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SADRŽAJ / CONTENTS

Ovogodišnja Nobelova nagrada za fiziologiju ili medicinu pripala je dvojici naučnika, Britancu ser Džonu Gurdonu (John B. Gurdon) (slika levo) i Japancu Šinji Jamanaki (Shinya Yamanaka) (slika desno) za otkriće da zrele ćelije mogu da budu reprogramirane u matične, pluripotentne ćelije koje imaju sposobnost da se diferenciraju i stvore drugi tip ćelija. Smatra se da će rezultati njihovih istraživanja omogućiti bolje razumevanje mehanizama razvoja ćelija i organizma u celini, mehanizama nastanka nekih bolesti, ali i obezbediti nove terapijske modalitete (vidi Uvodnik, str. 939).

The 2012 Nobel Prize in Physiology or Medicine has been jointly awarded to the two scientists, Sir John B. Gurdon (left) from Great Britain, and Shinya Yamanaka (right) from Japan, for the discovery that mature cells can be reprogrammed to become stem pluripotent ones that are able to develop into all types of cells in the body. It is considered that their research will offer a new view on the development of cells and organisms and understanding disease mechanisms, as well as to provide new therapeutic modalities (see Editorial, p. 939).

EDITORIAL

The 2012 Nobel Prize Laureates in Physiology or Medicine

Dobitnici Nobelove nagrade za fiziologiju ili medicinu u 2012.

Miodrag Čolić

Medical Faculty of the Military Medical Academy, University of Defense, Belgrade, Serbia

The Nobel Prize in Physiology or Medicine 2012 honors the discoveries in cell biology by two Laureates, Sir John B. Gurdon and Dr. Shinya Yamanaka. Their findings that mature, differentiated cells can be reprogrammed to a pluripotent stem cell state have introduced fundamentally new research areas and offered new opportunities to study disease mechanisms.

Sir John B Gurdon was born in Dippenhall, UK in 1933. He studied at the University of Oxford where he received his Doctorate in 1960. After finishing postdoctoral fellowship at California Institute of Technology, he joined the Cambridge University in 1972 as Professor of Cell Biology and Master of Magdalene College.

Attitude that the mature cell is permanently locked into the differentiated state and unable to return to a fully immature state was the common view during the first half of the 20th century. In 1962 Professor Gurdon considerably changed this view by discovering that the specialization of cells is reversible. Namely, he replaced the immature cells nucleus in an egg cell of a frog with the nucleus from a mature intestinal cell. Such treatment resulted in the development of a fully functional tadpole. Subsequent experiments demonstrated that cloned tadpoles can be developed into adult frogs. In addition, the DNA of the mature cell still had the code needed for differentiation of all cells in the frog. The discovery of Professor Gurdon broke the dogma that cellular differentiation could only be a unidirectional process. This finding initiated intense research in further techniques, leading to the cloning of mammals, including mouse, cow, sheep, pig, wolf and African wildcats. It is interesting, that even nuclei from T and B cells, in which antigen receptors are completely rearranged, could be reprogrammed to support the development of a mouse.

After this discovery, the question whether an intact differentiated cell could be fully reprogrammed to become pluripotent, remained. Many scientists considered that the process is impossible or accompanied with every complex reorganization in the cell to reverse its differentiated state. Shinya Yamanaka answered this question more than 40 years later and showed how intact mature cells in mice could be reprogrammed to become immature stem cells.

Shinya Yamanaka was born in Osaka, Japan in 1962. After obtaining MD in 1987 at Kobe University and training as an orthopedic surgeon, he switched to basic research. In 1993 he received his PhD at Osaka City University, after which he worked at the Gladstone Institutes in San Francisco, USA and Nara Institute of Science and Technology in Japan. He currently works as Professor at Kyoto University where directs the Center for Induced Pluripotent Stem Cell Research and Application.

His research concerned to the pluripotent stem cells that were isolated from the embryo and cultured in the laboratory. Such stem cells were initially isolated from mice by Martin Evans (Nobel Prize 2007) and Dr. Yamanaka tried to find the genes that kept them immature. After identification the set of 24 genes, encoding different transcription factors, Dr. Yamanaka and his co-workers introduced them into fibroblasts, as mature connective tissue cells, and discovered that the fibroblasts could be reprogrammed into immature stem cells. They identified a combination of only four transcription factor genes (Myc, Oct ¾, Sox 2 and Klf 4) that were sufficient for the reprogramming process in the differentiated cell toward its plurypotency. The discovery was published in 2006 and was immediately considered as a major breakthrough.

Taken together, the discoveries of Professors John Gurdon and Shinya Yamanaka have completely changed our understanding of cell and organism development and created new opportunities to study diseases and establish methods for diagnosis and therapy.

(Note: The author is a full member of the Serbian Academy of Sciences and Arts and a leading expert in the field of immunology).

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The Nobel Committee for Physiology and Medicine

The Nobel Committee for Physiology and Medicine at Karolinska Institutet is responsible for the selection of candidates from the names submitted by invited nominators. It consists of five members and the Secretary-General of the Nobel Assembly. Committee members are elected for a period of three years. Each year, ten associated memebers are elected for a term running from March to October.

Members of the Nobel Committee for Physiology or Medicine 2012 are (Figure 1):

1. Urban Lendhal, Professor of Genetics (Chairman)
2. Juleen Zierath, Professor in Clinical Integrative Physiology (Vice-chairman)
3. Jan Andersson, Professor of Infectious Disease
4. Thomas Perimann, Professor of Molecular Developmental Biology
5. Rune Toftgård, Professor of Environmental Toxicology
6. Göran K. Hansson, Professor of Experimental Cardiovascular Research (Secretary-General of the Nobel Assembly)

Fig. 1 – The Nobel Committee for Physiology or Medicine 2012
Seated left to right: Rune Toftgård, Juleen Zierath, Urban Lendhal, Thomas Perimann, Jan Andersson
Standing middle left to right: Carlos Ibáñez, Bo Angelin, Ole Kiehn, Göran K. Hansson, Anna Wedell, Björn Vennström (Chairman of the Nobel Assembly), Anders Hamsten, Klas Kärre
Standing back left to right: Patrik Ernfors, Christer Höög, Hans Forssberg, Karl Tryggvason (Deputy Chairman of the Noble Assembly); not in photo: Per-Olof Berggren

ORIGINAL ARTICLE

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Ocjena primjene rekombinantnog humanog tireotropina u praćenju bolesnika sa dobro diferentovanim karcinomom štitaste žlijezde

Assessment of recombinant human thyrotropin application in following-up patients with well-differentiated thyroid carcinoma

Zvezdana Rajkovača*, Pedja Kovačević†, Mirko Stanetić‡, Siniša Ristić§

***Zavod za nuklearnu medicinu i bolesti štitne žlijezde, †Jedinica intenzivne medicine, ‡Klinika za plućne bolesti, Klinički centar, Banja Luka, Bosna i Hercegovina; §Medicinski fakultet, Univerzitet u Istočnom Sarajevu, Foča, Bosna i Hercegovina**

Apstrakt

Uvod/Cilj. Najosjetljiviji indikatori otkrivanja recidiva dobro diferentovanog karcinoma štitaste žlijezde su scintigrafija cijelog tijela ^{131}I i mjerenje nivoa serumskog tireoglobulina (Tg). Za njihovo izvođenje potrebno je da se povisi nivo endogenog tireoidstimulišućeg hormona (TSH) što se može postići prekidom uzimanja terapije L-tiroksina tokom 3–5 nedelja ili davanjem rekombinantnog humanog tireotropina (rhTSH) bez prekidanja uzimanja terapije tireoidnim hormonima. Cilj ovog rada bio je ocjena upotrebe rhTSH u odnosu na tradicionalni prekid uzimanja supstitucionosupresorne terapije u praćenju bolesnika sa dobro diferentovanim karcinomom štitaste žlijezde. **Metode.** Ispitivanje je obavljeno kao retrospektivna studija kojom je bilo obuhvaćeno 44 bolesnika sa dobro diferentovnim karcinomom štitaste žlijezde podijeljenih u dve grupe. Grupu I činili su bolesnici (n = 31) kod kojih su analize za praćenje bolesti (scintigrafija cijelog tijela sa ^{131}I, mjrenje nivoa TSH, Tg i antiTgAt) urađene u hipotireoidnom stanju, a grupu II bolesnici (n = 13) kod kojih su iste analize obavljene nakon davanja rhTSH. Za procjenu pojave simptoma i znakova hipotireoidizma ispitivano je prisustvo 13 simptoma na dan davanja ^{131}I. Kvalitet života je ocenjen pomoću modifikovanog upitnika [Skala kvalitete života (SF-36)] ispunjenog na dan davanja ^{131}I. **Rezultati.** U obe grupe postignut je veoma dobar stimulativni nivo TSH, ali značajno viši u grupi II (grupa I 30,3–101,5 μlU/mL, grupa II 68,60–192,00 μlU/mL, $p < 0,05$). U obe grupe vrijednosti Tg bile su više tokom stimulacije TSH-om (grupa I 0,1–546,0 ng/mL, grupa II 0,1–7517 ng/mL) u odnosu na period uzimanja L-tiroksina (grupa I, 0,1–495,0 ng/mL, grupa II 0,1–2785 ng/mL). Nije bilo razlike u tehničkim kvalititetima dobijenih scintigrama cijelog tijela u obe grupe. Bolesnici iz grupe I imali su prisutno 8–13 simptoma hipotireoidizma, dok bolesnici iz grupe II nisu imali prisutne simptome hipotireoidizma. Znatno bolji kvalitet života imali su bolesnici poslije aplikacije rhTSH, 74 do 91 boda, u poređenju sa onima koji su prekinuli uzimanje L-tiroksina, 43 do 62 boda ($p < 0,05$). Bolesnici su dobro podnosili rhTSH; samo kod jednog bolesnika zabilježena je nauzeja. **Zaključak.** Upotreba rhTSH u praćenju bolesnika sa dobro diferentovanim karcinomom štitaste žlijezde sprečava pojavu simptoma hipotireoidizma doprinoseći održavanju metaboličke homeostaze organizma i očuvanju kvaliteta života. Rekombinantni humani tireotropin je bezbjedan, efikasan i jednostavan za upotrebu, ali za naše uslove još uvijek skup preparat.

Ključne reči:
tireoidna žlezda, neoplazme; dijagnoza; dijagnostičke tehnike, endokrine; scintigrafija; tireoglobulin; kvalitet života.

Abstract

Background/Aim. The most sensitive indicators for detecting recurrence of well-differentiated thyroid cancer (DTC) are ^{131}I whole body scintigraphy (WBS) and measurement of serum thyroglobulin (Tg). In order to perform it, it is necessary to raise the level of endogenous tiroidstimulating hormon (TSH), which can be achieved by L-thyroxine withdrawal for 3–5 weeks or administration of recombinant human thyrotropin (rhTSH) without requiring the discontinuation of thyroid hormone therapy. The aim of this study was to assess the effect of rhTSH using in comparison to the traditional thyroid hormone withdrawal in the follow-up of patients with DTC. **Methods.** This retrospective study included 44 patients, mean age 48.8 years, with DTC divided into 2 groups. The group I consisted of patients (n = 31) in which the analysis in the follow-up (WBS with ^{131}I, TSH, Tg and antiTgAt) made in the hypothyroid state, and group II patients (n = 13) in which they made after the administration of rhTSH. The presence of 13 symptoms and signs of hypothyroidism was investigated on the day of giving ^{131}I. Quality of life was evaluated using a modified form: the quality of life scale (SF-36) completed on the day of giving ^{131}I. **Results.** In both groups, serum

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TSH reached a very good stimulation level, but significantly higher in the group II (group I 30.3–101.5 μlU/mL, group II 68.6–192.0 μlU/mL, $p < 0.05$). In both groups, TSH-stimulated Tg was higher (group I 0.1–546.0 ng/mL, group II 0.1–7517 ng/mL) comapred to value during the L-thyroxine therapy (group I 0.1–495.0 ng/mL, group II 0.1–2785 ng/mL). There was no difference in technical quality of WBS obtained from both groups. The patients in the group I had attended 8–13 symptoms of hypothyroidism, while patients in group II did not have symptoms of hypothyroidism. The patients after application of rhTSH, showed statistically significantly better quality of life as compared with those who showed to have L-thyroxine withdrawal, (74–91 points *vs* 43–62 points; $p < 0.05$). The rhTSH was well tolerated, with nausea occurring in only one patient. **Conclusion.** Administration of rhTSH in the follow-up of patients with DTC prevents the debilitating effects of hypothyroidism contributing to the maintenance of metabolic homeostasis of the organism and preserves the quality of life. RhTSH is safe, effective and easy to use, but is still an expensive product in our country.

Key words:
thyroid neoplasms; diagnosis; diagnostic techniques, endocrine; radionuclide imaging; thyroglobulin; quality of life.

Uvod

U posljednih pet godina incidencija karcinoma štitaste žlijezde je u porastu [1, 2]. Dobro diferentovani tumori štitaste žlijezde, papilarni i folikularni, akumuliraju i metabolišu jod, pa se oni mogu detektovati i liječiti radioaktvnim jodom (^{131}I). Rizična dobna skupina za pojavu dobro diferentovanih karcinoma štitaste žlijezde je 20–60 godina starosti, tj. radno sposobne osobe. Smrtnost od karcinoma štitaste žlijezde je mala. Desetogodišnje preživljavanje iznosi 90–95%. Uspješno liječenje podrazumijeva totalnu tiroidektomiju, ablativnu dozu ^{131}I, supstituciono-supresivnu terapiju L-tiroksinom, po potrebi ponavljanje ablative doze ^{131}I i terapijske doze ^{131}I [3, 4].

Najosjetljiviji indikatori otkrivanja recidiva karcinoma štitaste žlijezde su scintigrafija cijelog tijela ^{131}I i mjerenje nivoa serumskog tireoglobulina (Tg), naročito kada se koriste u kombinaciji [5]. Scinitgrafija cijelog tijela sa ^{131}I pokazuje prisustvo/odsustvo recidiva ili jodoavidnih metastaza [6]. U praćenju bolesnika sa karcinomom štitaste žlijezde koristi se i ehotomogarfija vrata koja daje morfološke podatke o prisustvu/odsustvu lokalnog recidiva i metastaza u limfnim čvorovima vrata [3].

Da bi se mogli dobiti adekvatni nalazi nivoa Tg, kao i uraditi scintigrafija cijelog tijela sa ^{131}I, potrebno je povisiti nivo endogenog tireoidstimulišućeg hormona (TSH) u serumu iznad 30 μlU/mL. Najčešće, ovo se postiže prekidom uzimanja terapije L-tiroksina tokom 3–5 nedelja. Na ovaj način nastala kratkotrajna hipotireoza povezana je sa pojavom kognitivnih i fizičkih nedostataka i promjene kvaliteta života kod mladih i sredovječnih bolesnika. Kod starijih bolesnika, izostavljanje terapije L-tiroksinom može ugroziti kardiovaskularne, kognitivne i neurološke funkcije, kao i povećati rizik od nastanka oboljenja ovih sistema ili pogoršati već prisutna oboljenja [7, 8]. Nivo TSH ne može se povećati na ovaj način u slučajevima uporne proizvodnje tireoidnih hormona od strane velikih postoperativnih ostataka tkiva štitaste žlijezde, prisustva funkcionalnih metastaza, kod starijih bolesnika, kao i u prisustvu bolesti hipotalamusa/hipofize ili dugoročne terapije steroidima [9, 10].

Drugi način postizanja istog cilja bez izostavljanja terapije L-tiroksinom, jeste upotreba rekombinantnog humanog tireotropina (rhTSH). Na ovaj način izbjegava se pojava svih simptoma i rizika kratkotrajne hipotireoze i ne remeti radna sposobnost osobe. Danas je prihvaćen stav da je mjerilo svakog medicinskog tretmana kvaliteta života, koja se ne mijenja upotrebom rhTSH [11].

Cilj ovog rada bio je ocjena upotrebe rhTSH u odnosu na tradicionalni prekid uzimanja supstituciono-supresorne terapije u praćenju bolesnika sa dobro diferentovanim karcinomom štitaste žlijezde.

Metode

Ova retrospektivna studija sprovedena je u Zavodu za nuklearnu medicinu i bolesti štitaste žlijezde Kliničkog centra Banja Luka od januara 2009. do oktobra 2010. godine, na 44 bolesnika operisana od dobro diferentovanog karcinoma štitaste žlijezde. Podaci su dobijeni iz istorija bolesti, lista za štitastu žlijezdu, terapijskih kartona i otpusnih pisama.

Kriterijumi za uključivanje bolesnika u studiju bili su: patohistološki nalaz dobro diferentovanog karcinoma štitaste žlijezde, totalna ili skoro-totalna tiroidektomija, ablatvna doza ^{131}I nakon tiroidektomije, odsustvo drugih bolesti. Iz studije su isključeni bolesnici koji su izlagani rendgenskim kontrastnim pretragama ili se nisu pridržavali uputstava o niskojodnoj ishrani prije scintigrafije cijelog tijela ^{131}I.

Svi bolesnici bili su podijeljeni u dve grupe. Grupu I činili su bolesnici (n = 31) kod kojih su analize za praćenje bolesti (scintigrafija cijelog tijela sa ^{131}I, merenje nivoa TSH, Tg i antiTg antitela – antiTgAt) urađene u hipotireoidnom stanju, a grupu II bolesnici na supstitucionoj terapiji L-tiroksinom (n = 13) kod koji su analize urađene nakon davanja rhTSH.

Bolesnici iz grupe I nisu uzimali L-tirokisin četiri nedelje. Uzorak krvi za određivanje TSH, Tg i antiTgAt uziman je dan prije prekidanja terapije L-tiroksinom i na dan davanja ^{131}I. Nakon što je postignuta vrijednosti jutarnjeg TSH veća od 25 μlU/mL peroralno im je data kapsula sa 148 MBq (4 mCi) ^{131}I. Bolesnici iz grupe II su redovno uzimali L-tiroksin. Uzorak krvi za određivanje TSH, Tg i antiTgAt uziman je dan prije davanja rhTSH. Drugi uzorak za određivanje TSH uziman je dan nakon druge doze rhTSH, tj. na dan davanja ^{131}I, a uzorak za određivanje Tg i antTgAt trećeg dana od davanja druge doze rhTSH. Svi bolesnici iz grupe II primali su rhTSH intramuskularno prema protokolu: 0,9 mg rhTSH dva uzastopna dana; nakon 24 sata od druge doze dobili su peroralno 148 MBq (4 mCi) ^{131}I. Bolesnicima iz obe

grupe je 72 sata nakon ^{131}I rađena scintigrafija cijelog tijela na jednofotonskoj emisionoj kompjuterizovanoj tomografiji (SPECT) dvoglavoj gama kameri brzinom 10 cm/min kao i statička i SPECT scintigrafija vrata i drugih regija, ako je bilo potrebno, u trajanju od 300 sekundi Nalaze scintigrafija očitavala su tri specijalista nuklearne medicine nezavisno jedan od drugog. Konačna interpretacija nalaza je prihvatana, ako su dva od tri mišljenja bila ista.

Tireostsimulirajući hromon, Tg i antiTgAt određivani su elektrohemiluminiscentnom metodom. Referntne vrijednosti za TSH su 0,27 – 4,20 μlU/mL, za Tg 1,40 – 78,0 μg/mL i za antiTgA 0 - 115 IU/mL.

Za procjenu pojave simptoma i znakova hipotireoidizma ispitivano je prisustvo 13 simptoma na dan davanja ^{131}I: umor, pospanost, promjena boje glasa, nepodnošenje hladnoće, suhoća kože, promjena kvaliteta kose i noktiju, mišićni bolovi, dobijanje na težini, opstipacija, nadutost, poremećaj menstrualnog ciklusa, pojava edema, što ukupno iznosi 13 bodova. Kvalitet života ocenjivan je pomoću upitnika ispunjenog na dan davanja ^{131}I. Urađena je modifikacija kratke forme originalnog opšteg zdravstvenog upitnika SF-36 [*Medical Outcomes Study* (MOS) 36-*Item Short-Form Health Survey*] [12]. Upitnik SF-36 je tako sastavljen da daje uvid i u fizičke i u mentalne komponente zdravlja. Umjesto upoređivanja na godišnjem nivou, rađeno je poređenje sa periodom dva mjeseca prije aplikacije ^{131}I. Umjesto ocjene stanja u zadnje četiri nedelje rađena je procjena u odnosu na zadnju nedelju pre davanja ^{131}I. Upitnik sadrži pitanja koja se odnose na promjene kvaliteta života: ograničenja u fizičkim aktivnostima zbog zdravstvenog stanja; ograničenja uobičajenih svakodnevnih aktivnosti zbog fizičkih zdravstvenih problema; ograničenja uobičajenih svakodnevnih aktivnosti zbog emocionalnih problema; mentalno zdravlje; ograničenja u društvenim aktivnostima zbog fizičkih ili emocionalnih problema; prisustvo bola, vitalnost (energija i umor); percepciju opšteg zdravstvenog stanja.

Kvantitativni podaci su izraženi kao srednja vrijednost ± SD, a kvalitativni podaci kao procenat. Značajne razlike između vrijednosti TSH u obe grupe testirane su χ^2 testom, a ocjena kvaliteta života Wilcoxon-ovim testom.

Rezultati

Među 44 analizirana bolesnika bilo je 35 (79,5%) žena i 9 (20,5%) muškaraca (odnos 3,8 : 1). Starost bolesnika bila je od 24 do 72 godine, prosečno 48,8 ± 12,92 godina. Prosječna starost bolesnika u grupi I iznosila je 49,6 ± 12,83 godina, a u grupi II 47,5 ± 12,70 godina. Nije bilo statistički značajne razlike u starosnoj strukturi bolesnika među grupama ($p > 0,05$).

U grupi I bilo je 29 bolesnika (93,5%) sa papilarnim karcinomom štitaste žlijezde i 2 bolesnika (6,5%) sa folikularnim karcinomom štitaste žlijezde. U grupi II bilo je 12 bolesnika (92,3%) sa papilarnim karcinomom štitaste žlijezde i jedan bolesnik (7,7%) sa folikularnim karcinomom štitaste žlijezde. U tabeli 1 prikazana je tumor, nodus, metastaza (TNM) klasifikacija za obe grupe bolesnika.

U tabeli 2 prikazan je raspon vrijednosti TSH u grupi I i II kada su bolesnici bili na redovnoj supstitucionosupresivnoj terapiji, kao i na dan davanja ^{131}I za scintigrafiju cijelog tijela. Vidi se da je u obe grupe TSH postigao veoma dobar stimulativni nivo.

Tabela 1

Tumor, nodus, metastaza (TNM) klasifikacija operisanih tumora u obe grupe bolesnika

Stepen veličine tumora	Grupa I					Grupa II				
	N0	N1	Ukupno n (%)	M0	M1	N0	N1	Ukupno n (%)	M0	M1
T1	10	3	13 (41,9)	13	0	5	1	6 (46,1)	6	0
T2	8	3	11 (35,5)	11	0	3	1	4 (30,8)	4	0
T3	2	1	3 (9,7)	3	0	1	0	1 (7,7)	1	0
T4	0	4	4 (12,9)	4	0	0	2	2 (15,4)	1	1
Ukupno	20	11	31 (100)	31	0	9	4	13 (100)	12	1

T1, T2, T3, T4 – stepen veličine primarnog tumora; N0 – nema tumorskih ćelija u regionalnom limfnom čvoru; N1 – metastaze prisutne na nekoliko mesta u regionalnom limfnom čvoru; M0 – nema udaljenih metastaza; M1 – postojanje metastaze van regionalnih limfnih čvorova
Grupa I – bolesnici kod kojih su analize urađene u hipotireoidnom stanju
Grupa II – bolesnici na supstitucionoj terapiji L-tiroksinom kod kojih su analize urađene nakon primene rekombinantnog humanog tireoidstimulišućeg hormona (rsTSH)

Tabela 2

Vrijednost tireoidstimulišućeg hormona (TSH) prije i na dan primanja ^{131}I u obe grupe bolesnika

Vreme analize	TSH (μlU/mL), $\bar{x}$ ± SD (raspon)		*p*
	Grupa I	Grupa II	
Tokom supstituciono-supresivne terapije	0,27 ± 0,45 (0,02–1,1)	0,25 ± 0,47 (0,01–1,0)	< 0,05
Na dan aplikacije ^{131}I	67,73 ± 43,56 (30,3–101,5)	109,65 ± 31,69 (68,6–192,0)	> 0,05

Grupa I – bolesnici kod kojih su analize urađene u hipotireoidnom stanju
Grupa II – bolesnici na supstitucionoj terapiji L-tiroksinom kod kojih su analize urađene nakon primene rekombinantnog humanog tireoidstimulišućeg hormona (rsTSH)

U obe grupe bolesnika pozitivna antiTgAt bila su kod po jednog bolesnika, koji je imao visok nivo serumskog Tg, dok su kod svih ostalih bolesnika antiTgAt bila negativna. U tabeli 3 prikazan je broj negativnih vrijednosti Tg (< 0.9

Tabela 3

Vrijednosti tireoglobulina (Tg) prije i poslije aplikacije ^{131}I u obe grupe bolesnika

Tg	Grupa I		Grupa II	
	supstituciono-supresivna terapija	na dan aplikacije ^{131}I	supstituciono-supresivna terapija	nakon rhTSH
Negativan (n)	18	14	7	5
Nisko pozitivan (n)	9	8	3	4
Visoko pozitivan (n)	4	9	3	4
Raspon (ng/mL)	0,1–495,0	0,1–546,0	0,1–2785	0,1–7517

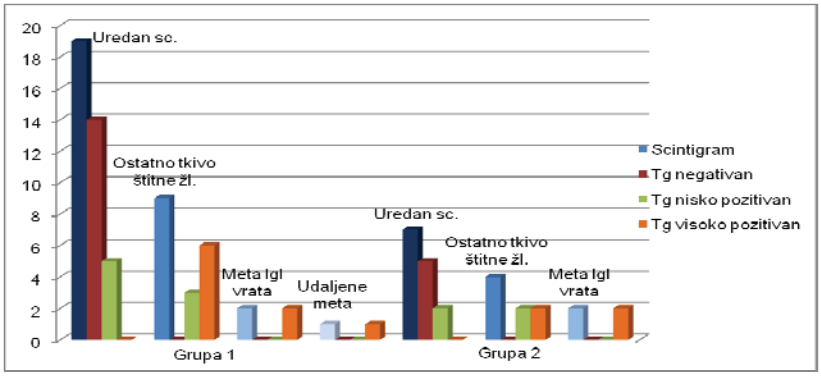
n – broj bolesnika
Grupa I – bolesnici kod kojih su analize urađene u hipotireoidnom stanju
Grupa II – bolesnici na supstitucionoj terapiji L-tiroksinom kod kojih su analize urađene nakon primene rekombinantnog humanog tireoidstimulišućeg hormona (rsTSH)

ng/mL), niskopozitivnih vrijednosti Tg (1-5 ng/mL), visokopozitivnih vrijednosti Tg (> 5 ng/mL) i raspon vrijednosti Tg u obe grupe bolesnika u vrijeme kada su bolesnici bili na supstituciono-supresivnoj terapiji i na dan aplikacije ^{131}I za grupu I, a za grupu II trećeg dana po dobijanju druge doze rhTSH.

