in function and all implants occurred well osseointegrated. When summarizing the Implant Stability Quotient (ISQ) values, it was noted that resonance frequency was significantly higher for mandibular implants. The results of this experimental

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setting showed that all evaluated surfaces achieved good implant stability. Increase of ISQ values was found for all implants in the mandible and partially decrease of ISQ values for maxillary implants after 6 months of functional loading with 4 unit bridges. **Conclusions.** Investigated endooseal implants did not show different degree of osseointegration, because there was not statisticaly significant difference among observed parameters (ISQh i ISQp) between implant systems.

Key words:
dental implants; dental implantation endosseous; osseointegration; denture, partial, fixed; dogs; denture retention.

Uvod

Oralna implantologija je, zahvaljujući multidisciplinarnom pristupu, razvila niz različitih terapijskih opcija, tako da endoosealni implantati imaju široko indikaciono područje, od zamene jednog izgubljenog zuba do zbrinjavanja različitih oblika krezubosti i bezubosti. Izrada zubnih nadoknada na implantatima danas je široko prihvaćen modalitet protetske terapije.

Brånemark i sar. [1] 1977. godine definisali su konvencionalni koncept zarastanja bez funkcionalnog opterećenja implantata u trajanju od četiri meseca za donju i šest meseci za gornju vilicu, kao najbolji način za promociju oseointegracije. Protetski protokol se pokazao uspešnim u višedecenijskoj kliničkoj praksi. Međutim, brojne histološke i kliničke studije omogućile su preispitivanje koncepta koji je utemeljila švedska škola.

Usavršavanjem implantatnih materijala i primenom atraumatske hirurgije sa novim tehnikama [2–4], kao i ugradnjom bez odizanja tkivnog režnja [5, 6], stvoreni su uslovi da se ubrza proces oseointegracije, a samim tim i ranije opterete implantati zubnom nadoknadom. Zahvaljujući tome, implantolozima su, zavisno od indikacije, danas na raspolaganju tri osnovna protokola: imedijatno, neposredno opterećenje implantata (*immediate loading*) privremenom ili definitivnom zubnom nadoknadom, neposredno po hirurškoj ugradnji i rano opterećenje implantata (*early loading*) koje podrazumeva definitivnu protetsku rehabilitaciju pacijenta za 6 do 8 nedelja postoperativno [7]. Stav grupe eminentnih stručnjaka [8] jeste da se svako zbrinjavanje implantata zubnom nadoknadom u vremenskom intervalu od tri dana do maksimum tri meseca nakon implantacije može smatrati ranim opterećenjem. Kasno, odloženo opterećenje implantata (*delayed loading*) zubnom nadoknadom nakon 3–4 meseca za donju vilicu i šest meseci za implantate u gornjoj vilici, odgovara konvencionalnom Brånemark-ovom konceptu.

Koncept imedijatnog opterećenja implantata zubnom nadoknadom je značajna inovacija oralne implantologije, jer nas približava ostvarenju ideala jednoseansne implantatne hirurško-protetske terapije, kojoj teži savremena rekonstruktivna stomatologija.

Za uspeh imedijatnog i ranog opterećenja implantata zubnom nadoknadom imperativ je primarna, inicijalna stabilnost postignuta u toku hirurške insercije. Primarna stabilnost podrazumeva odsustvo mobilnosti u momentu hirurške ugradnje implantata i zavisi od nivoa primarnog kontakta sa periimplantatnim koštanim tkivom [9]. Sekundarna implantatna stabilnost je zapravo klinička manifestacija oseointegracije, determinisana biološkim odgovorom perimplantatnog tkiva na hiruršku traumu i implantat.

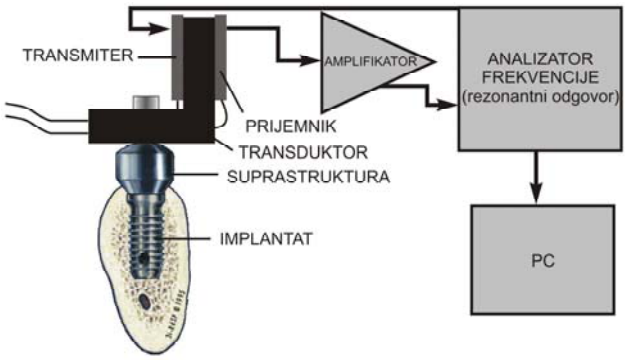
Trebalo bi, takođe, napomenuti da su kontrola okluzalnih sila i odsustvo štetnih navika i parafunkcija bitan faktor uspeha imedijatnog opterećenja implantata. Uravnotežena okluzija je rezultat uravnoteženja okluzalnih sila i otpornosti tkiva na koje deluju. Prema savremenim shvatanjima, fiziološki optimalna i stabilna okluzija je okluzija koja obezbeđuje nesmetano i efikasno odvijanje funkcija orofacijalnog sistema bez senzacija bola ili nelagodnosti, kao i bez znakova oštećenja orofacijalnih struktura [10].

Osnovni cilj ovog istraživanja, kao dela jedne obimnije studije, bio je da se ispitata mogućnost uspešne primene protokola imedijatnog opterećenja implantata različitog mikrodizajna fiksnim zubnim nadoknadama.

Metode

U istraživanju su korišćena četiri različita implantatna sistema vodećih svetskih proizvođača, predviđena za imedijatno i rano opterećenje: Mk III(RP) TiUnite (Nobel Biocare, Sweden), ITI-solid-Screw *Titanium plasma-sprayed* (TPS) (ITI Straumann, Switzerland), 3I-Osseotite (*Implant Innovation*, USA) i XiVE Cell plus (Dentsply, Germany). Osam implantata iz svakog sistema, ukupno 32, ugrađeno je u vilice eksperimentalnih životinja. U eksperimentalnu studiju uključena su dva psa mešanca, ženskog pola, mase približno od po 15 kg, starosti 20 meseci, koštano zreli, kompletnog zubala i pravilnog zagrižaja, vakcinisani, dehelmintisani i očišćeni od ektoparazita. Za vreme trajanja eksperimenta, životinje su držane u pojedinačnim kavezima, hranjene komercijalnom (peletiranom) hranom obročno jednom dnevno, s upotrebom vode *ad libitum*, uz svakodnevnu kliničku opservaciju. Merenje primarne i sekundarne implantatne stabilnosti učinjeno je metodom analize rezonantne frekvencije (RFA) (Osstell^{tm}, *Integration Diagnostics*, Sävedalen, Sweden), a dobijene vrednosti predstavljene su koeficijentom stabilnosti implantata (ISQ), kao hirurški (ISQh) i kao protetski (ISQp). Metoda je zasnovana na sofisticiranoj tehnologiji sa kompjuterskim merenjima rezonantne frekvencije (RF), koju određuju dva parametra: stepen gustine kosti na međuspoju implantat/kost i nivo marginalne alveolarne kosti oko transduktora.

Aparaturu čine Osstell transduktor i Osstell analizator koji je povezan sa personalnim računarom (slika 1). Izmerena amplituda rezonantne frekvencije prikazuje se numerički i grafički na Osstell analizatoru, a njen maksimum reprezentuje stabilnost implantata kvantifikovanu kroz ISQ jedinice. Vrednost ISQ je odraz krutosti sistema transduktor-implantat-kost i kalibracionih parametara transduktora. Merena na skali od 0 ISQ (3 500 Hz) do 100 ISQ jedinica (8 500 Hz), veća vrednost ISQ označava veću stabilnost implantata.



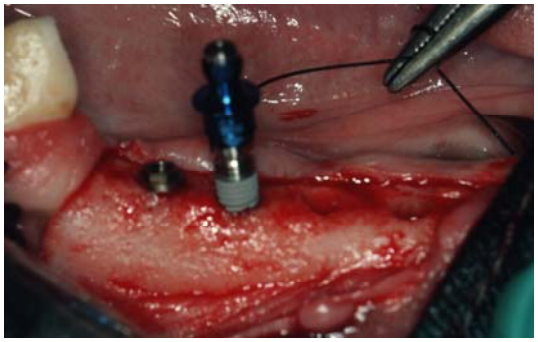
Sl. 1 – Šematski dijagram aparature za analizu rezonantne frekvencije implantata
PC – personalni računar

socijativni anestetik ketamin-hidrohlorid *im* 16,8 mg/kg TM, 5% Ralatek (Hemofarm, SCG), a za lokalnu anesteziju 2% lidokain (Xylocaine, Astra, Sweden).

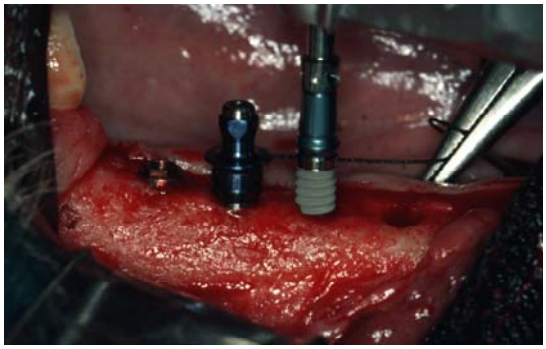
Zatim su ekstrahovana četiri premolara po svakom kvadrantu donje i gornje vilice. Hirurškoj fazi implantacije pristupljeno je tri meseca posle ekstrakcije zuba.

Nakon incizije i odizanja mukoperiostalnog režnja, izvršena je preparacija koštanih ležišta odgovarajućim svrdlima. Ugrađena su četiri različita implantata po kvadrantu u bezubim poljima premolarnih regija (slike 2–5). Intraoperativno, neposredno nakon implantacije, izmerena je primarna, hirurška stabilnost ugrađenih implantata, ISQh, RFA-metodom (analiza rezonantne frekvencije) (slika 6). Nakon operativnog zahvata adaptiran je tkivni režanj i zašiven pojedinačnim šavom sa resorptivnim koncem (Vicryl 2–0, Ethi-

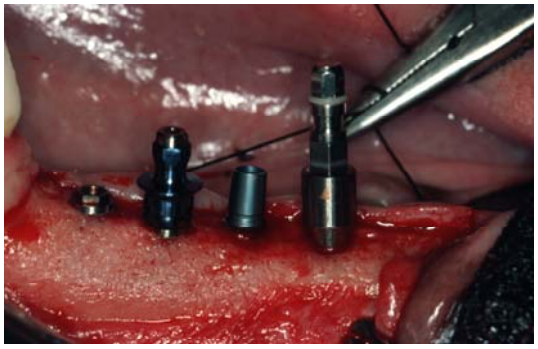
Sl. 2 – Ugradnja TiUnite implantata
(Nobel *Biocare*)



Sl. 3 – Ugradnja 3I-Osseotite implantata
(*Implant Innovation*)



Sl. 4 – Ugradnja XiVE implantata
(Dentsply Friadent)

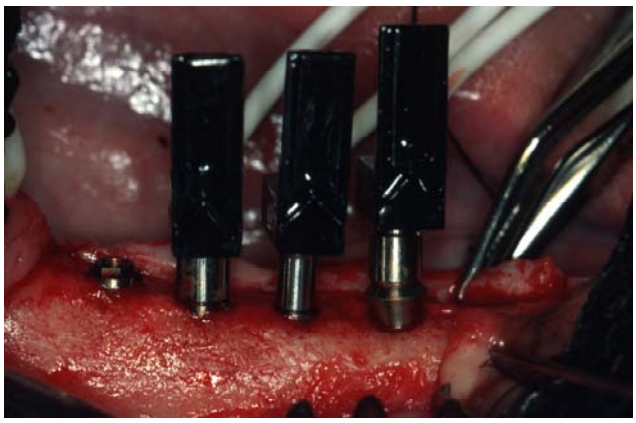


Sl. 5 – Ugradnja *Titanium Plasma-sprayed* (TPS) implantata
(ITI Straumann)

Eksperimentalni deo rada na psima urađen je na Fakultetu veterinarske medicine u Beogradu, a odobren od strane Etičkog komiteta Stomatološkog fakulteta u Beogradu.

Istraživanje na eksperimentalnim životinjama sprovedeno je prema sledećem protokolu: uzeti su otisci zuba gornje i donje vilice eksperimentalnih životinja kondenzacionim silikonom (Zetaplus, Zhermack®SpA, Italy) i izliveni studijski modeli od tvrdog gipsa; na modelima su izrađene individualne kašika od fotopolimerizujućeg akrilata (*light tray-light curing plates*, Ivoclar Vivadent AG, Germany) za potrebe kasnijeg otiskivanja implantata.

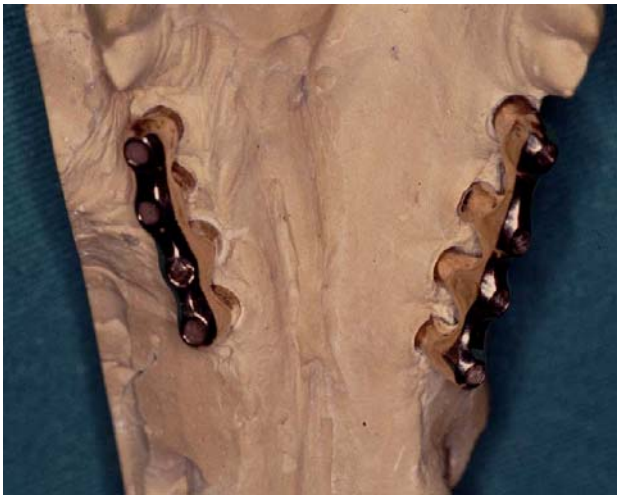
U premedikaciji ekperimentalnih životinja, korišćeni su atropin sulfat *sc* u dozi 0,5 mg/10 kg telesne mase (TM) (DP Sanofarm, Bosna) i propionil-promazin *iv* 4,25 mg/10 kg TM (Combelen, Germany). Za hiruršku anesteziju korišćen je di-



Sl. 6 – Transduktori Osstell instrumenta fiksirani za implantate tokom rezonantnih funkcija merenja (RFA)

con GmbH). Postavljene su transfer kapice (prenosnici položaja implantata) i uzeti su otisci za izradu definitivne zubne nadoknade. Implantati su otisnuti jednofaznom tehnikom, elastomernim materijalom iz grupe adicionih silikona srednje viskoznosti (Elite HD *regular body*, Zhermack®SpA, Italy). Za otiskivanje su korišćene individualne kašike sa međuprostorom. Celokupna procedura obavljena je pod opštom anestezijom i u maksimalno sterilnim uslovima. Hirurška i protetska procedura su izvedene instrumentima iz originalnih setova proizvođača, po standardnom protokolu predviđenom za svaki od korišćenih sistema.

Eksperimentalne životinje zbrinute su fiksnim zubnim nadoknadama dva dana posle implantacije. Nakon postavljanja suprastruktura, implantati su imedijatno opterećeni četvoročlanim mostovima od plemenite legure (*gold-platin* Legierung, type IV *extrahard*, Degulor®M, Degussa, Germany – AuPt) (slika 7). Mostovi su retinirani za suprastrukture na implantatima fiksacionim zavrtnjima, koji su moment ključem zategnuti silom od 24 Ncm za XiVE i ITI sistem, a 32 Ncm za MK III i Osseotite implantate, po preporuci proizvođača.



Sl. 7 – Izgled mostova na radnom modelu i u ustima eksperimentalnih životinja

Šest meseci nakon implantacije i imedijatnog opterećenja, uklonjene su fiksne zubne nadoknade i suprastrukture i sprovedena su RFA merenja sekundarne protetske stabilnosti svih implantata (ISQp).

Za statističku analizu dobijenih podataka korišćene su metode deskriptivne statistike: mere centralne tendencije (srednja vrednost i medijana), mere varijabiliteta (standardna devijacija, min i max), interval poverenja, i metode interferencijalne statistike (*t*-test, Mann Whitney *U*-test, jednofaktorska analiza varijanse, Kruskal Wallis-ov test). Statistička analiza obavljena je pomoću softverskog paketa SPSS 11.0.

Rezultati

Rezultati RFA merenja u donjoj vilici eksperimentalnih životinja, prikazani u tabeli 1, ukazuju da su svi implantati po hirurškoj ugradnji ostvarili stabilnost optimalnu za primenu protokola imedijatnog opterećenja (ISQ > 60).

Kod prvog psa vrednosti ISQh parametra bile su u intervalu od 65 do 81, a kod drugog od 62 do 80 ISQ jedinica. Nakon šestomesečnog funkcionalnog opterećenja fiksnim zubnim nadoknadama, implantatima u mandibuli eksperimentalnih životinja porasla je stabilnost. Svi implantati su dostigli odličnu stabilnost, ISQp > 80 i ostvarene su ujednačene vrednosti, bez obzira na tip površine. Najveća razlika između protetske i hirurške stabilnosti zabeležena je kod Osseotite (27 ISQ jed) i ITI TPS (24 ISQ jed), a najmanja kod XiVE (2 ISQ jed) i TiUnite sistema (7 ISQ jed).

Rezultati RFA merenja u gornjoj vilici eksperimentalnih životinja prikazani su u tabeli 2. Najveću stabilnost posle ugradnje ostvarili su implantati u I kvadrantu drugog psa od 72 do 77 ISQ jedinica. Najniža vrednost ISQh izmerena je kod Osseotite implantata, 59 ISQ jedinica. Implantati u gornjoj vilici druge eksperimentalne životinje postigli su veći protetski koeficijent implantatne stabilnosti (76–84 ISQ jed). U maksili prve eksperimentalne životinje, kod 50% implantata registrovan je pad stabilnosti posle perioda okluzalnog opterećenja implantata fiksnim zubnim nadoknadama.