Na scintigrafiji cijelog tijela sa ^{131}I nije viđena akumulacija radiofarmaka u regiji vrata kod 19 bolesnika grupe I i 7 bolesnika grupe II. Ostatno tkivo štitaste žlijezde bilo je prisutno kod devet bolesnika grupe I i četiri bolesnika grupe II. Prisustvo metastaza u limfnim čvorovima vrata utvrđeno je kod dva bolesnika grupe I i kod jednog bolesnika grupe II. Kod jednog bolesnika iz grupe II bile su prisutne multiple metastaze u limfnim čvorovima vrata, u plućima i kostima. Na slici 1 prikazan je odnos između vrijednosti nivoa serumskog Tg i nalaza scintigrafije cijelog tijela sa ^{131}I.

papilarni karcinom štitaste žlijezde, zastupljen u našem istraživanju kod preko 90% bolesnika iz obe grupe [1]. Epidemiološke studije pokazuju rast učestalosti malih i dobro lokalizovanih karcinoma štitaste žlijezde, što doprinosi malom mortalitetu. Podaci iz naše studije odgovoraju podacima iz literature. Kod više od 60% bolesnika iz obe grupe radilo se o pT1/pT2N0 stadijumu, dok su udaljene metastaze bile dijagnostikovane kod samo jednog bolesnika iz grupe 2 [1, 2].

Nasuprot relativno niskog mortaliteta bolesnika sa diferentovanim karcinomima štitaste žlijezde učestalost recidiva je 30–35% tokom prvih 30 godina, od čega je 2/3 u prvih 10 godina poslije inicijalne terapije. Ovo zahtijeva temeljite periodične kontrole bolesnika čija učestalost zavisi od korištenog protokola kao i individualnih faktora rizika. Periodične kontrole podrazumijevaju određivanje Tg, antiTgAt i scinti-



Sl. 1 – Odnos između nivoa tireoglobulina (Tg) i nalaza scintigrafije cijelog tijela sa ^{131}I

Grupa I – bolesnici kod kojih su analize urađene u hipotireoidnom stanju
Grupa II – bolesnici na supstitucionoj terapiji L-tiroksinom kod kojih su analize urađene nakon primene rekombinantnog humanog tireoidstimulišućeg hormona (rsTSH)
meta – metastaze; lgl – limfni čvorovi

Bolesnici iz grupe I imali su od osam do svih 13 simptoma hipotireoidizma, dok bolesnici iz grupe II nisu imali prisutne simptome hipotireoidizma. Znatno bolji kvalitet života imali su bolesnici poslije aplikacije rhTSH, 74 do 91 boda, u poređenju sa onima koji su prekinuli uzimanje L-tiroksina, 43 do 62 boda ($p < 0,05$). Od neželjenih efekata na rhTSH samo kod jednog bolesnika zabilježena je nauzeja.

Diskusija

Papilarni i folikularni karcinom štitaste žlijezde čini oko 80% svih malignih tumora štitaste žlijezde [1, 2]. U područjima sa deficitarnim jodom, u koja spada i naša zemlja, češći je

grafiju cijelog tijela sa ^{131}I radi otkrivanja funkcionalno aktivnog rezidualnog tkiva ili metastatskog tireoidnog tkiva koja se može uraditi samo ako postoji adekvatan nivo endogenog TSH [13].

Izostavljanje supstituciono-supresivne terapije je preduslov postizanja adekvatno visokog nivoa endogenog TSH. Kod nekih bolesnika endogeni odgovor TSH nije adekvatan ili pridružena oboljenja predstavljaju relativnu ili apsolutnu kontraindikaciju za prekid terapije L-tiroksinom. Na ovaj način nastala hipotireoza znatno ometa radnu sposobnost i kvalitet života bolesnika [9, 10].

Alternativni način postizanja adekvatno visokog nivoa TSH predstavlja egzogena stimulacija pomoću TSH. Nema

randomiziranih studija kojima se upoređuje izostavljanje terapije L-tiroksinom u odnosu na upotrebu rhTSH. Biohemijske osobine visokoprećišćenog rhTSH kompatibilne su sa osobinama humanog pituitarnog TSH. Njegovo vezivanje na receptore, kako u normalnom tkivu štitaste žlijezde tako i u tumorskom tkivu, stimuliše nakupljanje i organifikaciju joda i sintezu i sekreciju Tg i hormona štitaste žlijezde. Mogu se davati dve ili tri pojedinačne doze. U istraživanju je korišten protokol sa dve pojedinačne doze jer nisu opisane razlike u kvalitetu nalaza, a zbog jednostavnije primjene se preporučuje dvodnevni protokol [14].

Posmatrajući nivo TSH u grupi bolesnika koji su izostavili terapiju L-tiroksinom vidi se da je postignuta vrijednost niža od vrijednosti nakon davanja rhTSH. Viši nivo TSH omogućava bolju stimulaciju rezidualnog tkiva štitaste žlijezde kao i tumorskog tkiva. Nakon davanja rhTSH povećanje koncentracije TSH je kratkotrajno što minimizira uzrok ubrzanog rasta tumorskog tkiva.

Mnoge studije pokazale su da kod bolesnika nakon totalne tiroidektomije i ablativne doze ^{131}I prisustvo Tg upućuje na recidiv bolesti. Izuzetno je važno adekvatno određivanje Tg. Mjerenje se vrši u uslovima visokog TSH [15, 16]. Vrijednost Tg za vrijeme supstituciono-supresorne terapije nije kod svih bolesnika u našoj studiji bila jednaka vrijednosti u uslovima visokog TSH. U obe ispitivane grupe kod jednog broja bolesnika niska vrijednost Tg-a je prešla u niskopozitivnu vrijednost ili niskopozitivna vrijednost u visoku vrijednost Tg. Davanjem rhTSH adekvatno se stimuliše nivo Tg kao i prekidom uzimanja supstituciono-supresorne terapije.

Prisustvo antiTgAt javlja se kod 25% bolesnika sa karcinomom štitaste žlijezde i može uzrokovati lažno negativan nalaz Tg [5]. Za pravilnu interpretaciju vrijednosti Tg potrebno je znati i nivo antiTgAt.

Veliki broj autora su komparirali promjenu vrijednosti Tg sa scintigrafijom cijelog tijela ^{131}I. Cailleux i sar. [6] i Mazzaferi i Kloos [17] u svojim studijama čak navode nepotrebnost scintigrafije cijelog tijela ^{131}I ako je nakon rhTSH nivo Tg nemjerljiv, jer on adekvatno govori o odsutnosti lokalnog ili metastatskog tkiva štitaste žlijezde. Scintigrafija cijelog tijela sa ^{131}I pokazala je odsustvo bolesti kod bolesnika obe grupe kod kojih nije došlo do porasta negativne ili nisko pozitivne vrijednosti Tg. Kod bolesnika kod kojih je došlo do porasta vrijednosti Tg u obe grupe, na scintigrafiji cijelog tijela se vidjelo postojanje rezidualnog tkiva štitaste žlijezde, a kod tri bolesnika prisustvo metastaza u vratnim limfnim čvorovima. Jedan bolesnik je imao izrazito visoku vrijednost Tg, a na scintigrafiji cijelog tijela se vidjelo nakupljanje joda u mnogobrojnim metastazama u limfnim čvorovima vrata, plućima i kostima. Nije bilo razlike u tehničkim kvalititetima dobijenih scintigrama obe grupe. Rekombinantni humani tireotropin adekvatno stimuliše radiojodnu fiksaciju u preostalom tkivu štitaste žlijezde i u metastazama.

U izboru prioriteta kod planiranja terapijskih protokola značajna je i procjena kvaliteta života. Postoje mnoge definicije kvaliteta života. Najsveobuhvatnija je ona koju je dala Svjetska zdravstvena organizacija (WHO) po kojoj je to širok koncept koji čine fizičko zdravlje pojedinaca, psihološki status, materijalna nezavisnost, socijalni odnosi i njihovi odnosi prema značajnim karakteristikama spoljašnje sredine. Kvalitet života se izražava kao sposobnost za obavljanje životnih i radnih funkcija [18]. Upitnik SF-36 vrlo realno oslikava kvalitet života, a ima i odličnu korelaciju i sa fizičkim i psihičkim mogućnostima bolesnika.

Naši nalazi su u skladu sa drugim mnogobrojnim studijama koje pokazuju bolji kvalitet života bolesnika sa diferentovanim karcinomom štitaste žlijezde, koji su za praćenje bolesti koristili rhTSH. Prednost upotrebe rhTSH je izostanak simptoma hipotireoidizma što obezbjeđuje dobar kvalitet života i održavanja radne sposobnosti, a sve to smanjuje troškove za poslodavca i za društvo [19]. Schroeder i sar. [7] pokazali su da prekid uzimanja tireoidnih hormona djeluje mnogo više na kvalitet života nego što je to ranije dokumentovano, zbog uticaja na srčanu insuficijenciju, pojavu depresije i migrenozne glavobolje.

Rekombinantni humani tireotropin je siguran, jednostavan preparat za upotrebu koji daje minimalne nuzefekte [19, 20]. U našoj seriji bolesnika samo jedan je imao nauzeju. Neupotrebljavanje rhTSH kod većeg broja bolesnika u našoj ustanovi vezano je za ekonomski faktor tj. visoku cijenu rhTSH, a bolesnik mora sam da ga nabavi.

Zaključak

Upotreba rhTSH u praćenju bolesnika sa dobro diferentovanim karcinomom štitaste žlijezde sprečava pojavu simptoma hipotireoidizma doprinoseći održavanju metaboličke homeostaze organizma i očuvanju kvaliteta života. Rekombinantni humani tireotropin je bezbjedan, efikasan i jednostavan za upotrebu, ali za naše uslove još uvijek skup preparat.

L I T E R A T U R A

1. *Leenhardt L, Grosclaude P, Chérié-Challine L.* Increased incidence of thyroid carcinoma in france: a true epidemic or thyroid nodule management effects? Report from the French Thyroid Cancer Committee. Thyroid 2004; 14(12): 1056−60.
2. *Davies L, Welch HG.* Increasing incidence of thyroid cancer in the United States, 1973-2002. JAMA 2006; 295(18): 2164–7.
3. *Pacini F, Schlumberger M, Dralle H, Elisei R, Smit JW, Wiersinga W.* European consensus for the management of patients with differentiated thyroid carcinoma of the follicular epithelium. Eur J Endocrinol 2006; 154(6): 787–803.
4. *Cooper DS, Doherty GM, Haugen BR, Kloos RT, Lee SL, Mandel SJ* et al. Management guidelines for patients with thyroid nodules and differentiated thyroid cancer. Thyroid 2006; 16(2): 109–42.
5. *Eustatia-Rutten CF, Smit JW, Romijn JA, Van Der Kleij-Corssmit EP, Pereira AM, Stokkel MP,* et al. Diagnostic value of serum thyroglobulin. Clin Endocrinol (Oxf) 2004; 61(1): 61–74.
6. *Cailleux AF, Baudin E, Travagli JP, Ricard M, Schlumberger M.* Is diagnostic iodine-131 scanning useful after total thyroid ablation for differentiated thyroid cancer? J Clin Endocrinol Metab 2000; 85(1): 175−8.

7. *Schroeder PR, Haugen BR, Pacini F, Reiners C, Schlumberger M, Sherman SI,* at al. A Comparison of Short-Term Changes in Health-Related Quality of Life in Thyroid Carcinoma Patients Undergoing Diagnostic Evaluation with Recombinant Human Thyrotropin Compared with Thyroid Hormone Withdrawal. J Clin Endocrinol Metab 2006; 91(3): 878−84.
8. *Duntas LH, Biondi B.* Short-term hypothyroidism after Levothyroxinewithdrawal in patients with differentiated thyroid cancer: clinical and quality of life consequences. Eur J Endocrinol 2007; 156(1):13–9.
9. *Jarzab B, Handkiewicz-Junak D, Gawkowska-Suwinska M.* Recombinant human TSH in the diagnosis and treatment of disseminated differentiated thyroid cancer. Nucl Med Rev Cent East Eur 2000; 3(2): 82–8.
10. *Luster M, Lassmann M, Haenscheid M, Michalowski U, Incerti C, Reiners C.* Use of recombinant human thyrotropin before radioiodine therapy in patients with advanced differentiated thyroid carcinoma. J Clin Endocrinol Metab 2000; 85(5): 3640–45.
11. *Chen MK, Doddamane I, Cheng DW.* Recombinant human thyroid-stimulating hormone as an alternative for thyroid hormone withdrawal in thyroid cancer management. Curr Opin Oncol 2010; 22(1): 6−10.
12. *Ware JE, Sherbourne CD.* The MOS 36 item short-form health survey (SF-36). 1: Conceptual framework and item selection. Med Care 1992; 30: 473−80.
13. *Schlumberger M, Pacini F, Wiersinga WM, Toft A, Smit JW, Sanchez Franco F,* et al. Follow-up and management of differentiated thyroid carcinoma: a European perspective in clinical practice. Eur J Endocrinol 2004; 151(5): 539–48.
14. *Haugen BR, Pacini F, Reiners C, Schlumberger M, Ladenson PW, Sherman SI,* et al. A comparison of recombinant human thyrotropin and thyroid hormone withdrawal for the detection of thyroid remnant or cancer. J Clin Endocrinol Metab 1999; 84(11): 3877−85.
15. *Pelttari H, Valimaki MJ, Loyttyniemi E, Schalin-Jantti C.* Post-ablative serum thyroglobulin is an independent predictor of recurrence in low-risk differentiated thyroid carcinoma: a 16-year follow-up study. Eur J Endocrinol 2010; 163(5): 757−63.
16. *Mazzaferi EL, Robbins RJ, Spencer CA, Braverman LE, Pacini F, Wartofsky L,* et al. A consensus report of the role of serum thyroglobulin as a monitoring method for low-risk patients with papillary thyroid carcinoma. J Clin Endocrinol Metab 2003; 88(4): 1433−41.
17. *Mazzaferri ER, Kloos RT.* Is Diagnostic Iodine-131 Scanning with Recombinant Human TSH Useful in the Follow-Up of Differentiated Thyroid Cancer after Thyroid Ablation? J Clin Endocrinol Metab 2002; 87(4): 1490−8.
18. *Paterson C.* Quality of life measures. Br J Gen Pract 2010; 60(570): 53.
19. *Borget I, Corone C, Nocaudie M, Allyn M, Iacobelli S, Schlumberger M,* et al. Sick leave for follow-up control in thyroid cancer patients: comparison between stimulation with Thyrogen and thyroid hormone withdrawal. Eur J Endocrinol 2007; 156(5): 531−8.
20. *Hoftijzer HC, Heemstra KA, Corssmit EP, van der Klaauw AA, Romijn JA, Smit JW.* Quality of Life in Cured Patients with Differentiated Thyroid Carcinoma. J Clin Endocrinol Metab 2008; 93(1): 200−3.

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Application of alternative medicine in gastrointestinal cancer patients

Primena alternativne medicine kod bolesnika sa malignim oboljenjem digestivnog trakta

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Abstract

Bacground/Aim. Alternative medicine is a set of therapeutic procedures which are no part of official practice. At present, the use of alternative medicine among cancer patients is significant and the purpose of this study was to get more information on the methods and products of alternative medicine. Thus, the aim of the study was to determine the frequency of the use of alternative medicine among gastrointestinal cancer patients. **Methods.** The research was conducted using an anonymous questionnaire in writing. We included 205 patients with the diagnosis of gastrointestinal malignancy in the study but the questionnaire was fulfilled by 193 patients and the presented data were based on their answers. The questions were about the sociodemographic characteristics of the patients, the reasons for their use of alternative medicine, and their information sources about alternative medicine. We divided existing alternative therapies into 6 categories: herbal therapy, special diets, psychotherapy, body-mind therapy, spiritual therapy, and other supplements. **Results.** A total of 48 (24.9%) patients did not use any type of alternative therapy; 145 (75.1%) patients used at least one product and 124 (64.25%) patients used herbal preparations (beetroot juice was consumed by 110 [56.99%] patients); 136 (70.5%) patients were informed about alternative therapies by other patients.; 145 (75.1%) used alternative medicine to increase the chances for cure; 88 (45.6%) of interviewed patients would like to participate in future research in this field. **Conclusion.** The use of alternative medicine is evidently significant among cancer patients. Further research should be conducted in order to find out interactions of these products with other drugs and potential advantages and disadvantages of this form of treatment.

Key words:
neoplasms; gastrointestinal neoplasms; complementary therapies.

Apstrakt

Uvod/Cilj. Alternativna medicina je skup terapijskih postupaka koji, zbog nedostatka dokaza o delotvornosti, nisu deo klasične medicinske prakse. Alternativna medicina je danas značajno zastupljena među bolesnicima te je i cilj ove studije bio da se bliže informiše o pojedinostima njene upotrebe, kao i o metodama i proizvodima alternativne terapije. **Metode.** Istraživanje je sprovedeno uz pomoć anonimnog upitnika u pisanoj formi. Obuhvaćeno je 205 bolesnika sa dijagnostikovanim gastroenterološkim malignitetom, ali upitnik je ispunilo 193 bolesnika i svi podaci prikazani u radu se odnose na njih. Pitanja iz upitnika odnosila su se na sociodemografske karakteristike bolesnika, razloge upotrebe i izvore informacija o alternativnoj medicini. Oblici alternativne terapije bili su svrstani u šest kategorija: biljna terapija, specijalne dijete, psihoterapija, terapija koja deluje na um i telo, duhovna terapija i drugi suplementi. **Rezultati.** Ukupno 48 (24,9%) bolesnika nije koristilo nijedan od oblika alternativne terapije. Bar jedan vid terapije koristilo je 145 (75,1%) bolesnika, a njih 124 (64,25%) upotrebljavalo je biljne preparate (sok od cvekle 110 ili 56,99%). O alternativnoj terapiji 136 (70,5%) bolesnika informisalo se od drugih bolesnika. Da bi povećali šanse za izlečenjem, 145 (75,1%) koristilo je alternativnu medicinu, a 88 (45,6%) želelo bi da bude deo narednih istraživanja u ovoj oblasti. **Zaključak.** Zastupljenost alternativne medicine evidentno je značajna kod onkoloških bolesnika, te bi dalja istraživanja trebalo sprovoditi u pravcu otkrivanja interakcija ovih proizvoda sa drugim lekovima, kao i mogućih prednosti i nedostataka ovakvog oblika lečenja.

Ključne reči:
neoplazme; gastrointestinalne neoplazme; lečenje, komplementarno.

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Introduction

The use of alternative medicine (AM) among human beings dates back before Hippocrates and classical medicine. Each period in the evolution and development of human race has been characterized by its own diseases and their methods of cure. The lack of appropriate drugs made people rely on the powers of sorcerers and herbalists. However, in spite of the present use of modern diagnostic and therapeutic procedures, alternative medicine has not been abandoned. When we say alternative medicine, we refer to treatment that has not been founded on the evidence of its efficacy and tolerable harmfulness, which are the basics of classical medicine. According to the World Health Organisation (WHO) definition, alternative medicine refers to various measures of healthcare protection that are beyond the scope of official healthcare sector [1]. In recent years, the use of alternative medicine has tremendously been increased all over the world and it still has a rising trend [2]. In western countries, the increase is between 6 and 69 percent [3]. Several studies that were conducted in various regions of Turkey report the incidence of alternative medicine usage from 23 to 61 percent [4]. It should be mentioned that the concept of alternative medicine – its products and methods – differs from country to country. In the USA, biologically based therapy and acupuncture belong to alternative medicine while in China these methods are considered as the treatments of classical medicine [1]. In addition, psychotherapy (help rendered by psychiatrist, social worker, or psychologist) is a type of treatment that belongs to official healthcare sector; however, in earlier published papers its use in the treatment of cancer patients is mentioned in the context of alternative medicine [5].

Many cancer patients use the methods of alternative medicine concomitantly with conventional oncology treatments, *ie* radiotherapy, chemotherapy, and surgery. Why is the use of alternative medicine so extensive today? The answer can be found in the fact that human beings have innate instinct to alleviate suffering and pain by means of all possible treatments and in the existence of excellent media advertising of alternative medicine products and methods [6]. It seems that alternative medicine of today is an international phenomenon, which gradually turns into a powerful industry with an enormous turnover of the capital [5]. In addition, it gains an increasing medical, economical, and social significance.

The aim of the study was to determine the frequency of the use of alternative medicine among gastrointestinal cancer patients.

Methods

The study was conducted from October 2009 to June 2010 at the Oncology Institute of Vojvodina. A 205 patients diagnosed with gastroenterology malignancies were included in the study. All the included patients had metastatic disease stage 4 and all either used or were still using alternative medicine concomitantly with standard therapy. The investigation was anonymous and the patients were given a written questionnaire to circle the answers to each question. When a patient did not consent to participate in the study, the questionnaire was returned to the investigator. The questions were related to sociodemographic characteristics of the patients, their level of education, and disease stage. In addition, the patients were also asked to give the sources of alternative medicine information, about their wish to obtain new knowledge about AM, and their reason for AM usage. A group of questions covered the methods and products of AM that were grouped as: (1) biologically based therapy (special teas, Aloe vera, beetroot, blackberry wine, etc.); (2) special diets (macrobiotic, Breuss, vegetarian diets); (3) psychotherapy (psychiatrist, psychologist, social worker); (4) mind-body therapy (hypnotherapy, meditation, bioenergy, massage, yoga); (5) spiritual therapy (prayers); (6) other supplements (shark products, clay, petroleum, etc.).

As already mentioned, psychotherapy is a treatment that belongs to the official healthcare sector, but in earlier published papers, its use in the treatment of cancer patients is mentioned in the context of alternative medicine. We compared the obtained results with the results of other authors.

If more than one of abovementioned categories reported, an examinee was classified as the user of alternative medicine. Examinees were allowed to circle more than one of the suggested answers.

Results

A total of 12 (5.85%) of 205 included patients did not consent to participate in the investigation and returned the questionnaire. We found sex difference among participants not statistically significant. We included 120 (58.53%) men and 85 (41.46%) women, the median age was 63.5 years (range, 32–84 years). We did not find any statistically significant difference between sociodemographic characteristics (the place of residence and education level) of the patients and frequency of alternative medicine use ($p > 0.05$) (Table 1).

Table 1
The sociodemographic characteristics of the users of alternative medicine

Characteristics	Patients n (%)
Education level	
high	45 (31.03)
secondary	59 (40.68)
elementary	41 (28.27)
Residence area	
rural	68 (46.89)
urban	77 (53.10)

The examinees were classified as users of alternative medicine if they used at least one type of alternative medicine. Among 193 patients who answered the questionnaire 48 (24.9%) did not use any type or product of alternative medicine. The number of patients who used at least one AM

product/type was 145 (75.1%). The share between the use of biologically based therapies and other supplements was equal (124, 64.25%) (Table 2). Beetroot juice (110, 56.99%) was most commonly used from the group of biologically based therapies and whey (91, 47.15%) from the group of supplements (Table 3).

Table 2
The type and use of alternative medicine (AM)

Type of AM	Patients n (%)
Herbal therapy	124 (64.25)
Special diets	37 (19.2)
Psychotherapy	0 (0)
Mind-body therapy	32 (16.6)
Spiritual therapy	35 (18.1)
Other supplements	124 (64.25)

Table 3
The most often used products of alternative medicine (AM)

Products of AM	Patients n (%)
Tees	60 (30.95)
Aloe vera	51 (26.19)
Beetroot	110 (56.99)
Blackberry	89 (46.42)
Shark	86 (44.59)
Whey	91 (47.15)

The most common information sources on alternative were those provided by the relatives, other patients, and neighbours (136, 70.5%). Written information (magazines, brochures, and books) were reported by 61 (31.6%) of the examinees, while 49 (25.4%) of the study participants obtained information by means of electronic media (TV, Internet).

The enquiry regarding their willingness to participate in similar studies in future showed that 88 (45.6%) of examinees gave positive answer, 61 (31.6%) refused further involvement, and 44 (22.8%) were indecisive about their participation (Table 4).

Table 4
The attitude of the patients towards to participating in the study

Patients' attitude	Patients n (%)
Willing	88 (45.60)
Unwilling	61 (31.60)
Indecisive	44 (22.80)

Regarding the reason for using alternative medicine, 145 (75.1%) of interviewed patients reported increased chances for healing when combined with standard oncology treatments, 92 (47.7%) patients used AM to improve their immunity, 53 (27.5%) chose this way to prolong life, and 36 (18.6%) of patients believed that this would result in complete cure of malignant disease (Table 5).

Table 5
The reasons for using alternative medicine

Reasons	Patients n (%)
Increased chances for being cured	145 (75.1)
Improvement of immunity	92 (47.7)
Lifetime prolongation	53 (27.5)
Recovery from the disease	36 (18.6)

Discussion

The use of alternative medicine among oncology patients has been increased during the last decades [7]. In our study, 145 out of 193 interviewed patients were using or had used one of AM products. Numerous studies related the knowledge and use of AM among oncology patients have recently been conducted in different countries. Researching conducted in Canada showed that the frequency of AM use was 45% [8]. Studies conducted in 14 European countries reported that 36% of cancer patients use one or more types of AM [8]. The results obtained in our study showed that the share between the use of biologically based therapies and other supplements was equal. Beetroot juice, blackberry wine, and whey as dietary supplements were most commonly used among the interviewed patients. According to literature data, beetroot (*Beta vulgaris*) is an alternative medicine product with a history of longtime use in various world cultures. In ancient Rome, it was used for treating high temperature and constipation. In Eastern Europe, beetroot has been used for healing headache and toothache since 16th century. Its use in the treatment of cancers patients dates hundred years back. Beetroot is one of the most useful vegetables with great healing properties and high content of carbon hydrates, iron, calcium, phosphorus, potassium, and fibrous tissues. In addition, beetroot contains powerful inhibitors of carcinogenesis (betaine and betacyanin); it is a relatively low-price and easily accessible product that has no side effects after consuming. We believe that these characteristics are the main reason for its common use by oncology patients including our sample of patients from Vojvodina. Although beetroot and its products are consumed all over the world, we could not find explanation why beetroot, in spite of all its positive characteristics, was not among the top products of alternative medicine in surrounding and other countries of the world. A study performed in Turkey published similar results; biologically based AM products were on the top of use and nettle was the most popular plant [4]. In the United States, 58.78% of cancer patients prefer meditation, homeotherapy, and hypnotherapy as AM treatment [9, 10]. In our country, these methods are not so popular and are less used by cancer patients (16.6%). An interesting result of our study was the fact that none of interviewed patients mentioned the help of psychiatrist, social worker, psychologist, or prayers in handling mental health problems that always accompany any malignant disease. In Nigeria, the use of spiritual therapy – prayer – is in the second place among oncology patients [6].

Regarding the sources for obtaining information on alternative medicine the majority of patients (136, 70.5%) from our study relied on their relatives and other patients – users of AM. It shows the importance of interpersonal communication between the users of alternative medicine and the fact that patients, largely, believe in other people's experience when deciding about use of AM.

Many previous studies show that the use of AM is higher among female patients and patients with higher levels of education [11]. The results of our study failed to show statistically important difference among patients with gastrointestinal malignant disease in correlation to their age, sex, and sociodemographic characteristics; it reflects a universal wish of cancer patients to help them in any possible way including the methods that are not scientifically based. Because 88 (45.6%) of the examinees would like to participate in similar studies we concluded that cancer patients, in spite of the progress in medical treatment of malignant disease, had a great interest in alternative medicine therapies. Our study showed that cancer patients who use AM did not stop to believe in doctors' opinion. They wish to maximize their chances for the cure by combining the methods of alternative medicine with conventional oncology treatments. Our patients emphasized the importance for alleviation of disease symptoms, prolongation of life, and the improvement of their immunity by means of alternative medicine. In the USA and Turkey, 63% of patients wish to reach complete cure from the disease by means of alternative medicine; in our study, this reason was at the last place. It is also believed that many patients do not inform their physicians about the use of alternative medicine. The results of several studies conducted a few years ago found that half of the examined patients did not report the use of alternative medicine to their physicians [12]. As it is evident that alternative medicine is widely accepted among oncology patients, the oncologists should be timely informed about the use of AM among their patients. The boom of the alternative medicine during the last decade has partly been caused by increased number of people that want to take an independent part in their healthcare protection; and partly because of understanding that medical science has not succeed yet in the struggle to find the cure against malignant diseases for more than three decades [13, 14]. In addition, our results pointed out the importance of educating both patients and medical professionals about all aspects of alternative medicine, especially the risks of its use to prevent any possible side effect. The oncologists must inform their patients about benefits and side effects of alternative medicine. Future investigations related to AM should be targeted to clear up the safety and efficacy of AM treatments.

Conclusion

The use of alternative medicine is evidently significant among oncology patients (75.1%). Biologically based products in combination with standard oncology treatment are most frequently used to increase the chances for cure. Our patients obtained information on alternative medicine from their relatives or other patients with similar experiences. Future studies should be focused to patients' education provided by their physicians and other medical professionals, as well as to researching the interaction between antioneoplastic agents and products of alternative medicine.

REFERENCES

1. *Adams M, Jewell AP.* The use of Complementary and Alternative Medicine by cancer patients. Int Semin Surg Oncol 2007; 4: 10.
2. *Paltiel O, Avitzour M, Peretz T, Cherny N, Kaduri L, Pfeffer RM,* et al. Determinants of the use of complementary therapies by patients with cancer. J Clin Oncol 2001; 19(9): 2439−48.
3. *Xue CC, Zhang AL, Lin V, Da Costa C, Story DF.* Complementary and alternative medicine use in Australia: a national population-based survey. J Altern Complement Med 2007; 13(6): 643−50.
4. *Tarhan O, Alacacioglu A, Somali I, Sipahi H, Zencir M, Oztop I,* et al. Complementary-alternative medicine among cancer patients in the western region of Turkey. J BUON 2009; 14(2): 265−9.
5. *Richardson MA, Sanders T, Palmer JL, Greisinger A, Singletary SE.* Complementary/alternative medicine use in a comprehensive cancer center and the implications for oncology. J Clin Oncol 2000; 18(13): 2505−14.
6. *Ezeome ER, Anarado AN.* Use of complementary and alternative medicine by cancer patients at the University of Nigeria Teaching Hospital, Enugu, Nigeria. BMC Complement Altern Med 2007; 7: 28.
7. *Boon H, Stewart M, Kennard MA, Gray R, Sawka C, Brown JB,* et al. Use of complementary/alternative medicine by breast cancer survivors in Ontario: prevalence and perceptions. J Clin Oncol 2000; 18(13): 2515−21.
8. *Leis A, Verhoef MJ, Deschamps M, Doll R, Tan L, Dewar R.* What determines use of complementary therapies by Canadian cancer patients. Focus Alternat Complement Therapy 2003; 8**:** 149.
9. *Bott J.* An analysis of paper-based sources of information on complementary therapies. Complement Ther Clin Pract 2007; 13(1): 53−62.
10. *Verhoef MJ, Balneaves LG, Boon HS, Vroegindewey A.* Reasons for and characteristics associated with complementary and alternative medicine use among adult cancer patients: a systematic review. Integr Cancer Ther 2005; 4(4): 274−86.
11. *Mao JJ, Farrar JT, Xie SX, Bowman MA, Armstrong K.* Use of complementary and alternative medicine and prayer among a national sample of cancer survivors compared to other populations without cancer. Complement Ther Med 2007; 15(1): 21−9.
12. *Ernst E.* Why alternative medicines are used. Pharm J 2005; 275: 55.
13. *Paltiel O, Avitzour M, Peretz T, Cherny N, Kaduri L, Pfeffer RM,* et al. Determinants of the use of complementary therapies by patients with cancer. J Clin Oncol 2001; 19(9): 2439−48.
14. *Nassr-Allah AA, Aboul-Enein AA, Aboul-Enein KM, Lightfoot DA, Coccheto A, El-Shemy HA.* Anti-cancer and anti-oxidant activity of some Egyptian medicinal plants. J Med Plants Res 2009; 3(10): 799−808.

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Influenza A (H1N1) – past season's wonder flu in Vojvodina

Influenca A (H1N1) – čudo od gripa u Vojvodini prošle sezone

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Abstract

Background/Aim. Influenza A (H1N1) re-emerged in the human population during 2009. The aim of this study was to describe characteristics, laboratory findings, clinical presentation and treatment outcome among patients with influenza A (H1N1) infection. **Methods.** The study was performed at the Institute for Pulmonary Diseases of Vojvodina including all the patients hospitalized at the Intensive Care Unit or High Dependency Unit with confirmed, probable or suspected Influenza A (H1N1) infection between November 6th, 2009 and April 13th, 2010. **Results.** Among 64 patients Influenza A (H1N1) infection was confirmed by rt-PCR in 50, defined as probable in 7 and as suspected in 6 patients. There was an equal number of male and female patients. Their mean age was 46 years (SD ± 12.1). None of the patients were vaccinated against influenza. Comorbidities were present in 37 (58%) patients. There were 29 (45%) obese patients. Three patients were pregnant. The median time from symptom onset to hospital admission was 5 days (IQR 4–7). At admission, the median Modified Early Warning Score (MEWS) was 4 (IQR 3–6). The most common presenting symptoms were cough (100%) and fever (89%). The mean oxygen saturation at admission was 85.3% (SD 9.0). Auscultatory finding of wheesing in the absence of a chronic lung disease was found in 10 (15.6%) patients. Leukopenia was noted in 23 (35.9%) patients, and thrombocytopenia in 14 (21.9%) patients. Aspartate aminotransferase values were elevated in 41 (64.1%) patients, alanine aminotransferase in 32 (50%) patients, and creatine kinase in 36 (56.2%) patients. Opacities on an initial chest radiograph were predominantly patchy and the median number of the lung fields involved was 1 (IQR = 0–3). The non-survivors had statistically significantly higher MEWS at admission (p = 0.0001), lower oxygen saturation (p = 0.001), more lung fields involved on an initial chest radiograph (p = 0.006), wheezing in the absence of chronic lung disease (p = 0.02) and elevated aspartat aminotransferase (p = 0.02) and creatine kinase (p = 0.03). Acute respiratory disstress developed in 21 (32.9%) patients, and mechanical ventilation was required in 23 (36.1%) patients. Septic shock developed in 12 (18.7%) patients, and 19 (29.7%) patients had a multi-organ dysfunction. The overall hospital mortality was high – 20.3% (95% CI, 11.3%–32.2%; n = 13), and especially so among the patients who required mechanical ventilation – 56.5% (95% CI, 36.8%–74.4%). **Conclusion.** Timely initiation of antiviral therapy and early recognition of critically ill are important factors for reducing mortality.

Key words:
influenza A virus, H1N1 subtype; risk factors; signs and symptoms; diagnosis; treatment outcome.

Apstrakt

Uvod/Cilj. Virus Influenca A (H1N1) ponovo se pojavio u humanoj populaciji tokom 2009. godine. Cilj ove studije bio je da se opišu osobine, laboratorijski nalazi, klinička slika i ishod lečenja bolesnika sa infekcijom virusa influenca A (H1N1). **Metode.** Ovo istraživanje je sprovedeno u Institutu za plućne bolesti Vojvodine i uključivalo je bolesnike hospitalizovane u Jedinici intezivne nege ili Odeljenju poluintezivne nege sa potvrđenom, verovatnom ili suspektnom infekcijom virusa influenca A (H1N1) u periodu od 6. novembra 2009. do 13. aprila 2010. **Rezultati.** Od ukupno 64 bolesnika infekcija virusom influenca A (H1N1) potvrđena je metodom rt-PCR kod 50 bolesnika, a definisana kao verovatna kod sedam i kao suspektna kod šest bolesnika. Broj muškaraca i žena bio je jednak. Prosečno životno doba bolesnika bilo je 46 godina (SD ± 12.1). Niko od bolesnika nije bio vakcinisan protiv gripa. Kod 37 (58%) bolesnika evidentirani su komorbiditeti. Gojazno je bilo 29 (45%) bolesnika. Tri bolesnice bile su trudne. Prosečno vreme od pojave simptoma do hospitalizacije bilo je pet dana (IQR = 4–7). Prosečna vrednost modifikovanog skora za rano upozorenje (*Modified Early Warning Score* – MEWS) pri prijemu bila je 4 (IQR = 3–6). Najčešći simptomi bili su kašalj (100%) i febrilnost (89%). Prosečna saturacija kiseonikom pri prijemu bila je 85,3% (SD 9,0). Auskultatorni nalaz u vidu zvižduka u odsustvu hroničnog plućnog oboljenja bio je prisutan kod 10 (15,6%) bolesnika. Leukopenija je bila evidentirana kod 23 (35,9%) bolesnika, a trombocitopenija kod 14 (21,9%). Aspartat aminotransferaza bila je povišena kod 41 (64,1%) bolesnika, alanin

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aminotransferaza kod 32 (50%), a kreatin kinaza kod 36 (56,2%) bolesnika. Zasenčenja na prvom radiogramu bila su u najvećem broju slučajeva mrljasta, a prosečan broj zasenčenih plućnih polja bio je 1 (IQR = 0–3). Bolesnici kod kojih je došlo do smrtnog ishoda imali su stastistički značajno viši MEWS skor na prijemu (p = 0,0001), nižu saturaciju (p = 0,001), više zasenčenih plućnih polja na inicijalnom radiografu (p = 0,006), zvižduke u odsustvu hroničnog plućnog oboljenja (p = 0,02) i povišenu vrednost aspartat aminotransferaze (p = 0.02) i kreatin kinaze (p = 0,03) u serumu. Akutni respiratorni distres razvio se kod 21 (32,9%) bolesnika, a mehanička ventilacija bila je neophodna kod 23 (36,1%) bolesnika. Septični šok razvio se kod 12 (18,7%) bolesnika, a 19 (29,7%) njih je imalo multiorgansku disfunkciju. Ukupan bolnički mortalitet bio je visok – 20,3% (95% CI, 11,3–32,2%; n = 13), a naročito među bolesnicima kod kojih je bila neophodna mehanička ventilacija – 56,5% (95% CI, 36,8–74,4%). **Zaključak.** Pravovremeno započinjanje antivirusne terapije i rano prepoznavanje kritično obolelih su važni faktori za smanjenje mortaliteta.

Ključne reči:
influenca A virus, podtip H1N1; faktori rizika; znaci i simptomi; dijagnoza; lečenje, ishod.

Introduction

Although the year 2009 was largely marked by Influenza A pandemic, this was not neither the first nor the most serious manifestation of this communicable disease. During the year 1918 Spanish flu infected one third of the world population and took the toll of 40 million lives [1]. The expected trend of three pandemics in one hundred years was confirmed by the flu pandemics in 1957 and 1968 [2]. As for Influenza A (H1N1) or the swine flu virus re-emergence during the past 50 years, it first infected about 200 people in New Jersey during 1976. The fear of pandemic resulted in a rigorous vaccine campaign in the USA. Consequently, in October of 1976 more than 40 million people were vaccinated against Influenza A-New Jersey-1976-H1N1 virus [3]. Then followed an epidemic caused by Influenza A/USSR/90/77 (H1N1) virus in Russia during the flu season 1977–1978, when mostly people younger than 23 years of age were infected. The explanation lies in the fact that similar virus subtype had circulated during 1947 flu season, thus providing an acquired immunity for older people.[4] Influenza A (H1N1) re-emerged in the human population during 2009. Patient zero is believed to be a six-month old girl from northern Mexico, who was hospitalized on February 24. The disease spread rapidly, so that on June 11, 2009 the World Health Organization (WHO) declared phase 6 pandemic [5].

Flu symptoms are well-known but non-specific: cough, sore throat, fever, fatigue, headache, and sometimes nausea and diarrhea. During the epidemic laboratory confirmation by the real time polymerase chain reaction (PCR) test (rt-PCR) was necessary only in severe forms of the disease, while every patient with Influenza-like-illness who was positive for Influenza A was considered a probable case of novel Influenza A (H1N1) virus infection. A suspected case of novel Influenza A (H1N1) included persons with an influenza-like-illness who lived in a region with one or more confirmed or probable cases [6]. The infection is airborne, and the critical distance from the source to the potential recipient is set at 1.83 m. Incubation period is from one to seven days. It is believed that most of the infected people are contagious up to seven days after the first symptoms, while some patients remain contagious up to 10 days. Severe forms of infection require hospitalization, and they are characterised by dyspnea, hypoxia, cyanosis, hemoptysis, chest pain and hypotension [7]. In these patients respiratory insufficiency can progress quickly, leading to acute respiratory distress syndrome, which requires the use of mechanical ventilation.

The aim of this study was to describe characteristics, laboratory findings, clinical presentation and treatment outcome in hospitalized patients with Influenza A (H1N1) infection.

Methods

The study was performed at the Institute for Pulmonary Diseases of Vojvodina in the town of Sremska Kamenica. Data were collected retrospectively or prospectively on all patients with 2009 Influenza A (H1N1) infection hospitalized at the Intensive Care Unit (ICU) or High Dependency Unit (HDU) between November 6, 2009 and April 13, 2010. There was no need for *a priori* informed consent because of the non-interventional study design.

Eligible patients included all the patients admitted to the ICU or HDU with confirmed, probable or suspected infection, according to the case definitions developed by WHO [7]. Nasopharyngeal swabs, that is tracheal aspirates in the intubated patients, were taken upon admission and sent to the referent national laboratory at the Serbian Institute for Public Health "Dr Milan Jovanović Batut". Laboratory confirmation was performed by the rt-PCR method.

The score used for a quick evaluation of disease severity at admission was modified early warning score (MEWS). MEWS is a simple physiological score which includes all the vital parameters (heart rate, respiratory rate, arterial blood pressure, body temperature, consciousness level, diuresis). It is accepted that the MEWS value above 4 at admission or an increase by two points during the hospitalization demands urgent physician's attention [8].

All the patients uderwent chest radiography at admission. Patchy opacities were noted and their distribution recorded among the six lung fields: left and right basal, middle and upper field, where an imaginary line is drawn along the lower margin of anterior portions of the 2nd and the 4th rib.

In order to identify the risk factors for death, the patients were classified as "survivors" and "non-survivors" and tested for the differences in the baseline characteristics: a 2-sample *t*-test or the Mann Whitney test was used for continuous variables as appropriate and the χ^2 test or Fisher exact

test was used for discrete variables. Descriptive statistics included frequency analysis (percentages) for categorical variables and means and standard deviations or medians and interquartile ranges (IQRs) for continuous variables.

Results

This study included 64 patients. Influenza A (H1N1) infection was confirmed by the rt-PCR in 50 (78.1%) patients, 7 (10.9%) patients had a probable infection and 6 (9.4%) patients suspected infection, according the the WHO case definitions [7]. There was an equal number of male and female patients. Their mean age was 46 years (SD ± 12.1year). Age distribution of the patients is shown in Figure 1.

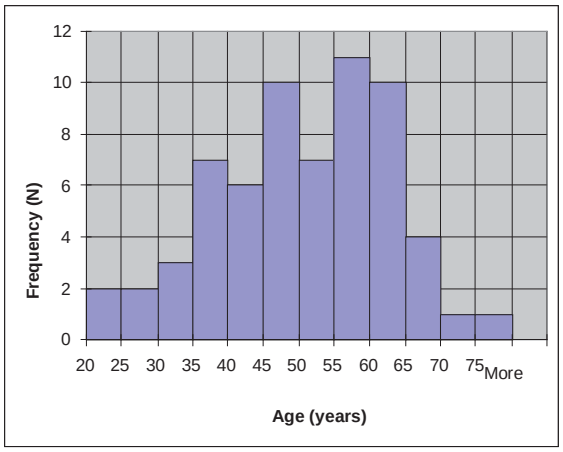

Fig. 1 – Age distribution of 64 patients with Influenza A (H1N1) infection

At presentation, comorbidities were present in 37 (58%) patients. Chronic lung disease was present in 15 (23.4%) patients, arterial hypertension in 22 (34.4%) patients and cardiac disease in 7 (10.9%) patients. There were 4 (6.2%) patients with diabetes and 3 (4.7%) patients with malignancy. Seventeen (27%) patients were smokers. None of the patients were vaccinated against influenza.

Also, there were 29 (47.5%) obese patients. This number excludes three pregnant patients, since body mass index (BMI) cut-off value is not applicable in pregnancy. One patient was in the fourth gestation week, and two were in the 33rd week.

The median time from symptom onset to hospital admission and antiviral therapy initiation was 5 days (IQR = 4–7 days). At admission, the median MEWS was 4 (IQR = 3–6). The most common presenting symptoms were cough (100%) and fever (89%). Median body temperature of febrile patients at admission was 39 ^{o}C (IQR 38.8 ^{o}C–40 ^{o}C). Mean oxygen saturation at admission was 85.3% (SD ± 9.0).

The diverse auscultatory findings included: only crackles in 41 (53.1%) patients, crackles and wheezing in 10 (15.6%) patients, only wheezing in 5 (7.8%) patients, and normal auscultatory finding in 8 (12.5%) patients. Out of the 15 patients with wheezing, only five had either asthma or a chronic obstructive pulmonary disease.

The predominant radiographic findings on initial chest radiographs were patchy consolidations, which were present in 45 (71.4%) patients. Other 18 (28.6%) patients only had marked interstitium on initial chest radiographs. Patchy opacities were noted and their distribution recorded among the six lung fields, where the median number of the lung fields involved was 1 (IQR = 0–3). Figure 2 shows the initial chest radiograph of a patient with acute respiratory distress syndrome (ARDS).

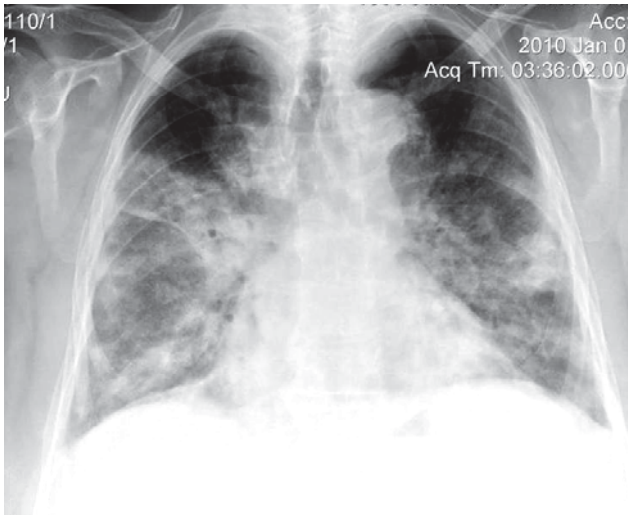

Fig. 2 – Initial chest radiography of a patient with acute respiratory distress syndrome (ARDS) caused by Influenza A (H1N1)

Leukopenia at admission was noted in 23 (35.9%) patients, and thrombocytopenia in 14 (21.9%) patients. Leukocytosis was recorded in 10 (15.6%) patients, 9 of which also had high procalcitonin and relative neutrophilia in differential blood count, which was interpreted as a marker of secondary bacterial infection. Aspartate aminotransferase (AST) values were elevated in 41 (64.1%) patients, alanine aminotransferase (ALT) in 32 (50%) patients, while creatine kinase (CK) was elevated in 36 (56.2%) patients.

Invasive mechanical ventilation was required in 23 (35.9%) patients. Non-invasive ventilation was attempted in 2 patients with ARDS, but these patients ultimately required invasive ventilation. ARDS was noted in 21 (32.8%) patients, while 19 (29.7%) patients had a multi-organ dysfunction syndrome (MODS). Septic shock developed in 12 (18.7%) patients. High frequency oscillatory ventilation was necessary in 9 (14.1%) patients. Continuous renal replacement therapy was given to 2 (3.1%) patients. Overall hospital mortality was high – 20.3% (95% CI, 11.3%–32.2%; n = 13), and especially so among the patients who required mechanical ventilation – 56.5% (95% CI, 36.8%–74.4%).

There were three pregnant patients with severe form of the infection – one in the 4rth and two in the 33rd week. The first pregnant patient died. Other two patients in the 33rd week gave birth to healthy babies by Cesarean section. Cesarean section was performed by obstetricians from Vojvodina Clinical Center in an operation room of the Clinic for Thoracic Surgery at the Institute for Pulmonary Diseases of

Vojvodina. Both babies were born healthy. One patient died nine days after the Cesarean section.

Table 1 shows the differences between survivors and non-survivors. The patients who died had a statistically significant higher MEWS at admission, lower oxygen saturation, more lung fields involved on a chest X-ray, wheezing in the absence of chronic lung disease, elevated creatine kinase and aspartate aminotransferase. As expected, a necessity for an invasive mechanical ventilation, development of ARDS, MODS or septic shock resulted in the increased mortality rate.