Tabela 1

Prikaz rezultata analize rezonantnih frekvencija (RFA) dobijenih merenjima u donjoj vilici eksperimentalnih životinja

Vilica	Pas	Položaj	Kvadrant	Sistem	ISQh	ISQp	Razlika
Mandibula	1	1P	III	ITI	68	88	20
		2P		XiVE	78	85	7
		3P		Osseotite	65	92	27
		4P		TiUnite	81	88	7
		1P	IV	Osseotite	74	84	10
		2P		TiUnite	76	83	7
		3P		ITI	73	85	12
		4P		XiVE	72	82	10
	2	1P	III	XiVE	79	81	2
		2P		ITI	62	86	24
		3P		TiUnite	66	84	18
		4P		Osseotite	69	81	12
		1P	IV	TiUnite	70	85	15
		2P		Osseotite	69	81	12
		3P		XiVE	80	86	6
		4P		ITI	74	86	12

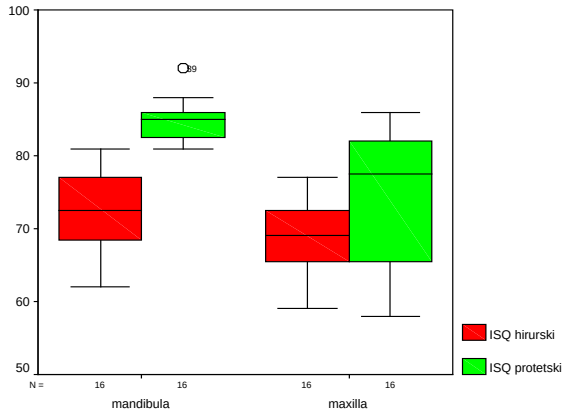
Tabela 2

Prikaz rezultata analize rezonantnih frekvencija (RFA) dobijenih merenjima u gornjoj vilici eksperimentalnih životinja

Vilica	Pas	Položaj	Kvadrant	Sistem	ISQh	ISQp	Razlika
Maksila	1	1P	I	TiUnite	69	62	-7
		2P		XiVE	73	85	12
		3P		Osseotite	69	86	17
		4P		ITI	68	58	-10
		1P	II	Osseotite	59	65	6
		2P		ITI	63	67	4
		3P		TiUnite	69	66	-3
		4P		XiVE	68	63	-5
	2	1P	I	XiVE	76	78	2
		2P		TiUnite	72	82	10
		3P		ITI	77	77	0
		4P		Osseotite	74	76	2
		1P	II	ITI	63	84	21
		2P		Osseotite	59	82	23
		3P		XiVE	69	78	9
		4P		TiUnite	68	82	14

Rezultati statističke analize vrednosti hirurškog i protetskog koeficijenta imlantatne stabilnosti ISQh i ISQp, kao i njihove razlike (ISQp – ISQh), kod različitih implantatnih sistema u gornjoj i donjoj vilici eksperimentalnih životinja prikazani su u tabeli 3. Između vrednosti ISQh u mandibuli i maksili nije uočena statistički značajna razlika (*t*-test, p = 0,064). Vrednost ISQp statistički se značajno razlikovala između donje i gornje vilice (Mann-Whitney *U*-test, p = 0,000) Vrednosti ISQh i ISQp za implantate u donjoj i gornjoj vilici prikazane su na slici 8. Hirurški koeficijent implantatne stabilnosti (ISQh) nije se statistički značajno razlikovao između različitih implantatnih sistema (jednofaktorska analiza varijanse, p = 0,053).

Analizom vrednosti ISQp različitih implantatnih sistema nije nađena statistički značajna razlika (Kruskal Wallisov test, p = 0,958). Vrednosti ISQh i ISQp po sistemima im-



Sl. 8 – Vrednosti koeficijenta stabilnosti implantata-hirurškog (ISQh) i implantata-protetskog (ISQp) za implantate u donjoj i gornjoj vilici

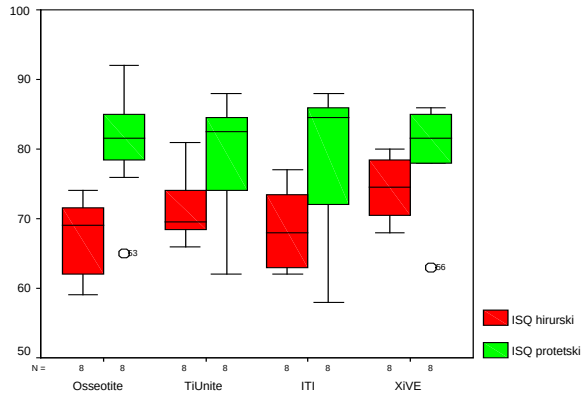
Tabela 3

Rezultati statističke obrade vrednosti koeficijenata stabilnosti implantata-hirurških (ISQh) i implantata-protetskih (ISQp) i njihove razlike

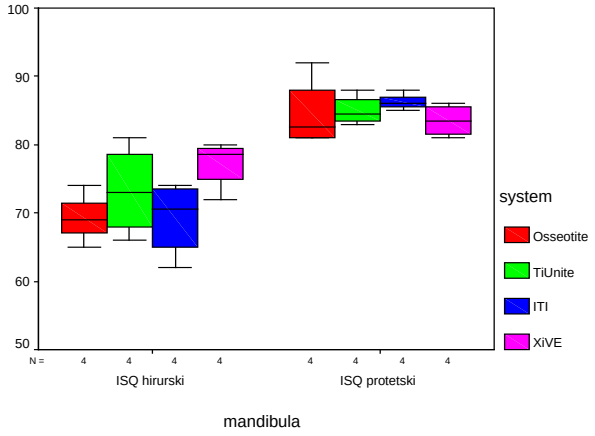
Posmatrani parametri	x̄	Med	SD	Min	Max	CI 95%
ISQ hirurški (ISQh)	70,38	69,00	5,75	59	81	68,30–72,45
Mandibula	72,25	72,50	5,64	62	81	69,25–75,25
Maksila	68,50	69,00	5,38	59	77	65,63–71,37
Osseotite	67,25	69,00	5,87	59	74	62,34–72,16
TiUnite	71,38	69,50	4,90	66	81	67,28–75,47
TPS	68,50	68,00	5,68	62	77	63,75–73,25
XiVE	74,38	74,50	4,57	68	80	70,56–78,19
ISQ protetski (ISQp)	79,63	82,00	8,63	58	92	76,51–82,74
Mandibula	84,81	85,00	2,97	81	92	83,23–86,40
Maksila	74,44	77,50	9,36	58	86	69,45–79,42
Osseotite	80,88	81,50	7,90	65	92	74,27–87,48
TiUnite	79,00	82,50	9,52	62	88	71,04–86,96
TPS	78,88	84,50	10,88	58	88	69,78187,97
XiVE	79,75	81,50	7,44	63	86	73,53–85,97
Razlika: ISQp-ISQh	9,25	10,00	8,98	-10	27	6,01–12,49
Mandibula	12,56	12,00	6,79	2	27	8,94–16,18
Maksila	5,94	5,00	9,86	-10	23	0,68–11,19
Osseotite	13,63	12,00	8,37	2	27	6,63–20,62
TiUnite	7,63	8,50	8,75	-7	18	0,31–14,94
TPS	10,38	12,00	11,71	-10	24	0,59–20,16
XiVE	5,38	6,50	5,50	-5	12	0,78–9,97

X – aritmetička sredina; Med – medijana; SD – standardna devijacija; Min – minimum; Max – maksimum; CI – interval poverenja

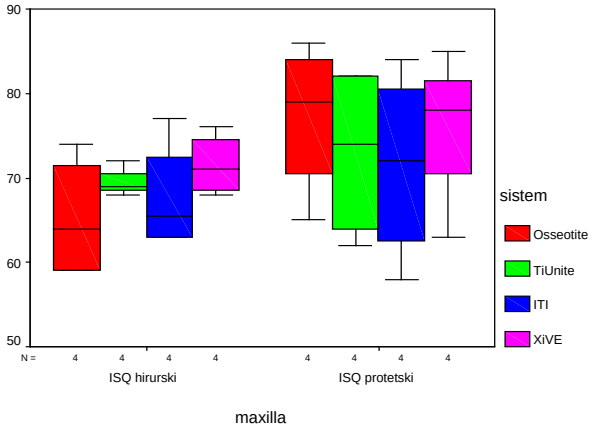
plantata prikazane su na slici 9, a vrednosti ISQ implantatnih sistema u mandibuli i maksili na slikama 10 i 11. Analizom razlike protetskog i hirurškog koeficijenta implantatne stabilnosti, ISQp–ISQh, utvrđena je statistički značajna razlika između mandibularnih i maksilarnih implantata (*t*-test, $p = 0{,}035$) (slika 12). Statistički značajna razlika nije uočena kod parametra ISQp–ISQh, posmatrano kod korišćenih implantatnih sistema (jednofaktorska analiza varijanse, $p = 0{,}296$).



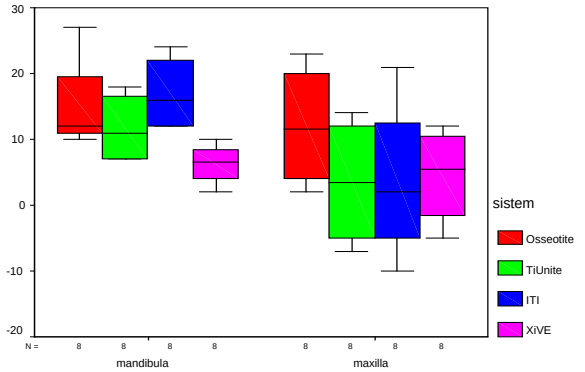
Sl. 9 – Vrednosti koeficijenta stabilnosti implantata-hirurškog (ISQh) i implantata-protetskog (ISQp) po sistemima implantata



Sl. 10 – Vrednosti koeficijenta stabilnosti implantata (ISQ) implantatnih sistema u mandibuli



Sl. 11 – Vrednosti koeficijenta stabilnosti implantata (ISQ) implantatnih sistema u maksili



Sl. 12 – Distribucija vrednosti razlike koeficijenta stabilnosti implantata-protetskog (ISQp) i implantata-hirurškog (ISQh) po sistemima i vilicama

Diskusija

Imedijatno opterećenje implantata već duže vreme zaokuplja pažnju implantološke stručne javnosti, ali i pobuđuje izvesne nedoumice zbog pitanja razlika između imedijatne implantacije/restauracije, imedijatnog/ranog i odloženog opterećenja.

Bez obzira na činjenicu da proces oseointegracije zahteva prosečno šest meseci u gornjoj i tri meseca u donjoj vilici, ipak postoji potreba za imedijatnim opterećenjem, zbog brže rehabilitacije bezubih i krezubih pacijenata [11, 12]. Visok stepen predvidivosti konvencionalnog koncepta opterećenja implantata u mandibuli, doveo je do reevaluacije hirurškog i protetskog protokola [13].

Brojna klinička [14–23] i eksperimentalna istraživanja [24–27] pokazala su da RFA tehnika može biti uspešno korišćena u proceni implantatne stabilnosti odmah posle ugradnje, u toku zarastanja i u funkciji implantata sa zubnom nadoknadom. Preporuka implantologa [28] sa značajnim iskustvom u primeni RFA metode je da se implantati ne opterećuju neposredno posle ugradnje ako je inicijalna stabilnost manja od 45 ISQ jedinica. U ISQ intervalu od 45 do 60 imedijatno opterećenje je moguće, ali sa izvesnim rizikom, uz neophodnu kontrolu pacijenata i ponovna merenja, kako bi se pratila stabilnost funkcionalno opterećenih implantata. Empirijski podaci sugerišu da su vrednosti ISQ $\geq$ 65 optimalne za opterećenje implantata posle hirurške ugradnje [29, 30]. Iskustvo u primeni RFA metode u ovom istraživanju pokazalo je da ISQ vrednosti realnije prikazuju stabilnost implantata u donjoj nego u gornjoj vilici, najverovatnije zbog kompaktnije građe koštanog tkiva mandibule, što se odražava na kvalitet merenja. Kod implantata ugrađenih u kost lošeg kvaliteta, mogu se dobiti dve potpuno različite ISQ vrednosti, grafički predstavljene sa dva maksimuma, što je znak netačnog merenja. Brojne kliničke [31–38] i eksperimentalne studije [39, 40] pokazale su da se imedijatno opterećenje dentalnih implantata može uspešno ostvariti i sa mobilnim [41, 42] i fiksnim zubnim nadoknadama [43–45]. Mikronaprezanja mogu biti stimulativna za vreme perioda zarastanja, jer mogu da doprinesu povećanju gustine i mineralizaciji periimplantatne kosti, što je poznato iz istraživanja osteoporotične kosti [46]. Postoje i studije [39] u

kojima imedijatno opterećenje nije doprinelo porastu gustine periimplantatnog koštanog tkiva, ali nije ni narušilo oseointegraciju implantata. Poznato je da endoosealni implantati prenose okluzalno opterećenje na periimplantatno ležište u vidu kompresivnih sila i sila smicanja malog intenziteta, bez pojave intenzivnih naprezanja u zoni međuspoja implantata i kosti [47]. Ova činjenica još jednom ističe da je za imedijatno opterećenje implantata fiksnim zubnim nadoknadama, sem primarne stabilnosti, potrebno rigidno povezati implantate blok konstrukcijom i isključiti parafunkcionalne aktivnosti mandibule.

Adekvatna oralna higijena je, takođe, bitan faktor tokom zarastanja imedijatno opterećenih implantata [40]. Za vreme trajanja naše studije nije sprovođena kontrola plaka, što se nije negativno odrazilo na tok oseointegracije, pa se čini da kod životinja, kao eksperimentalnih modela, oralna higijena nije presudna za uspešnost implantacije.

U našem istraživanju, poređenjem rezultata implantatne stabilnosti različitih površina implantata, nisu utvrđene statistički značajne razlike između ispitivanih sistema. Vrednosti ISQh nije se značajno razlikovala između sistema, što navodi na zaključak da različit tip površinske hrapavosti ne uslovljava različite vrednosti primarne stabilnosti implantata, što je u skladu sa istraživanjem koje su sproveli Rompen i sar. [48]. Rezultati naših eksperimentalnih merenja u donjoj vilici životinja pokazali su, da su svi implantati po hirurškoj ugradnji ostvarili stabilnost optimalnu za primenu protokola imedijatnog opterećenja (ISQ > 60). Neposredno nakon ugradnje implantata najveće vrednosti primarne stabilnosti pokazali su implantati XiVE sistema i u donjoj (77,25 ± 3,59) i u gornjoj vilici (71,50 ± 3,70) eksperimentalnih životinja.

U studiji na zečevima, koja je metodološki slična našoj, Gottlow i sar. [49] su dobili statistički značajno veće vrednosti primarne i sekundarne stabilnosti nakon šest nedelja zarastanja, kod TiUnite u poređenju sa Osseotite implantatima. Sennerby i Miyamoto [50] poredili su stabilnost implantata sa TiUnite (NobelBiocare) i SLA (ITI Straumann) površinom, posle ugradnje i nakon tri nedelje zarastanja. Nisu utvrdili statistički značajnu razliku između ovih implantatnih sistema, što potvrđuju i rezultati našeg istraživanja.

Nakon šest meseci okluzalnog opterećenja zubnom nadoknadom u našem istraživanju najveće vrednosti ISQp su zabeležene kod ITI TPS implantata u mandibuli (86,25 ± 1,26) i Osseotite sistema u maksili (77,25 ± 9,14) eksperimentalnih životinja. Najmanja varijacija ISQ očitavanja nađena je kod XiVE sistema, koji je ujedno ostvario najveću srednju vrednost ISQh parametra 74,38 ± 4,57. Maksimalan rezultat za ISQp, ostvarili su Osseotite implantati (80,88 ± 7,90).

Merenjima RFA u istraživanju utvrđena je razlika između protetske i hirurške stabilnosti implantata. Svi implantati ugrađeni u donju vilicu eksperimentalnih životinja imali su veću stabilnost nakon 6-mesečnog funkcionalnog opterećenja od one izmerene posle hirurške ugradnje, ISQp > ISQh. Porast ISQ vrednosti imedijatno opterećenih implantata tokom vremena, zabeležen je i u sličnim istraživanjima [18, 26, 48]. Implantati su, takođe, dostigli ujednačene vrednosti protetskog koeficijenta stabilnosti (ISQp > 80), bez obzira na različite početne vrednosti hirurškog ISQh. Veći porast stabilnosti zabeležen je kod implantata koji su imali niže vrednosti ISQh, što potvrđuju i rezultati studije koju su sproveli Friberg i sar. [51].

Kod pojedinih implantata u gornjoj vilici (TiUnite, XiVE i ITI TPS sistema) registrovan je pad ili stagnacija stabilnosti i pored uspešne oseointegrisanosti. Najveća razlika između protetskog i hirurškog koeficijenta implantatne stabilnosti utvrđena je kod Osseotite sistema, a najmanja kod XiVE implantata.

Veći ISQ su ostvarili implantati u donjoj vilici, što je i očekivano, zbog kompaktnije koštane građe mandibule. Sumiranjem rezultata RFA merenja, ustanovljena je veća stabilnost implantata u mandibuli u poređenju sa implantatima u maksili, statistički značajna za ISQp. U donjoj vilici prosečna vrednost za ISQh bila je 72,25 ± 5,64, a za ISQp 84,81 ± 2,97, u poređenju sa implantatima u gornjoj vilici kod kojih je ISQh iznosio 68,50 ± 5,38, a ISQp 74,44 ± 9,36. Nakon perioda okluzalnog opterećenja standardna devijacija kod implantata u donjoj vilici redukovala se, a kod onih u maksili povećala.

Rompen i sar. [24] su u svojoj studiji RFA metodom merili primarnu stabilnost TiUnite MKIII i MKIV(NBC) implantata ugrađenih u mandibule pasa. Dobijene ISQ vrednosti MKIII (80,0 ± 5,9) i MKIV (77,8 ± 5,7) komparabilne su sa rezultatima našeg istraživanja za TiUnite implantate ISQh, 71,38 ± 4,90.

Dosadašnja istraživanja pokazala su da primena protokola imedijatnog opterećenja implantata zubnom nadoknadom u svakodnevnom kliničkom radu, zahteva opreznost u proceni opravdanosti i rizika. Prednosti analize rezonantne frekvencije su jednostavnost primene u kliničkim uslovima, prilagođenost svakom implantološkom sistemu i odsustvo senzacija pacijenta za vreme merenja, koje traje nekoliko sekundi. Značajna prednost RFA metode u odnosu na ostale je, što se merenja mogu izvoditi u bilo kom trenutku za vreme zarastanja ili opterećenja implantata mobilnom ili fiksnom zubnom nadoknadom fiksiranom zavrtnjem, što omogućava kontinuiranu opservaciju razvoja oseointegracije implantata.

U prethodnim istraživanjima nedovoljno je razmatran uticaj okluzalnog faktora na odgovor periimplantatnog tkiva u procesu zarastanja. Takođe, nedostaju studije u kojima bi se analizirale razlike u stabilnosti implantata imedijatno opterećnih mobilnim *vs* fiksnim zubnim nadoknadama, pojedinačnim krunama sa povezivanjem implantata u blok rigidnom vezom. Pouzdanost primene protokola imedijatnog opterećenja implantata mora se potvrditi rezultatima dugoročnih, multicentričnih studija, jer se retko prikazuje detaljna klinička i radiografska procena implantata praćenih tokom pet i više godina. Za vrednovanje dobijenih rezultata neophodna je standardizacija kriterijuma za procenu uspešnosti implantatne terapije, hirurške i protetske faze.

Zaključak

Na osnovu dobijenih rezultata ovog istraživanja može se zaključiti da ispitivani endoosealni implantati nisu pokazali različit stepen oseointegracije jer se rezultati merenih parametara (ISQh i ISQp) nisu statistički značajno razlikovali između implantatnih sistema, kao i da lokalizacija implantata u gornjoj ili

donjoj vilici statistički značajno utiče na merene parametre; analiza rezonantne frekvencije omogućava merenje implantatne stabilnosti, ali samo kao pomoćna dijagnostička metoda pri izboru protokola opterećenja. Konačnu odluku o primeni protokola imedijatnog opterećenja implantološki tim bi trebalo da donese na osnovu procene svih faktora oseointegracije, naročito očekivanog individualnog odgovora zarastanja tkiva i uslova funkcionalnog opterećenja kod svakog pacijenta.