Table 1

Comparison of survivors and non-survivors

Parameters	Survivors (n = 51)	Non-survivors (n = 13)	*p*
Age (years), mean (SD)	45 (11.7)	49 (13.7)	0.33
Male sex, n (%)	25 (49)	7 (53.8)	1.0
Comorbidities, n (%)	31 (60.8)	6 (46.1)	0.36
Chronic pulmonary disease, n (%)	11 (21.6)	4 (30.8)	0.48
Ever smoker, n (%)	15 (29.4)	2 (15.4)	0.48
Obesity, n (%)[§]	21 (41.2)	8 (61.5)	0.09
Duration of symptoms prior to hospital admission/antiviral therapy initiation, median (IQR)	5 (4–7)	7 (5.5–8)	0.03*
Involved lung fields, median (IQR)	1 (0–3)	3 (1–4)	0.006*
MEWS at admission, median (IQR)	4 (3–5)	6 (5–7)	0.0001*
Initial O_2 saturation, mean (SD)	87 (7.9)	78 (9.8)	0.0008*
Wheezing, without chronic pulmonary disease, n (%)	5 (9.8)	5 (38.5)	0.02*
Leukopenia, n (%)	16 (31.4)	7 (53.8)	0.19
Thrombocytopenia, n (%)	11 (21.6)	3 (23.1)	1.0
Elevated AST, n (%)	29 (56.9)	12 (92.3)	0.02*
Elevated CK, n (%)	26 (50.9)	11 (84.6)	0.03*
ARDS, n (%)	8 (15.7)	13 (100)	0.0001*
Mechanical ventilation, n (%)	10 (19.6)	13 (100)	0.0001*
Septic shock, n (%)	2 (3.9)	10 (43.5)	0.0001*
MODS, n (%)	6 (11.7)	13 (100)	0.0001*

***Statistically significant; [§]Three pregnant patients excluded; IQR – interquartile ranges; MEWS – modified early warning; AST – aspartat aminotransferase; CK – creatine kinase; ARDS – acute respiratory distress syndrome; MODS – multi-organ dysfunction syndrome**

All the patients received oseltamivir. The dosage and the length of therapy was doubled for the patients with ARDS (150 mg twice a day for 10 days compared to 75 mg twice a day for 5 days). Empiric antibiotic therapy for community acquired pneumonia included combination of a parenteral 3rd generation cephalosporin and azithromycin. Moderate dose of parenteral corticosteroids was given to 18 patients with severe ARDS [partial pressure of oxygen / fraction of inspired oxygen (PaO2/FiO2 < 120 on positive end-expiratory pressure (PEEP ≥ 12)]. All the patients received prophylactic doses of low molecular weight heparin as well as stress ulcus prophylaxis.

Discussion

At the Institute for Pulmonary Diseases of Vojvodina, Influenza A (H1N1) pandemics started on Nov 6, 2009, when the first patient with a severe form of infection was hospitalized. The first patient was young, which turned out to be characteristic for novel swine flu, unlike the previous seasonal influenza. The man age of the patients included in our study was 46, since the Institute provides health care only for adults over 18 years of age. In the study conducted by Dawood et al. [9] the mean age of hospitalized patients was 20, and mean age of those who died 37. The ascceptable explanation is that similar sub-types of viruses were circulating in population until the year 1957, leaving the protective immunity to the persons born before that time [10].

Obesity has been recognized as a risk factor for the severe form of infection [11, 12]. In our study 45% of the patients had a BMI of over 30. Even though we have not been able to prove statistical significance of obesity as a risk factor for lethal outcome, Smith et al. [13] proved, using a rodent model, that Influenza mortality was significantly higher among the rats fed with high lipid diet. Our assumption is that abdominal type of obesity is a risk due to the presence of hypoventilation syndrome. Similarly, late pregnancy has the same effect on lung mechanics as abdominal obesity. Of course, further research is necessary to confirm this hypothesis.

WHO and Center for Disease Control (CDC) stated that patients with chronic diseases have a higher risk of contracting the novel flu. We had 58% patients with at least one chronic condition, of which 41% had a chronic lung disease. Jain et al. [14] studied 272 critically ill patients and found that 73% had a chronic condition, while 38% had asthma. Still, the presence of comorbidities has not proven to be a risk factor for death in our study, which is what made this flu different from the past seasonal flues.

The median MEWS score upon admission was 4 (IQR = 3–6). The score was significantly higher among the non-survivors, which confirms that MEWS is a useful tool for timely recognition of critically ill patients. This was especially important during the pandemic, since our ICU has only five beds, and all other patients had to be placed either in the HDU or in the General Hospital Ward. At one point, there were 8 patients on mechanical ventilators at the same time. Thus, the MEWS helps to identify patients at risk of deterioration who require increased levels of care in the HDU or ICU [8].

As for the initial chest radiographs, our study showed that non-survivors had significantly higher number of the involved lung fields than survivors, proving that the initial chest radiographs have significance in predicting clinical outcome, similar to the study results of Aviram et al. [15].

The median time from the onset of symptoms until hospitalization and initiation of antiviral therapy was 5 days, and there was a statistically significant difference between survivors and non-survivors. Jain et al. [14] found that the only factor related to positive outcome was the initiation of antiviral therapy within 48 hours of symptoms onset. However, only 5 of our patients received antiviral treatment in the mentioned period, and all survived. On the other hand, the use of corticosteroids in severe ARDS is a moot topic. They were recomended in low to moderate dosage regimens in a recent study [16]. However, this remains a contraversial issue.

The only therapy that has proven beneficial for patients with ARDS is "lung protective", mechanical ventilation. Out of 21 patients with ARDS, non-invasive ventilation was initially attempted in 2, but these patients ultimately required invasive ventilation. In a Canadian study of critically ill patients with H1N1 infection, non-invasive mechanical ventilation was atempted in 55 patients, while 85% of them ultimately required invasive ventilation [17]. The use of non invasive mechanical ventilation in ARDS may be an option for conscious patients with hemodynamic stability, but this is a moot topic and further research is warranted.

Conclusions

The patients with severe forms of 2009 influenza A H1N1 infection in Vojvodina were younger than the patients with severe forms of influenza infection during the previous years. Chronic pulmonary diseases and obesity were predominant among the hospitalized patients. None of the patients with a severe form of the disease were vaccinated against influenza. Non-survivors had a significantly higher MEWS score at admission, lower oxygen saturation, more lung fields involved on an initial chest radiograph, wheezing in the absence of a chronic lung disease and elevated serum levels of AST and CK. ARDS, MODS or septic shock were frequently observed. The overall hospital mortality was high. Timely initiation of antiviral therapy and early recognition of critically ill are important factors for reducing mortality.

Comment

The pandemic was declared over on Aug 10, 2010. It is expected that the same virus will continue to circulate in the upcoming flu seasons. However, thanks to the newly acquired protective immunity in the majority of population, the percentage of severe forms of the disease should be smaller, and the age pattern of critically ill is expected to return to the previous seasonal flu range.

REFERENCES

1. *Taubenberger JK, Morens DM.* 1918 Influenca: the mother of all pandemics. Emerg Infect Dis 2006; 12(1): 15−22.
2. *Morens DM, Taubenberger JK, Fauci AS.* The persistent legacy of the 1918 influenza virus. N Eng J Med 2009; 361(3): 225−9.
3. *Bartlett JG.* 2009 H1N1 Influenza - Just the facts: Clinical Features and Epidemiology. Available from : http://www.medscape.com/viewarticle/709540.
4. H1N1 Influenza (Swine Flu): eMedicine Infectious Diseases [updated 2012 May 9]. Available from: http//www.emedicine.medscape.com/article/1807048.
5. *World Health Organization.* Current WHO phase of pandemic alert. Geneva: world Health Organization; 2009.
6. CDC Interim guidance for clinicians on identifying and caring for patients with swine-origin Influenza A (H1N1) virus infection. [cited 2009 May 4]. Available from: *http://www.cdc.gov/flu/keyfacts.htm.*
7. *World Health Organization.* Clinical management of human infection with new influenza A (H1N1) virus initial guidance 2009. Geneva: World Health Organization; 2009.
8. *Subbe CP, Kruger M, Rutherford P, Gemmel L.* Validation of a modified Early Warning Score in medical admissions. QJM 2001; 94(10): 521−6.
9. *Dawood FS, Jain S, Finelli L, Shaw MW, Lindstrom S, Garten RJ,* et al. Emergence of a novel swine-origin influenza A (H1N1) virus in humans. N Engl J Med 2009; 360(25): 2605−15.
10. *Itoh Y, Shinya K, Kiso M, Watanabe T, Sakoda Y, Hatta M,* et al. In vitro and in vivo characterisation of new swine-origin H1N1 influenza viruses. Nature 2009; (460): 1021−5.
11. *Louie JK, Acosta M, Winter K, Jean C, Gavali S, Schechter* R, et al. Factors associated with death or hospitalization due to pandemic 2009 influenza A(H1N1) infection in California. JAMA 2009; 302(17): 1896−902.
12. *Kelly HA, Grant KA, Williams S, Fielding J, Smith D.* Epidemiological characteristics of pandemic influenza H1N1 2009 and seasonal influenza infection. Med J Aust 2009; 191(3): 146−9.
13. *Smith AG, Sheridan PA, Harp JB, Beck MA.* Diet-induced obese mice have increased mortality and altered immune responses when infected with influenza virus. J Nutr 2007; 137(5): 1236−43.
14. *Jain S, Kamimoto L, Bramley AM, Schmitz AM, Benoit SR, Louie J,* et al. Hospitalized patients with 2009 H1N1 influenza in the United States, April-June 2009. N Engl J Med 2009; 361(20): 1935−44.
15. *Aviram G, Bar-Shai A, Sosna J, Rogowski O, Rosen G, Weinstein I,* et al. H1N1 influenza: initial chest radiographic findings in helping predict patient outcome. Radiology 2010; 255(1): 252−9.
16. *Meduri GU, Golden E, Freire AX, Taylor E, Zaman M, Carson SJ,* et al. Methylprednisolone infusion in early severe ARDS: results of a randomized controlled trial. Chest 2007; 131(4): 954−63.
17. *Kumar A, Zarychanski R, Pinto R, Cook DJ, Marshall J, Lacroix J,* et al. Critically ill patients with 2009 influenza A(H1N1) infection in Canada. JAMA 2009; 302(17): 1872−9.

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The effects of physical training on cardiovascular parameters, lipid disorders and endothelial function

Uticaj fizičkog treninga na kardiovaskularne parametre, lipidne poremećaje i endotelnu funkciju

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Abstract

Bacground/Aim. Regular physical activity is widely accepted as factor that reduces all-cause mortality and improves a number of health outcomes. The aim of this study was to investigate the effects of aerobic exercise training on cardiovascular parameters, lipid profile and endothelial function in patients with stable coronary artery disease (CAD). **Methods.** The study included seventy patients with stable CAD. All the patients were divided into two groups: the group I – 33 patients with CAD and with regular aerobic physical training during cardiovascular rehabilitation program phase II for 3 weeks in our rehabilitation center and 3 weeks after that in their home setting, and the group II (control) – 37 patients with CAD and sedentary lifestyle. Exercise training consisted of continual aerobic exercise for 45 minutes on a treadmill, room bicycle or walking, three times a week. We determined lipid and cardiovascular parameters and nitric oxide (NO) concentration at the beginning and after a six-week of training. **Results.** There were no significant differences in body weight, waist circumference and waist/hip ratio at the start and at the end of physical training program. Physical training significantly reduced body mass index after six weeks compared to the initial and control values. Physical training significantly reduced systolic and diastolic blood pressure and heart rate after a six-week training period ($p < 0.05$). Heart rate was significantly lower after a training period as compared to the control ($p < 0.05$). A significant reduction of triglyceride and increased high density lipoprotein cholesterol (HDL-C) concentration after cardiovascular rehabilitation were registered ($p < 0.05$). The concentration of triglycerides was significantly lower while NO and HDL-C were higher after six weeks in the exercise training group ($p < 0.05$). **Conclusion.** Dynamic training can improve blood pressure in patients with moderate to severe hypertension and reduce the need for medication. Exercise programs induced favorable adaptations on lipoproteins profile, cardiovascular parameters and endothelial function which are clinically desirable in primary and secondary prevention of CAD.

Key words:
exercise; cardiovascular system; coronary artery disease; blood pressure; risk assessment; obesity; body mass index.

Apstrakt

Uvod/Cilj. Redovna fizička aktivnost je dobro poznati i prihvaćeni faktor koji snižava ukupan mortalitet i poboljšava ishod mnogobrojnih oboljenja. Cilj rada bio je da se ispita efekat umerenog aerobnog fizičkog treninga na kardiovaskularne pokazatelje, lipidni status i endotelnu funkciju kod bolesnika sa stabilnom koronarnom bolešću (SKBS). **Metode.** Istraživanje je obuhvatilo 70 bolesnika od koronarne bolesti podeljenih u dve grupe [33 bolesnika sa SKBS i redovnim aerobnim fizičkim treningom (tri puta nedeljno po 45 minuta hodanja na traci, vožnje bicikla ili hodanja) tokom šest nedelja i kontrola – 37 bolesnika sa SKBS koji u poslednjih 6 meseci, osim osnovnih kućnih fizičkih aktivnosti, nisu upražnjavali fizički trening)]. Kardiovaskularni, lipidni parametri i koncentracija azot-oksida (NO) određivani su na početku i na kraju ispitivanog perioda i upoređivani između grupa. **Rezultati.** Efekat fizičkog treninga nije se odrazio na promenu telesne mase, obima struka i odnosa struk/kuk. Fizički trening značajno je redukovao indeks telesne mase u odnosu na početne vrednosti i kontrolu ($p < 0,05$). U grupi bolesnika sa fizičkim treningom došlo je do značajnog pada sistolnog i dijastolnog krvnog pritiska i srčane frekvencije ($p < 0,05$) nakon programa rehabilitacije sprovedenog fizičkom aktivnošću. Srčana frekvencija u grupi sa fizičkim treningom bila je značajno manja nakon spro-

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vedenog programa ($p < 0,05$) nego u kontrolnoj grupi. Efekat šestonedeljnog programa kardiovaskularne rehabilitacije na lipidne parametre ogledao se u značajnoj redukciji triglicerida i u porastu NO i lipoproteina velike gustine u grupi sa fizičkim treningom u odnosu na početne vrednosti i vrednosti u kontroloj grupi ($p < 0,05$). **Zaključak.** Fizička aktivnost izaziva povoljne promene kardiovaskualrnih i lipidnih pokazatelja i popravlja parametare endotelne funkcije u sekundarnoj prevenciji koronarne bolesti.

Ključne reči:
vežbanje; kardiovaskularni sistem; koronarna bolest; krvni pritisak; rizik, procena; gojaznost; indeks telesne mase.

Introduction

Regular physical activity and good physical fitness are widely accepted as factors that reduce all-cause mortality and improve a number of health outcomes [1]. Exercise training, major component of cardiac rehabilitation, reduces risk factors, improves functional capacity and prognosis, and enhances psychosocial well-being and quality of life in patients suffering from coronary artery disease (CAD) [2]. It has been shown that low maximal aerobic capacity is closely related to an increase of untoward cardiac events [3]. Therefore, physical training has been proposed to reduce these events by improving aerobic capacity. Nevertheless, a paradox seems to arise when considering that aerobic exercise is closely related to higher oxygen consumption and, thus, more pronounced oxidative stress which can be defined as an increase in the intracellular steady state concentration of oxidants over physiological values [4].

In the groups of well-trained persons, the prooxidant state was counteracted by an increase in hydrosoluble, liposoluble and enzymatic antioxidants. The other observed change was the increase in high density lipoproteins (HDL) capacity to inhibit low density hipoproteins (LDL) oxidation. These change were attributed to an adaptative response, to the deleterious effect associated to aerobic physical activity [5].

However, the association of training response and cardiovascular autonomic function is largely unknown. Laitinen et al. [6] have shown that blood pressure (BP) variability (BPV) represents predominantly sympathetic modulation of cardiovascular regulation. In addition, they found that age, gender, body mass index (BMI) and BP are significant independent determinants of BPV. BPV and heart rate variability are shown to be an independent predictors of cardiovascular mortality and indicators of autonomic cardiovascular function [6].

In assessing exercise, both the frequency and the intensity of exercise are important. Exercise training is generally categorized as being of: low intensity (less than 45% of maximal oxygen uptake), moderate intensity (45%–60% of maximal oxygen uptake), vigorous intensity (61%–75% of maximal oxygen uptake) and strenuous intensity (greater than 75% of maximal oxygen uptake). Moderate-intensity exercise, for example, corresponds to an exercise that elicits 60%–70% of maximal heart rate (about 110 to 125 beats/min) [7].

With the growing knowledge of the endothelial function importance, the endothelium has become a major target for therapeutic interventions. Apart from pharmacological interventions with ACE inhibitors and statins, exercise training has evolved as an accepted therapy to improve endothelial function. The results show that only regular moderate physical activity promotes an antioxidant state and preserves endothelial function. Thus, exercise may have a beneficial effect on the development of cardiovascular disease through preserving endothelial function [8].

Impaired endothelial function is detectable in patients with diseases associated with vascular complications. An important functional consequence of endothelial dysfunction is the inability to release nitric oxide (NO), the vasodilator of the underlying vascular smooth muscle cells [9].

The aim of this study was to investigate the effects of aerobic exercise training on cardiovascular parameters, lipid profile and endothelial function in patients with stable CAD participating in a cardiovascular rehabilitation exercise program.

Methods

The study included 70 patients with stable coronary heart disease who had been accepted into the outpatient phase II cardiovascular rehabilitation program at the Institute for Treatment and Rehabilitation of Cardiovascular Diseases Niška Banja, Niš, Serbia. All the patients were divided into two groups: the group I – 33 patients with stable coronary heart disease who had regular aerobic physical training during 3 weeks of cardiovascular rehabilitation program phase II in cardiovascular rehabilitation center and 3 weeks after that at their homes; the group II (control) – 37 patients with stable coronary heart disease who practiced only usual housework without recommended aerobic physical training 6 weeks before examination.

The patients participated in the study if they had a history of any of the following: myocardial infarction (MI), coronary revascularization, angiographic evidence of more than 50% stenosis in one or more coronary vessels. The subjects were excluded from the study if they had uncontrolled arrhythmias, hypertension (systolic BP >180 mmHg or diastolic BP >100 mmHg), unstable angina pectoris, poorly controlled congestive heart failure, abnormal hemodynamic response or ischemic electrocardiogram changes during stage 1 of the exercise tolerance test (Bruce protocol), or uncontrolled metabolic disease (e.g. uncontrolled diabetes or thyroid disease).

All cardiac-related medication doses were kept constant throughout the study. All the subjects used beta-blockers, ACE inhibitors and statins in their therapy. The patients were required to refrain from any change in their habitual diet.

Exercise training protocol

The patients underwent 6 weeks of aerobic exercise training consisting of 45 min sessions of continuous aerobic

exercise on a treadmill, ergo bicycle or walking. The intensity was maintained at 70%–80% of the individual maximum heart rate obtained in the pre-study graded exercise test. All the patients trained 3 times a week for 6 weeks.

Biochemical examinations were done at the beginning and after 6 weeks of physical exercise training and compared with the control group. In the first group blood samples were collected at least 24 h after the last bout of exercise in order to avoid immediate effects of exercise. The lipids status comprised determination of total cholesterol, LDL cholesterol, HDL cholesterol and triglycerides.

Cardiovascular parameters comprised measuring of systolic and diastolic arterial tension. Upon arrival, the subjects remained in sitting position for 5 min before the start of measuring. BP was measured in the left upper arm by auscultation (sphygmomanometer and stethoscope, Becton Dickinson, USA) three times accordingly to the American Heart Association procedure and the average values were adopted [6].

Besides arterial tension, measurements simultaneously determined heart rate.

Anthropometry

Fasting body weight, body height, waist circumference and hip circumference were measured. BMI was calculated as kg/m^2. We also calculated waist/hip ratio.

Endothelial function

NO release was determined spectrophotometrically by measuring the accumulation of its stable degradation products nitrite and nitrate. Total nitrite was then determined spectrophotometrically by using the Navarro-Gonzalvez et al, reaction based on Griess reaction [10]. A total nitrate and nitrite concentration was given in nmol/mg protein.

The data were analyzed by standard descriptive methods (mean, standard deviation and percent frequency). The results were analyzed by using the Student's *t*-test, χ^2 test and Fisher test depending on specimens and the type of data. Statistical analyzes were done by the software package SPSS 11.0.

Results

The patients in both groups had similar age, gender distribution and risk factors profile (Table 1).

There were no significant changes in body weight, waist circumference and waist/hip ratio before and after the exercise training program as well as in their values between the groups. Physical training significantly reduced BMI after 6 weeks compared to the initial and control values ($p < 0.05$) (Table 2).

Table 1

Characteristics of the patients

Parameters	The exercise training group	The control group	*p*
Male/female (n)	14/19	18/19	ns
Age (years)	57.2 ± 5.9	59.3 ± 7.1	ns
MI, n (%)	22 (66)	26 (70)	ns
CAB, n (%)	5 (15)	4 (11)	ns
PTCA, n (%)	6 (19)	7 (19)	ns
Risk factors for CAD, n (%)			
smokers	13 (39.4)	16 (43.2)	ns
hypertension	28 (84.8)	30 (81.1)	ns
dyslipidemia	14 (42.4)	15 (40.5)	ns
diabetes mellitus	11 (33.3)	13 (35.1)	ns
obesity	10 (30.3)	10 (27.0)	ns
family history for CAD	14 (42.4)	15 (40.5)	ns

Data are presented as the mean ± SD or n (%); MI – myocardial infarct; PTCA – percutaneous transluminal coronary angioplasty; CAB – coronary artery bypass; CAD – coronary artery disease; ns – non significant

Table 2

Anthropometric data, cardiovascular lipid parameters and NO concentrations

Parameters	The exercise training group ($\bar{x}$ ± SD)		The control group ($\bar{x}$ ± SD)	
	before	after	before	after
Body weight (kg)	79.1 ± 12.1	74.7 ± 11.3	73.3 ± 10.6	68.9 ± 10.3
Body hight (cm)	163.5 ± 7.1	-	165.9 ± 5.4	-
Body mass index (kg/m^2)	29.7 ± 5.2	28.2 ± 4.4*†	26.3 ± 4.7	24.8 ± 3.2
Waist circumference (cm)	94.9 ± 10.4	93.7 ± 9.6	88.2 ± 7.6	88.8 ± 6.5
WHR	0.88 ± 0.04	0.87 ± 0.04	0.89 ± 0.05	0.88 ± 0.04
Sistolic arterial tension (mmHg)	143.4 ± 5.6	135.7 ± 4.2†	138.8 ± 4.3	134.1 ± 6.2
Diastolic arterial tension (mmHg)	90.2 ± 6.5	82.8 ± 5.1†	87.3 ± 6.3	84.7 ± 7.1
Heart rate (/min)	78.6 ± 11	72.3 ± 5.6*†	76.1 ± 9.2	77.4 ± 6.8
Total cholesterol (mmol/L)	5.4 ± 0.8	5.2 ± 0.7	5.3 ± 0.4	5.1 ± 0.9
LDL-C (mmol/L)	3.3 ± 0.6	3.0 ± 0.28	3.6 ± 0.3	3.4 ± 0.6
HDL-C (mmol/L)	0.98 ± 0.2	1.21 ± 0.2*†	1.0 ± 0.3	0.99 ± 0.2
Triglicerides (mmol/L)	1.78 ± 0.5	1.58 ± 0.3*†	1.7 ± 0.2	1.8 ± 0.4
NO (nmoL/mg prot.)	64.95 ± 23.15	72.43 ± 28.65*†	68.5 ± 17.9	65.7 ± 19.6

***$p < 0.05$ *vs* control; †$p < 0.05$ *vs* initial values; WHR – waist/hip ratio; LDL-C – low density lipoprotein cholesterol; HDL-C – high density lipoprotein cholesterol; NO – nitric oxide**

Exercise training induced significant reduction in systolic and diastolic BP and heart rate after 6 weeks of cardiovascular rehabilitation ($p < 0.05$). Similar trends were not registered in the control group. In the patients with moderate aerobic physical training significantly lower heart rate was registered after a 6-week follow-up compared to the group with sedentary lifestyle ($p < 0.05$) (Table 2).

The effects of a 6-week cardiovascular rehabilitation on lipid parameters were visible in a significant reduction in triglycerides and significant increase in HDL cholesterol concentration ($p < 0.05$). The concentrations of triglycerides were significantly lower and HDL cholesterol significantly higher after a 6-week follow-up in the exercise training group as compared to sedentary patients ($p < 0.05$) (Table 2).

Regular moderate exercise training within 6 weeks induced favorable increase in NO compared with its initial level and the level in the sedentary control group (Table 2).

Discussion

Anthropometric parameters did not show any significant difference in the groups of patients with similar age and gender distribution. Meanwhile, comparison of the initial values with those at the end of a 6-week follow-up showed a significant reduction in obesity without changes in the degree of visceral obesity. Similar findings of anthropometric parameters value after 6 weeks of physical training are presented by other authors [11], showing no important reduction in the degree of visceral obesity measured by waist circumference and waist/hip ratio.

Moderate aerobic physical training reduced systolic and diastolic BP. The BP values were very similar between the examined groups at the start of examination. Only significantly lower heart rate was registered in the group with physical training. BP reduction trend was obvious in both groups but a significant reduction was registered only in the group with physical training.

There is consistent evidence that regular rhythmic physical exercise of the lower extremities decreases both systolic and diastolic BP by 5–7 mmHg independently of weight loss, alcohol intake or salt intake [12]. These results of exercise training did not seem to be affected by the type of aerobic training because several studies that used home training programs found reductions in BP comparable to those in which subjects trained under staff supervision [13].

Thus, it appears that antihypertensive effects of exercise were additive with those of most used antihypertensive medications [14]. It has been shown that chronic NO-deficient hypertension is associated with depletion of antioxidants and oxidative injury of the cardiovascular system. Exercise training normalizes BP by scavenging free radicals/reactive oxygen species (ROS), through up-regulation of cardiac NO and antioxidant systems [15]. It is hypothesized that interaction of exercise training and chronic nitroglycerin treatment would maintain BP through the up-regulation of NO and cardiac antioxidant system in rat [16].

Heart rate was lower in the group with moderate aerobic physical training after 6 weeks compared to starting values and the control group. One similar study conducted by Anton et al. [17] found a reduction of BP and decrease of heart rate in physically-active smokers compared to sedentary ones.