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ORIGINAL ARTICLE

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Prognostic significance of tympanosclerotic plaques localization and their morphological and histological characteristics for the outcome of surgical treatment

Prognostički značaj lokalizacije, morfoloških i histoloških karakteristika timpanosklerotičnih plakova na ishod operativnog lečenja

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Abstract

Background/Aim. Tympanosclerosis is a sequela of inflammation of the middle ear usually causing conductive hearing loss. The aim of the study was to determine the significance of tympanosclerotic plaques localization in the middle ear and their morphological and histological characteristics for surgical treatment outcome. **Methods.** This retrospective study included a total of 73 patients operated on for tympanosclerosis in the Clinic for Otorhinolaryngology, Military Medical Academy (MMA) in a period 1996–2010. The results of surgical treatment as well as the last audiometry findings were analyzed. considering follow-up periods of 6 months to 8 years. The patients were divided into 4 groups according to tympanosclerotic plaques localization in the middle ear and the classification suggested by Wieling and Kerr. The patients were also divided based on intraoperatively noticed morphological characteristics of tympanosclerotic plaques, while the third division was done as per histological findings. Surgical success was assessed using the suggestions of the Japan Otological Society. **Results.** The analyzed results showed the surgical success especially in the group II according to Wieling and Kerr, while histological findings had no impact on the outcome of the surgery. **Conclusion.** Surgical treatment has good results especially in patients with the mobile stapes. Results are satisfactory in other localizations, while various morphological and histological characteristics do not have impact on the surgery outcome.

Key words:
tympanoplasty; hearing disorders; audiometry; histology; prognosis.

Apstrakt

Uvod/Cilj. Timplanoskleroza nastaje kao posledica upale srednjeg uva i najčešće izaziva konduktivni gubitak sluha. Cilj ovog rada bio je ispitivanje uticaja lokalizacije, morfoloških i histoloških karakteristika timpanosklerotičnih plakova u srednjem uvu na ishod operativnog lečenja timpanoskleroze. **Metode.** Retrospektivna studija sprovedena je na 73 bolesnika operisana zbog timpanoskleroze u Klinici za otorinolaringologiju VMA u periodu od 1996. do 2010. god. Posmatrani su rezultati hirurškog lečenja kroz nalaz zadnje dostupne audiometrije. Period praćenja bio je od 6 meseci do 8 godina. Bolesnici su bili podeljeni u četiri grupe u odnosu na lokalizaciju timpanosklerotičnih plakova u srednjem uvu prema klasifikaciji koju su predložili Wielinga i Kerr. Druga podela učinjena je na osnovu intraoperativno uočenih morfoloških karakteristika timpanosklerotičnih plakova, a treća na osnovu histološkog nalaza. Procena operativnog uspeha izvršena je na osnovu predloga Japanskog udruženja otologa. **Rezultati.** Analizirani rezultati pokazali su operativni uspeh naročito u grupi II prema Wieilinga i Kerr klasifikaciji dok morfološke karakteristike i histološki nalazi nisu imali uticaj na ishod operativnog lečenja. **Zaključak.** Hirurško lečenje timpanoskleroze daje dobre rezultate naročito u grupi bolesnika sa mobilnim stapesom. Zadovoljavajući rezultati postižu se i kod ostalih lokalizacija, dok varijacije morfoloških i histoloških karakteristika nisu imale uticaj na ishod operativnog lečenja.

Ključne reči:
timpanoplastika; sluh, poremećaji; audiometrija; histologija; prognoza.

Introduction

Tympanosclerosis is a consequence of inflammation of the middle ear often resulting in conductive hearing loss [1]. When localized only in the tympanic membrane it refers to myringosclerosis and does not cause a significant hearing loss, while localized in the *cavum tympani* often affects the ossicular chain followed by hearing loss.

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Tympanosclerosis is clinically characterized by hyaline changes or calcifications on the tympanic membrane, commonly in the shape of a horseshoe. They are caused by hyalinization of fibrous and elastic fibres in the lamina propria of the tympanic membrane. Similar changes can happen in the submucosal layer of the cavum. Collagen of the fibrous tissue loses its structure and merges with homogeneous mass. By the progression of the process calcium deposits causing ossification of the changes. Depending on the extent of calcification and localization of the process there comes to ossicular chain fixation and conductive hearing loss of various levels and, in rare cases, to deafness [2]. There are numerous controversies regarding ethiology, pathogenetic and histological aspects of the disease as well as disagreement about surgical treatment and the choice of an optimal approach [3]. Some of the leading otologists, Austin, Sheehy, House, Tos advocate surgical treatment, while opponents, Schukhnecht, Morrison and Smyth, who even consider tympanosclerosis the last disease of the middle ear to be surgicaly managed [4]. There is a general agreement, however, only about nonspecificity of the process resulting from inflammation or infection of the middle ear.

The aim of the study was to determine the significance of tympanosclerotic plaques localization in the middle ear and histological findings for surgical treatment outcome.

Methods

This retrospective study included a total of 73 patients with the diagnosed tympanosclerosis who had been operated on in the Clinic for Otorhynolaringology, Military Medical Academy (MMA), in Belgrade within a period from 1996 to 2010. Postoperatively, the patients were followed up from 6 months to 8 years. Operative success was assessed by the means of the last available audiometry. The average follow-up period was 18 months, while the first audiometry was performed 3 weeks following the surgery. The study included patients with the primary operation no matter if tympanosclerosis was comorbide with a kind of chronic inflammation of the middle ear.

Regarding localization of tympanosclerotic process, the classification suggested by Wieling and Kerr [5] was performed: the group I – the process affects pars tensa intacta or perforated tympanic membranes, the group II – the process fixes the incudomalleolar complex while the stapes is mobile, the group III – fixed or absent stapes and the mobile incudomalleolar complex if there is one, and the group IV – a completely fixed ossicular chain.

Division of the patients regarding the noticed morphological characteristics was made in accordance with the recommendations given by Selcuk et al [6].

The group I included causes similar to cholesteatoma matrix, of soft consistency easy to be surgically detached. Cheese-like characteristics are imputed to them due to their hardness and appearance.

The group II included moderately hard changes removed like a leaf from the bones of hearing, promontorium or some other localization in the cavum.

The group III consisted of extremely hard plaques that looked like osseous tissue difficult to be removed from the mucose in the cavum or the ossicular chain.

Based on histological examination of the sent material the 3 groups were also formed: the group I included connective tissue loss and fibroblast and collagen fibres proliferation with the appearance of rare calcium cristals.

The group II included changes characterized by further proliferation of fibroblasts and masses of irregular collagen fibres with focal calcification.

The group III included chondroblasts-like cells, round in form, localized in lacunae and the process of intense calcifications.

Preoperatively, hearing condition was determined on the basis of tone liminal audiometry in accordance with the suggestions of the Japan Otological Society. Only the values measured at 500 Hz, 1 000 Hz and 2 000 Hz were considered.

Postoperatively, surgical success was determined according to the same criteria that assumes tympanoplasty successful if one of the 3 conditions is met: air-bone gap less than 15 dB; hearing improvement of more than 15 dB; and postoperative air conduction hearing threshold less than 30 dB.

In case no of the 3 conditions fulfilled tympanoplasty is considered failed.

Old principles of middle ear surgery accepted in the Clinic for Otorhinolaryngology, MMA were applied in the surgical approach to tympanosclerosis.

In the group I clssified according to Wielinge and Kerr, myringoplasty was performed using a temporalis muscle fascia graft placed at underlay or inlay position.

Calcificates not affecting more than one quadrant of the tympanic membrane were not excised if not affecting the *annulus tympanicus* towards the atticus so as to fix a part of ossicular chain.

In the group II including the patients with lateral intact ossicular chain fixation and mobile stapes we performed ossicular chain mobilization, modelled incus interposition in case of second arm necrosis or partial ossicular prosthesis placement between the mobile stapes and tympanic membrane in cases of manubrium malleus absence.

Combined or transmeatal approach to the *cavum tympani* was used.

In the group III including the patients with fixed stapes, tympanosclerotic plaques were removed layer by layer and within the same act partial stapedectomy and partial stapedoplasty were performed stabilising the placed prosthesis by fibrous tissue or perichondrium.

In the group IV including patients with multicentric localization the prime aim was to preserve intact ossicular chain if present at all.

Absence of the stapes suprastructure required, except for mobilization, both partial stapedectomy and stapedoplasty. A total ossicular replacement prosthesis (TORP) was placed in one case.

Results

Table 1 shows the frequency of some tympanosclerotic plaques localizations in a group of 73 patients.

Table 1

Tympanosclerotic plaques localizations

Localization	Patients [n (%)]
Tympanic membrane	5 (6.8)
Lateral attic	37 (50.7)
Oval window and stapes	18 (24.7)
Multicentric localisation	13 (17.8)

Each of the 5 patients in the group with tympanic membrane plaques had open type tympanosclerosis audiometricly characterized by air-bone gap up to 20 dB. The patients with lateral fixation (n = 37) (50.7%) were dominant in the studied series. Hearing damage in this group was classified in the group with air-bone gap of more than 30 dB, and in the group with air-bone gap between 20 and 30 dB. In the group of patients with stapes fixation the highest number of patients had hearing damage with air-bone gap of more than 30 dB. Table 2 shows the frequency of intraoperatively found morphological charactristics of tympanosclerotic plaques. Table 3 shows the histological plaque distribution, and Table 4 pre- and posstoperative hearing condition regarding localization of tympanosclerotic process.

Analysis of operatively seen morphological characteristics revealed that a higher number of the patients in the groups 1 and 2 were characterized with the absence or the biginning of the process of calcification. Histological findings in the majority of cases confirmed operatively seen characteristics of plaques (Figures 1 and 2).

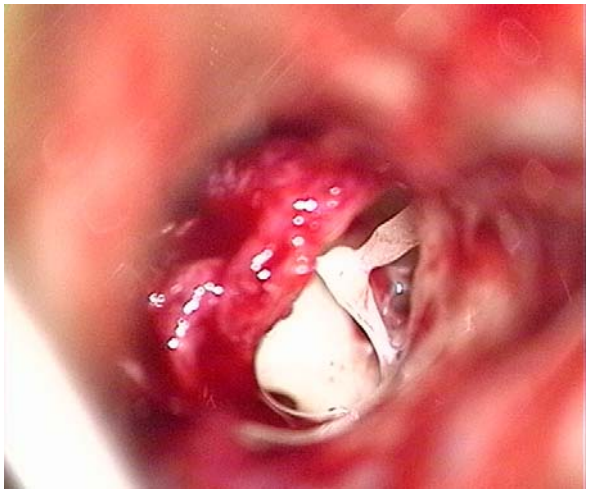

Fig. 1 – Erosion of the second arm caused by tympanosclerotic process

Table 2

Intraoperative morphological characteristics of tympanosclerotic plaques

Intraoperative morphological characteristics	Patients [n (%)]	Preoperative hearing condition (dB)	Postoperative hearing condition (dB)
Soft cheese-like plaques similar to cholesteatoma matrix	25 (34.25)	Air 50.8 ± 6.2 Bone 27.3 ± 6.4	Air 38.6 ± 3.8 Bone 23.7 ± 4.4
Leaf-like moderately hard plaques	29 (39.7)	Air 61.5 ± 10.5 Bone 25.7 ± 5.6	Air 38.6 ± 4.3 Bone 20.7 ± 2.4
Bone-hard calcified plaques	19 (28.1)	Air 65.2 ± 8.5 Bone 28.7 ± 3.7	Air 44.8 ± 7.3 Bone 25.8 ± 3.2

Table 3

Histological findings in tympanosclerotic plaques

Histological finding	Patients [n (%)]	Preoperative hearing condition (dB)	Postoperative hearing condition (dB)
Connective tissue loss, proliferation of fibroblast and collagen fibres	29 (39.7)	Air 52.8 ± 8.2 Bone 28.3 ± 8.4	Air 39.6 ± 5.8 Bone 22.7 ± 4.6
Focal calcification of irregularly distributed collagen fibres	27 (37.0)	Air 62.5 ± 12.5 Bone 26.7 ± 3.6	Air 38.6 ± 4.3 Bone 21.7 ± 1.6
Chondroblastoma-like cells and calcifications	17 (23.3)	Air 65.5 ± 12.5 Bone 29.7 ± 2.8	Air 42.8 ± 8.2 Bone 24.7 ± 2.2

Table 4

Preoperative hearing condition and postoperative success regarding localization of tympanosclerotic process

Localization	Preoperative hearing condition (dB)	Postoperative hearing condition (dB)
Tympanic membrane	Air 32.8 ± 6.12 Bone 16.4 ± 13.5	Air 15.8 ± 4.6 Bone 14.6 ± 3.4
Attic	Air 52.3 ± 6.12 Bone 26.6 ± 12.7	Air 39.1 ± 12.5 Bone 24.8 ± 9.8
Oval window	Air 65.5 ± 12.5 Bone 38.2 ± 14.6	Air 58.5 ± 13.2 Bone 35.2 ± 11.74
Multicentric localizations	Air 62.6 ± 14.3 Bone 35.4 ± 16.62	Air 57.3 ± 15.6 Bone 31.8 ± 12.9

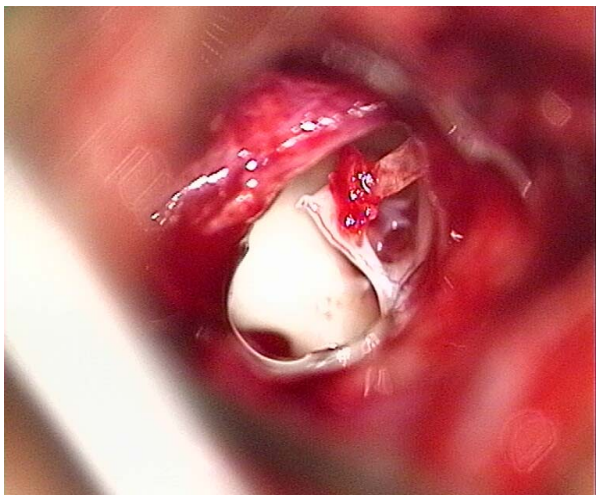

Fig. 2 – Ossiculoplasty by mastoid cortex plasty

Discussion

There have been many controversies regarding etiology, pathogenesis, diagnostics and the treatment of tympanosclerosis since the beginning. The use of surgical microscope that has made middle ear surgery possible contributed especially to dilemmas about surgical treatment. The questions about the need at all to surgically manage tympanosclerosis then about surgical technique in cases that have to be surgically managed have been put.

A long-standing experience in middle ear surgery in the Military Medical Academy, Belgrade led to the attitude that tympanosclerosis need to be surgically managed sticking to a certain extent to the modified basic otosurgery principles, including firstly, eradication of pathological process as a whole, and secondly restitution of the transmission apparatus. Considering that just a small number of patients with tympanosclerosis show symptoms such are otorrhea, instability in walking, that might disturb daily activities of patients with tympanosclerosis, hearing improvement by the use of surgical treatment should be the major aim. Especially if we consider the fact that tympanosclerotic process does not complicate as other forms of chronic inflammatory processes, such are active squamous ones, for example. The basic motive for the group of 73 patients to present to the doctor was a hearing improvement possibility.

Tympanosclerosis open type localized only to the tympanic membrane damage hearing less than other three groups. Calcificates on the tympanic membrane that need annulus should be removed in the same way as those that affect more than a half of the tympanic membrane, thus considerably disturbing its mobility. According to our experiance, temporalis muscle fascia is the best material available for perforation reconstruction, the ideal position being that between the cutane layer and lamina propria which, however, is not always easy to realize. In the 3 patients of this group perforations required posterior quadrants that is a precondition for this so-called inlay fascia position. In other patients fascia was placed below the remnants of the tympanic membrane. According to the criterion of the Japan Otological Society, surgical treatment of all of the 5 patients was classified as successful. They, also, met more strict evaluation criterion that assumed only air conductivity of less than 30 dB as socially acceptable hearing.

Lateral atticotomy and mobilization of the ossicular chain lead to surgical success in case of incudomalleolar complex and intact ossicular chain mobilization. It is a known fact that immediate postoperative results are good tending, however, to refix due to the formation of scarf tissue in a period up to 2 years following the surgery. Prevention is possible by placing silastics or gel foams onto the potential contact sites, that was applied in the group of the studied patients in all the cases in which refixation was considered possible. Topical application of corticosteroid drops and the use of various laser types (argonne, CO_2) have also been presented in papers of many authors [3, 7–11]. When it was impossible to mobilize the ossicular chain to a certain satisfying extent or when the chain was disrupted, we applied disarticulation of the incudomalleolar joint and interposition of previously modelled incus.

In the study we did not compare postoperative success between mobilization and interposition. Considering data of other authors, e.g. Albu et al. [4], however, there is no statistisally significant difference, although the results are slightly better in the group of patients with mobilization. This agrees with our attitude to keep intact ossicular chain whenever it is possible. It was the most frequent localization in the studied group (n = 37, 50.7%) of the patients. There was a significant correlation found between this localization and surgical success, explained by the significance of the intact and mobile stapes in sound transmission.

There are the most pronounced controversies regarding surgical approach to tympanosclerosis, no matter if there are suprastructures of the stapes or not. The question remains if mobilization of the fixed stapes plate is enough or if there is a need for stapedectomy, i.e. stapedoplasty.

Tos et al. [7] suggest in case of fixed plate and intact ossicular chain to keep it along with mobilization of the stapes. They approve stapes ligaments resection in case of uneasy approach to the oval niche during removal the plaques, but with no fenestration of the plate nor its removal. In case of the stapes suprastructures absence and if the plaques fix the plate they recommend stapedoplasaty in 2 acts: in act 1 to remove plaques from the plate, and in act 2 to do stapedotomy and stapedoplasty. Numerous authors share the same opinion, advocating mobilization, too, agreeing with Tos et al. [7] that its highest advantage is one act performance, while Smyth [8] disagrees with that, thinking that the possibility of damaging the inner ear during mobilization either with hydric blast or perilymphatic fistula is very great, leading together to sensorineural hearing damage. In his serie he reports that it ranges up to 36%. Giddings and House [9] has the same opinion giving the priority to stapedectomy in managing tympanosclerosis of the stapes. There is not the small number of authors who accept both attitudes, documenting no difference between mobilization and stapedectomy or stapedoplasty [10], but who do differentiate stapedotomy from stapedectomy in favor of the second due to better results in

speaking discrimination and hearing condition in stapedectomy. Tuefert and De La Cruz [3] make a difference between mobilization and stapedectomy. In their series of 73 patients, too, followed up within a longer time period (average 1.6 years) they report equally good results for both procedures giving more significance to the surgeon skill than to the choice of the method.

In a part of the patients of our series with the fixed stapes we performed fenestration of the stapes footplate and stapedoplasty along with the use of various types piston stapes prosthesis (Fisch, House, Kurtz). Of the whole group, in one patient there was a more serious sensorineural damage requering hearing amplyfication.