Slight reduction in triglyceride concentration and HDL cholesterol level rising in the group with physical training are in concordance with literature results. Training programs revealed positive adaptations of body composition and lipid profile total cholesterol and triglycerides. Literature data showed decrease in body weight by 1.7%–2.0% after 16 weeks of physical training. Also, exercise groups showed a significantly reduced total cholesterol (-7.0 mg%) and triglycerides (-14.5 mg%) [18]. Similar findings are registred in diabetics with physical acitvity [19].

LDL oxidation is a crucial step in atherosclerosis, process which can be inhibited by HDL by its ROS capable components or associated enzymes like paraoxonase [5].

Exercise training consistently improves NO bioavailability, and the number of endothelial progenitor cells. It also diminishes the level of inflammatory markers, namely proinflammatory cytokines and C-reactive protein [20]. A significantly higher NO concentration in the exercise training group found in this study indicates an improvement in endothelial function. Knowing that exercise and statins therapy reduce inflammatory response and improve other indicators of endothelial function [21] obtained results pushed exercise training programe in front of other therapy modalities in secondary prevention.

Conclusion

Dynamic training can improve BP in patients with moderate to severe hypertension and reduce the need for medication.

Exercise programs induced favorable adaptations to HDL cholesterol, triglycerides, and NO bioavailability.

This study adds an important piece of evidence to the rationale for exercise training in patients with stable CAD: it documents that an optimized medical therapy, along with exercise training as a lifestyle intervention, can be an alternative approach to an interventional strategy in selected motivated patients with stable CAD.

REFERENCES

1. *Ascensão A, Ferreira R, Magalhães J.* Exercise-induced cardioprotection--biochemical, morphological and functional evidence in whole tissue and isolated mitochondria. Int J Cardiol 2007; 117(1): 16−30.

2. *Leon AS, Franklin BA, Costa F, Balady GJ, Berra KA, Stewart KJ,* et al. Cardiac rehabilitation and secondary prevention of coronary heart disease: an American Heart Association scientific statement from the Council on Clinical Cardiology (Subcommittee on Exercise, Cardiac Rehabilitation, and Prevention)

and the Council on Nutrition, Physical Activity, and Metabolism (Subcommittee on Physical Activity), in collaboration with the American association of Cardiovascular and Pulmonary Rehabilitation. Circulation 2005; 111(3): 369−76.

3. *Myers J, Prakash M, Froelicher V, Do D, Partington S, Atwood JE.* Exercise capacity and mortality among men referred for exercise testing. N Engl J Med 2002; 346(11): 793−801.
4. *Banerjee AK, Mandal A, Chanda D, Chakraborti S.* Oxidant, antioxidant and physical exercise. Mol Cell Biochem 2003; 253(1−2): 307−12.
5. *Brites F, Zago V, Verona J, Muzzio ML, Wikinski R, Schreier L.* HDL capacity to inhibit LDL oxidation in well-trained triathletes. Life Sci 2006; 78(26): 3074−81.
6. *Laitinen T, Hartikainen J, Niskanen L, Geelen G, Länsimies E.* Sympathovagal balance is major determinant of short-term blood pressure variability in healthy subjects. Am J Physiol 1999; 276(4 Pt 2): H1245−52.
7. *Pickering TG, Hall JE, Appel LJ, Falkner BE, Graves J, Hill MN,* et al. Recommendations for blood pressure measurement in humans and experimental animals: Part 1: blood pressure measurement in humans: a statement for professionals from the Subcommittee of Professional and Public Education of the American Heart Association Council on High Blood Pressure Research. Hypertension 2005; 45(1): 142−61.
8. *Di Francescomarino S, Sciartilli A, Di Valerio V, Di Baldassarre A, Gallina S.* The effect of physical exercise on endothelial function. Sports Med 2009; 39(10): 797−812.
9. *Fuchsjäger-Mayrl G, Pleiner J, Wiesinger G, Sieder EA, Quittan M, Nuhr MJ,* et al. Exercise training improves vascular endothelial function in patients with type 1 diabetes. Diabetes Care 2002; 25(10): 1795−801.
10. *Navarro-Gonzalvez AJ, Garcia-Benayas C, Arenas J.* Semiautomated Measurement of Nitrate in Biological Fluids. Clin Chem 1998; 44(3): 679−81.
11. *Uusitalo A, Laitinen T, Väisänen S, Länsimies E, Rauramaa R.* Physical training and heart rate and blood pressure variability: a 5-yr randomized trial. Am J Physiol Heart Circ Physiol 2004; 286(5): H1821−6.
12. *Whelton SP, Chin A, Xin X, He J.* Effect of aerobic exercise on blood pressure: a meta-analysis of randomized, controlled trials. Ann Intern Med 2002; 136(7): 493−503.
13. *Nemoto K, Genno H, Masuki S, Okazaki K, Nose H.* Effects of high-intensity interval Walking training on physical fitness and blood pressure in middle-aged and older people. Mayo Clin Proc 2007; 82(7): 803−11.
14. *Nelson ME, Rejeski WJ, Blair SN, Duncan PW, Judge JO, King AC,* et al. Physical activity and public health in older adults: recommendation from the American College of Sports Medicine and the American Heart Association. Circulation 2007; 116(9): 1094−105.
15. *Husain K.* Exercise conditioning attenuates the hypertensive effects of nitric oxide synthase inhibitor in rat. Mol Cell Biochem 2002; 231(1−2): 129−37.
16. *Husain K, Hazelrigg SR.* Oxidative injury due to chronic nitric oxide synthase inhibition in rat: effect of regular exercise on the heart. Biochim Biophys Acta 2002; 1587(1): 75−82.
17. *Anton MM, Cortez-Cooper MY, DeVan AE, Neidre DB, Cook JN, Tanaka H.* Cigarette smoking, regular exercise, and peripheral blood flow. Atherosclerosis 2006; 185(1): 201−5.
18. *Volaklis KA, Spassis AT, Tokmakidis SP.* Land versus water exercise in patients with coronary artery disease: effects on body composition, blood lipids, and physical fitness. Am Heart J 2007; 154(3): 560.e1−6.
19. *Đinđić B, Janković R, Savić T, Bojanić V.* Antilipemic therapy and low total cholesterol problems. Acta Medica Medianae 2004; 43(1): 43−7. (Serbian)
20. *Ribeiro F, Alves AJ, Duarte JA, Oliveira J.* Is exercise training an effective therapy targeting endothelial dysfunction and vascular wall inflammation? Int J Cardiol 2010; 141(3): 214−21.
21. *Djindjić B, Ranković G, Zivić M, Savić T, Spasić M, Bubanj M.* Gender difference in hipolipemic and anti-inflammatory effects of statins in diabetics with coronary artery disease. Vojnosanit Pregl 2009; 66(12): 966−72. (Serbian)

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Evaluation of lipid parameters and bioindices in patients with different stages of chronic renal failure

Određivanje lipidnih parametara i bioindeksa kod bolesnika u različitim stadijumima hronične bubrežne insuficijencije

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Abstract

Background/Aim. Cardiovascular morbidity and mortality are markedly increased in chronic renal failure (CRF). The aim of this study was to evaluate lipid parameters and bioindices in patients with different stages of CRF. **Methods.** In 46 hemodialysed (HD), 50 CRF patients with II, III and IV stage of CRF (non-HD) and 48 control subjects triglycerides (TG), total cholesterol (C), HDL-C, urea, creatinine, creatinuria (standard biochemical methods), apolipoprotein (apo) A-I, apo B, lipoprotein(a), cystatin C (immunoturbidimetric method) were evaluated, and LDL-C, non-HDL-C, LDL-C/HDL-C, non-HDL-/HDL-C, TG/HDL-C, and new bioindices, LTI (lipid tetrad index), logLTI, LPI (lipid pentad index), logLPI, AIP (atherogenic index of plasma), and creatinine clearance were calculated. **Results.** There were significant differences in the levels of TG, HDL-C, LDL-C, non-HDL-C, total C and apo A-I between the HD and non-HD patients, and the HD patients and the controls. LTI and LPI were significantly higher in the HD and non-HD patients compared to the controls ($p < 0.05$), without a good separation by the Box-Whisker plots. The values of TG/HDL-C ratio and AIP were significantly higher in the HD and non-HD-patients compared to the controls ($p < 0.05$), and significantly higher in the HD compared to non-HD patients ($p < 0.05$). AIP > 0.11 was found in 71.7% of the HD, 56% of non-HD and 31.3% of the controls. **Conclusion.** Among lipid parameters and indices, AIP and TG/HDL-C ratio are most suitable for evaluation of lipid disturbances in different stages of CRF. In addition to, non-HDL-/HDL-C, and apoB/A-I ratios, apo A-I, HDL-C and TG are important markers in HD patients. Non-HDL-C is not a suitable marker. LTI and LPI need to be further investigated.

Key words:
kidney failure, chronic; renal, dialysis; lipids; metabolism.

Apstrakt

Uvod/Cilj. U hroničnoj bubrežnoj insuficijenciji (HBI) naročito su povećani kardiovaskularni morbiditet i mortalitet. Cilj studije bio je ispitivanje lipidnih parametara i bioindeksa u različitim stadijumima HBI. **Metode.** Kod 46 bolesnika na hemodijalizi (HD), 50 bolesnika u II, III i IV stadijumu HBI (ne-HD), kao i kod 48 osoba kontrolne grupe određivane su serumske koncentracije ukupnog holesterola (H), HDL-H, triglicerida (TG), ureje, kreatinina, kao i nivo kreatinurije (standardnim biohemijskim metodama), apolipoproteini A-I i B, lipoprotein(a), cistatin C (imunoturbidimetrijskom metodom), i izračunavani su LDL-H, non-HDL-H, odnosi LDL-/HDL-H, nonHDL-/HDL-H, TG/HDL-H, novi bioindeksi lipid tetrada indeks (LTI), logLTI, lipid pentada indeks (LPI), logLPI, AIP (aterogeni indeks plazme), kao i klirens kreatinina. **Rezultati**. Nivoi TG, ukupnog H, HDL-H, LDL-H, non-HDL-H, i apoA-I značajno su se razlikovali kod HD u odnosu na ne-HD bolesnike, kao i u odnosu na kontrolnu grupu. Lipid tetrada indeks i lipid pentada indeks bili su viši kod HD i ne-HD bolesnika u poređenju s kontrolom ($p < 0,05$), ali bez dobrog razdvajanja korišćenjem Box-Whisker grafika. Odnos TG/HDL-H i AIP bili su viši kod HD i ne-HD bolesnika nego kod kontrole ($p < 0,05$), i kod HD nego kod ne-HD ($p < 0,05$). Aterogeni indeks plazme > 0,11 utvrđen je kod 71,7% HD, 56% ne-HD bolesnika, kao i kod 31,3% osoba iz kontrolne grupe. **Zaključak.** Aterogeni indeks plazme i TG/HDL-C najpogodniji su parametri za procenu poremećaja metabolizma lipida u različitim stadijumima HBI. Osim toga, odnosi ne-HDL-/HDL-H, apoB/A-I, kao i vrednosti apoA-I, HDL-H i TG su značajni markeri kod HD bolesnika. LTI i LPI zahtevaju dalja istraživanja.

Ključne reči:
bubreg, hronična insuficijencija; bubreg, dijaliza; lipidi; metabolizam.

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Introduction

Epidemiological data indicate that approximately 10% of adult population have some form of chronic renal disease that eventually may progress into a complete loss of kidney function [1]. However, before that, the majority of patients will die from fatal cardiovascular events, particularly from coronary heart disese [2]. Moreover, cardiovascular mortality is 10 to 30 times higher in hemodialysis patients compared to general population [3].

Dyslipidemia is a well-known traditional risk factor for premature atherosclerosis. According to the current guidelines for the diagnosis and treatment of cardiovascular disease (CVD), it is considered a major risk factor [4]. In renal patients, dyslipidemia is being present in 40–60% [5].

Lipid disturbance in chronic renal failure (CRF) are markedly expressed and specific. Due to the dysregulation of numerous enzymes, apolipoproteins and receptors, maturation and metabolism of HDL and metabolism of triglyceride-rich lipoproteins are decreased [5, 6]. A typical lipid profile in CRF is thus characterized by a combination of quantitative and qualitative abnormalities, including hypertriglyceridemia, decreased HDL-C levels, normal, mildly increased, or even mildly decreased total and LDL cholesterol levels, and an abnormal lipid subfraction profile with a predominance of atherogenic low-dense LDL (sd LDL) and HDL particles [7]. The retention of lipoprotein particles of modified structure and size occurs already in the early, pre-dialysis stage, before the elevation of plasma lipids, and persists even after successful transplatation [8]. Plasma lipids and apolipoproteins may hence be unreliable predictors of the cardiovascular risk in CRF-induced dyslipidemia, especially in the early stages of kidney disease.

The mechanism underlying the atherogenic lipoprotein profile associated with renal failure involves delicate metabolic interrelations within the whole lipoprotein system, which is typically present when major traditional risk factors such as hypertension, glucose intolerance and abdominal obesity co-occur [9, 10].

For such patient populations calculations of novel bioindices have recently been proposed, such as lipid tetrad index (LTI), lipid pentad index (LPI) [11, 12] and atherogenic index of plasma (AIP) [13], whose formulas comprise several lipid parameters (total cholesterol, triglycerides, HDL-C, apo A, apo B and Lp(a)). In addition, AIP is an indirect indicator of sdLDL levels [13].

Considering that there are no data about these approaches in CRF patients, the aim of this research was to evaluate the new lipid bioindices (LPI, LTI and AIP) and traditional lipid parameters and ratios (total cholesterol, triglycerides, LDL-C, HDL-C, non-HDL-C, Lp(a), apo A-I and B, apo B/A-I, LDL-C/HDL-C, non-HDL-/HDL-C, triglycerides/HDL-C) in patients with different stages of CRF (hemodialysed and non-hemodialysed).

Methods

The study was carried out in the Clinical Center of Vojvodina, Novi Sad, Serbia in the period January–December 2009. The study had previously been approved by an institutional ethics committee and all the subjects included had consented to participate. A total of 144 subjects were included – 96 with CRF [46 on chronic hemodialysis (HD) and 50 non-hemodialysed (non-HD) patients in different stages of CRF] and 48 controls with normal creatinine clearance (CrCl) and without hypolipemic or antihypertensive therapy.

The inclusion criteria were CRF of various etiologies and stages and chronic hemodialysis treatment lasting more than 18 months. Blood samples were collected on the first day of weekly hemodialysis, before connecting the patient with the dialyser and administering heparin. Patients with nephrotic syndrome, acute infection, liver disease, malignancies, previous or recent myocardial infarction, stroke or peripheral arterial disease were excluded from the study.

Analyses were performed immediately after blood-sampling. Serum levels of creatinine, urea, total C, and HDL-C, TG and creatinuria were measured using the standard biochemical methods, and apo A-I and B, Lp(a) and cystatin C by immunoturbidimetry.

Creatinine clearance was calculated by the formula:

$CrCl = [U_{Cr} \times 24\ h\ urine\ volume\ (ml)]/[S_{Cr} \times 1440\ (min)]$; Note: U_{Cr} urine creatinine (μmol/L) and S_{Cr} – serum creatinine (μmol/L)

The obtained values were normalized to 1.73 m^2 body surface area, sex and age. A deviation above 10% from the expected value in the past 6 months was taken as a criterion for the existence of CRF.

We calculated the values of LDL-C, non-HDL-C, and traditional indICes LDL-/HDL-C, non-HDL-/HDL-C, TG/HDL-C, and new bioindexes Lipid tetrad index (LTI) = TG x total C x Lp(a)/HDL-C; Lipid pentad index (LPI) = TG x total C x Lp(a) x apo B/A-I; Atherogenic index of plasma (AIP) = log (TG/HDL-C). All the parameters were translated into the SI units. The patients were classified accordingly into three risk categories for AIP: low < 0.11, intermediate 0.11–0.21, and high > 0.21 [14]. Since the indices LPI and LTI did not have normal distributions, we used variables logLPI and logLTI.

In addition, body mass index (BMI) was calculated for all subjects [15].

Descriptive statistics, including median, arithmetic mean, standard deviation (SD) and standard error (SE) were used to describe the studied parameters. The differences in distributions of individual parameters between study groups were analyzed using the parametric Student's *t*-test, or the non-parametric Mann-Whitney test in case a distribution showed a significant deviation. Linear regression analysis and Pearson coefficient of linear correlation were used to study the correlation between variables. The differences between the groups were illustrated using Box and Whisker plots and empirical distribution functions. Statistical analysis was performed using the Statistica 8.0 software. A value of $p < 0.05$ was considered statistically significant.

Results

The main characteristics of the study subjects are shown in Table 1. There were significant differences in age and BMI between the HD and non-HD patients.

Laboratory parameters and the calculated indices are presented in Tables 2 and 3.

Total C, TG, HDL-C, LDL-C, non-HDL-C, apo A-I, and non-HDL-/HDL-C levels, and apo B/A-I and TG/HDL-C ratios were significantly different in the HD patients compared with the other two groups. TG/HDL-C ratio was significantly higher in the non-HD patients compared with controls.

Table 1

Basic characteristics of subjects

Basic characteristics	HD patients	Non-HD patients	Control subjects
Number of subjects (f/m)	46 (17/29)	50 (21/29)	48 (19/29)
Age (years), $\bar{x} \pm SD$ (range)	50.4 ± 13.1* (27–76)	56.8 ± 12.1 (24–72)	52.4 ± 10.7 (32–73)
BMI (kg /m^2), $\bar{x} \pm SD$	24.7 ± 5.0*	26.8 ± 4.7	25.8 ± 2.8
Nephroangiosclerosis, n (%)	16 (34.6)	26 (52)	–
Glomerulonephritis chr, n (%)	9 (20)	5 (10)	–
Diabetes mellitus type 2, n (%)	8 (17.3)	3 (6)	–
Nephrolithiasis, n (%)	4 (8.6)	3 (6)	–
IgA nephropathy, n (%)	3 (6.5)	1 (2)	–
Pyelonephritis chr, n (%)	1 (2.2)	5 (10)	–
Other causes, n (%)	5 (10.8)	7 (14)	–

HD – hemodialysis; BMI – Body mass index; *$p < 0.05$ compared to non-hemodialysed patients; Other causes – analgesic nephropathy, polycystic kidney disease, not-recognized nephropaties

Table 2

Laboratory parameters

Parameters	HD patients ($\bar{x} \pm SD$)	Non-HD patients ($\bar{x} \pm SD$)	Control subjects ($\bar{x} \pm SD$)
Urea (mmol/L)	26.6 ± 7.5 ¶,‡	7.9 ± 4.6 * (med. 7.15)	5.3 ± 1.3
Creatinine (μmol/L)	976.9 ± 204.1 ¶,‡	140.6 ± 88.5 ‡ (med. 124.5)	91.9 ± 14.5
CrCl (ml/min/1.73m^2)	–	59.9 ± 26.7 ‡ (med. 57.5)	101.4 ± 15.5
Cystatin C (mg/L)	7.7 ± 1.6 ¶,‡	1.22 ± 0.6 ‡	0.92 ± 0.34
Total cholesterol (mmol/L)	4.6 ± 0.9 ¶,‡	5.33 ± 1.0	5.45 ± 0.79
Triglycerides (mmol/L)	2.1 ± 1.2 †,§	1.79 ± 1.10	1.38 ± 0.73
HDL-C (mmol/L)	1.0 ± 0.2 ¶,‡	1.3 ± 0.5	1.34 ± 0.32
LDL-C (mmol/L)	2.8 ± 0.7 II,‡	3.31 ± 0.8	3.48 ± 0.67
non-HDL-C (mmol/L)	3.6 ± 0.9 §,†	4.03 ± 0.8	4.15 ± 0.72

HD – hemodialysis; CrCl – creatinine clearance; HDL-C – high density lipoprotein cholesterol; LDL-C – low density lipoprotein cholesterol; *$p < 0.05$, †$p < 0.01$, ‡$p < 0.001$ compared to control subjects; §$p < 0.05$, II$p < 0.01$, ¶$p < 0.001$ compared to non-HD patients

Table 3

Laboratory parameters and the calculated ratios

Parameters	HD patients ($\bar{x} \pm SD$)	Non-HD patients ($\bar{x} \pm SD$)	Control subjects ($\bar{x} \pm SD$)
Apo A-I (g/L)	1.1 ± 0.2 ¶,‡	1.3 ± 0.2	1.28 ± 0.14
Apo B (g/L)	0.96 ± 0.2	1.0 ± 0.2	1.03 ± 0.2
Lp (a) (g/L)	0.21 ± 0.3	0.18 ± 0.2	0.2 ± 0.3
LDL-C/HDL-C	2.9 ± 1.1	2.74 ± 0.8	2.71 ± 0.73
nonHDL-C/HDL-C	3.8 ± 1.3 §,*	3.31 ± 0.9	3.31 ± 0.92
Apo B/A-I	0.88 ± 0.3 §	0.76 ± 0.2	0.8 ± 0.22
Triglycerides/HDL-C	2.27 ± 1.7 §,‡	1.6 ± 1.2 *	1.1 ± 0.66
AIP	0.27 ± 0.3 §,‡	0.1 ± 0.3 *	- 0.03 ± 0.27
LTI	1979.2 ± 3118.5 *	2285.2 ± 6248.6 *	1210.8 ± 2412.2
log LTI	6.5 ± 1.6 *	6.4 ± 1.5 *	5.8 ± 1.5
LPI	80.1 x 10^3 ± 15.5 x 10^{5*}	78.1 x 10^3 ± 21 x 10^{5*}	49 x 10^3 ± 107 x 10^5
log LPI	9.98 ± 1.8 *	9.97 ± 1.4 *	9.3 ± 1.7

HD – hemodialysis; HDL-C – high density lipoprotein cholesterol; LDL-C – low density lipoprotein cholesterol; AIP – atherogenic index of plasma, LTI – lipid tetrad index, LPI – lipid pentad index; *$p < 0.05$, †$p < 0.01$, ‡$p < 0.001$ compared to control subjects; §$p < 0.05$, II$p < 0.01$, ¶$p < 0.001$ compared to non-hemodialysed patients.

Distribution of patological values of LDL-/HDL-C, apoB/A-I and non-HDL-/HDL-C ratios in non-HD and HD patients are presented in Table 4.

The values below the upper limit of the confidence interval for the mean of the log LTI (6.23) were recorded in 70.83% of the controls, 46% of the non-HD patients and 36.9% of the HD patients. The values below the upper limit of the confidence interval for the mean of the log LPI (9.80) were recorded in 68.75%, of the controls, 46% of the non-HD patients and 39.13% of the HD patients.

Table 4

Distribution of the patients according to patological values of some lipid indices

Parameters	Categories	non-HD (%)	HD (%)
AIP	> 0.11	46.0	71.7
LDL-/HDL-C	> 3.4	16.0	17.4
Apo B/A-I	> 0.63 (m) > 0.54 (f)	30.0	54.3
Non-HDL-/HDL-C	> 3.25	54.0	62.5

AIP – atherogenic index of plasma; LDL-C – low density lipoprotein cholesterol; HDL-C – high density lipoprotein cholesterol; non-HD – non-hemodialysed patients; HD – hemodialysed patients; m – male; f – female

There was a significant difference in the mean logLPI and logLTI between the controls and the two groups of patients, but not between the HD and non-HD patients (Table 2). This was supported by the non-parametric Mann-Whitney test for LPI and LTI. However, considering great dispersion around both indices their values overlapped. This was illustrated on empirical function distribution plots in Figures 1 and 2.

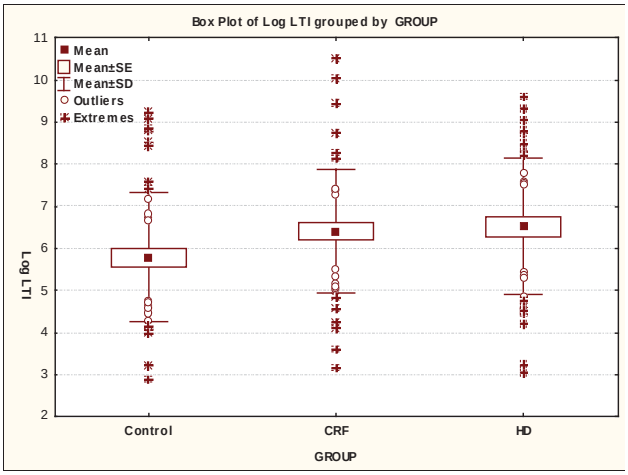

Fig.1 – Box plot of log lipid tetrad index (log LTI)

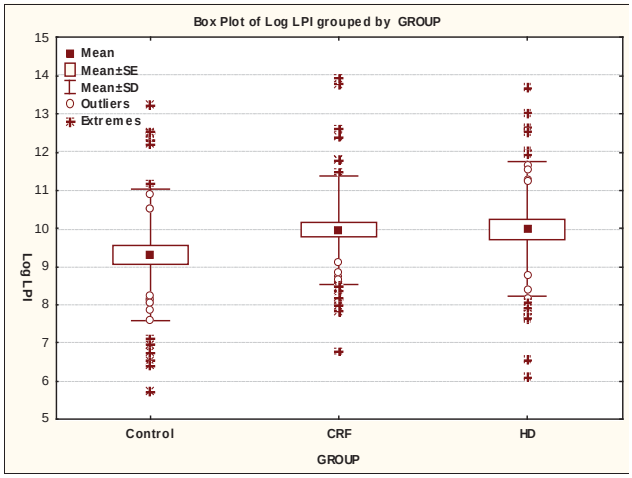

Fig. 2 – Box plot of log lipid pentad index (log LPI)

Similar results were obtained for AIP, however, the significance of the difference in the means in the controls and the HD patients ($p < 0.001$) was higher than in the case of LPI and LTI ($p < 0.05$). Box-plots (Figure 3) showed that most HD patients had AIP over 0.11 (71.7%), which was significantly higher ($p = 0.0001$) compared with the control group, where 31.3% of the subjects had this finding (Table 3).

In the non-HD patients, however, the percentage was insignificantly higher in comparison with the control group (46%, $p = 0.067$).

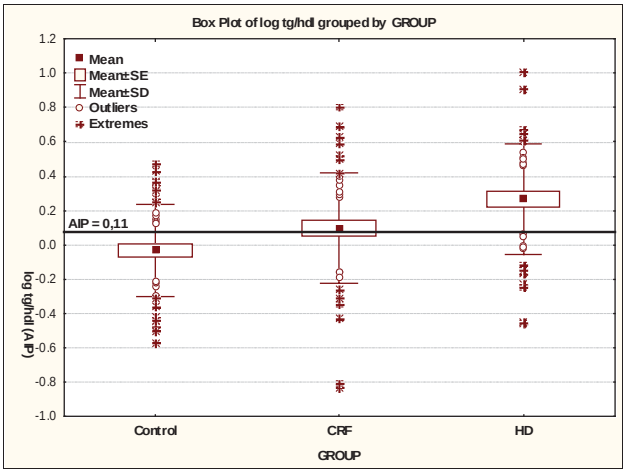

Fig. 3 – Box plot of atherogenic index of plasma (AIP)

Discussion

Cardiovascular morbidity and mortality are present already in mild renal impairment [16, 17] and increased in end-stage renal disease [18].

Among many factors contributing to accelerated atherosclerosis in CRF, dyslipidemia is found in 40–60% patients [5]. Regardless the etiology, patients with CRF develop complex qualitative and quantitative abnormalities, predominantly hypertriglyceridemia and HDL-decrease. In the present

study, the highest triglyceride levels, the lowest HDL-C levels, and the highest values of TG/HDL-C ratio were observed in HD patients. Beside this, the values of TG/HDL-C ratio, that is one of the most potent predictors of cardiovascular disease [8], were significantly higher in the non-HD patients compared with the controls.

More than half of the HD group had high-risk HDL-C levels, which is similar to the results of other authors [19, 20].

Total and LDL cholesterol levels were lowest in the HD patients, compared to the controls and non-HD patients ($p < 0.001$), whereas the levels in the non-HD patients were similar to those of the controls. Most previous studies have also reported similar or lower values [9, 19, 21–23]. Unlike general population, lower plasma cholesterol in CRF has been associated with a higher cardiovascular mortality [24].

Similarly to the results of Schreier at al. [25], the results of our study show that non-HDL-C may not be appropriate marker for risk assessment among CRF patients. The level of non-HDL-C in the HD patients was significantly lower compared with the controls, with no differences among the other groups. The level of non-HDL-C/HDL-C ratio was significantly higher in the HD patients compared with the others groups, mainly due to low HDL-C levels.

More recent research indicates that apolipoproteins are more effective atherogenic markers than plasma lipids: apoA-I is a useful summary index of the antiatherogenic properties of HDL [26–28], apoB of total atherogenic particle number [29–32], and apo B/A-I ratio is better than any cholesterol ratio [26, 33, 34]. In our study, apoA-I was significantly lower only in the HD patients, whereas apoB did not differ significantly, similarly to the results of Alabakovska et al. [19]. The frequency of pathological ratio apoB/A-I was significantly higher than LDL-/HDL-C in both groups of patients in our study.

Although relations between Lp(a) and renal functional status in patients with CRF have been explored in numerous studies, the results obtained are contradictory [19, 35, 36]. In our study, Lp(a) levels in the HD and non-HD patients did not differ significantly from the controls. Furthermore, there was no significant correlation between Lp(a) levels and CrCl ($r = 0.12$, $p = 0.51$), contrary to some other studies dealing with early stages of CRF [37].

Literature data on LTI and LPI in CRF patients are scarce. Despite a significant difference in bioindices between healthy controls and patients with CRF, we did not obtain adequate delineations, which prevented us from determining reference values. An explanation could lie in similar distributions of Lp(a) levels and the existence of extreme pathological values of certain lipid parameters in both controls and patients that is characteristic for our geographic area.

Furthermore, there are no literature data on AIP in patients with CRF. We found the values of > 0.11, which indicated moderate and high atherogenic risk, in 31.3% of the control subjects, 56% of non-HD patients and 71.7% of HD patients. It is significantly different between the groups, particularly controls and HD patients. These findings were expected, since CRF is characterized by the predominance of atherogenic sdLDL particles.

Conclusion

In conclusion, TG, HDL-C, apo A-I, non-HDL-/HDL-C, and apoB/A-I ratios, are important lipid markers only in HD patients. Non-HDL-C is not a suitable marker in CRF patients. Calculations of bioindices LPI and LTI did not show significant benefits in our study population. In contrast, significantly increased pathological values of AIP that we registered in our study in both HD and non-HD patients suggest that this index may have a potential application in routine clinical practice as an indirect indicator of sdLDL levels. Besides AIP, TG/HDL-C ratio could also be a suitable marker for evaluation of lipid disturbances in different stages of CRF.

Acknowledgements

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REFERENCES

1. *Coresh J, Selvin E, Stevens LA, Manzi J, Kusek JW, Eggers P*, et al. Prevalence of chronic kidney disease in the United States. JAMA 2007; 298(17): 2038−47.
2. *Hage FG, Venkataraman R, Zoghbi GJ, Perry GJ, DeMattos AM, Iskandrian AE*. The scope of coronary heart disease in patients with chronic kidney disease. J Am Coll Cardiol 2009; 53(23): 2129−40.
3. *Yilmaz FM, Akay H, Duranay M, Yilmaz G, Oztekin PS, Koşar U*, et al. Carotid atherosclerosis and cardiovascular risk factors in hemodialysis and peritoneal dialysis patients. Clin Biochem 2007; 40(18): 1361−6.
4. *National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III)*. Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report. Circulation 2002; 106(25): 3143−421.
5. *Đurđević-Mirković T, Ćurić S*. Lipid metabolism disturbance in kidney diseases. In: *Đerić M, Stokić E, Todorović-Đilas Lj,* editors. Hyperlipoproteinemias-current aspects. Novi Sad: Physicians Society of Vojvodina-Serbian Physicians Society; 2005. p. 255−60. (Serbian)
6. *Vaziri ND*. Dyslipidemia of chronic renal failure: the nature, mechanisms, and potential consequences. Am J Physiol Renal Physiol 2006; 290(2): F262−72.
7. *Rajman I, Harper L, McPake D, Kendall MJ, Wheeler DC*. Low-density lipoprotein subfraction profiles in chronic renal failure. Nephrol Dial Transplant 1998; 13(9): 2281−7.
8. *da Luz PL, Favarato D, Faria-Neto JR Jr, Lemos P, Chagas AC*. High ratio of triglycerides to HDL-cholesterol predicts extensive coronary disease. Clinics (Sao Paulo) 2008; 63(4): 427−32.
9. *Bayés B, Pastor MC, Bonal J, Juncà J, Hernandez JM, Riutort N*, et al. Homocysteine, C-reactive protein, lipid peroxidation and mortality in haemodialysis patients. Nephrol Dial Transplant 2003; 18(1): 106−12.

10. *Herzog CA, Ma JZ, Collins AJ.* Poor long-term survival after acute myocardial infarction among patients on long-term dialysis. N Engl J Med 1998; 339(12): 799−805.
11. *Das B, Daga MK, Gupta SK.* Lipid Pentad Index: A novel bioindex for evaluation of lipid risk factors for atherosclerosis in young adolescents and children of premature coronary artery disease patients in India. Clin Biochem 2007; 40(1−2): 18−24.
12. *Bogavac-Stanojević N, Jelić-Ivanović Z, Spasojević-Kalimanovska V, Spasić S, Kalimanovska-Ostrić D.* Lipid and inflammatory markers for the prediction of coronary artery disease: a multi-marker approach. Clin Biochem 2007; 40(13−14): 1000−6.
13. *Dobiásová M, Frohlich J.* The plasma parameter log (TG/HDL-C) as an atherogenic index: correlation with lipoprotein particle size and esterification rate in apoB-lipoprotein-depleted plasma (FER(HDL)). Clin Biochem 2001; 34(7): 583−8.
14. *Holmes DT, Frohlich J, Buhr KA.* The concept of precision extended to the atherogenic index of plasma. Clin Biochem 2008; 41(7−8): 631−5.
15. *Tsigos C, Hainer V, Basdevant A, Finer N, Fried M, Mathus-Vliegen E,* et al. Management of obesity in adults: European clinical practice guidelines. Obes Facts 2008; 1(2): 106−16.
16. *Go AS, Chertow GM, Fan D, McCulloch CE, Hsu CY.* Chronic kidney disease and the risks of death, cardiovascular events, and hospitalization. N Engl J Med 2004; 351(13): 1296−305.
17. *Foley RN, Wang C, Collins AJ.* Cardiovascular risk factor profiles and kidney function stage in the US general population: the NHANES III study. Mayo Clin Proc 2005; 80(10): 1270−7.
18. *Chan DT, Irish AB, Dogra GK, Watts GF.* Dyslipidaemia and cardiorenal disease: mechanisms, therapeutic opportunities and clinical trials. Atherosclerosis 2008; 196(2): 823−34.
19. *Alabakovska SB, Todorova BB, Labudovic DD, Tosheska KN.* LDL and HDL subclass distribution in patients with end-stage renal diseases. Clin Biochem 2002; 35(3): 211−6.
20. *Rajman I, Harper L, McPake D, Kendall MJ, Wheeler DC.* Low-density lipoprotein subfraction profiles in chronic renal failure. Nephrol Dial Transplant 1998; 13(9): 2281−7.
21. *Ozsoy RC, Kastelein JJ, Arisz L, Koopman MG.* Atorvastatin and the dyslipidemia of early renal failure. Atherosclerosis 2003; 166(1): 187−94.
22. *Guerrero A, Montes R, Muñoz-Terol J, Gil-Peralta A, Toro J, Naranjo M,* et al. Peripheral arterial disease in patients with stages IV and V chronic renal failure. Nephrol Dial Transplant 2006; 21(12): 3525−31.
23. *Parsons DS, Reaveley DA, Pavitt DV, Misra M, Brown EA.* Lipoprotein (a) levels in those with high molecular weight apo (a) isoforms may remain low in a significant proportion of patients with end-stage renal disease. Nephrol Dial Transplant 2003; 18(9): 1848−53.
24. *Iseki K, Yamazato M, Tozawa M, Takishita S.* Hypocholesterolemia is a significant predictor of death in a cohort of chronic hemodialysis patients. Kidney Int 2002; 61(5): 1887−93.
25. *Schreier L, González AI, Elbert A, Berg G, Wikinski R.* Utility of non-high-density lipoprotein cholesterol in hemodialyzed patients. Metabolism 2004; 53(8): 1013−5.
26. *Walldius G, Jungner I, Aastveit AH, Holme I, Furberg CD, Sniderman AD.* The apoB/apoA-I ratio is better than the cholesterol ratios to estimate the balance between plasma proatherogenic and antiatherogenic lipoproteins and to predict coronary risk. Clin Chem Lab Med 2004; 42(12): 1355−63.
27. *Francis MC, Frohlich JJ.* Coronary artery disease in patients at low risk--apolipoprotein AI as an independent risk factor. Atherosclerosis 2001; 155(1): 165−70.
28. *Luc G, Bard JM, Ferrières J, Evans A, Amouyel P, Arveiler D,* et al. Value of HDL cholesterol, apolipoprotein A-I, lipoprotein A-I, and lipoprotein A-I/A-II in prediction of coronary heart disease: the PRIME Study. Prospective Epidemiological Study of Myocardial Infarction. Arterioscler Thromb Vasc Biol 2002; 22(7): 1155−61.
29. *Vaverkova H, Karasek D, Novotny D, Jackuliakova D, Lukes J, Halenka M,* et al. Apolipoprotein B versus LDL-cholesterol: Association with other risk factors for atherosclerosis. Clin Biochem 2009; 42(12): 1246−51.
30. *Benn M.* Apolipoprotein B levels, APOB alleles, and risk of ischemic cardiovascular disease in the general population, a review. Atherosclerosis 2009; 206(1): 17−30.
31. *Benn M, Nordestgaard BG, Jensen GB, Tybjaerg-Hansen A.* Improving prediction of ischemic cardiovascular disease in the general population using apolipoprotein B: the Copenhagen City Heart Study. Arterioscler Thromb Vasc Biol 2007; 27(3): 661−70.
32. *Chien KL, Hsu HC, Su TC, Chen MF, Lee YT, Hu FB.* Apolipoprotein B and non-high density lipoprotein cholesterol and the risk of coronary heart disease in Chinese. J Lipid Res 2007; 48(11): 2499−505.
33. *Sniderman AD, Furberg CD, Keech A, Roeters van Lennep JE, Frohlich J, Jungner I,* et al. Apolipoproteins versus lipids as indices of coronary risk and as targets for statin treatment. Lancet 2003; 361(9359): 777−80.
34. *Walldius G, Jungner I.* Apolipoprotein B and apolipoprotein A-I: risk indicators of coronary heart disease and targets for lipid-modifying therapy. J Intern Med 2004; 255(2): 188−205.
35. *Labudovik D, Tosheska K, Alabakovska S, Bogdanska J, Todorova BB.* Apo pro tein(a) isoforms and plasma LP(a) concentration in members of four families. J Med Biochem 2008; 27: 439–46.
36. *Cauza E, Kletzmaier J, Bodlaj G, Dunky A, Herrmann W, Kostner K.* Relationship of non-LDL-bound apo(a), urinary apo(a) fragments and plasma Lp(a) in patients with impaired renal function. Nephrol Dial Transplant 2003; 18(8): 1568−72.
37. *Sechi LA, Zingaro L, De Carli S, Sechi G, Catena C, Falleti E,* et al. Increased serum lipoprotein(a) levels in patients with early renal failure. Ann Intern Med 1998; 129(6): 457−61.

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Hemodinamska stabilnost tokom totalne intravenske anestezije propofolom uz koindukciju midazolamom i opšte balansirane anestezije kod laparoskopske holecistektomije

Hemodynamic stability in total intravenous propofol anesthesia with midazolam coinduction *versus* general balanced anaesthesia in laparoscopic cholecystectomy

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Apstrakt

Uvod/Cilj. Iako se smatra da je laparoskopska holecistektomija (LH) minimalno invazivna procedura za hirurga, ona kod bolesnika sa pratećim kardiovaskularnim oboljenjima može biti izazov za anesteziologa. Cilj našeg istraživanja bio je da utvrdimo koji od dva primenjena metoda anestezije, totalna intravenska anestezija propofolom uz koindukciju midazolamom (TIVA) i opšta balansirana anestezija (OBA), obezbeđuje veću hemodinamsku stabilnost tokom LH kod bolesnika koji pripadaju grupi III prema klasifikaciji Američkog društva anesteziologa (ASA). **Metode.** Ispitivanje je obuhvatilo dve grupe po 30 bolesnika. Prva (TIVA) grupa bila je podvrgnuta LH u totalnoj intravenskoj anesteziji propofolom uz koindukciju midazolamom, a druga (OBA) u opštoj balansiranoj anesteziji (midazolam, tiopenton, azot-oksidul i kiseonik). U pet vremenskih intervala praćeni su hemodinamski parametri: srčana frekvencija, sistolni, dijastolni i srednji arterijski pritisak. **Rezultati.** Stepen hemodinamske stabilnosti (odstupanje svakog parametra za manje od 10% od početnih vrednosti) postignut je u grupi TIVA kod svih bolesnika u svim vremenskim intervalima za razliku od OBA grupe ($p < 0,01$). **Zaključak.** Totalna intravenska anestezija obezbeđuje mnogo bolju hemodinamsku stabilnost kod bolesnika grupe III prema klasifikaciji ASA sa pratećim kardiovaskularnim oboljenjima nego OBA.

Ključne reči:
holecistektomija, laparoskopska; intraoperativni period; postoperativne komplikacije; kardiovaskularne bolesti; anestezija, opšta

Abstract

Background/Aim. Laparoscopic cholecystectomy can be a greater challenge for anesthesiologist than for surgeon if the patient is ASA III with concomitant cardiovascular diseases. The aim of our study was to compare the effect of total intravenous anesthesia (TIVA – propofol with midazolam) and general balanced anesthesia (GBA – midazolam, thiopenton, nitrous oxide and O2) on hemodynamic stability in the ASA III patients who underwent laparoscopic cholecystectomy. **Methods.** In our study, 60 patients were randomized into two groups depending on whether they received TIVA or GBA. Heart rate, systolic, diastolic and mean arterial pressure were monitored continuously and recorded in five time intervals. **Results.** Statistical analysis showed that TIVA with propofol provides better hemodynamic stability (less than 10% deviation from basal values for each measured parameter) then GBA group ($p < 0.01$). **Conclusion.** Total intravenous anesthesia with propofol provides better hemodynamic stability for ASA III patients with concomitant cardiovascular diseases then GBA.

Key words:
cholecystectomy, laparoscopic; intraoperative period; postoperative complications; cardiovascular diseases; anesthesia, general.

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Uvod

Laparoskopska holecistektomija (LH) uvedena je u hirušku i anesteziološku praksu krajem osamdesetih godina od strane nemačkih i francuskih autora. Phillipe Mouret je opisao prvu laparoskopsku holecistektomiju u Francuskoj 1987. godine; o prvoj seriji izvestio je Perissat i sar. [1], a tehnika je uvedena u SAD 1988. godine od strane Reddick-a i Olsen-a. Upotreba kompjuterizovane, video-endoskopske opreme omogućila je brzi razvoj endoskopske hirurgije.

Sniženje postoperativnog bola, manja trauma, brži oporavak i kraća hospitalizacija učinili su ovu metodu široko prihvaćenom, tako da holecistektomija izvedena laparoskopskom hirurškom tehnikom predstavlja metod izbora za lečenje nekomplikovane holelitijaze. Laparoskopska holecistektomija je najčešća operacija koja se izvodi laparoskopskom hirurškom tehnikom. Procenat laparoskopske holecistektomije porastao je od 0% 1987. do 80% 1992. godine. S obzirom na smanjen mortalitet i morbiditet kod laparoskopskih procedura, uveden je i prihvaćen pojam „minimalne invazivne terapije". Time se naglašava da je trauma organizma znatno smanjena, uz zadržavanje zadovoljavajućeg terapijskog efekta.

Međutim, postoje specifičnosti anestezije kod endoskopske holecistektomije. Ključni momenti koji izazivaju različite patofiziološke promene kod laparoskopske u odnosu na klasičnu holecistektomiju jesu položaj bolesnika na operacionom stolu i stvaranje povećanog intraabdominalnog pritiska (IAP), takozvanog pneumoperitoneuma insuflacijom ugljen-dioksida u trbušnu duplju. Ovo negativno utiče na kardiovaskularni sistem, odnosno hemodinamsku stabilnost.

Jasno je da će već postojeća ishemija miokarda, insuficijencija ili valvularna mana srca kod bolesnika grupe III prema klasifikaciji ASA (*American Society of Anesthesiologists*) biti pogoršana ekstenzivnošću i specifičnostima laparoskopske holecistektomije. Zbog toga, od izuzetnog značaja je da se utvrdi koji metod anestezije obezbeđuje najveću hemodinamsku stabilnost, o čemu, sudeći prema podacima iz literature, još ne postoji saglasnost autora [2, 3].

Cilj našeg istraživanja bio je da utvrdimo koji od dva primenjena metoda anestezije, totalna intravenska anestezija propofolom uz koindukciju midazolamom (TIVA) i opšta balansirana anestezija (OBA) obezbeđuje veću hemodinamsku stabilnost tokom laparoskopske holecistektomije kod bolesnika koji pripadaju grupi III prema klasifikaciji ASA.

Metode

Ispitivanje je obavljeno u Klinici za anesteziologiju i intenzivnu terapiju Vojnomedicinske akademije (VMA) u saradnji sa Klinikom za abdominalnu i endokrinu hirurgiju VMA na ukupno 60 ispitanika, oba pola, kod kojih je postavljena indikacija za hirurško lečenje holelitijaze laparoskopskom hirurškom tehnikom.

Kriterijumi za izbor ispitanika bili su: dijagnostikovana hronična holelitijaza i postavljenja indikacija za njeno hirurško lečenje laparoskopskom operativnom tehnikom; pripadnost ispitanika grupi III prema klasifikaciji ASA na osnovu prethodno izvršene procene opšteg zdravstvenog stanja (svi ispitanici pripadali su ovoj grupi zbog teškog poremećaja kardiovaskularne funkcije, a neki od njih su, pored navedene, imali i poremećaje funkcije drugih organskih sistema).

Ispitanici su bili podeljeni u dve grupe po 30, prema metodi opšte anestezije koju primaju. Podela ispitanika u grupe obavljena je metodom slučajnog izbora, neposredno po pozivanju bolesnika u operacionu salu.

Prva grupa, TIVA, dobijala je totalnu intravensku anesteziju propofolom uz koindukciju midazolamom i dalje ventilacijom 100% kiseonikom.

Druga grupa, OBA, primala je opštu balansiranu anesteziju (koindukcija midazolam – tiopenton) i dalje inhalaciju kiseonika i azot-oksidula u odnosu 50% : 50%.

Svi ispitanici premedicirani su benzodiazepamom u dozi od 10 mg *im* pola sata pre početka operacije.

Za analgeziju korišćen je sufentanil u dozi od 0,5 μg/kg telesne težine, a prema potrebi tokom operacije dodavano je 50% od početne doze.

Za relaksaciju korišćen je rokuronijum-hlorid u bolus dozi od 0,6 mg/kg telesne mase, prema potrebi tokom operacije dodavano je 10–30 mg u bolus dozi.

U grupi TIVA (n = 30) uvod u anesteziju izveden je koindukcijom: prvo je intravenski dat midazolam u dozi 0,05 mg/kg telesne mase tokom 10 sekundi; posle 1,5–2 minuta lagano intravenski dat je propofol u prosečnoj dozi od 1,2 mg/kg telesne mase tokom 30 sekundi. Održavanje anestezije izvršeno je kontinuiranom intravenskom infuzijom propofola putem mikrokompjuterski kontrolisane infuzione pumpe (Graseby 3400 Syringe Pump). Koncentracije su iznosile 8–10 mg/kg/h uz smanjivanje doze za 2 mg/kg/h na svakih 10 minuta. Bolesnici su ventilisani 100% kiseonikom.

U grupi OBA (n = 30) uvod u anesteziju izveden je koindukcijom: prvo je intravenski dat midazolam u dozi 0,05 mg/kg telesne mase tokom 10 sekundi; posle 1,5–2 minuta lagano intravenski dat je tiopenton u prosečnoj dozi od 3,4 mg/kg telesne mase. Bolesnici su ventilisani mešavinom kiseonika i azot-oksidula u odnosu 50% : 50%.

Kod svih bolesnika praćene su vrednosti hemodinamskih parametara (vrednosti frekvencije srčanog rada, sistolnog, dijastolnog i srednjeg arterijskog pritiska). Vrednosti navedenih parametara registrovane su korišćenjem Datex-Engstrom AS/3 monitora u šest vremena.

Za svakog bolesnika izračunato je procentualno odstupanje od početnih vrednosti i registrovana učestalost odstupanja većih ili manjih od 20% u odnosu na početne vrednosti.

Kriterijumi za stepenovanje hemodinamske stabilnosti bili su: prvi stepen: promena od 0 do 10% u odnosu na početne vrednosti; drugi stepen – promena od 11 do 20% u odnosu na početne vrednosti; treći stepen: promena od 21 do 30% u odnosu na početne vrednosti; četvrti stepen: promena preko 30% u odnosu na početne vrednosti.

Merenja hemodinamskih parametara (frekvencija srčanog rada, sistolni, dijastolni i srednji arterijski pritisak) izvršena su u sledećim vremenskim intervalima: t_o – pre uvoda u anesteziju; t_1 – posle uvoda u anesteziju, pre incizije kože; t_2 – posle incizije kože; t_3 – 5 minuta posle stvaranja pneumo-

peritoneuma; t_4 – tokom pneumoperitoneuma; t_5 – 5 minuta posle oslobađanja od pneumoperitoneuma.

Vrednosti obeležja posmatranja prikazane su kao srednje vrednosti i standardne devijacije. Kao analitička metoda primenjeni su Friedman-ov χ^2 test, χ^2 test i Studentov *t*-test. Analiza je vršena primenom kompjuterskog programa SPSS for Windows (verzija 7.2).

Rezultati

Demografske karakteristike bolesnika i pridružena oboljenja kardiovaskularnog sistema prikazana su u tabeli 1.

Distribucija bolesnika prema stepenu hemodinamske nestabilnosti u odnosu na srčanu frekvenciju, po vremenima u obe grupe prikazana je u tabeli 2.

U svim vremenima postojala je statistički visokoznačajna razlika u stepenu hemodinamske nestabilnosti između grupa. Do ove razlike došlo je zbog toga što su svi bolesnici iz grupe TIVA pripadali prvom, najblažem stepenu hemodinamske nestabilnosti. U grupi OBA različit broj bolesnika (čak do preko 30%) pripadao je drugom stepenu, a u t_5 jedan bolesnik bio je u trećem, najtežem stepenu hemodinamske nestabilnosti.