In the group with multicentric localization we performed the principles common for the two previous groups. Removal of plaques and mobilization of the chain if intact is the primary aim which we obtained in the 6 out of the 13 patients. The process in the medial attic requires disarticulation of the incudomalleolar joint and reconstruction of the chain [11]. The procedure of a modelled incus interpositioning between the manubrium malleus and stapes suprastructures, if any present, gives good results. In one case we placed a total ossicular replacement prosthesis (TORP), and the result of the most recent audiometry 12 months following the surgery was not satisfactory according to the requirements of the Japan Otological Society.

Statistical data analysis of histological changes in tympanosclerosis by the use of the χ^2 test did not reveal any statistically significant dependence regarding the success of surgical treatment. A possible explanation is the fact that histological changes characteristic for all the three groups could be found in one patient suggesting dynamic process and a long evolution of the disease. According to data of other authors [4] immediate postoperative results are better in a group of patients with proliferation of fibroblast and collagen fibers. At the same time, in each patient there is a higher possibility of conductive hearing loss recurrence than in a group of patients with more pronounced calcification.

Conclusion

Surgical treatment of tympanosclerosis leads to good results regardless location and the extent of the process affecting the middle ear, however, especially good results are obtained in a group of patients with the mobile stapes footplate and suprastructures present. The variety of morphological changes seen intraoperatively in the studied series of patients did not show a statistically significant correlation with the success of surgical treatment. The same conclusion applies to the results of pathohistological findings which, inspite of the obvious differences, do not influence the outcome of surgical treatment.

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CURRENT TOPICS

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Should we prescribe "vasodilating" beta-blockers in Marfan syndrome to prevent aortic aneurysm and dissection?

Da li bi trebalo da propisujemo „vazodilatirajuće" beta blokatore kod sindroma Marfan za sprečavanje aneurizme i disekcije aorte?

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Key words:
marfan syndrome; aortic aneurysm; aortic rupture; therapeutics; adrenergic beta-antagonists.

Ključne reči:
marfanov sindrom; aorta, aneurizma; aorta, ruptura; lečenje; adrenergički beta blokatori.

Introduction

The Marfan syndrome (MFS) was named after Antoine Bernard-Jean Marfan (1858–1942), a French pediatrician, who described the syndrome in a 5-year-old girl in 1896. [1]. There is a great deal of interest in medical therapy for MFS, which protects the aorta and prevents or delays surgery [2].

MFS is a hereditary connective tissue disorder, with the incidence of 2–3 in 10,000 live births worldwide [3, 4]. Approximately 15%–30% of MFS patients are due to *de novo* genetic mutations, but it is mostly inherited as an autosomal dominant trait, due to mutations in the fibrillin-1 gene [5]. Fibrillin-1 is the major constituent of microfibrils, which are components of extracellular matrix, as well as of elastic fibers [4]. Thus, in MFS connective tissues are looser than usual, damaging the support structures of the entire body. Elastin fibers are found throughout the body, but are particularly abundant in the aorta, ligaments and the eye; consequently skeletal, cardiovascular (CV) and ocular systems are among the worst affected in MFS patients [4, 6]. The diagnosis of MFS is based on the revised Ghent criteria [3, 7, 8].

Elastic fibers are abundant in elastic arteries: up to 40% of the wall of the thoracic aorta [9]. In MFS the amount of elastin in the aortic wall is decreased (quantitative disorder), together with a loss of elastin's normally highly organized structure (qualitative disorder) [10]. In systole, aorta normally expands, stretching the elastic fibers and enables a portion of a stroke volume to be stored. The aorta recoils during diastole (i.e. elastic fibers return to their original size, bringing back the aorta to its unexpanded diameter), so that blood continues to flow forward from the aorta to the periphery during diastole, thus creating a nearly continuous peripheral blood flow [9]. This is named the buffering (Windkessel) function of the aorta. The proximal aorta provides more than half of the "buffering" capacity of the entire arterial system, and the aorta and some of the proximal large vessels store about 50% of the left ventricular (LV) stroke volume during systole [9, 11]. The Windkessel model was proposed in 1899 by Frank [12, 13]. Buffering function allows blood flow to be converted from an intermittent, pulsatile flow to a more steady and laminar one, protecting sensitive end-organs from the detrimental effects of excessive pressure pulsatility [11, 14].

Aortic elasticity with consequent buffering function is very useful: it protects ascending aorta from an abrupt increase of wall tension during systole, reduces LV afterload, and improves both LV relaxation and coronary blood flow [9]. Thus, fibrillin is the primary component of the microfibrils that allow tissues to stretch repeatedly without weakening. In MFS, the impaired microfibrils do not help the elastic fibers spring back and the vulnerable, weak aorta gets stretched out over time by the force of the blood. As it widens, the aorta weakens additionally.

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Aortic dissection is major cause of death in Marfan syndrome

At 30 years of age, men with MFS have an annual risk of death of about 2%, and women have a risk of about 1%, 20–40-fold increased risk compared with the United Kingdom population of the same age. The mean age at death in affected people is 44 years for men and 47 years for women, and about 70% die from acute CV complications, mainly AoD [3].

It is not a surprise, having in mind that in MFS aorta is impaired and aorta is the largest and the strongest artery in the body, carrying roughly 200 million liters of blood through the body in an average lifetime [10]. A clinical hallmark and the major cause of morbidity and premature death in MFS was and remains aortic root dilation and associated aortic regurgitation, dissection, and rupture [1, 6, 15, 16]. Vice versa, MFS is one of the most important risk factors for AoD especially in the young [10, 17, 18].

Beta-blockers are standard therapy for aortic dissection

As a result of earlier detection, better follow-up and both surgical and medical treatment, average life expectancy in MFS have been increased by 30 years or more [19]. Beta-blockers (BBs) retard the rates of aortic dilatation and AoD in MFS [1, 3, 15, 20]. The story started in 1959, when it was announced that reserpine had prevented aortic rupture in susceptible turkeys, and continued in 1965, with successful treatment of AoD without surgery, i.e. with antihypertensive drugs. The experience with propranolol ability to slow aortic root dilatation and dissection in MFS was published in 1971. BBs are the standard treatment for MFS, since the only randomized clinical trial of Shores et al [2, 16, 21].

BBs have three actions, considered useful in the prevention of AoD in MFS: diminishing blood pressure (BP), heart rate (HR) and LV contractility (rate of pressure change, dP/dt) [1, 4, 6, 19, 22, 23]. It is recommended in a nonpregnant patient that dosage be titrated to a resting HR of < 60 beats per minute [8]. If there is a contraindication for BB, verapamil has theoretical grounds for expecting benefit in MFS, since it has negative inotropic and chronotropic action and produces generalized arterial and arteriolar dilatation, with consequent BP reduction [3, 19]. Finally, BBs have been recommended for MFS also in the Guidelines [24–26].

Antiarhythmic and antifibrillatory effect of BBs may also be useful in MFS [21]. Tachyarrhythmias may result from mitral valve prolapse, which is very common in MFS (60%–80%) [4, 21]. Even in the absence of regurgitant lesions, LV dilation occurs commonly in patients with MFS, and it generates arrhythmias [27]. The incidence of ventricular arrhythmias is significant with a mortality rate from presumed arrhythmogenic death of 4%, which might exceed the rate of aortic rupture as Yetman et al. [27] suggested.

From a pragmatic standpoint, additional benefit may be expected from BBs which act longer (once-a-day formula), because they may be less dangerous if the dose is omitted. Namely, BBs are a typical example for the drugs capable of inducing rebound phenomenon [28]. There is a case report of a patient who got AoD following the BBs dose omission and consequent BBs rebound effect [29]. It is not surprising, because sympathetic surge has been known as the important physiopathologic mechanism for AoD since it augments the pressures in aorta and both the number and the intensity of strokes into the aortic wall [30].

On the other hand, it is unacceptable that MFS patients with increased aortic diameters are given BBs treatment based on one unblinded trial, published over 10 years ago and two retrospective trials in children [31, 32].

However, pending the outcome of randomized controlled trials and based on the limited published data, Williams et al. [3] suggest that BBs should remain the first-line treatment of aortic dilatation in MFS. In a small (17 patients) randomized double-blind trial, the ACE inhibitor perindopril reduced aortic stiffness and even aortic root diameter compared to placebo when given to adult MFS patients in addition to BB treatment for 24 weeks [33].

To our knowledge, there is no more randomized, double-blind clinical trials with other drugs published, but a couple of them (mostly with angiotensin receptor blockers) are ongoing. One interesting trial compares effects of losartan *vs.* nebivolol vs. the association of both on the progression of aortic root dilation in MFS [34]. Losartan antagonizes TGF-β, which has been shown to prevent aortic elastic fibre degeneration. Nebivolol exerts antistiffness effects [34], and aortic stiffness is increased and relates to aortic disease progression in MFS [35].

BBs in uncomplicated arterial hypertension: not the first-line antihypertensives anymore

BBs have been used in arterial hypertension (AHT) for decades, but their role in uncomplicated AHT was challenged for the first time in 1998 [36]. "The time has come to admit that BBs should no longer be considered appropriate for the first-line therapy of uncomplicated AHT" [37]. Compared with other antihypertensives, BBs are less effective for preventing CV events in patients with uncomplicated AHT [38, 39]. Moreover, two recent meta-analyses showed that despite reducing brachial BP, BBs was not effective in reducing CV events when compared with either placebo or other antihypertensive agents [40–42]. BBs increases peripheral vascular resistance, which in turn may increase central aortic pressure and wall stress [3, 22, 43].

BBs lower central aortic BP to a lesser degree even when BP measured by sphygmomanometry is reduced substantially. Given the strong relationship between central aortic BP and target organ damage, the effectiveness of BBs may be overestimated in practice on the basis of conventional BP measurements alone [38]. Despite a "beneficial" effect on the brachial BP, which is surrogate end point, BBs failed to favorably affect the clinical end point, *i.e.*, coronary artery disease and CV mortality and all-cause mortality [36]. The increase in the augmentation index reported after BBs results in increased central systolic BP in hypertensive patients. Thus, BBs could have a deleterious effect on LV-aortic coupling, LV afterload, LV hypertrophy, and, ultimately, the risk of CV events [44]. The fall in pulse

rate is an obvious mechanism for the higher central BP with BB–based therapy noted in the Conduit Artery Function Evaluation (CAFE) study [45].

BBs' side effects are also important, including: precipitation of diabetes mellitus, little effect on regression of LV hypertrophy, likely failure to improve endothelial function, weight gain and decrease in exercise endurance [45, 46]. Thus, National Institute for Clinical Excellence downgraded BBs from the first-line drug choice for uncomplicated AHT. In head-to-head trials, BBs were usually less effective than a comparator drug at reducing major CV events, in particular stroke. Atenolol was the BBs used in most of these studies and, in the absence of substantial data on other agents, it is unclear whether this conclusion applies to all BBs. BBs are not a preferred initial therapy for AHT. However, BBs may be considered in younger people, particularly: those with an intolerance or contraindication to angiotensin converting enzyme (ACE) inhibitors and angiotensin receptor blockers, or women of child-bearing potential, or people with evidence of increased sympathetic drive [47].

On the contrary, European Society of Hypertension and of the European Society of Cardiology recommended: "BBs may still be considered an option for initial and subsequent antihypertensive treatment strategies. …they should not be preferred, however, in hypertensives with multiple metabolic risk factors including the metabolic syndrome…" [48]. Contemporary titles in leading medical journals give us also picture about current status of BBs: "Beta-blockers in hypertension: adding insult to injury" [45]. "Hypertension in the elderly: a compelling contraindication for β-blockers?" [49]. "Beta-blockers in hypertension-the emperor has no clothes" [37].

Importance of central aortic (and carotid) blood pressure

Although the differences between central (aortic and carotid) and peripheral BP have been known for decades, the consequences of decision-making based on peripheral rather than central BP have only recently been recognized [50]. Although brachial measurement may accurately determine diastolic BP, it does not accurately reflect systolic BP. This is mainly attributed to the fact that BP waveform is distorted as it travels outward from the heart due to the presence of wave reflections from the peripheral arteries [51]. Brachial systolic and pulse pressures tend to overestimate central systolic and pulse pressures, especially in younger subjects who have more pronounced amplification, but also in older people, especially with tachycardia, exercise, use of vasoactive agents, or in those with systolic heart failure [52, 53]. The superior prognostic utility of central compared with brachial BP was demonstrated in an unselected geriatric population[54] in patients with coronary artery disease, in patients with end-stage renal failure, etc [52]. Moreover, young African-American men have greater central BP, despite comparable brachial BP compared with young white men [14].

Central (aortic and carotid) pressures are pathophysiologically more relevant than peripheral pressures for the pathogenesis of CV disease. It is aortic systolic BP that the LV encounters during systole (afterload), and the aortic BP during diastole is a determinant of coronary perfusion [14, 51, 52]. More and more clinical studies suggest that central BP may provide additional information regarding CV risk beyond peripheral BP [50–52, 55–58]. Recent findings suggest that the pulsatile component of BP (when represented by central pulse pressures or central pulsatility) is one of the most important factors determining event-free survival, because it accelerates atherosclerosis and leads to plaque rupture in coronary arteries [50]. Recent large-scale trials have shown that central hemodynamics may provide a worthwhile treatment target [52]. ACE inhibitors, angiotensin receptor blocketrs and dihydropyridine calcium blockers diminish central BP [59].

Effects on central pressures may not be evident by pressure measurements in the periphery, because the reflected wave is added to a different part of the central waveform. This may explain why drugs with similar reduction in peripheral pressures have a differential impact on CV outcomes [52]. Important multicenter trials gave rise to the hypothesis that blockers of the renin–angiotensin system, may reduce CV outcomes beyond (peripheral) BP control, perhaps by decreasing also central BP and protecting from subclinical organ damage [52]. Besides, there is compelling evidence regarding the detrimental effect of BBs (mainly atenolol) on central BP [59]. However, the prognostic role of central as opposed to peripheral BP needs to be further confirmed in more large-scale observational and interventional studies [48].

Blood pressure in the aorta (central blood pressure) is important for aortic dissection genesis

The strong argument for the abovementioned statement comes from the fact that AHT is recognized as the most important cause of AoD [24]. Central pulse pressure is a major determinant of ascending aorta dilation in MFS [60]. Over time, and presumably as a consequence of central BP and waves acting on the stiff aortic wall, the aortic diameter enlarges, which increases the risk of AoD [3]. In patients with malignant AHT, reducing BP to normal but not reducing the rate of change in the central arterial pressure with respect to time (dP/dt) did not prevent AoD but apparently increased its risk [21, 61]. The theoretical reason suggested for the beneficial effect of BBs on Marfan aortas was the decrease in the rate of change in central arterial pressure (dP/dt) [3]. Central pulse pressure, which takes into account wave reflections and aortic stiffness, is a better determinant of ascending aorta diameter than brachial pulse pressures in MFS patients, independently of age and body surface area [6].

Vasodilating BBs decrease also central (aortic) blood pressure and they are recommended in arterial hypertension

Atenolol may even increase central BP [59, 62]. Hemodynamic effects of vasodilating BBs clearly differ. Carvedilol and labetalol appear to cause vasodilation through α-1 receptor blockade; nebivolol induces endothelium-dependent vasodilation by stimulating nitric oxide bioactivity. Their favorable hemodynamic profile includes reduction of pulmonary vascular resistance, while maintaining or improving cardiac output, stroke volume, and LV function, whereas nonvasodilating BBs tend to raise pulmonary vascular resis-

tance and reduce cardiac output and LV function. Compared with conventional BBs, vasodilating BBs have beneficial hemodynamic effects including decreased pressure wave reflection from the periphery, leading to decreases in central aortic BP [42, 63–65]. Nebivolol improves endothelial function, leading to a reduction in arterial stiffness, with beneficial hemodynamic effects including reductions in central aortic BP [42, 62, 63]. Central hemodynamic at rest and during exercise were recorded 1 h and 2 h after carvedilol tablet and the results indicated a combined BBs and vasodilating effect [66].

Besides, vasodilating BBs have been preferred in AHT due to metabolic profile [48, 67, 68]. Namely, non-vasodilating BBs have higher potential to elevate blood glucose and cholesterol level. The risk which should not be neglected, as Messerli et al. [69] warn: in uncomplicated AHT, diuretics and BBs should no longer be considered for the first-line treatment. The trade-off of lowering BP at the expense of increasing risk for diabetes mellitus by up to 10% yearly is not acceptable. The risk for diabetes mellitus is greater with atenolol, in the elderly, and in studies in which BBs were less efficacious antihypertensive agents and increased exponentially with longer duration on BBs [67]. BBs have been shown to inhibit pancreatic insulin secretion (*via* β-2 receptors), worsen insulin resistance, cause weight gain, diminish peripheral blood flow, and lead to increased glycogenolysis (by unopposed α-2 action), all of which are implicated in adverse glycemic control. This is not a class effect, and BBs with intrinsic sympathomimetic effects, β-1 selective blockers with β-2 agonist properties, and newer noncardioselective BBs with vasodilating properties (such as carvedilol) have minimal effects on glycemic control [67].

Metabolic studies evaluated the effects of vasodilating BBs, such as dilevalol, carvedilol and celiprolol, on insulin sensitivity and the atherogenic risk factors. None of them decreased insulin sensitivity, as has been described for the BBs with and without β-1 selectivity. This supports the idea that peripheral vascular resistance and peripheral blood flow play a central role in mediating the metabolic side effects of the BBs, as the vasodilating action (either *via* β-2 stimulation or α-1 blockade) seems to more than offset the detrimental effects of the blockade of β (or β-1) receptors [70]. Indeed long-term CV outcome in AHT treated with carvedilol or nebivolol is still not known [65].

Vasodilating BBs decrease also central (aortic) blood pressure: should not they also be considered in Marfan syndrome to prevent aortic aneurysm and aortic dissection?

Our idea is: if BBs with better impact upon central BP are preferred in AHT, why should not they also be preferred in MFS to prevent aortic aneurysm and AoD? Namely, central BP means BP in the aorta, where also the prevention target in MFS is. If vasodilating BBs have the advantage in AHT due (among others) to better performances at this particular site (aorta), they may be also better suited for MFS as well. Metabolic profile of vasodilating BBs may be additional argument to consider them as the first line choice for patients with MFS, because prevention is expected to be prolonged, usually life-long.

A word of caution is needed, because neither we have a definite proof from large trials that central BP is clearly superior prognosticator, nor that vasodilating BBs improve outcomes in terms of survival and freedom from myocardial infarctions and strokes better than classical BBs.

Finally, there are two premises, i.e. sentences from the literature. The first is: central pulse pressure is a major determinant of ascending aorta dilation in MFS [6]. The second follows: compared with conventional BBs, vasodilating BBs have beneficial hemodynamic effects including decreased pressure wave reflection from the periphery, leading to decreases in central aortic BP [63]. The conclusion is obvious from the premises: vasodilating BBs may have the advantage in preventing aortic complications in MFS. Indeed, to obtain a valid conclusion, promises should be checked.

A PubMed search for terms: "central blood pressure Marfan" retrieved 11 papers, and for "central blood pressure Marfan beta blocker" only two (30st March. 2010). None of them had evaluation of the potential role of vasodilatatory BBs in the prevention of aortic aneurysm and AoD in MFS.