Tabela 1

Demografske karakteristike bolesnika i prateća kardiovaskularna oboljenja

Karakteristike bolesnika	TIVA (n = 30)	OBA (n = 30)
Godine života (raspon)	61,4 (41–73)	61,2 (42–71)
Pol		
muški (n)	13	14
ženski (n)	17	16
Prateća kardiovaskularna oboljenja (n)		
hronična kompenzovana kardiomiopatija	11	10
arterijska hipertenzija	10	11
ishemijska bolest srca	9	9

TIVA – totalna *iv* anestezija propofolom uz koindukciju midazolamom; OBA – opšta balansirana anestezija

Vrednosti srčane frekvencije, kao hemodinamskog parametra merene su kontinuirano, a evidentirane su u vremenskim intervalima od t_0 do t_5. U svakoj grupi ponaosob testirana je značajnost razlike promena srčane frekvencije u funkciji vremena od t_0 do t_5 Friedman-ovim χ^2 testom. Primena ovog testa pokazala je da u obe grupe dolazi do statistički visokoznačajne promene srčane frekvencije (TIVA: $\chi^2_F = 33,7$, $p < 0,01$; OBA: $\chi^2_F = 37,7$, $p < 0,01$). Ova razlika nastala je zbog promena u intervalu t_0–t_1 tokom kojeg dolazi do porasta srčane frekvencije i u intervalu t_4–t_5 kada dolazi do sniženja vrednosti srčane frekvencije.

Vrednosti sistolnog, dijastolnog i srednjeg arterijskog pritiska kao hemodinamskih parametara, takođe su merene kontinuirano, a evidentirane su u vremenskim intervalima od t_0 do t_5.

U svakoj grupi ponaosob testirana je značajnost razlike promena sistolnog pritiska u funkciji vremena od t_0 do t_5 Friedman-ovim χ^2 testom. Primena ovog testa pokazala je da u obe grupe dolazi do statistički visoko značajne promene sistolnog pritiska (TIVA: $\chi^2_F = 66,8$, $p < 0,01$; OBA: $\chi^2_F = 83,5$, $p < 0,01$). Ova razlika nastala je zbog promena u intervalima t_0–t_1 i t_4–t_5 tokom kojih dolazi do pada vrednosti sistolnog pritiska.

Tabela 2

Distribucija bolesnika prema stepenu hemodinamske nestabilnosti u odnosu na srčanu frekvenciju

Vreme i stepen hemodinamske nestabilnosti	TIVA (n)	OBA (n)	TIVA : OBA *p*
t_1			
I stepen	30	24	< 0,01
II stepen	0	6	
III sepen	0	0	
t_2			
I stepen	30	20	< 0,01
II stepen	0	10	
III sepen	0	0	
t_3			
I stepen	30	19	< 0,01
II stepen	0	11	
III sepen	0	0	
t_4			
I stepen	30	23	< 0,01
II stepen	0	7	
III sepen	0	0	
t_5			
I stepen	30	24	< 0,01
II stepen	0	5	
III sepen	0	1	

TIVA – totalna *iv* anestezija propofolom uz koindukciju midazolamom; OBA – opšta balansirana anestezija

Takođe, u svakoj grupi ponaosob testirana je značajnost razlike promena dijastolnog pritiska u funkciji vremena od t_0 do t_5 Friedman-ovim χ^2 testom. Primena ovog testa pokazala je da u obe grupe dolazi do statistički visoko značajne promene dijastolnog pritiska (TIVA: $\chi^2_F = 41,0$, $p < 0,01$; OBA: $\chi^2_F = 49,5$, $p < 0,01$). Ova razlika nastala je zbog promena u intervalima t_0–t_1 i t_4–t_5 tokom kojih dolazi do pada vrednosti dijastolnog pritiska.

Značajnost razlike promena srednjeg arterijskog pritiska u funkciji vremena od t_0 do t_5 Friedman-ovim χ^2 testom testirana je u svakoj grupi ponaosob. Primena ovog testa pokazala je da u obe grupe dolazi do statistički visokoznačajne promene srednjeg arterijskog pritiska (TIVA: $\chi^2_F = 46.7$, $p < 0,01$; OBA: $\chi^2_F = 52,5$, $p < 0,01$). Ova razlika nastala je zbog promena u intervalima t_0–t_1 i t_4–t_5 tokom kojih dolazi do pada vrednosti srednjeg arterijskog pritiska.

Distribucija bolesnika prema stepenu hemodinamske nestabilnosti u odnosu na sistolni, dijastolni i srednji arterijski pritisak, po vremenima u obe grupe prikazana je u tabeli 3.

U vremenima t_1 i t_5 postajala je statistički visokoznačajna razlika u stepenu hemodinamske nestabilnosti između grupa u odnosu na sistolni pritisak. Do ove razlike je došlo zbog toga što su svi bolesnici iz grupe TIVA pripadali prvom, najblažem stepenu hemodinamske nestabilnosti. U grupi OBA, različit broj bolesika (oko 30%) pripadao je drugom stepenu u t_1 i t_5, a u t_1 jedan bolesnik bio je u trećem, najtežem stepenu hemodinamske nestabilnosti. U ostalim vremenima nije bilo statistički značajne razlike.

U svim vremenima postojala je statistički visokoznačajna razlika u stepenu hemodinamske nestabilnosti između grupa u odnosu na dijastolni pritisak. Do ove razlike došlo je zbog toga što su svi bolesnici iz grupe TIVA pripadali prvom, najblažem, stepenu hemodinamske nestabilnosti. U grupi OBA, različit broj bolesnika (25–50%) pripadao je drugom stepenu, a u t_1 jedan bolesnik bio je u trećem, najtežem stepenu hemodinamske nestabilnosti.

Takođe, u svim vremenima postojala je statistički značajna razlika u stepenu hemodinamske nestabilnosti između grupa koja je u vremenima t_1 i t_5 dostizala vrednost visoke značajnosti u odnosu na srednji arterijski pritisak. Do ove razlike došlo je zbog toga što su svi bolesnici iz grupe TIVA pripadali prvom, najblažem stepenu hemodinamske nestabilnosti. U grupi OBA različit broj bolesnika (25–30%) pripadao je drugom stepenu, a tri bolesnika bila su u trećem, najtežem stepenu hemodinamske nestabilnosti (dva u t_1 i jedan u t_5).

Diskusija

Laparoskopska holecistektomija je najčešća operacija koja se izvodi laparoskopskom hirurškom tehnikom i predstavlja metod izbora za lečenje nekomplikovane holelitijaze.

Tabela 3

Distribucija bolesnika prema stepenu hemodinamske nestabilnosti u odnosu na sistolni, dijastolni i srednji arterijski pritisak

Vreme i stepen hemodinamske nestabilnosti	Sistolni pritisak			Dijastolni pritisak			Srednji arterijski pritisak		
	TIVA (n)	OBA (n)	*p*	TIVA (n)	OBA (n)	*p*	TIVA (n)	OBA (n)	*p*
t_1									
I stepen	30	20	< 0,01	30	14	< 0,01	30	20	< 0,01
II stepen	0	9		0	15		0	8	
III sepen	0	1		0	1		0	2	
t_2									
I stepen	30	30		30	20	< 0,01	30	26	< 0,05
II stepen	0	0		0	10		0	4	
III sepen	0	0		0	0		0	0	
t_3									
I stepen	30	29		30	23	< 0,01	30	27	< 0,05
II stepen	0	1		0	7		0	3	
III sepen	0	0		0	0		0	0	
t_4									
I stepen	30	29		30	23	< 0,01	30	27	< 0,05
II stepen	0	1		0	7		0	3	
III sepen	0	0		0	0		0	0	
t_5 *									
I stepen	30	22	< 0,01	30	17	< 0,01	30	23	< 0,01
II stepen	0	8		0	13		0	6	
III sepen	0	0		0	0		0	1	

TIVA – totalna *iv* anestezija propofolom uz koindukciju midazolamom; OBA – opšta balansirana anestezija

Jasno je da laparoskopska hirurgija smanjuje postoperativni i ukupni operativni rizik bolesnika. Nedovoljno je, međutim, istaknuta činjenica da postoje intraoperativni rizici i specifičnosti anestezije kod endoskopske holecistektomije. Zbog položaja bolesnika na operacionom stolu i jatrogeno izazvanog pneumoperitoneuma kompromituje se hemodinamska i respiratorna funkcija što komplikuje vođenje anestezije[2]. Danas se starosna granica bolesnika koji se operišu laparoskopskom tehnikom pomera naviše. Time se dodatno povećavaju rizici u anesteziji, jer za razliku od mlađe populacije bolesnika koji su podvrgavani laparoskopskoj holecistektomiji danas su pacijenti stariji, sa značajnim kardiovaskularnim i respiratornim oboljenjima (hipertenzija, ishemijska bolest srca, miokardiopatija, hronična opstruktivna bolest

pluća, emfizem). Ovi bolesnici pripadaju grupi III prema klasifikaciji ASA. Njihov sve veći broj nametao je niz novih problema u vođenju anestezije zbog manje funkcionalne rezerve i mogućnosti da kompenzuju dodatna hemodinamska i respiratorna opterećenja kojima su izloženi tokom laparoskopske holecistektomije.

Stvaranje umerenog intraabdominalnog pritiska (IAP) kod zdravih i/ili mladih osoba ima minimalne efekte na udarni i minutni volumen srca. Dodatkom anti-Trendelenburgovog položaja dolazi do značajnijih smanjenja ovih srčanih parametara.

Stvaranje povišenog IAP većeg od 10 mmHg, negativno utiče na kardiovaskularni sistem. Smanjenje volumnog opterećenja (*preload*) i povećanje opterećenja srca pritiskom (*afterload*) kao rezultat povišenog IAP izaziva negativne efekte kod bolesnika sa oštećenom funkcijom srca, anemijom ili hipovolemijom. Poremećaji se karakterišu smanjenjem minutnog volumena srca, povećanjem arterijskog pritiska i povećanjem sistemske i pulmonalne vaskularne rezistencije. Nedavno sprovedena studija pokazala je smanjenje hepatičnog i renalnog protoka krvi prouzrokovanog stvaranjem pneumoperitoneuma pod pozitivnim pritiskom kod laparoskopske holecistekotmije [3].

Bolesnici koji imaju kongestivnu srčanu insuficijenciju sigurno su skloniji razvijanju srčanih komplikacija nego oni sa ishemijskom bolešću srca tokom laparoskopije. Minutni volumen srca značajno je smanjen tokom pneumoperitoneuma u poređenju sa preoperativnim vrednostima uprkos hirurškom stresu, dok povišenje arterijskog krvnog pritiska i srčane frekcencije, koji su potencijalno štetni kod bolesnika sa oboljenjem koronarnih arterija, nije toliko naglašeno. Da li je laparoskopija opasnija nego laparotomija kod ovih bolesnika još nije istraženo i zaslužuje pažljivo razmatranje. Za ove bolesnike postoperativne koristi od laparoskopije moraju biti izbalansirane u odnosu na intraoperativne rizike.

Do pre 20 godina pripadnost bolesnika grupi ASA III bila je prvo apsolutna, a kasnije relativna kontraindikacija za laparoskopsku holecistektomiju zbog visokog intraoperativnog rizika. Iako je broj bolesnika grupe ASA III koji se operišu metodom laparoskopske holecistektomije sve veći, još nije postignut konsenzus o metodi opšte anestezije koja kod njih omogućava najveći stepen hemodinamske stabilnosti [4–10].

Prema našem mišljenju do sada korišćen metod OBA kod bolesnika grupe ASA III nije omogućavao hemodinamsku stabilnost u dovoljnom stepenu. Zbog svojih karakteristika metod TIVA anestezije trebalo bi teoretski da obezbedi najveći stepen hemodinamske stabilnosti, o čemu prema podacima iz literature još ne postoji saglasnost autora [2–4].

Merenja hemodinamskih parametara (frekvenca srčanog rada, sistolni, dijastolni i srednji arterijski pritisak) izvršena su u sledećim vremenskim intervalima: t_o – pre uvoda u anesteziju; t_1 – posle uvoda u anesteziju, pre incizije kože; t_2 – posle incizije kože; t_3 – 5 minuta posle stvaranja pneumoperitoneuma; t_4 – tokom pneumoperitoneuma; t_5 – 5 minuta posle oslobađanja od pneumoperitoneuma.

Ovi vremenski intervali odabrani su jer se u njima događaju ključne patofiziološke promene koje utiču na hemodinamsku stabilnost. Zbog toga su navedeni hemodinaski parametri praćeni u funkciji vremena od t_0 do t_5.

Vremenski interval t_0 je početna, osnovna, vrednost u odnosu na koju je određivana hemodinamska stabilnost u ostalim intervalima za svaki parametar.

U t_1 – vremenu posle uvoda u anesteziju, a pre incizije kože, ispoljavaju se eventualni negativni efekti datih anestetika i intubacije na kardiovaskularnu funkciju.

U t_2 – vremenu posle incizije kože hemodinamski parametri određivani su jer je incizija kože prvi hirurški stimulus koji utiče na stabilnost kardiocirkulatorne funkcije.

U t_3 – vremenu 5 minuta posle stvaranja pneumoperitoneuma ispoljvaju se prvi negativni efekti povećanog intraabdominalnog pritiska. Srčane smetnje javljaju se najčešće rano u insuflaciji, kada su patofiziološke hemodinamske promene najintenzivnije.

Vreme t_4 bitno je zbog toga što su intraabdominalni organi i krvni sudovi već duže vreme izloženi povećanom IAP, pa samim tim i negativni efekti IAP na hemodinamsku stabilnost održavaju se u dužem vremenskom periodu u zavisnosti od trajanja hirurške intervencije.

Vreme t_5 – 5 minuta posle oslobađanja od pneumoperitoneuma važno je jer brza CO_2 eksuflacija pospešuje akutnu hipotenziju, bradikardiju i hipoksemiju.

U nama dostupnoj literaturi nismo pronašli poređenje uticaja TIVA i OBA anestezije na hemodinamsku stabilnost tokom laparoskopske holecistektomije kod bolesnika iz grupe ASA III u navedenim vremenskim intervalima.

Praćenjem vrednosti srčane frekvencije zaključili smo da u obe grupe ispitanika dolazi do statistički visokoznačajne promene vrednosti srčane frekvencije. Međutim, u grupi TIVA svi ispitanici bili su u prvom stepenu hemodinamske nestabilnosti, odnosno kod svih je došlo do promene srčane frekvence do 10% u odnosu na početne vrednosti (t_0). U grupi OBA u vremenu t_2 bilo je 10 ispitanika u drugom stepenu hemodinamske nestabilnosti, u t_3 11, a u t_5 pet ispitanika u drugom, a jedan u trećem stepenu hemodinamske nestabilnosti. Zaključili smo da TIVA metod anestezije daje visoku hemodinamsku stabilnost i u vremenima u kojima je negativan uticaj pneumoperitoneuma i položaja bolesnika maksimalno ispoljen, za razliku od OBA metoda anestezije.

Analiza vrednosti sistolnog pritiska u obe grupe po vremenima pokazala je sledeće: u grupi TIVA svi ispitanici u svim vremenima bili su u prvom stepenu hemodinamske nestabilnosti. U grupi OBA u t_1 devet ispitanika je bilo u drugom stepenu hemodinamske nestabilnosti, a jedan u trećem stepenu. U t_5 u grupi OBA osam ispitanika je bilo u drugom stepenu hemodinamske nestabilnosti. U t_1 u grupi TIVA svi bolesnici su bili hemodinamski stabilni. U tom vremenskom periodu, posle uvoda u anesteziju, a pre incizije kože, ispoljili su se negativni efekti anestetika za uvod u anesteziju u grupi OBA.

Poređenjem vrednosti dijastolnog pritiska između grupa prema vremenima utvrđena je statistički visokoznačajna razlika u t_1 i t_5. U grupi TIVA svi ispitanici bili su u prvom stepenu hemodinamske nestabilnosti. U grupi OBA u t_1 15 ispitanika bilo je u drugom stepenu hemodinamske nestabilnosti, a jedan u trećem. U t_2 u drugom stepenu hemodinamske nestabilnosti bilo je 10 ispitanika, u t_3 i t_4 po sedam ispitanika,

a u t_5 13 ispitanika. Tokom čitavog intraoperativnog perioda bolesnici iz grupe TIVA bili su hemodinamski stabilni, a u grupi OBA hemodinamska nestabilnost bila je izražena u svim vremenima u različitim stepenima. To znači da su anestetici upotrebljeni u grupi TIVA ispoljili minimalne negativne kardiocirkulatorne efekte za razliku od onih koji su upotrebljeni u grupi OBA.

Poređenjem vrednosti srednjeg arterijskog pritiska između grupa po vremenima utvrđena je statistički visokoznačajna razlika u svim vremenima osim t_4 gde nije bilo statistički značajne razlike. To se može objasniti time što su u svim drugim vremenskim periodima negativni efekti na hemodinamsku stabilnost mnogo izraženiji nego tokom samog trajanja pneumoperitoneuma. U grupi TIVA svi bolesnici u svim vremenskim periodima bili su hemodinamski stabilni, a u grupi OBA u t_1 osam ispitanika bilo je u drugom stepenu, a dva u trećem stepenu hemodinamske nestabilnosti, u t_2 je bilo četiri ispitanika u drugom stepenu, a u t_3 i t_4 po tri ispitanika u drugom stepenu hemodinamske nestabilnosti. U t_5 šest ispitanika je bilo u drugom stepenu a jedan u trećem stepenu hemodinamske nestabilnosti. To objašnjavamo činjenicom da anestetici upotereblјeni u metodi OBA obezbeđuju mnogo manju hemodinamsku stabilnost kod bolesnika iz grupe ASA III koji, i inače, imaju manju funkcionalnu rezervu kardiovaskularnog sistema.

Istraživanje je pokazalo da su bolesnici iz grupe TIVA u svim vremenima i svim hemodinamskim parametrima bili u I stepenu hemodinamske nestabilnosti, odnosno odstupanje od osnovne vrednosti svakog parametra u svim vremenima bilo je manje od 10% od početnih vrednosti. To pokazuje veliku hemodinamsku stabilnost postignutu ovom metodom anestezije, za razliku od grupe OBA u kojoj su bolesnici u svim parametrima i u većini vremenskih intervala pokazali statistički visokoznačajnu hemodinamsku nestabilnost, odstupanja od početnih vrednosti su bila veća od 10%.

Hemodinamska nestabilnost u smislu hipotenzije može biti problem u vođenju anestezije. Zbog toga je važno kako bolesnici reaguju na primenu vazopresora. Iako je efekat anestetika na kardiovaskularne efekte vazopresora nedovoljno proučen, pokazalo se da je lakše izvršiti reverziju anestezijom indukovane hipotenzije efedrinom, kada je primenjen propofol nego kada je u pitanju isparljivi anestetik [4]. To ide u prilog našim rezultatima koji su pokazali da je primena TIVA anestezije propofolom obezbeđivala veću hemodinamsku stabilnost. Istraživanje koje su vršili Atallah i Othman. [5] pokazalo je da je TIVA bolja od opšte balansirane inhalacione anestezije za laparoskopske procedure kao i u našem istraživanju.

Neke studije pokazale su da nema značajnije razlike u hemodinamskoj stabilnosti kada se primenjuje isparljiva anestezija u poređenju sa propofolom [6, 7].

Neki autori došli su do rezultata suprotnih našim. U njihovim istraživanjima isparljivi anestetici pružali su bolju hemodinamsku stabilnost u odnosu na primenu propofola i TIVE [8–10]. Jedno od objašnjenja ovog neslaganja moglo bi biti to što je u tim studijama primenjivan sevofluran kao isaprljivi anestetik koji u našem istraživanju nije korišćen.

Zaključak

Naše istraživanje pokazalo je da metod TIVA anestezije obezbeđuje najveći stepen hemodinamske stabilnosti tokom laparoskopske holecistektomije kod bolesnika iz grupe ASA III sa pratećim kardiovaskularnim oboljenjima.

L I T E R A T U R A

1. *Perissat J, Collet D, Belliard R.* Gallstones: laparoscopic treatment--cholecystectomy, cholecystostomy, and lithotripsy. Our own technique. Surg Endosc 1990; 4(1): 1−5.
2. *Sudheer PS, Logan SW, Ateleanu B, Hall JE.* Haemodynamic effects of the prone position: a comparison of propofol total intravenous and inhalation anaesthesia. Anaesthesia 2006; 61(2): 138−41.
3. *Bickel A, Loberant N, Bersudsky M, Goldfeld M, Ivry S, Herskovits M*, et al. Overcoming reduced hepatic and renal perfusion caused by positive-pressure pneumoperitoneum. Arch Surg 2007; 142(2): 119−24; discussion 125.
4. *Kanaya N, Satoh H, Seki S, Nakayama M, Namiki A.* Propofol anesthesia enhances the pressor response to intravenous ephedrine. Anesth Analg 2002; 94(5): 1207-11, table of contents.
5. *Atallah MM, Othman MM.* Robotic laparoscopic radical cystectomy inhalational versus total intravenous anesthesia: a pilot study. Middle East J Anesthesiol 2009; 20(2): 257−63.
6. *Watson KR, Shah MV.* Clinical comparison of 'single agent' anaesthesia with sevoflurane versus target controlled infusion of propofol. Br J Anaesth 2000; 85(4): 541−6.
7. *El-Orbany MI, Wafai Y, Joseph NJ, Salem MR.* Tracheal intubation conditions and cardiovascular effects after modified rapid-sequence induction with sevoflurane-rapacuronium versus propofol-rapacuronium. J Clin Anesth 2002; 14(2): 115−20.
8. *Ozkose Z, Ercan B, Unal Y, Yardim S, Kaymaz M, Dogulu F,* et al. Inhalation versus total intravenous anesthesia for lumbar disc herniation: comparison of hemodynamic effects, recovery characteristics, and cost. J Neurosurg Anesthesiol 2001; 13(4): 296−302.
9. *Matute E, Alsina E, Roses R, Blanc G, Pérez-Hernández C, Gilsanz F.* An inhalation bolus of sevoflurane versus an intravenous bolus of remifentanil for controlling hemodynamic responses to surgical stress during major surgery: a prospective randomized trial. Anesth Analg 2002; 94(5): 1217−22, table of contents.
10. *Husedzinović I, Tonković D, Barisin S, Bradić N, Gasparović S.* Hemodynamic differences in sevoflurane versus propofol anesthesia. Coll Antropol 2003; 27(1): 205−12.

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Risk factors for the appearance of minimal pathologic lesions on vocal folds in vocal professionals

Faktori rizika od nastanka minimalnih patoloških lezija na glasnim žicama vokalnih profecionalaca

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Abstract

Background/Aim. An excessive use or misuse of voice by vocal professionals may result in symptoms such are husky voice, hoarse voice, total loss of voice, or even organic changes taking place on vocal folds – minimal pathological lesions – MAPLs. The purpose of this study was to identify the type of MAPLs which affects vocal professionals, as well as to identify the risk factors that bring about these changes. **Methods.** There were 94 vocal professionals who were examined altogether, out of whom 46 were affected by MAPLs, whereas 48 of them were diagnosed with no MAPLs, so that they served as the control group. All these patients were clinically examined (anamnesis, clinical examination, bacteoriological examination of nose and pharynx, radiography of paranasal cavities, allergological processing, phoniatric examination, endo-video-stroboscopic examination, as well as gastroenterologic examination, and finally endocrinological and pulmological analyses). **Results.** The changes that occurred most often were identified as nodules (50%; n = 23/46) and polyps (24%; n = 11/46). Risk factors causing MAPLs in vocal professionals were as follows: age, which reduced the risk by 23.9% [OR 0.861 (0.786–0.942)] whereas the years of career increase the risk [OR 1.114 (1.000–1.241)], as well as the presence of a chronic respiratory disease [OR 7.310 (1.712–31.218)], and the presence of gastro-oesophageal reflux disease [OR 4.542 (1.263–16.334)]. The following factors did not contribute to development of MAPLs in vocal professionals: sex, a place of residence, irritation, smoking, endocrinologic disease and the presence of poly-sinusitis. **Conclusion.** It is necessary to introduce comprehensive procedures for prevention of MAPLs, particularly in high-risk groups. Identification of the risk factors for MAPLs and prevention of their influence on vocal professionals (given that their income depends on their vocal ability) is of the highest importance.

Key words:
vocal cords; occupational exposure; risk factors; polyps.

Apstrakt

Uvod/Cilj. Prekomerna upotreba ili zloupotreba glasa kod vokalnih profesionalaca može dovesti do pojave simptoma (slabosti glasa, promuklosti i gubitka glasa), kao i do razvoja organskih promena na glasnim žicama (MAPLs – *minimal pathological lesions*). Cilj rada bio je utvrditi vrstu MAPLs kod vokalnih profesionalaca, kao i faktore rizika koji dovode do njihove pojave. **Metode.** Ova prospektivna studija obuhvatila je 94 vokalna profesionalaca i to 46 sa MAPLs i 48 bez MAPLs, koji su činili kontrolnu grupu. Kod svih bolesnika bilo je sprovedeno osnovno kliničko ispitivanje [anamneza, klinički pregled, bakteriološki pregled nosa i ždrela, radiografija paranazalnih šupljina, alergološka obrada, fonijatrijski pregled, endovideostroboskopski pregled (Storz), kao i gastroenterološki pregled, endokrinološka i pulmološka obrada]. **Rezultati.** Najčešće utvrđene promene kod vokalnih profesionalaca bili su: noduli (50%, n = 23/46), a zatim polipi (24%, n = 11/46). Faktori koji su imali uticaja na pojavu MAPLs kod vokalnih profesionalaca su: godine starosti koje smanjuju rizik za 23,9% [OR 0,861 (0,786–0,942)], dok rizik povećevaju godine staža [OR 1,114 (1,000–1,241)], postojanje hronične respiratorne bolesti [OR 7,310 (1,712–31,218)] i postojanje gastroezofagusne bolesti [OR 4,542 (1,263–16,334)]. Na pojavu MAPLs kod vokalnih profesionalaca nisu imali uticaja pol, mesto življenja, postojanje iritacije, pušenje, endokrinološka bolest, kao i postojanje polisinuzitisa. **Zaključak.** Neophodno je uvođenje sveobuhvatnih preventivnih postupaka za sprečavanje nastanka MAPLs, posebno u visokorizičnim grupama. Naročito je važno utvrđivanje faktora rizika od nastanka MAPLs i otklanjanje njihovog uticaja u grupi vokalnih profesionalaca, s obzirom da je njihova sposobnost zarade u negativnoj vezi sa gubitkom kvaliteta glasa.