Conclusion

In the recent guidelines for arterial hypertension BBs (in the absence of compelling indications) have been removed from the first-line antihypertensive therapy – in part due to insufficient efficacy in decreasing central BP. It is probable that central (aortic) BP reduction is central for the prevention of aortic dilatation, AoD and rupture in MFS. Thus, the same inefficacy of classic BBs to decrease central BP may be the reason to consider them less effective in the prevention of aortic aneurysm and AoD in MFS. On the other hand, vasodilating BBs might have larger efficacy in decreasing central BP and thus in delaying aortic complications in MFS. Metabolic profile of vasodilating BBs should be another argument to use them in MFS, because a prolonged application is expected. This issue deserves more attention, in order to better prevent catastrophic diseases (aortic aneurysm and AoD) in MFS. Indeed, randomized controlled trials as well as large registries' data are needed to obtain a more precise answer.

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C A S E R E P O R T S

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Depressive symptoms as a side effect of the sustained release form of methylphenidate in a 7-year-old boy with attention-deficit hyperactivity disorder

Depresivni simptomi kao neželjeni efekat dejstva sporooslobađajuće forme metilfenidata kod 7-godišnjeg dečaka sa hiperkinetičkim poremećajem i poremećajem pažnje

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Abstract

Introduction. Hyperkinetic disorder or attention-deficit hyperactivity disorder (ADHD) is a clinical entity consisting of a cluster of symptoms including hyperactivity, attention disorder and impulse control disorder group. In the context of ADHD etiology we may say that genetic, clinical and imaging studies point out a disruption of the brain dopamine system, which is corroborated by the clinical effectiveness of stimulant drugs, which increase extracellular dopamine in the brain. Basically, it is a biological and not psychological disorder, which is important both for the comprehension and therapeutical approach to this problem. Today, the best recommended approach regarding children with ADHD is a combination of two therapeutic modalities: pharmacotherapy and behavioral treatment. The first-choice drugs for this disorder belong to the group of sympathomimetics – psychostimulants and atomoxetine (more recently). As the first-choice therapy, methylphenydate in sustained release form has numerous advantages. Like all drugs, methylphenidate has its unwanted side effects. Most common are: loss of appetite, weight loss, sleeping disorders, irritability, headache. These side effects are well-known and documented in the literature. By analysing the available literature we have found cases of psychiatric side effects such as: psychosis, mania, visual hallucinations, agitation, suicidal ideas. We have not found examples of ADHD in children who use increased dosage of sustained release of methylphenidate leading to depressive symptomatology. On the other side, methylphenidate may be prescribed for off-label use in treatment-resistant cases of depression. **Case report.** The case of a 7-year-old boy diagnosed with ADHD was on a minimal dose of sustained release form of methylphenidate. After initial titration of the drug, i.e. after raising the dose to the next level the boy developed clinical signs of depression. The treatment was ceased and depressive symptoms were withdrawed. **Conclusion.** Manifestation of depressive symptomatology after dose increasement of sustained release form of methylphenidate in a 7-year-old boy with ADHD represents an uncommon side effect. Precise drug activity mechanisms responsible for the appearance of these symptoms remains to be explained.

Key words:
metylphenidate; depression; attention deficit disorder with hyperactivity; treatment outcome.

Apstrakt

Uvod/Cilj. Hiperkinetički poremećaj ili poremećaj pažnje sa hiperaktivnošću (ADHD) je klinički entitet čiju suštinu čini grupa simptoma koji uključuju hiperaktivnost, poremećaj pažnje i impulsivnost. U kontekstu etiologije ADHD može se reći da genetske, kliničke i radiološke studije ukazuju na poremećaj u dopaminskom sistemu mozga, što potvrđuje efikasnost psihostimulanasa koji dovode do povišenja ekstraćelijskog dopamina u mozgu. Bazično, ovo je biološki, a ne psihološki poremećaj, što je važno i za razumevanje, ali i za terapijski pristup problemu. Danas, najbolji preporučeni pristup deci sa ADHD je kombinacija dva terapijska modaliteta: farmakoterapijskog (psihofarmaci) i bihevioralnog tretmana. Psihofarmaci prvog izbora za ovaj poremećaj su simpatikomimetici i to: psihostimulansi i atomoksetin (u poslednje vreme). Terapija prvog izbora je primena metilfenidata u formi sa produženim oslobađanjem i ona ima mnogobrojne prednosti. Slično drugim lekovima i metilfenidat ima neželjena dejstva. Najčešća su: gubitak apetita, gubitak telesne mase, poremećaj spavanja, iritabilnost, glavobolja. Ova neželjena dejstva su dobro dokumentovana u literaturi. Analizom dostupne literature pronašli smo i psihijatrijska neželjena dejstva kao što su: psihoza, manija, vi-

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zuelne halucinacije, agitacija, suicidne ideje. Nismo naišli na prikaze dece sa ADHD kod koje se, pri povećanju doze metilfenidata u sporoslobađajućoj formi, razvila depresivna simptomatologija. S druge strane, metilfenidat se propisuje nestandardno za lečenje rezistentnih slučajeva depresije. **Prikaz bolesnika.** U radu je prikazan 7-godišnji dečak sa ustanovljenim ADHD. Dečak je inicijalno primao najnižu dozu sporooslobađajuće forme metilfenidata. Nakon podizanja doze leka na sledeći nivo, kod prikazanog dečaka ispoljili su se klinički znaci depresije. Posle obustave tretmana, došlo je do povlačenja simptoma depresije. **Zaključak.** Pojava depresivne simptomatologije kod 7-godišnjeg dečaka sa ADHD nakon povišenja doze sporooslobađajuće forme metilfenidata predstavlja nepoznat neželjeni efekat ovog leka. Precizan mehanizam dejstva leka koji je odgovoran za pojavu ovih simptoma ostaje nerazjašnjeno.

Ključne reči:
metilfenidat; depresija; hiperkinetički sindrom; lečenje ishod.

Introduction

Hyperkinetic disorder [1] or attention-deficit hyperactivity disorder (ADHD) [2] is a clinical entity consisting of a cluster of symptoms including hyperactivity, attention disorder and impulse control disorder group [3–6]. Actually, it is a syndrome of attention disorder, hyperactivity and other deficits of executive functions. It includes a damaged capability of planning "one`s tasks and executing them" [7].

These symptoms must be expressed in that particular degree and range (be general), so as to provoke important difficulties in functionning in different areas of everyday life (home, school, work, social relations, etc.).

The disorder appears at around two-three years of age but is not recognized in most cases before starting to attend school, where a high degree of self-control is requested concerning behavior, attention and perseverance in activities which demand longer cognitive engagement. The leading place in the origin of this disorder (etyology) have biological mechanisms of neurotransmission regulation in the central nervous system (CNS) [8, 9]. In the context of ADHD etiology we may say that genetic, clinical and imaging studies point out a disruption of the brain dopamine system, which is corroborated by the clinical effectiveness of stimulant drugs, which increase extracellular dopamine in the brain. Basically, it is a biological and not psychological disorder, which is important both for the comprehension and therapeutical approach to this problem.

Today, the best recommended approach regarding children with ADHD is a combination of two therapeutic modalities: pharmacotherapy (sympathomimetics – psychostimulants and atomoxetine) and behavioral treatment [10–15]. The first choice drugs for this disorder belong to the group of sympathomimetics as follows: psychostimulants and atomoxetine (more recently) [12–15]. As the first choice therapy, methylphenydate in a sustained release form has numerous advantages [17]. It helps to preserve one's privacy and avoid stigmatization at school, which considerably improves the compliance.

Case report

We presented a 7-year-old boy referred to the Clinic when he was 6. The boy was not able to sit still (in continuous movement), stay at one place for a longer period of time. The speech-language therapists could not work with him, his mother "did not know what to do" and "was constantly running after him".

The boy was born as a second child, his parents were young and healthy. The mother had a regular pregnancy, delivery was "somehow difficult". At birth, the boy did not breathe for a couple of minutes (APGAR score 7) and spent 10 days in the incubator. Weight at birth was 4 kg. Cranial ultrasonography presented no abnormalities. At the age of one and half a month the boy was diagnosed with hypotonia and torticolis by the pediatric neurologist. The repeated cranial ultrasonography test was normal. Electroencephalogram (EEG) showed the presence of epileptic paroxysmally dysrythmic activity. The boy had no seizures. Antiepileptic treatment "for preventive reasons" (valproic acid) started from the 2nd year of age.

Motoric development milestones were at the limits for the boy's age, but there was slowness in speech and cognitive development, so the boy was submitted to continuous development stimulation and speech-language treatment. Valproic acid was withrowed after a 3.5-year period.

Family history showed no important signs of inheritance-relevant health problems. At admission the boy was well developed for his age and well-brought-up. The boy was in constant movement, used to open closet doors and drawers, run through the office, it was not possible to keep him at the same place for a longer period of time. The boy was motorically agile, smiling, in a good mood, and emotionally warm. His non-verbal communication was short, and verbal communication difficult. Speech was dysphasic (dysphasia expressiva) with frequent repeating of words, from time to time speaking in the third person.

Fascinated by the strip, the boy was constantly rolling it around his hand. Neurological finding was regular.

The finding of neurological examination was regular; psychological assessment showed that intellectually the boy was at the level of mild mental retardation; laboratory analyses of blood and urine showed results in the referential limits; screening for ADHD-IOWA Conner's scale was 29; electroencephalogram background activity was regular; magnetic resonance imaging (MRI) showed no abnormalities; no drug treatment, while reeducation and stimulation of development such as speech language treatment in continuity were recommended.

As a result of analyses and differential-diagnostic considerations of DSM/IV&MKB/10 criteria the patient was diagnosed with: ADHD, mild mental retardation, and dysphasia evolutionis expressiva.

The diagnosis of ADHD was confirmed. The benefits and risks of the proper use of methylphenidate (sustained release form) as well as alternative treatments were discussed with the mother before prescribing stimulants. We started with a low dose. Therapeutic response was positive. The mother said that: "Now the boy can sit still even for half an hour, his words fund has increased, he rediscovered his toys from the past, wants to draw and write, is more reasonable, with less compulsive repeating of what has nothing to do with the environment". The mother was satisfied, looked at him for the first two days with belief. The doctor and speech-language therapist were very satisfied with him. After starting the therapy, the boy had (during 7 days) transitory sleep disorders (woke up early) and eating disorder (loss of appetite). Laboratory analyses of blood and urine, body weight and hight and screening for ADHD-IOWA Conner's scale were monitored monthly: low and transient loss of appetite and loss of weight (3 kg for 5 months) growing up 2 cm and ADHD-IOWA Conner's scale index 18 were notified.

For five months the boy was at the same lowest dose. The mother reported a certain "activity progression, the boy was getting better, but is constantly moving". With mother's agreement, the dosage was increased (the next dose entity).

Three days after the increased therapy the mother phoned disturbed saying that: "The boy is sad, but not calm, cries a lot, eats less, is more anxious, wakes up at night, never has been like this, she cannot recognize him." At the next control, 4 days after introducing this treatment: "He looks sad, speaks with a low wailing voice; continually repeats: "I cried, I cried, I am sad", being in continuous movement, with something in his hand, irritable. Mother said: "When he is not sad, he is angry". The treatment of a sustained release form of methylphenidate was interrupted. At the control two days later, the mother said "He is as before, unrestrained, nothing can be done with him, but he is in a good mood again, he laughs". The patient remained further at the lowest dose of the drug which he still takes, attending a pre-school institution.

Discussion

Methylphenidate is a psychostimulant drug approved for treatment of ADHD, postural orthostatic tachicardia syndrome and narcolepsy. It may also be prescribed for off-label use in treatment-resistant cases of lethargy, depression, obesity. The accepted model of dopamine deficits in brain is the most probable cause of ADHD dictate therapeutical approach.

Methylphenidate increases levels of dopamine and norepinephrine in the brain through reuptake inhibition of the monoamine transporters [8, 9].

Like all drugs, methylphenidate has its unwanted side effects. Most common are: loss of appetite, weight loss, sleeping disorders, irritability, headache, stomach ache, skin rash, development or worsening of tics, slow growing [17, 18]. These side effects are well-known and documented in the literature. Most side effects are minor and disappear over time or the dosage level is lowered. By analysing the available literature we have found cases of psychiatric side effects such as: psychosis, mania, visual hallucinations, agitation, suicidal ideas. We have not found cases of ADHD in children with an increased dosage of sustained release of methylphenidate leading to depressive symptomatology. On the other side, methylphenidate may be prescribed for off-label use in treatment-resistant cases of depression.

A dramatic switch in behaviour in our patient due to medication (dose increase and drug interruption) gave a clear confirmation of the biologic basis of the disorder. At the same time, it opened a question: How can (dose-dependent) methylphenidate lead to the appearance of depressive symptoms when its basic activity is absolutely opposed?

Is this maybe a paradoxical effect which has not been recognized till now and which represents the expression of individual hypersensitivity? Or is it a heterogeneous group of disorders where the same symptoms were acquired by other biological means? The conceptualisation of ADHD over time (postencephalitic parkinsonism, minimal cerebral dysfunction…) sustains this possibility [5].

Differential-diagnostic considerations include the possibility of comorbidity in affective disorder which, although rarely, appears in children [3–5, 19]. It is known that stimulant drugs may exacerbate symptoms and reveal them for the first time in children with previously unrecognozed psychiatric illnessess. The presented patient had neither signs nor anamnestic data regarding possible elements of affective disorder. No cases of affective disorder in family history had been found, as well.

Conclusion

Manifestation of depressive symptomatology after dose increasement of the sustained release form of methylphenidate in a 7-year-old boy with ADHD represents an uncommon adverse effect of the drugs. Precise mechanisms responsible for the appearance of these adverse effects cannot be explained, at the moment.

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CASE REPORT

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Reversal deterioration of renal function accompanied with primary hypothyrodism

Reverzibilno akutno smanjenje bubrežne funkcije udruženo sa primarnom hipotireozom

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Abstract

Introduction. Hypothyroidism is often accompanied with decline of kidney function, or inability to maintain electrolyte balance. These changes are usually overlooked in everyday practice. Early recognition of this association eliminates unnecessary diagnostic procedures that postpone the adequate treatment. **Case report.** Two patients with elevated serum creatinine levels due to primary autoimmune hypothyroidism, with complete recovery of creatinine clearance after thyroid hormone substitution therapy are presented. The first patient was a young male whose laboratory tests suggested acute renal failure, and the delicate clinical presentation of reduced thyroid function. The second patient was an elderly woman with a history of a long-term signs and symptoms attributed to ageing, including the deterioration of renal function, with consequently delayed diagnosis of hypothyroidism. **Conclusion.** Serum thyrotropin and thyroxin levels measurement should be done in all cases of renal failure with undefined renal desease, even if the typical clinical presentation of hypothyroidism is absent. Thyroid hormone assays sholud also be performed in all patients with chronic kidney disease whose kidney function is rapidly worsening.

Key words:
hypothyroidism; renal insufficiency; diagnosis; thyrotropin; thyroxine; treatment outcome.

Apstrakt

Uvod. Hipotireoza je često udružena sa smanjenjem bubrežne funkcije ili poremećajem u održavanju elektrolitnog balansa. U svakodnevnoj kliničkoj praksi, ove promene najčešće ostaju neprimećene. Ranim prepoznavanjem ove udruženosti smanjuje se broj nepotrebnih dijagnostičkih procedura koje odlažu blagovremeno lečenje bolesnika sa hipotireozom. **Prikaz bolesnika.** Prikazana su dva bolesnika kod kojih je novootkrivena autoimunska hipotireoza bila praćena biohumoralnim pokazateljima akutnog smanjenja bubrežne funkcije, uz kompletno obnavljanje klirensa kreatinina do fizioloških nivoa posle adekvatne supstitucijske terapije. Prvi bolesnik bila je mlađa osoba sa netipičnom kliničkom manifestacijom hipotireoze. Drugi bolesnik bio je osoba starijeg životnog doba sa višemesečnim simptomima i znacima hipotireoze koji su se pripisivali starenju, uključujući i slabljenje bubrežne funkcije, što je sprečilo blagovremenu dijagnostiku i lečenje. **Zaključak.** Preporučujemo proveru vrednosti tireotropnog hormona i tiroksina kod svih bolesnika sa akutnom bubrežnom insuficijencijom nejasne etiologije, ili kod naglog pogoršanja bubrežne funkcije kod osoba sa hroničnom bubrežnom bolesti, čak i bez tipične kliničke manifestacije hipotireoze.

Ključne reči:
hipotireoidizam; bubreg, insuficijencija; dijagnoza; tireotropin; tiroksin; lečenje, ishod.

Introduction

Thyroid hormones are nesessary for growth and developement of the kidney. They are also involved in maintenance of water and electrolyte homeostasis in different organs and tissue departments. Hypothyroidism is often accompanied with decline of kidney function, or failure of electrolyte balance [1]. These changes are very subtle and usually overlooked in everyday clinical practice. We described two patients with impaired renal function due to primary hypothyroidism, and complete recovery of creatinine clearance after thyroid hormone replacement therapy.

Case reports

Case 1

A 23-year-old male was referred to the outpatient department for elevated serum creatinine levels with the history of the mild muscle weakness and tiredness over the few months. Physical examination revealed normal anthropomet-

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ric parameters, body mass index (BMI) of 24 kg/m^2, body temperature of 36.5C°, dry skin with no edema. He was normotensive with pulse rate of 64 beats per minute, and showed no hepatomegaly.

Laboratory blood tests revealed an elevated serum creatinine level of 168 mmol/L (normal range: 50–75 mmol/L), creatine kinase (CK) level of 430 U/L (normal range 21–294 U/L) and serum total cholesterol level: 8.4 mmol/L (normal range < 5.2 mmol/L), while serum liver enzymes levels were slightly elevated: aspartate aminotransferase (AST) 44 U/L (normal range 0–34 U/L), alanine aminotransferase (ALT) 63 U/L (normal range 7–49 U/L) and lactate dehydrogenase (LDH) 420 U/L (normal range 208–378 U/L). Biochemistry data included normal concentration of serum urea, triglyceride, bilirubine, albumin, potassium, sodium, glucose, hemoglobin, platelet and white blood count. Urine tests showed no proteinuria or hematuria. There were no casts in the urine sediment. Serum and urine myoglobin levels were not determinated. Creatinine clearance estimated using the Cockroft-Gault formula was reduced to the value of 68 mL/min (normal range for males 97–137 mL/min), suggesting renal failure. An abdominal ultrasonography showed normal morphology and volumen of both kidneys. Due to the constant presence of muscle weakness, the thyroid function test were performed. Thyroid stimulating hormone or thyrotropin (TSH) level was elevated: 56 mU/L (normal range 0.35–5.5 mU/L), serum free thyroxine (FT4) level was lower: 9.5 pmol/L (normal range 11.5–22.7 pmol/L) and free triiodothyronine (FT3) level was at the lower limit of the normal range: 3.6 pmol/L (normal range 3.5–6.5 pmol/L). The thyroperoxidase antibodies titer were elevated, 1: > 1 300 (normal range 0–60.0), while antithyroglobulin antibodies titer were not determined. The ultrasonography of the thyroid showed a slight enlargement of the gland with the heterogenous texture suggesting autoimmune thyroiditis.

After the primary hypothyroidism was detected, the patient was recommended to take levothyroxine replacement therapy at 100 mcg daily dose (1.6 mcg/kg). One week after starting the levothyroxine therapy, the patient felt better. After two months of hormone substitution, all biochemical parameters returned to the normal levels: TSH level was 3.5 mU/L, serum creatinine level was 94 mmol/L and the creatinine clearance was 117 mL/min (Table 1). The patients went back to his everyday activities.

Case 2

An 81-year-old female patient was referred to our department for the presence of clinical and humoral parameters suggesting primary hypothyroidism. The patient complained about tiredness, muscle pain, dry skin and the presence of elastic edema of the lower limbs and ankcles, not responsive to furosemid therapy over the last eight months. The patient also had the history of arterial hypertension and ischemic heart disease, ordinarily treated with adequate therapy recomended by the cardiologist. Physical examination revealed bradycardia (heart rate of 55 beats per minute), increased blood preassure of 160/100 mmHg, dry skin, palor, elastic edema of the ankles, with no hepatomegaly or other signs of cardiac failure. The patient had slightly enlarged palpable thyroid with hard consistency. Laboratory data noted mild normochromic and normocytic anemia with hemoglobin levels of 105 g/L (normal range 130–180 g/L); serum glycemia was 7 mmol/L (normal range 4.1–5.9 mmol/L) with elevated level of total cholesterol 9.6 mmol/L (normal range < 5.2 mmol/L) and CK 480 U/L (normal range 21–294 U/L). Serum biochemistry included high levels of serum creatinine of 180 mmol/L (normal range 80–124 mmol/L) and slightly elevated serum urea levels of 11.6 mmol/L (normal range 3.2–8.2 mmol/L). Serum levels of other liver enzymes, triglyceride, albumin and bilirubin were normal. Creatinine clearence, assesed using the Cockroft-Gault formula, was reduced to the value of 35 mL/min (normal values for female 88–128 mL/min) suggesting renal failure. Ultrasonography of the kidneys or other abdominal organs were not performed.

Diagnosis of primary hypothyroidism was based on the elevated TSH levels: 65.6 mU/L (normal range 0.35–5.5 mU/L), decreased FT4: 10.4 pmol/L (normal range 11.5–22.7 pmol/L) and FT3 levels: 0.9 pmol/L (normal range 3.5–6.5 pmol/L). The thyroperoxidase antibodies titer were elevated (1 : 600). The ultrasonography of the thyroid gland showed that its volume was at the lower limit of normal values with heterogenous structure suggesting autoimmune thyroiditis. Levothyroxine replacement therapy started with the dose of 100 mcg daily (gradually increasing dose by 25 mcg weekly). Two months after starting the substitution therapy, the patient's edema retreated following the decrease of plasma TSH levels. After four months of the therapy, thy-

Table 1

Comparation of laboratory data before and after the L-thyroxine replacement therapy in case 1

Biochemical parameters	At diagnosis	Two weeks later	Two months later
TSH (mU/L)	56	41	3.5
Free T4 (pmol/L)	9.5	12	17.5
Free T3 (pmol/L)	3.6	4.1	5.4
Creatinine (mmol/L)	168	130	94
Total cholesterol (mmol/L)	8.4	6.1	5.3
LDL cholesterol (mmol/L)	4.9	3.5	2.95
CK (U/L)	430	300	115
LDH (U/L)	420	305	277
AST (U/L)	44	/	20
ALT (U/L)	63	/	34
Creatinine clearance (mL/min)	68	/	117

TSH – thyrotropin; T4 – thyroxine; T3 – triiodothyronine; LDL – low density lipoprotein; CK – creatine kinase; LDH – lactate dehydrogenase; AST – aspartate aminotransferase; ALT – alanine aminotransferase

roid hormon levels returned to the normal level. At the same period, plasma creatinine values decreased to 81 mmol/L while creatinine clearance increased up to 72 mL/min (Table 2). The patient continued to feel well.

Table 2

Comparation of laboratory data before and after the L-thyroxine replacement therapy in case 2

Biochemical parameters	At diagnosis	2 months later	4 months later
TSH (mU/L)	65.6	32.8	3.8
Free T4 (pmol/L)	10.4	12.2	19.3
Free T3 (pmol/L)	0.9	2.1	4.5
Hemoglobin (g/L)	105	108	119
Total cholesterol (mmol/L)	9.6	7.2	6.1
CK (U/L)	480	290	180
Creatinine (mmol/L)	180	115	81
BUN (mmol/L)	11.6	9.0	7.9
Creatinine clearance (mL/min)	35	63	72

TSH – thyrotropin; T4 – thyroxine; T3 – triiodothyronine; CK – creatine kinase; BUN – blood urea nitrogen

Discussion

The functional association between hypothyroidism and kidney failure has been described many times in the literature [2–5], and it seems to be reversibile after hormone substitution therapy. Montenegro et al. [6] showed a decrease in glomerular filtration rate (GFR) in all of their hypothyroid patients, whereas only 55% had an increase in serum creatinine levels. A few years later, Villabona et al. [7] described the decrease in effective renal blood flow and GFR in hypothyroid patients with chronic renal disease. Karanikas et al. [8] performed isotopic renal function studies in thyroidectomized patients showing that the hemodynamic changes in severe hypothyroidism mainly affect the glomerular function.

The most common kidney derangements associated with hypothyroidism are an increase in serum creatinine levels, reduction in GFR and renal plasma flow, decreased capacity of free water excretion and hyponatremia [1]. About one half of patients with autoimmune thyroid disease have positive circulating immunocomplexes that are in correlation with the presence of thyroid peroxidase antibodies, but not with their titer [9]. Immunocomplexes deposits in the basement membrane of the glomeruli have been also reported in patients with Hashimotho thyroiditis; still, no casual relationship between the presence of immunocomplexes and antibodies has been proved so far [1].

Although the exact mechanism of these changes has not been defined yet, it seems that kidney failure secondary to hypothyroidism involves heterogenous processes based on the direct or indirect effects of thyroid hormones on renal hemodynamics [1, 10, 11]. Thyroid hormone deficiency decreases myocardial contractility and cardiac output. On the other hand, an impaired endothelial-mediated vasodilatation in hypothyroidism increases peripheral and renal vascular resistance [11]. These effects reduce renal plasma flow and GFR, resulting in free water overload and decrease in creatinine clearance. Consequently, elevation of plasma creatinine levels might happen. Decrease in GFR produces a diminished water delivery to distal tubular segments that is partly responsible for the hyponatremia. Hyponatremia appears in 45% of hypothyroid patients who have elevated serum creatinine levels and in about 21% of those with normal creatinine levels [1]. Thyroid hormones also have a hold upon tubular transport of sodium *via* their actions on the sodium-potassium adenosine triphosphate pump (Na/K ATP-ase) and on the potassium permeability in the membrane of the proximal tubules.

Levothyroxine is a synthetic product identical to natural thyroxine, produced by the thyroid gland. After the normalisation of serum thyroxine levels, cardiac output and myocardial contractility recover, leading to the increase in renal plasma flow and creatinine clearance.

Systemic manifestations of hypothyroidism vary considerably, depending on the duration and severity of the hypothyroid state. Gradual and imperceptible onset somethimes account for the inconclusive clinical diagnosis of hypothyroiditis. We described two patients with autoimmune hypothyroidism presented with elevated serum creatinine levels and reverse deterioration of renal function.

The first patient was a young male with a delicate clinical presentation of reduced thyroid function. Due to nonspecific symptoms and signs associated with laboratory parameters suggesting acute renal failure, clinical findings were not easy to intreprete. Our examination results could not reveal any kidney disease.

Hypothyroidism is known to be associated with elevated serum CK levels along with other muscle enzymes (LDH). Due to the myopathy, hypercholesterolemia and elevated levels of CK in this young patient, we performed thyroid hormone testing. High serum thyrotropin and decreased serum free thyrotropin levels, accompained with elevated titer of antyperoxidase antibodies were adequate to define primary autoimmune hypothyrodism. After the beginning of thyroid replacement therapy, thyroid status improved and kidney function progressively recovered. This observation is consistent with the previously published data [2–5].

In elderly patients with various illnesses, symptoms and signs of hypothyrodism could be easily confused with the usuall signs or effects of aging such as cold intolerance, dry, pale, thick and rough skin, intestinal constipation, non-depressive edema, mental slowness or increased body weight [12]. In the

second case, we described the elderly patient with the history of long-term edema and simptoms similar to aging. Deterioration of renal function, followed with moderate renal atrophy and elevated serum creatinine levels are not unusual in an 81-year-old patient with the hystory of arterial hypertension or ischemic heart disease. Creatinine clearance appears to decrease with age (each decade corresponds to a decrease of about 6.5 mL/min/1.73 m^2). After establishing the diagnosis of primary hypothyrodism, hormonal treatment with levothyroxin was started. After four months of the treatment, adequate control of hypothyroidism was seen with progressive recovery of kidney function and restore of serum creatinine levels, that was somewhat unexpected. Still, similar findings has been observed by other autors: in older patients with various illnesses, even with the moderate renal atrophy on ultrasound images, thyroid replacement therapy recovered renal creatinine clearance to physiological values [4, 5, 12].

Hyponatremia, as the commonest electrolyte derangement in hipothyrodism, did not appear in any of our patients. Because of the mild elevation of serum CK levels and the mild form of myalgia, we did not find it necessary to perform measurement of serum and urinary myoglobine levels. Both of our patients were presented with elevated CK and cholesterol levels. A deficit in the expression of the hepatic low density lipoprotein (LDL) receptor gene in hypothyroidism diminishes LDL cholesterol clearance which results in hypercholesterolemia. A degree of metabolic dysfunction in skeletal muscle was seen even in subclinical hypothyrodism [13]. Similary to kidney disfunction, this could be reversed with thyroid hormone treatment. In the presented cases, a few months after the introduction of thyroxine replacement therapy and normalisation of serum TSH levels, creatinine clearance, serum cholesterol and serum CK levels recovered to the normal vaelues, no matter of the clinicial presentation of hypothyroid state, or the age of the patients.

There are some published clinical case reports confirming stabilisation of kidney function in patients with chronic kidney disease after correction of thyroid function [1, 5, 14]. There are also described cases of acute deterioration of kidney function in patients with chronic kidney disease and unrecognised hypothyroidism [15]. We therefore recommends measurement of serum thyrotropin and thyroxin levels in all cases of renal failure with undefined renal desease, even if the typical clinical presentation of hypothyroidism is absent. We also recommend that thyroid hormone assays should be performed in all patients with chronic kidney disease whose kidney function is rapidly worsening.

Conclusion

The presence of reversal renal failure as the consequence of hypothyrodisms is usually subtle and frequently overlooked. Knowledge of the association between hypothyrodism and deterioration of renal function is very important in clinical practice.

This association must be recognized in time, avoiding the unnecessary diagnostic procedures that postpone adequate treatment.

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CASE REPORT

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Multiple myeloma invasion of the central nervous system

Zahvatanje centralnog nervnog sistema multiplim mijelomom

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Abstract

Introduction. Multiple myeloma (MM) is characterized by the presence of neoplastic proliferating plasma cells. The tumor is generally restricted to the bone marrow. The most common complications include renal insufficiency, hypercalcemia, anemia and reccurent infections. The spectrum of MM neurological complications is diverse, however, involvement of MM in the cerebrospinal fluid (CSF) and leptomeningeal infiltration are rare considered. In about 1% of the cases, the disease affects the central nervous system (CNS) and presents itself in the form of localized intraparenchymal lesions, solitary cerebral plasmocytoma or CNS myelomatosis (LMM). **Case report**. We presented the clinical course of a 55-year-old man with MM and LMM proven by malignant plasma cells in the CSF, hospitalized with the pain in the thoracic spine. His medical history was uneventful. There had been no evidence of mental or neurological impairment prior to the seizures. Physical examination showed no abnormalities. After a complete staging, the diagnosis of MM type biclonal gammopathia IgG lambda and free lambda light chains in the stage III was confirmed. The treatment started with systemic chemotherapy (with vincristine, doxorubicin plus high-dose dexamethasone – VAD protocol), radiotherapy and bisphosphonate. The patient developed weakness, nausea, febrility, dispnea, bilateral bronchopneumonia, acute renal insufficiency, confusions, headaches and soon thereafter sensomotor aphasias and right hemiparesis. The patient was treated with the adequate therapy including one hemodyalisis. His neurological status was deteriorated, so Multislice Computed Tomography (MSCT) of the head was performed and the findings were normal. Analysis of CSF showed pleocytosis, 26 elements/mL and increased concentrations of proteins. Cytological analysis revealed an increased number of plasma cells (29%). Electrophoretic analysis of proteins disclosed the existance of monoclonal components in the serum, urine and CSF. Immunofixation electrophoretic and quantitative nephelometric tests confirmed Biclonal multiple myeloma of IgG lambda and light chain lambda isotypes. Analysis of neurothropic viruses with ELISA methods was negative. Once the presence of LMM was confirmed, the patient received intrathecal chemotherapy with methotrexate, cytosine arabinoside, dexamethasone three times a week, and systemic high doses of dexamethasone *iv* like a single agent without craniospinale irradiations. Despite the treatment, the patient died one month after the diagnosis. Autopsy was not performed. **Conclusion.** Presented patient, as well as most other patients with MM progressing to CNS infiltration was in the stage III. In addition to the detailed clinical examination, and all investigations required for MM diagnosis and staging of the disease, we introduced the additional CSF examination and calculation of kappa lambda ratio, that helped us make an early diagnosis and prognosis of MM with LMM. Although LMM had a low prevalence, it could be more frequent than expected especially in patients with high risk. CSF examination with positive plasma cells and abnormal morphology remains the hallmark for diagnosing CNS infiltration.

Key words:
multiple myeloma; neoplasm metastasis; brain; diagnosis, differential; immunoglobulins.

Apstrakt

Uvod. Multipli mijelom (MM) karakteriše prisustvo neoplastičnih proliferišućih plazma ćelija, koje se najvećim delom nalaze u kostnoj srži. Najčešća komplikacija oboljenja je pojava renalne insuficijencije, hiperkalcemije, anemije i rekurentnih infekcija. Postoje različite neurološke komplikacije kod bolesnika sa MM, a zahvatanje cerebrospinalnog likvora (CSF) i leptomeninga je retko. Kod oko 1% slučajeva bolest zahvata centralni nervni sistem (CNS) u vidu pojave lokalizovanih intraparenhimskih lezija, solitarnog cerebralnog plazmocitoma ili leptomeningealne mijelomatoze (LMM). **Prikaz bolesnika.** U ovom radu prikazali smo bolesnika, starog 55 godina, sa dokazanim MM i LMM koji je primljen u Kliniku

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za hematologiju VMA zbog bolova u torakalnom delu kičme, bez ranijih značajnijih oboljenja i prethodne neurološke simptomatologije. Nakon učinjenog ispitivanja dokazan je MM tipa biklonalne gamapatije (IgG tipa lambda i slobodnih lakih lanaca tipa lambda) u III kliničkom stadijumu. Nakon primene hemioterapije prema protokolu VAD (vinkristin, doksorubicin plus visoke doze deksametazona), uz radioterapiju i bisfosfonate dolazi do razvoja slabosti, muke, povišene telesne temperature, dispneje, obostrane bronhopneumonije, akutne bubrežne insuficijencije, konfuzije i glavobolje, a brzo posle toga i do senzomotorne afazije i desnostrane hemipareze. Primenjena je adekvatna terapija i jedna hemodijaliza, ali je zbog daljeg pogoršanja neurološkog statusa učinjena multislajsna kompjuterska tomografija (MSCT) glave, čiji je nalaz bio uredan. U daljem toku, zbog sumnje u zahvaćenost CNS osnovnim oboljenjem učinjena je lumbalna punkcija. Analizom likvora viđen je povećan broj ćelijskih elemenata, povećana koncentracija proteina i oko 29% patoloških plazma ćelija. Elektroforezom proteina seruma, urina i likvora potvrđena je monoklonska komponenta, a imunofiksacijom dokazano da se radi o biklonalnoj gamapatiji (IgG tipa lambda i slobodni laki lanaci tipa lambda), uz negativan nalaz neurotropnih virusa, čime je potvrđeno prisustvo LMM. Dalje lečenje sprovođeno je trojnom intratekalnom terapijom uz visoke doze deksametazona, bez primene kraniospinalne iradijacije. Uprkos primenjenom lečenju bolesnik je umro mesec dana nakon dijagnoze MM, a obdukcija nije urađena. **Zaključak.** Većina bolesnika sa MM, kao i prikazani bolesnik, kod kojih je dokazana LMM, nalaze se u III kliničkom stadijumu. Pored detaljne kliničke obrade potrebne za dijagnozu i stepenovanje MM učinili smo ispitivanje likvora i odredili kapa/lambda odnos, što je značajno pomoglo u ranoj dijagnozi LMM. Iako je prevalencija LMM niska, može se češće dokazati kod bolesnika sa MM koji imaju faktore visokog rizika. Ispitivanje likvora sa dokazanim plazma ćelijama koje imaju abnormalnu morfologiju ostaje najznačajniji dijagnostički test za dijagnozu LMM.

Ključne reči:
multipli mijelom; neoplazme, metastaze; mozak; dijagnoza, diferencijalna; imunoglobulini.

Introduction

Multiple myeloma (MM) is characterized by monoclonal paraprotein production, lytic lesions and increased plasma cells in the bone marrow [1]. The most common complications include renal insufficiency, hypercalcaemia, anemia and recurrent infection [2, 3]. Patients often have neurological complications, either due to metabolic disorders such as hypercalcaemia, uremia and hyperviscosity or due to peripherial neuropathy, spinal cord compression and cranial nerve infiltration. The most common are cord compression and peripheral neuropathy [4]. Involvement of the CNS in MM is very rare. Leptomeningeal involvement in MM is the most frequent type of CNS MM reported in the literature. Approximately 70 cases have been reported and published in the literature in the last 20 years [5]. Despite aggressive systemic and local treatment, the outcome was poor [6]. In this study we reported the neurological symptoms and signs, imaging, cerebrospinal fluid (CSF) findings and the clinical course of a 55-year-old man with MM and CNS myelomatosis (LMM). LMM was proven by malignant plasma cells, protein electrophoresis and immunofixation tests of the CSF in the presence of CNS symptoms.

Case report

A 55-year-old man was hospitalized because of thoracic spine pain. His medical history had been uneventful. There had been no evidence of mental or neurological impairment prior to the seizures. Physical examination showed no abnormalities. Initial blood chemistry analyses showed mild anemia (hemoglobine 102 g/L), elevated erythrocyte sedimentation rate of 62 mm/h, fibrinogen 3.59 g/L, increase in serum creatinine, 178 μmol/L, and blood urea nitrogen (BUN) 10.5 mmol/L. The level of β_2-microglobulin was highly increased, 7.99 mg/L, compared to the normal range of 0.7–1.8. Serum albumin and calcium concentrations were normal, but proteinuria was 4.34 g/24 h. Serum protein electrophoresis (PE) on a "Sebia" cappillarys/hydrasys demonstrated a spike in the gamma fraction, as well as in the beta region of urine and CSF PE. Immunofixation electrophoresis (IFE) confirmed that serum M-component belonged to IgG lambda isotype, with concentration of 38.6 g/L on admission, suppressed IgA (< 0.33 g/L) and IgM (< 0.16 g/L), as well as increased value of total lambda free chain (9.2 mg/L). Urinary homogen M-fraction was due to owerflow type of proteinuria and existence of free light chain (FLC) of immunoglobulin (Ig) molecules or Bence Jones proteins. FLC lambda concentration was dramatically elevated to 2490 mg/L as compared to the reference interval of 5.7–26.2 mg/L. Bone marrow aspiration revealed plasma cell infiltration (40%), confirming the diagnosis of multiple myeloma. Magnetic resonance imaging of thoracic spine showed a pathology fracture of Th8 with a decrease in bone intesity near the thoracic spine. Craniogram revealed multiple lytic skull lesions. After a complete staging, we retained the diagnosis of MM type biclonal gammopathia (IgG lambda and free lambda light chains) in the III B clinical stage (CS), and soon, we introduced systemic chemotherapy (VAD protocol) combining vincristine, doxorucin and dexamethasone with bisphosphonate and radiotherapy (single shoot of Th8). Soon thereafter, the patient developed confusions, headaches, weakness, nausea, febrility, dispnea and later sensomotor aphasias and right hemiparesis. After complete examinations (lab analysis, chest rentgenography, echocardiography, microbiology analysis of sputum, bronchoscopy) we concluded that the patient developed bilateral bronchopneumonia and acute renal insufficiency. The patient was treated with adequate antibiotics and other therapy including one hemodyalisis. Because his neurological status deteriorated, we did multislice computed tomography of the head, which was normal. CSF analysis showed pleocytosis (26 elements/mL), increased concentrations of proteins in the CSF, without hypoglicorahia. Cytological analysis revealed an increased number of plasma cells (29%)

(Figure 1). Analysis of neurotropic viruses with ELISA methods was negative twice.

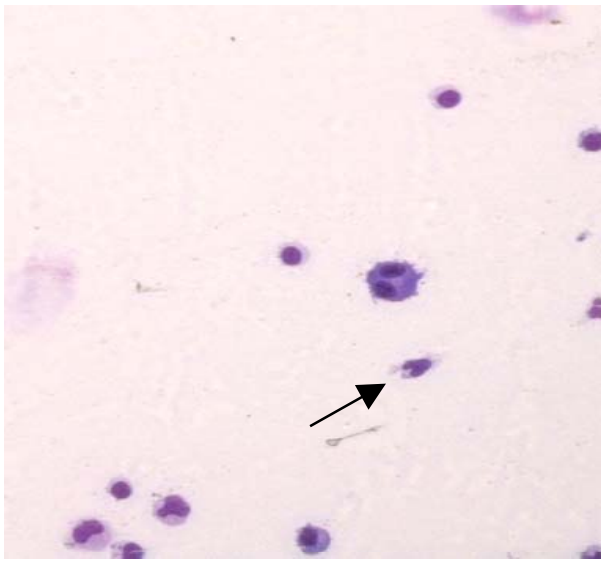

Fig. 1 – High-power view of cytological findings of cerebrospinal fluid (CSF): binuclear plasma cells and mononuclear plasma cells are seen. Other cells are lymphocytes and monocytes

Increased concentration of all three immunoglobulins in CSF and elevated CSF/serum albumin ratio were also detected. Protein imunofixation electrophoresis showed a homogene fraction in CSF for all three immunoglobulines, as mono- or oligoclonal bands (Figure 2), but in serum only for IgG. Besides that, the calculation of kappa/lambda free light chain ratio was decreased (0.006). The concentration of all the mentioned proteins was determined by immunonephelometry ("SIEMENS" DADE BNII) method (Table 1).

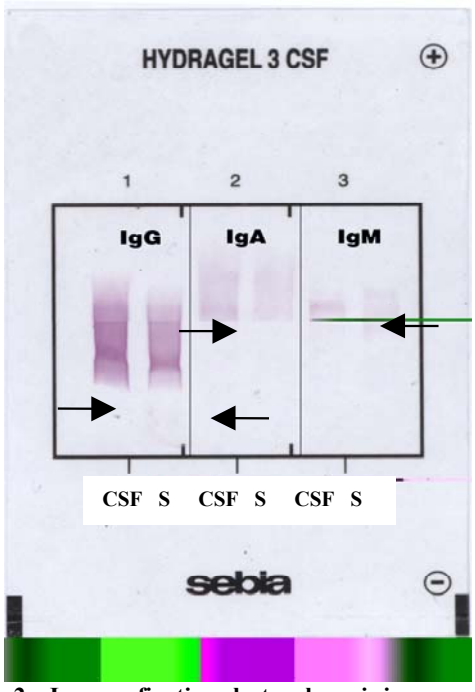

Fig. 2 – Immunofixation electrophoresis in serum (S) and cerebrospinal fluid (CSF)

Table 1
Concentrations of immunoglobulines, Ig fragments and albumin in serum and synovial fluid

Body fluid	Values	(Ref. values)
Cerebrospinal fluid (CSF)		
IgG (mg/L)	330	0–5)
IgM (mg/L)	6.3	0–1
IgA (mg/L)	33	0–5
Albumin	788	0–1
Serum (s)		
IgG (mg/L)	26.7	8–17
albumin (g/L)	35.5	37–53
albumin quotient	22.2	< 5.4
IgG index	0.55	< 0.7
IgG *de novo*		
Free kappa (mg/L)	14.9	
Free lambda (mg/L)	2490	
k/l ratio	0.006	0.26–1.65
CSF/s protein ratio		
IgA index	0.26	0–0.15
IgM index	2.4	0–0.1

After the diagnosis of LMM, the patient received intrathecal chemotherapy with 20 mg methotrexate, 20 mg cytosine arabinoside and 20 mg dexamethasone three times a week, with systemic high doses of dexamethasone *iv* as a single agent without craniospinal irradiations. In spite of the treatment, the patient died one month after being diagnosed. Autopsy was not performed.

Discussion

Patients with MM often have neurologic complications, either due to metabolic disordes such as hypercalcemia, uremia and hyperviscosity or due to peripheral neuropathy, spinal radiculopathies, spinal cord compression and cranial nerve infiltration. While infiltration of leptomeninges by various malignancies including acute lymphocytic leukemia, diffuse lymphomas, breast cancer and small-cell lung cancer with the rates of 2%–75% is well-known, invasion of the CNS in MM is rare, either as presumed localized intraparenchymal lesions, solitary cerebral plasmocytom or as LMM. The overall incidence of CNS involvement was only about 1%. At the time of LMM diagnosis, there was a diffuse array of neurologic symptoms and signs, including cerebral symptoms, neurologic findings referable to cranial neuropathies and to the spinal cord or spinal nerve roots. Presenting symptoms of CNS involvement most commonly include headaches, limb weakness, mental changes and cranial nerve palsies [7–10]. Approximately 70 cases, mostly case reports, have been reported in the English-language literature in the past century. The largest series, 23 patients out of 2,000 patients with LMM was reported by Schluterman et al. [5] from the University of Arcansas Medical Center over a 13-year period. All 23 patients presented with symptoms suggestive of CNS involvement that prompted neurological investigations: 15 patients had cerebral symptoms, including headaches, mental status changes and seizures, 12 had cranial neuropathy, while 18 patients had motor and sensory disturbances due to spinal nerve root involvement. The median

survival from the MM diagnosis to the development of LMM was 3 months. The 23 cases had intrathecal chemotherapy twice a week with methotrexate, cytosine arabinoside plus hydrocortisone. Cytologic sterilization of CSF was achived in 11 patients. Systemic chemotherapy was given to 18 patients and 5 patients who did not receive systemic chemotherapy eather died soon after the diagnosis or refused further treatment. The authors concluded that the reasons underlying a relative paucity of CNS invasion by MM in comparison with other tumors remain unknown, but might be the result of the underlying biological characteristics, or lack thereof, of malignant plasma cells [5].

Pizzuti et al. [11] reported 3 cases and made a retrospective review of 18 cases with MM infiltration. They found that meningeal involvement occurred in patients with initially stage III MM in 85% of cases, and it was associated with the occurrence of plasma cell leukaemia in 20% of cases. The most frequent neuorological signs were confusion (60%), altered consciousness (25%), gait disorder (25%) and cranial nerve (25%) palsy.

If survival was prolonged, the prevalence of LMM might be higher. There are 2 reported cases of patients with MM in which LMM developed after 7 and 10 years suggesting that it can occur after a longer period. However, it does not confirm that a long survival time might increase the prevalence, and a large series over several years might be needed to confirm this statement [8]. Involvement of the CNS in MM is determined by the detection of malignant plasma cells in CSF, with the presence of symptoms suggestive of MM [5]. Dispenzieri and Kyle [10] in a review of the neurological aspects of MM, classified intracranial plasmacytomas or myelomas into 4 groups: those extending from the skull pressing inward; those growing from the dura mater or the leptomeninges; those arising from the mucous membranes of a nasopharyngeal plasmacytoma; and intraparenchymal lesions without evidence of extencion from any of the other 3 sites.

Movsas et al. [12] reported cases with sixth-nerve palsies as a presenting sign for intracranial plasmacytoma in MM. Kyle and Dispenzieri [9] noted that the involvement of cranial nerves and their divisions is a rare complication of MM, which occurs most commonly at the time of a progressive disease. Other rare type of presentation was a 42-year-old woman with Bence-Jones-type MM who developed ocular abnormalities as described by Tuncbilek et al [13]. Haegelen et al. [14] reported a 72-year-old woman presented with headaches and left hemiparesis, and was diagnosed with dural plasmacytoma; further investigations showed systemic MM. Specific magnetic resonance imaging suggestive of CNS invasion included leptomeningeal contrast enhancement and the evidence of meningeal-based lesions sometimes masquerading as intraparenchymal lesions [14]. MSCT of the head in our case raport showed no abnormality.

CSF examination remains the definitive test for diagnosing LMM, usually exhibiting pleocytosis or elevated protein content plus positive cytologic findings [15]. Cytology showed eritrocytes, lymphocytes, and monocytes, but there were also 29% of pleomorphic plasma cells. Pathologic, binucelar plasma cells were also seen.

We systematically measured immunoglobulin levels in sera and CSF samples, and decreased concentration of IgG was expected to the level of 16 g/L after introducing the therapy. Elevated CSF/serum albumin ratio and a significant decrease in filtration capability of blood brain bariere was due to involvement of the CNS in MM. Findings of homogenic fractions in CSF only for IgA and IgM but not in sera, could be connected with their probable local production inside CNS, similar as in cases of Ig intrathecal synthesis [16]. Besides that, extremely low free k/L ratio, as a marker for fast progression and short survival time was also confirmed [17, 18]. These results are in complete concordance with others [19] that imply the quantifications of serum free light chains and calculating FLC k/λ ratio as a useful diagnostic tool for the course and survival.

Merelli et al. [20] reported 3 cases with IgD MM. They detected and identified IgD paraprotein in CSF and concluded that there was a correlation between the presence of paraprotein in CSF and the possible neurological involvement.

The presence of CNS symptoms in MM will usually lead to further investigations, including CSF examination and radiological testing for restaging if the patient was known (diagnosed) with MM. Detection of malignant plasma cells in the CSF is considered the hallmark of the diagnosis. At diagnosis of LMM, intrathecal chemotherapy was given once or twice weekly ranging from 3 to 12 doses, depending on the clinical neurologic response. Systemic treatment consisted of intermediate–dose chemoterapy, high-dose chemoterapy followed by autologous stem cell transplant or followed by allogeneic stem cell transplant. In this case, a standard VAD therapy was introduced with bisphosphonate and radiotherapy (single shoot of Th8). After diagnosis of LMM, we administered three times a week intrathecal chemotherapy with 20 mg methotrexate, 20 mg cytosine arabinoside and 20 mg dexamethasone, with systemic high doses of dexamethasone *iv* like a single agent without craniospinal irradiations.

The prognosis of patients with LMM is poor, despite aggressive local and systemic treatment. The median survival has been estimated at approximately 4–5 months. Even when sterilization of CSF was achieved, patient's survival was limited by the aggressive systemic disease. The most common are high-risk cytogenetic abnormalities in both bone marrow and CSF, plasmablastic morphology, extramedullary manifestations, plasma cell leukemia and high serum lactate dehydrogenase levels. Our previous experience with other Ig isotypes of MM could elicit consideration, that, in this case report, the existence of the second LCD lambda type with CNS involment was more significant for fast progression and worse prognosis. Thus, leptomeningeal seeding is a concomitant of aggressive MM. We believe that the treatment of LMM is indicated, given its potential for symptomatic relief and improvement in the quality of life. Better understanding of the biology of LMM may allow prospective and earlier recognition and treatment of patients at risk for this complication.

Conclusion

Presented patient, as well as most other patients with MM progressing to CNS infiltration was in the stage III. Besides detailed clinical examination, with all the required investigations for MM diagnosis and staging of the disease, we introduced additional CSF examination and calculation of kappa/lambda ratio, that would help us in making an early diagnosis and prognosis of MM with LMM. Although LMM has a low prevalence, it could be more frequent than expected, especially in high-risk patients. CSF examination with positive plasma cells and abnormal morphology remains the hallmark for the diagnosis of CNS infiltration.

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CASE REPORT

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Reversal deterioration of renal function accompanied with primary hypothyrodism

Reverzibilno akutno smanjenje bubrežne funkcije udruženo sa primarnom hipotireozom

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Abstract

Introduction. Hypothyroidism is often accompanied with decline of kidney function, or inability to maintain electrolyte balance. These changes are usually overlooked in everyday practice. Early recognition of this association eliminates unnecessary diagnostic procedures that postpone the adequate treatment. **Case report.** Two patients with elevated serum creatinine levels due to primary autoimmune hypothyroidism, with complete recovery of creatinine clearance after thyroid hormone substitution therapy are presented. The first patient was a young male whose laboratory tests suggested acute renal failure, and the delicate clinical presentation of reduced thyroid function. The second patient was an elderly woman with a history of a long-term signs and symptoms attributed to ageing, including the deterioration of renal function, with consequently delayed diagnosis of hypothyroidism. **Conclusion.** Serum thyrotropin and thyroxin levels measurement should be done in all cases of renal failure with undefined renal desease, even if the typical clinical presentation of hypothyroidism is absent. Thyroid hormone assays sholud also be performed in all patients with chronic kidney disease whose kidney function is rapidly worsening.

Key words:
hypothyroidism; renal insufficiency; diagnosis; thyrotropin; thyroxine; treatment outcome.

Apstrakt

Uvod. Hipotireoza je često udružena sa smanjenjem bubrežne funkcije ili poremećajem u održavanju elektrolitnog balansa. U svakodnevnoj kliničkoj praksi, ove promene najčešće ostaju neprimećene. Ranim prepoznavanjem ove udruženosti smanjuje se broj nepotrebnih dijagnostičkih procedura koje odlažu blagovremeno lečenje bolesnika sa hipotireozom. **Prikaz bolesnika.** Prikazana su dva bolesnika kod kojih je novootkrivena autoimunska hipotireoza bila praćena biohumoralnim pokazateljima akutnog smanjenja bubrežne funkcije, uz kompletno obnavljanje klirensa kreatinina do fizioloških nivoa posle adekvatne supstitucijske terapije. Prvi bolesnik bila je mlađa osoba sa netipičnom kliničkom manifestacijom hipotireoze. Drugi bolesnik bio je osoba starijeg životnog doba sa višemesečnim simptomima i znacima hipotireoze koji su se pripisivali starenju, uključujući i slabljenje bubrežne funkcije, što je sprečilo blagovremenu dijagnostiku i lečenje. **Zaključak.** Preporučujemo proveru vrednosti tireotropnog hormona i tiroksina kod svih bolesnika sa akutnom bubrežnom insuficijencijom nejasne etiologije, ili kod naglog pogoršanja bubrežne funkcije kod osoba sa hroničnom bubrežnom bolesti, čak i bez tipične kliničke manifestacije hipotireoze.

Ključne reči:
hipotireoidizam; bubreg, insuficijencija; dijagnoza; tireotropin; tiroksin; lečenje, ishod.

Introduction

Thyroid hormones are nesessary for growth and developement of the kidney. They are also involved in maintenance of water and electrolyte homeostasis in different organs and tissue departments. Hypothyroidism is often accompanied with decline of kidney function, or failure of electrolyte balance [1]. These changes are very subtle and usually overlooked in everyday clinical practice. We described two patients with impaired renal function due to primary hypothyroidism, and complete recovery of creatinine clearance after thyroid hormone replacement therapy.

Case reports

Case 1

A 23-year-old male was referred to the outpatient department for elevated serum creatinine levels with the history of the mild muscle weakness and tiredness over the few months. Physical examination revealed normal anthropomet-

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ric parameters, body mass index (BMI) of 24 kg/m^2, body temperature of 36.5C°, dry skin with no edema. He was normotensive with pulse rate of 64 beats per minute, and showed no hepatomegaly.

Laboratory blood tests revealed an elevated serum creatinine level of 168 mmol/L (normal range: 50–75 mmol/L), creatine kinase (CK) level of 430 U/L (normal range 21–294 U/L) and serum total cholesterol level: 8.4 mmol/L (normal range < 5.2 mmol/L), while serum liver enzymes levels were slightly elevated: aspartate aminotransferase (AST) 44 U/L (normal range 0–34 U/L), alanine aminotransferase (ALT) 63 U/L (normal range 7–49 U/L) and lactate dehydrogenase (LDH) 420 U/L (normal range 208–378 U/L). Biochemistry data included normal concentration of serum urea, triglyceride, bilirubine, albumin, potassium, sodium, glucose, hemoglobin, platelet and white blood count. Urine tests showed no proteinuria or hematuria. There were no casts in the urine sediment. Serum and urine myoglobin levels were not determinated. Creatinine clearance estimated using the Cockroft-Gault formula was reduced to the value of 68 mL/min (normal range for males 97–137 mL/min), suggesting renal failure. An abdominal ultrasonography showed normal morphology and volumen of both kidneys. Due to the constant presence of muscle weakness, the thyroid function test were performed. Thyroid stimulating hormone or thyrotropin (TSH) level was elevated: 56 mU/L (normal range 0.35–5.5 mU/L), serum free thyroxine (FT4) level was lower: 9.5 pmol/L (normal range 11.5–22.7 pmol/L) and free triiodothyronine (FT3) level was at the lower limit of the normal range: 3.6 pmol/L (normal range 3.5–6.5 pmol/L). The thyroperoxidase antibodies titer were elevated, 1: > 1 300 (normal range 0–60.0), while antithyroglobulin antibodies titer were not determined. The ultrasonography of the thyroid showed a slight enlargement of the gland with the heterogenous texture suggesting autoimmune thyroiditis.

After the primary hypothyroidism was detected, the patient was recommended to take levothyroxine replacement therapy at 100 mcg daily dose (1.6 mcg/kg). One week after starting the levothyroxine therapy, the patient felt better. After two months of hormone substitution, all biochemical parameters returned to the normal levels: TSH level was 3.5 mU/L, serum creatinine level was 94 mmol/L and the creatinine clearance was 117 mL/min (Table 1). The patients went back to his everyday activities.

Case 2

An 81-year-old female patient was referred to our department for the presence of clinical and humoral parameters suggesting primary hypothyroidism. The patient complained about tiredness, muscle pain, dry skin and the presence of elastic edema of the lower limbs and ankcles, not responsive to furosemid therapy over the last eight months. The patient also had the history of arterial hypertension and ischemic heart disease, ordinarily treated with adequate therapy recomended by the cardiologist. Physical examination revealed bradycardia (heart rate of 55 beats per minute), increased blood preassure of 160/100 mmHg, dry skin, palor, elastic edema of the ankles, with no hepatomegaly or other signs of cardiac failure. The patient had slightly enlarged palpable thyroid with hard consistency. Laboratory data noted mild normochromic and normocytic anemia with hemoglobin levels of 105 g/L (normal range 130–180 g/L); serum glycemia was 7 mmol/L (normal range 4.1–5.9 mmol/L) with elevated level of total cholesterol 9.6 mmol/L (normal range < 5.2 mmol/L) and CK 480 U/L (normal range 21–294 U/L). Serum biochemistry included high levels of serum creatinine of 180 mmol/L (normal range 80–124 mmol/L) and slightly elevated serum urea levels of 11.6 mmol/L (normal range 3.2–8.2 mmol/L). Serum levels of other liver enzymes, triglyceride, albumin and bilirubin were normal. Creatinine clearence, assesed using the Cockroft-Gault formula, was reduced to the value of 35 mL/min (normal values for female 88–128 mL/min) suggesting renal failure. Ultrasonography of the kidneys or other abdominal organs were not performed.

Diagnosis of primary hypothyroidism was based on the elevated TSH levels: 65.6 mU/L (normal range 0.35–5.5 mU/L), decreased FT4: 10.4 pmol/L (normal range 11.5–22.7 pmol/L) and FT3 levels: 0.9 pmol/L (normal range 3.5–6.5 pmol/L). The thyroperoxidase antibodies titer were elevated (1 : 600). The ultrasonography of the thyroid gland showed that its volume was at the lower limit of normal values with heterogenous structure suggesting autoimmune thyroiditis. Levothyroxine replacement therapy started with the dose of 100 mcg daily (gradually increasing dose by 25 mcg weekly). Two months after starting the substitution therapy, the patient's edema retreated following the decrease of plasma TSH levels. After four months of the therapy, thy-

Table 1

Comparation of laboratory data before and after the L-thyroxine replacement therapy in case 1

Biochemical parameters	At diagnosis	Two weeks later	Two months later
TSH (mU/L)	56	41	3.5
Free T4 (pmol/L)	9.5	12	17.5
Free T3 (pmol/L)	3.6	4.1	5.4
Creatinine (mmol/L)	168	130	94
Total cholesterol (mmol/L)	8.4	6.1	5.3
LDL cholesterol (mmol/L)	4.9	3.5	2.95
CK (U/L)	430	300	115
LDH (U/L)	420	305	277
AST (U/L)	44	/	20
ALT (U/L)	63	/	34
Creatinine clearance (mL/min)	68	/	117

TSH – thyrotropin; T4 – thyroxine; T3 – triiodothyronine; LDL – low density lipoprotein; CK – creatine kinase; LDH – lactate dehydrogenase; AST – aspartate aminotransferase; ALT – alanine aminotransferase

roid hormon levels returned to the normal level. At the same period, plasma creatinine values decreased to 81 mmol/L while creatinine clearance increased up to 72 mL/min (Table 2). The patient continued to feel well.

Table 2

Comparation of laboratory data before and after the L-thyroxine replacement therapy in case 2

Biochemical parameters	At diagnosis	2 months later	4 months later
TSH (mU/L)	65.6	32.8	3.8
Free T4 (pmol/L)	10.4	12.2	19.3
Free T3 (pmol/L)	0.9	2.1	4.5
Hemoglobin (g/L)	105	108	119
Total cholesterol (mmol/L)	9.6	7.2	6.1
CK (U/L)	480	290	180
Creatinine (mmol/L)	180	115	81
BUN (mmol/L)	11.6	9.0	7.9
Creatinine clearance (mL/min)	35	63	72

TSH – thyrotropin; T4 – thyroxine; T3 – triiodothyronine; CK – creatine kinase; BUN – blood urea nitrogen

Discussion

The functional association between hypothyroidism and kidney failure has been described many times in the literature [2–5], and it seems to be reversibile after hormone substitution therapy. Montenegro et al. [6] showed a decrease in glomerular filtration rate (GFR) in all of their hypothyroid patients, whereas only 55% had an increase in serum creatinine levels. A few years later, Villabona et al. [7] described the decrease in effective renal blood flow and GFR in hypothyroid patients with chronic renal disease. Karanikas et al. [8] performed isotopic renal function studies in thyroidectomized patients showing that the hemodynamic changes in severe hypothyroidism mainly affect the glomerular function.

The most common kidney derangements associated with hypothyroidism are an increase in serum creatinine levels, reduction in GFR and renal plasma flow, decreased capacity of free water excretion and hyponatremia [1]. About one half of patients with autoimmune thyroid disease have positive circulating immunocomplexes that are in correlation with the presence of thyroid peroxidase antibodies, but not with their titer [9]. Immunocomplexes deposits in the basement membrane of the glomeruli have been also reported in patients with Hashimotho thyroiditis; still, no casual relationship between the presence of immunocomplexes and antibodies has been proved so far [1].

Although the exact mechanism of these changes has not been defined yet, it seems that kidney failure secondary to hypothyroidism involves heterogenous processes based on the direct or indirect effects of thyroid hormones on renal hemodynamics [1, 10, 11]. Thyroid hormone deficiency decreases myocardial contractility and cardiac output. On the other hand, an impaired endothelial-mediated vasodilatation in hypothyroidism increases peripheral and renal vascular resistance [11]. These effects reduce renal plasma flow and GFR, resulting in free water overload and decrease in creatinine clearance. Consequently, elevation of plasma creatinine levels might happen. Decrease in GFR produces a diminished water delivery to distal tubular segments that is partly responsible for the hyponatremia. Hyponatremia appears in 45% of hypothyroid patients who have elevated serum creatinine levels and in about 21% of those with normal creatinine levels [1]. Thyroid hormones also have a hold upon tubular transport of sodium *via* their actions on the sodium-potassium adenosine triphosphate pump (Na/K ATP-ase) and on the potassium permeability in the membrane of the proximal tubules.

Levothyroxine is a synthetic product identical to natural thyroxine, produced by the thyroid gland. After the normalisation of serum thyroxine levels, cardiac output and myocardial contractility recover, leading to the increase in renal plasma flow and creatinine clearance.

Systemic manifestations of hypothyroidism vary considerably, depending on the duration and severity of the hypothyroid state. Gradual and imperceptible onset somethimes account for the inconclusive clinical diagnosis of hypothyroiditis. We described two patients with autoimmune hypothyroidism presented with elevated serum creatinine levels and reverse deterioration of renal function.

The first patient was a young male with a delicate clinical presentation of reduced thyroid function. Due to nonspecific symptoms and signs associated with laboratory parameters suggesting acute renal failure, clinical findings were not easy to intreprete. Our examination results could not reveal any kidney disease.

Hypothyroidism is known to be associated with elevated serum CK levels along with other muscle enzymes (LDH). Due to the myopathy, hypercholesterolemia and elevated levels of CK in this young patient, we performed thyroid hormone testing. High serum thyrotropin and decreased serum free thyrotropin levels, accompained with elevated titer of antyperoxidase antibodies were adequate to define primary autoimmune hypothyrodism. After the beginning of thyroid replacement therapy, thyroid status improved and kidney function progressively recovered. This observation is consistent with the previously published data [2–5].

In elderly patients with various illnesses, symptoms and signs of hypothyrodism could be easily confused with the usuall signs or effects of aging such as cold intolerance, dry, pale, thick and rough skin, intestinal constipation, non-depressive edema, mental slowness or increased body weight [12]. In the

second case, we described the elderly patient with the history of long-term edema and simptoms similar to aging. Deterioration of renal function, followed with moderate renal atrophy and elevated serum creatinine levels are not unusual in an 81-year-old patient with the hystory of arterial hypertension or ischemic heart disease. Creatinine clearance appears to decrease with age (each decade corresponds to a decrease of about 6.5 mL/min/1.73 m^2). After establishing the diagnosis of primary hypothyrodism, hormonal treatment with levothyroxin was started. After four months of the treatment, adequate control of hypothyroidism was seen with progressive recovery of kidney function and restore of serum creatinine levels, that was somewhat unexpected. Still, similar findings has been observed by other autors: in older patients with various illnesses, even with the moderate renal atrophy on ultrasound images, thyroid replacement therapy recovered renal creatinine clearance to physiological values [4, 5, 12].

Hyponatremia, as the commonest electrolyte derangement in hipothyrodism, did not appear in any of our patients. Because of the mild elevation of serum CK levels and the mild form of myalgia, we did not find it necessary to perform measurement of serum and urinary myoglobine levels. Both of our patients were presented with elevated CK and cholesterol levels. A deficit in the expression of the hepatic low density lipoprotein (LDL) receptor gene in hypothyroidism diminishes LDL cholesterol clearance which results in hypercholesterolemia. A degree of metabolic dysfunction in skeletal muscle was seen even in subclinical hypothyrodism [13]. Similary to kidney disfunction, this could be reversed with thyroid hormone treatment. In the presented cases, a few months after the introduction of thyroxine replacement therapy and normalisation of serum TSH levels, creatinine clearance, serum cholesterol and serum CK levels recovered to the normal vaelues, no matter of the clinicial presentation of hypothyroid state, or the age of the patients.

There are some published clinical case reports confirming stabilisation of kidney function in patients with chronic kidney disease after correction of thyroid function [1, 5, 14]. There are also described cases of acute deterioration of kidney function in patients with chronic kidney disease and unrecognised hypothyroidism [15]. We therefore recommends measurement of serum thyrotropin and thyroxin levels in all cases of renal failure with undefined renal desease, even if the typical clinical presentation of hypothyroidism is absent. We also recommend that thyroid hormone assays should be performed in all patients with chronic kidney disease whose kidney function is rapidly worsening.

Conclusion

The presence of reversal renal failure as the consequence of hypothyrodisms is usually subtle and frequently overlooked. Knowledge of the association between hypothyrodism and deterioration of renal function is very important in clinical practice.

This association must be recognized in time, avoiding the unnecessary diagnostic procedures that postpone adequate treatment.

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ERRATUM

UVODNIK/EDITORIAL

Dobrić S.
Nova godina – novi izazovi
The New Year – new challenges
Vojnosanit Pregl 2012; 69(1): 5–8

U Uvodniku citiranom gore potkrala se greška u imenu recenzenta u Tabeli 2, kolona IV (str. 8). Umesto imena **Radonjić Vida**, treba da stoji **Radonjić Vidosava**.

In the Editorial cited above, Table 2, column 4 (page 8), instead of the name **Radonjić Vida** it should be the name **Radonjić Vidosava.**

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Na drugoj stranici nalazi se strukturisani apstrakt sa naslovom rada. Kratkim rečenicama na srpskom i engleskom jeziku iznosi se **uvod i cilj** rada, osnovne procedure - **metode** (izbor ispitanika ili laboratorijskih životinja; metode posmatranja i analize), glavni nalazi - **rezultati** (konkretni podaci i njihova statistička značajnost) i glavni **zaključak**. Naglasiti nove i značajne aspekte studije ili zapažanja. Strukturisani apstrakt (**250** reči) ima podnaslove: *uvod/cilj, metode, rezultati* i *zaključak*. Za apstrakte na engleskom dozvoljeno je i do **450** reči. Strukturisani apstrakt je obavezan za metaanalize (istog obima kao i za originalne članke) i kazuistiku (do 150 reči, sa podnaslovima *uvod, prikaz slučaja i zaključak*). Ispod apstrakta, pod podnaslovom „Ključne reči" predložiti 3–10 ključnih reči ili kratkih izraza koji oslikavaju sadržinu članka.

3. ***Tekst članka***

Tekst sadrži sledeća poglavlja: **uvod**, **metode**, **rezultate** i **diskusiju**. **Zaključak** može da bude posebno poglavlje ili se iznosi u poslednjem pasusu diskusije. U **uvodu** ponovo napisati naslov rada, bez navođenja autora. Navesti hipotezu (ukoliko je ima) i ciljeve rada. Ukratko izneti razloge za studiju ili posmatranje. Navesti samo strogo relevantne podatke iz literature i ne iznositi opširna razmatranja o predmetu rada, kao ni podatke ili zaključke iz rada o kome se izveštava.

Metode. Jasno opisati izbor metoda posmatranja ili eksperimentnih metoda (ispitanici ili eksperimentne životinje, uključujući kontrolne). Identifikovati metode, aparaturu (ime i adresa proizvođača u zagradi) i proceduru, dovoljno detaljno da se drugim autorima omogući reprodukcija rezultata. Navesti podatke iz literature za uhodane metode, uključujući i statističke. Tačno identifikovati sve primenjene lekove i hemikalije, uključujući generičko ime, doze i načine davanja. Za ispitivanja na ljudima i životinjama navesti saglasnost etičkog komiteta.

Rezultate prikazati logičkim redosledom u tekstu, tabelama i ilustracijama. U tekstu naglasiti ili sumirati samo značajna zapažanja.

U **diskusiji** naglasiti nove i značajne aspekte studije i izvedene zaključke. Posmatranja dovesti u vezu sa drugim relevantnim studijama, u načelu iz poslednje tri godine, a samo izuzetno i starijim. Povezati zaključke sa ciljevima rada, ali izbegavati nesumnjive tvrdnje i one zaključke koje podaci iz rada ne podržavaju u potpunosti.

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Literatura se u radu citira kao superskript, a popisuje rednim brojevima pod kojima se citat pojavljuje u tekstu. Navode se svi autori, ali ako broj prelazi šest, navodi se prvih šest i dodaje et al. Svi podaci o citiranoj literaturi moraju biti tačni. Literatura se u celini citira na engleskom jeziku, a iza naslova se navodi jezik članka u zagradi. Ne prihvata se citiranje apstrakata, sekundarnih publikacija, usmenih saopštenja, neobjavljenih radova, službenih i poverljivih dokumenata. Radovi koji su prihvaćeni za štampu, ali još nisu objavljeni, navode se uz dodatak „u štampi". Rukopisi koji su predati, ali još nisu prihvaćeni za štampu, u tekstu se citiraju kao „neobjavljeni podaci" (u zagradi). Podaci sa *Interneta* citiraju se uz navođenje datuma.

Primeri oblika referenci:

Đurović BM. Endothelial trauma in the surgery of cataract. Vojnosanit Pregl 2004; 61(5): 491–7. (Serbian)

Balint B. From the haemotherapy to the haemomodulation. Beograd: Zavod za udžbenike i nastavna sredstva; 2001. (Serbian)

Mladenović T, Kandolf L, Mijušković ŽP. Lasers in dermatology. In: *Karadaglić Đ*, editor. Dermatology. Beograd: Vojnoizdavački zavod & Verzal Press; 2000. p. 1437–49. (Serbian)

Christensen S, Oppacher F. An analysis of Koza's computational effort statistic for genetic programming. In: *Foster JA, Lutton E, Miller J, Ryan C, Tettamanzi AG,* editors. Genetic programming. EuroGP 2002: Proceedings of the 5th European Conference on Genetic Programming; 2002 Apr 3-5; Kinsdale, Ireland. Berlin: Springer; 2002. p. 182-91.

Abood S. Quality improvement initiative in nursing homes: the ANA acts in an advisory role. Am J Nurs [serial on the Internet]. 2002 Jun [cited 2002 Aug 12]; 102(6): [about 3 p.]. Available from: http://www.nursingworld.org/AJN/2002/june/Wawatch.htm

Tabele

Sve tabele štampaju se sa proredom 1,5 na posebnom listu hartije. Obeležavaju se arapskim brojevima, redosledom pojavljivanja, u desnom uglu (**Tabela 1**), a svakoj se daje kratak naslov. Objašnjenja se daju u fus-noti, ne u zaglavlju. Za fus-notu koristiti sledeće simbole ovim redosledom: ***, †, ‡, §, ||, ¶, **, ††, ...** Svaka tabela mora da se pomene u tekstu. Ako se koriste tuđi podaci, obavezno ih navesti kao i svaki drugi podatak iz literature.

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Discussion is to emphasize the new and important aspects of the study and the conclusions that result from them. Relate the observations to other relevant studies. Link the conclusions with the goals of the study, but avoid unqualified statements and conclusions not completely supported by your data.

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Examples of references:

Jurhar-Pavlova M, Petlichkovski A, TrajkovD, Efinska-Mladenovska O, Arsov T, Strezova A, et al. Influence of the elevated ambient temperature on immunoglobulin G and immunoglobulin G subclasses in sera of Wistar rats. Vojnosanit Pregl 2003; 60(6): 657–612.

DiMaio VJ. Forensic Pathology. 2nd ed. Boca Raton: CRC Press; 2001.

Blinder MA. Anemia and Transfusion Therapy. In: Ahya NS, Flood K, Paranjothi S, editors. The Washington Manual of Medical Therapeutics, 30th edition. Boston: Lippincot, Williams and Wilkins; 2001. p. 413-28.

Christensen S, Oppacher F. An analysis of Koza's computational effort statistic for genetic programming. In: *Foster JA, Lutton E, Miller J, Ryan C, Tettamanzi AG,* editors. Genetic programming. EuroGP 2002: Proceedings of the 5th European Conference on Genetic Programming; 2002 Apr 3-5; Kinsdale, Ireland. Berlin: Springer; 2002. p. 182-91.

Abood S. Quality improvement initiative in nursing homes: the ANA acts in an advisory role. Am J Nurs [serial on the Internet]. 2002 Jun [cited 2002 Aug 12]; 102(6): [about 3 p.]. Available from: http://www.nursingworld.org/AJN/2002/june/Wawatch.htm

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Type each table double-spaced on a separate sheet. Number tables consecutively in the order of their first citation in the text in the upper right corner **(Table 1)** and supply a brief title for each. Place explanatory matter in footnotes, using the following symbols, in this sequence: ***, †, ‡, §, ||, ¶, **, ††, ...** Each table has to be mentioned in the text. If you use data from another source, acknowledge fully.

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