Ključne reči:
glasne žice; profesionalna izloženost; faktori rizika; polipi.

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Introduction

It has been determined that 3%–9% of the population is diagnosed with voice disorder [1]. According to Sataloff et al. [2], the term "vocal professionals" refers to anyone whose income is likely to be affected by the loss of quality or resilience of their voice. Vocal professionals use their voice to a larger extent, over an extended period of time, in a higher intensity, and within various circumstances including different psycho-physical and microclimatic conditions. There are four widely-accepted levels of voice disorder and voice usage nowadays (Wake Forest University) [3]: level I – minimal voice lesions could have serious professional consequences (opera singers, other types of singers); level II – moderate voice lesions may prevent a vocal professional from carrying out their professional activities (educators, anchors, politicians, receptionists, priests, etc.); level III – serious voice lesions found in non-professionals, which may hinder their professional activities (lawyers, judges, doctors, businessmen); level IV – non-vocal, and non-professional voice usage associated with people whose voice quality is unlikely to affect their professional life (officers, workers).

Two comprehensive studies carried out in the US and Sweden showed that singers are at the highest risk of being affected by disphonia, and then other professionals follow: social workers, teachers, lawyers, priests, telephonists. Teaching profession is of particular socio-medical interest, given the fact that teachers comprised the majority of patients who reported having problems with hoarse voice, which hindered their ability to work and made them take a leave from work [4, 5].

Inadequate vocal techniques may refer to overused voice, or too loud voice (Lombard effect – noisy speech), as well as they may be associated with an inadequate vocal hygiene [6], which may cause the symptoms of voice pathology to appear (weak voice, hoarse voice, or the loss of voice) or it may result in organic changes on vocal folds [7]. The term of minimal pathological lesions (MAPLs) has been defined so as to denote any changes on vocal folds which do not require primary surgical treatment, but are treated by phoniatric methods to a satisfactory degree [6]. According to Kotby [7], organic disphonies coming together with MAPLs are identified as follows: polyps, vocal fold knots (juvenile and adult types), Reinke's edema, contact granuloma, vocal cord cysts and ventricular disphonia with hypertrophy.

The factors which may cause the occurrence of MAPLs, apart from those mentioned above, including voice overuse or vocal traumatism, include thyroid gland dysfunction, allergic diseases of voice activator, generator and resonator, smoking, the existence of chemical and mechanical irritations in working and living environments, alcohol consummation, laryngeal reflux, etc [8].

The aim of this study was to identify the type of MAPLs which affect vocal professionals, as well to identify the risk factors that bring about these changes.

Methods

In a prospective study carried out at the Phoniatrics Department of the Institute for Otorhinolaryngology and Maxillofacial Surgery of the Clinical Centre of Serbia in Belgrade, 94 vocal professionals were observed. Out of them 46 were affected by MAPLs, whereas 48 didn't have MAPLs, so that they were the control group.

All these patients were clinically examined: anamnesis, clinical examination, bacteoriological examination of nose and pharynx, radiography of paranasal cavities, allergological processing, phoniatric examination, endo-videostrobos-copic examination, as well as gastroenterologic examination, and finally endocrinological and pulmological analyses.

According to Kotby [7], organic disphonies coming together with MAPLs are identified as the following: polyps, vocal fold knots (juvenile and adult types), Reinke's edema, contact granuloma, vocal cord cysts, and ventricular disphony (with hypertrophy).

The group with MAPLs included examinees out of whom 21% (n = 20/94) had a job associated with the level I, 52% (n = 49/94) of them were engaged in professional activities belonging to the level II, whereas 27% of them (n = 25/94) had a career associated with the level 3 of voice usage [9]. Patients with MAPLs, befalling to voice usage level IV, were not included in the study.

The observation was carried out in line with ethical standards of the Declaration of Helsinki.

The methods of descriptive statistics were used, Student's *t*-test and χ^2 test were used to test the dependability of category variables. ROC curve was used to examine if age could play a role of a marker for MAPLs. Binary logistic regression helped us determine dependability of MAPLs on those variables that the χ^2 test identified as being associated with MAPLs. The degree of risk was expressed by odds ratio (OR). For statistically important difference we took the difference $p < 0.05$.

Results

The frequency and the type of MAPLs changes in the MAPLs group (n = 46) are presented in the Figure 1. The most frequent changes were found to be nodules (50%, n = 23/46), and polyps (24%, n = 11/46).

In the whole group of examinees it was determined that MAPLs were more frequently found in female vocal professionals (74%, n = 34/46), but it can be observed that, when it comes to sex, there was no statistically important difference in comparison to the control group (70.8%, n = 34/48) (p = 0.173).

However, there was a statistically important difference when it comes to the average age of examinees in the MAPLs group and those in the control one. The average age of the patients in the MAPLs group was 39, whereas the average age of the patients in the control group was 47 (39 *vs* 47, $p < 0.005$, cut off = 42.5 years).

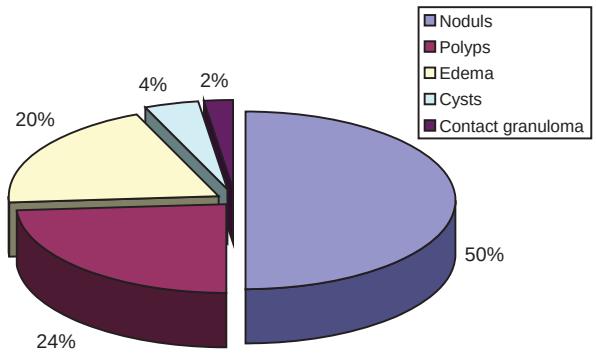

Fig. 1 – The frequency and type of the minimal pathological lesions (MAPLs) in the vocal professionals (the type of MAPLs is expressed in percentages)

The frequency of MAPLs declines in the patients whose age was over 42.5 (for the cut-off of 42.5 sensitivity equaled 62.5%, while the specificity was 65.7%).

There was a statistically important difference between the MAPLs and the control group in terms of the length of professional career. The patients in the MAPLs group had a shorter career (15 years), while the patients in the control group spent averagely 19 years in office ($p = 0.022$).

In the group of elite vocal professionals with level I it was observed that 50% of the examinees had MAPLs diagnosed (n = 10/20), whereas 59% of them were diagnosed with MAPLs in the group of professionals associated with the level II changes (n = 29/49). The lowest percentage was observed in patients whose career was associated with the level III, where 28% of them were diagnosed with MAPLs (n = 7/25). A statistically important difference has been found in terms of different levels of voice usage and their role in generating MAPLs ($p = 0.040$).

In the MAPLs group 19% (n = 9/46) of patients were exposed to chemical irritation at work, whereas only 8% of patients in the control group experienced a similar exposure (n = 4/48), but a statistically significant difference between the MAPLs and the control group has not been established ($p = 0.100$). In the MAPLs group, smokers (39%, n = 18/46) were more frequently found than non-smokers (33%, n = 16/48), but this variable was proved to be statistically insignificant ($p = 0.356$).

Chronical obstructive pulmonary diseases statistically correlated with MAPLs ($p = 0.003$). In the MAPLs group, 32.6% (n = 15/46) of the examinees were diagnosed with asthma or COPD, while 8% (n = 4/48) of the examinees from the control group had the same diagnosis. It was determined that all patients with asthma had MAPLs (n = 3/3) and 75% (n = 12/16) of them were diagnosed with COPD.

Gastro-oesophageal reflux disease (GERD) proved to be statistically significant for MAPLs found in vocal professionals ($p = 0.008$). GERD was diagnosed in 32% (n = 15/46) cases in the observed group, whereas this was the case with 10.4% (n = 5/48) of the examinees in the control group.

Nasal swab findings ($p = 0.380$) and radiography and computed tomography scan of paranasal cavities ($p = 0.539$), as well as the endocrine dieseases of thyroid gland ($p = 0.318$), were identified as no significant risk factors for MAPLs.

Considering the variables for which χ^2 test and *t*-test showed that they correlated with MAPLs, binary logistic regression helped us prove a mutual interdependance between MAPLs and the following: age ($p = 0.001$), the length of career ($p = 0.049$), chronical respiratory diseases ($p = 0.007$), and gastro-oesophageal reflux disease ($p = 0.020$). The risk values as related to the above-mentioned factors are showed in Table 1.

Table 1

Odds ratio for the occurence of minimal pathological lesions (MALPs) in relation to the exposition to the observed risk factors

Risk factors	Odss ratio (OR)	MAPLs risk description
Age (years)	0.861 (0.786 – 0.942)	Each year of age decreases the risk by 23.9%
Years in career	1.114 (1.000 – 1.241)	Each year in career increases the risk by 11.4%
Chronic respiratory diseases	7.310 (1.712 – 31.218)	The existence of chronic respiratory disease increases the risk for MALPs by 7.3%
Gastro-oesophageal reflux disease	4.542 (1.263 – 16.334)	The presence of gastro-oesophageal reflux disease increases the risk for MAPLs by 4.5%

Our findings show that the following observed risk factors do not correlate with MAPLs: sex, place of residence, the presence of chemical and mechanical irritations, smoking, thyroid gland disfuncion, and the diagnosis of rhinosinusitis.

Discussion

Vocal professionals account for about a half of working population. In 30% of them there is a functional disphonia observed, and its long-term presence may result in MAPLs [10]. Morphological changes that are identified to occur most often in vocal professionals are the following: fibrovascular lesions on vocal folds (nodules, polyps), cysts, scars on vocal folds, changes in vocal fold mobility, the presence of laryngopharyngeal reflux and muscle tension dysphonia [11]. In our MAPLs group 70% of vocal profession-

als diagnosed with MAPLs had nodules and polyps. Not all vocal professionals are equally sensitive to vocal trauma, which means that some of them are more likely to develop organic lesions than the others [12].

Many studies carried out in China, America, Spain, and Slovenia, whose aim was to examine disphonia and organic lesions on vocal folds in vocal professionals, were based on profession classification according to which one's voice is one's primary working tool [4, 8, 13–15]. Chinese authors pointed out that one's profession may play an important role of risk factor for generation of benign lesions on vocal folds, with a risk being up to 2.6 times higher in professions where one's voice is more extensively used than in those where it is not [8]. Hocevar-Boltazer [15] reported that 85.6% of priests had voice-related problems adding that 15.9% of them had reported health problems related to voice loss to their physician at a regular basis [15]. Miller and Verdolini [16] found that 16% of music (singing) teachers (level I), in the autoanamnesis, reported voice-related problems. Phyland et al [17] reported findings which showed that 44% of singers (level I) had problems with their voice [17]. Smith et al [18] claimed that 32% of teachers (level II) had problems with their voice. Spanish authors carried out a comprehensive study which observed 905 teachers (level II) showing that 57% of them had voice disorder, out of whom 14% were diagnosed with nodules and 2.5% of them with polyps [13, 14]. Our research, also, showed that there was a statistical correlation between one's profession and organic lesions. Half of vocal professionals, who are solo singers, choir singers, actors/actresses (level I) were diagnosed with MAPLs, while on the other hand, there were 59% of examinees who worked as children care professionals, teachers and priests (level II). A high friquency of MAPLs in vocal professionals associated with level II was attributed to lower level of oral hygiene as compared to elite vocal professionals (level I), who got MAPLs due to high vocal demands. A quarter of examinees who were lawyers, judges, doctors, and command staff (level III) displayed MAPLs, as well.

Pérez Fernández and Preciado Lopéz [19] pointed out that vocal professionals with a shorter professional life were more likely to develop nodules than their colleagues with a longer career. This comes as a consequence of voice overuse by the young in comparison to the older people, who have been trained in using their voice. This matches our findings, where vocal professionals with MAPLs in the observed group were, at average 8 years younger than the examinees in the control group.

Spanish authors did not identify any statistically significant difference in the presence of disphonia among examinees of different sex, based on the sample of 300 teachers; but on the extended sample of 905 examinees, they managed to conclude that women are three times more likely to be affected by organic lesions than men [13, 14]. Phyland et al. [17] concluded that sex (female) can pose a risk to voice-related problems (OR = 1.4). According to Preciado et al. [20] one of the reasons why women (19.3%) experience problems with their voice more often than men (15.6%) may be found in a higher number of women performing as vocal professionals. According to other authors, this may result from women being the ones who visit their doctor more often than men, because they are concerned about their voice more than men are about their own, regardless of whether the problem is related to acute or chronic voice problems [12, 18]. Our research has shown that sex did not influence the occurrence of organic lesions on vocal folds, although women were found to be more susceptible to MAPLs.

Some associated diseases which contribute to disorders in voice production – whether they affect the controlled expirium (which provides for voice activation) or the sufficient glottis occlusion (the level of voice generator) – are listed as risk factors for MAPLs occurence in literature [21]. Professional singers and other elite vocal professionals are susceptible to alterations of vocal aparatus and pulmonary functions, because the voice activator represents the essential element in production of sound and speech [22]. The presence of asthma or COPD disables the adequate amount of expiratory air flow from leaving lungs, which hinders voice production, as well. Thus, patients suffering from asthma or COPD are more likely to experience disphonia.

Videostroboscopic findings of asthma-suffering patients, show that there is a reduction in the amplitude of vibrations and reductions in expanding mucus flow, resulting in hyperemia of vocal folds, or the occurence of changes similar to plaque on vocal folds [23]. Our study shows that vocal professionals with chronic respiratory diseases are 7.3 times more likely to develop MAPLs than vocal professionals with no chronic respiratory diseases. On the other hand, a number of authors point out that it is possible to draw lines between MAPLs in asthma-suffering patients and the therapy they get to cure the disease. For instance, Del Gaudio [24] noticed more frequent occurence of mucus edema, eritema, and candidiasis on the epiderm of larynx in asthmatic patients whose disphonia was treated with inhalatory corticosteroids. Disphonia in these patients disappeared after therapy with inhalatory corticosteroids was discontinued [24].

Arabian authors noticed that 80% of patients with benign vocal fold lesions were diagnosed with GERD [25]. Makhadoom et al. [25] pointed out the influence of gastro-oesophageal reflux disease on the pathogenesis of MAPLs. Kuhn et al. [26] pointed out the influence that GERD may have on the occurence of nodules on vocal folds. Our results show that one third of vocal professionals diagnosed with MAPLs suffer from GERD, as well. Our tests showed that vocal professionals diagnosed with GERD were 4.5 times more likely to develop MAPLs, as opposed to the patients not been diagnosed with GERD. Given the influence of gastro-oesophageal reflux disease on the occurence of MAPLs, there are numerous authors who emphasize the need for pulmonologists, allergologists, and ORL specialists, and other physicians who treat chronic cough or asthma non-responsive to the therapy (uncontrolled asthma), to be familiar with diagnosis and treatment of these diseases, in order to provide efficient treatment to their patients [27].

Among the vocal professionals whose working ability is negatively affected by their voice-related problems, there is a more frequent decline in self-esteem and self-confidence, as

well as moodiness due to disphonia. For this reason, it is necessary to take a more comprehensive approach to examining voice in vocal professionals, which would be directed towards individuals so as to include the following: a phoniatric examinations of individuals who want to start a career of a vocal professional (systematic examinations of schoolchildren and students, as well as the young individuals who seek employment, etc); education of vocal professionals providing advice on voice hygiene (proper usage of their voice); identification and removal of a wide spectrum of additionally listed risk factors which may result in MAPLs, such as: noise level, classroom size, the number of workload hours in class, the presence of air dryness and dust at the place of work; development of general and individual protocols in treating disphonia and MAPLs in vocal professionals.

Conclusion

It is necessary to introduce comprehensive procedures for prevention of MAPLs, particularly in high-risk groups. Identification of the risk factors for MAPLs and prevention of their influence on vocal professionals (given that their income depends on their vocal ability) is of the highest importance.

REFERENCES

1. *Ramig LO, Verdolini K.* Treatment efficacy: voice disorders. J Speech Lang Hear Res 1998; 41(1): S101−16.
2. *Sataloff RT, Divi V, Heman-Ackah YD, Hawkshaw MJ.* Medical history in voice professionals. Otolaryngol Clin North Am 2007; 40(5): 931−51, v.
3. *Fritzell B.* Voice disorders and occupations. Log Phon Vocol 1996; 21**:** 7–12.
4. *Titze IR, Lemke J, Montequin D.* Populations in the U.S. workforce who rely on voice as a primary tool of trade: a preliminary report. J Voice 1997; 11(3): 254−9.
5. *Mumović MG.* Conservative treatment of dysphonia. Novi Sad: Medicinski fakultet; 2004. (Serbian)
6. *Williams NR.* Occupational groups at risk of voice disorders: a review of the literature. Occup Med (Lond) 2003; 53(7): 456−60.
7. *Kotby MV.* Voice disorders: Recent diagnostic advances. Egyptian J Otolaryngol 1986; 3(1): 69−98.
8. *Huang DY, Yang WY, Yu P, He Y, Han DY.* Case-control survey on risk factors of benign vocal fold lesions. Zhonghua Er Bi Yan Hou Tou Jing Wai Ke Za Zhi 2008; 43(2): 120−4. (Chinese)
9. *Koufman JA, Blalock PD.* Functional voice disorders. Otolaryngol Clin North Am 1991; 24(5): 1059−73.
10. *Van Houtte E, Van Lierde K, D'Haeseleer E, Claeys S.* The prevalence of laryngeal pathology in a treatment-seeking population with dysphonia. Laryngoscope 2010; 120(2): 306−12.
11. *Franco RA, Andrus JG.* Common diagnoses and treatments in professional voice users. Otolaryngol Clin North Am 2007; 40(5): 1025−61, vii.
12. *Eckley CA, Swensson J, Duprat Ade C, Donati F, Costa HO.* Incidence of structural vocal fold abnormalities associated with vocal fold polyps. Braz J Otorhinolaryngol 2008; 74(4): 508−11.
13. *Preciado J, Pérez C, Calzada M, Preciado P.* Frequency and risk factors of voice disorders among teaching staff of La Rioja, Spain. Clinical study: questionnaire, function vocal examination, acoustic analysis and videolaryngostroboscopy. Acta Otorrinolaringol Esp 2005; 56(4): 161−70. (Spanish)
14. *Preciado-López J, Pérez-Fernández C, Calzada-Uriondo M, Preciado-Ruiz P.* Epidemiological study of voice disorders among teaching professionals of La Rioja, Spain. J Voice 2008; 22(4): 489−508.
15. *Hocevar-Boltezar I.* Prevalence and risk factors for voice problems in priests. Wien Klin Wochenschr 2009; 121(7−8): 276−81.
16. *Miller MK, Verdolini K.* Frequency and risk factors for voice problems in teachers of singing and control subjects. J Voice 1995; 9(4): 348−62.
17. *Phyland DJ, Oates J, Greenwood KM.* Self-reported voice problems among three groups of professional singers. J Voice 1999; 13(4): 602−11.
18. *Smith E, Lemke J, Taylor M, Kirchner HL, Hoffman H.* Frequency of voice problems among teachers and other occupations. J Voice 1998; 12(4): 480−8.
19. *Pérez Fernández CA, Preciado López J.* Vocal fold nodules. Risk factors in teachers. A case control study design. Acta Otorrinolaringol Esp 2003; 54(4): 253−60. (Spanish)
20. *Preciado JA, García Tapia R, Infante JC.* Prevalence of voice disorders among educational professionals. Factors contributing to their appearance or their persistence. Acta Otorrinolaringol Esp 1998; 49(2): 137−42. (Spanish)
21. *Milutinović Z.* Clinical atlas of voice disorders – theory and practice. Belgrade: Zavod za udžbenike i nastavna sredstva; 1997. (Serbian)
22. *Cohn JR, Sataloff RT, Branton C.* Response of asthma-related voice dysfunction to allergen immunotherapy: a case report of confirmation by methacholine challenge. J Voice 2001; 15(4): 558−60.
23. *Mirza N, Kasper Schwartz S, Antin-Ozerkis D.* Laryngeal findings in users of combination corticosteroid and bronchodilator therapy. Laryngoscope 2004; 114(9): 1566−9.
24. *DelGaudio JM.* Steroid inhaler laryngitis: dysphonia caused by inhaled fluticasone therapy. Arch Otolaryngol Head Neck Surg 2002; 128(6): 677−81.
25. *Makhadoom N, Abouloyoun A, Bokhary HA, Dhafar KO, Gazzaz ZJ, Azab BA.* Prevalence of gastroesophageal reflux disease in patients with laryngeal and voice disorders. Saudi Med J 2007; 28(7): 1068−71.
26. *Kuhn J, Toohill RJ, Ulualp SO, Kulpa J, Hofmann C, Arndorfer R,* et al. Pharyngeal acid reflux events in patients with vocal cord nodules. Laryngoscope 1998; 108(8 Pt 1): 1146−9.
27. *Franco RA Jr.* Laryngopharyngeal reflux. Allergy Asthma Proc 2006; 27(1): 21−5.

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Factors influencing a patient's decision to choose the type of treatment to improve dental esthetics

Faktori koji utiču na pacijentov izbor terapije za poboljšanje estetike zuba

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Abstract

Background/Aim. Interest in dental esthetics has increased rapidly during the last few decades among both patients and dentists, and the creation of a natural dental appearance has become an important task in all fields of dentistry, especially in prosthodontics and restorative dentistry. The aim of this research was to investigate factors influencing a patient's decision to choose the type of treatment to improve dental esthetics. **Methods.** A total of 700 Caucasian subjects participated in the cross-sectional study (261 men, 439 women, aged 18–86 years, mean age 46.2 ± 18.6). The study included clinical examination and a self-administrated questionnaire based on self-perceived esthetics, satisfaction with the appearance of their maxillary anterior teeth and previous dental experience. Multiple logistic regression was used in statistical analysis. **Results.** Hiding teeth during smile was the most important predictor for choosing fixed prosthetic restorations (OR 9.1), followed by self-perceived bad fixed prosthesis, malpositioned teeth and female gender (OR 2.9, 2.4, and 1.5, respectively). The increase in satisfaction with dental appearance and previous orthodontic therapy reduced chances for seeking prosthetic therapy (each OR 0.4). The significant predictors for bleaching choosing were hiding teeth during smiling, already done bleaching, female gender, lower levels of satisfaction with dental appearance and the absence of the previous orthodontic therapy (OR 5.8, 2.4, 1.8, 0.5 and 0.4, respecitively). Hiding teeth during smile, self-perceived malposition and crowding, and lower levels of satisfaction, were significant predictors for choosing orthodontic treatment (OR 3.1, 2.4, 2.2 and 0.6, respectively). None of current dental statuses was statistically significant predictor for choosing prosthodontic, bleeching nor orthodontic therapy. **Conclusion.** The psychological elements and female gender are the main predictors of seeking dental therapy. Understanding the prevalence of dissatisfaction with the present esthetics and desired treatments to improve esthetics can be a guide for strategies for intervention to improve esthetics.

Key words:
patient satisfaction; esthetics, dental; crowns; tooth bleaching; orthodonics.

Apstrakt

Uvod/Cilj. U poslednjih nekoliko decenija značajno se povećava interesovanje za dentalnu estetiku kako ispitanika tako i stomatologa. Postizanje prirodnog izgleda je važan zadatak u svim poljima stomatologije, naročito protetike i restorativne stomatoglogije. Cilj ovog istraživanja bio je da se utvrdi koji faktori utiču na izbor terapije za poboljšanje zubne estetike kod ispitanika. **Metode.** Istraživanjem je bilo obuhvaćeno 700 ispitanika (261 muškarac, 439 žena, prosečne starosti 46,2 ± 18,6 godina, srednje godine 45). Istraživanje je bilo zasnovano na kliničkom pregledu i ispunjavanju upitnika koji je uključivao pitanja zasnovana na samoproceni zadovoljstva pojavnošću gornjih prednjih zuba, te prethodnim dentalnim iskustvima. U statističkoj obradi podataka korišćena je multipla logistička regresija. **Rezultati.** Skrivanje zuba tokom smejanja je najvažniji prediktor za izbor fiksnih protetskih nadomestaka (OR 9.1), potom loše percipirani fiksni protetski nadomesci, loše pozicionirani zubi, te ženski pol (OR 2.9, 2.4, i 1.5 respektivno). Povećanje zadovoljstva dentalnom estetikom i prethodna ortodontska terapija smanjuju šansu za traženjem protetske terapije (svaki OR 0.4). Značajni prediktori za traženje postupka izbeljivanja zuba su: skrivanje zuba tokom smejanja, prethodni postupak izbeljivanja, ženski pol, niže razine zadovoljstva dentalnom estetikom, te odsutnost prethodne ortodontske terapije (OR 5.8, 2.4, 1.8, 0.5 i 0.4 respektivno). Skrivanje zuba tokom osmeha, samopercipirani loše pozicionirani i zbijeni zubi te niža razina zadovoljstva dentalnom estetikom bili su prediktori tra-

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ženja ortodontske terapije (OR 3.1, 2.4, 2.2 i 0.6 respektivno). Niti jedan od postojećih dentalnih statusa nije bio značajan prediktor traženja protetske terapije, izbeljivanja ili ortodontske terapije. **Zaključak.** Psihološki elementi i ženski pol glavni su prediktori traženja dentalne terapije. Razumevanje prevalencije nezadovoljstva dentalnom estetikom i željenih tretmana za poboljšanje iste glavni su vodiči strategije za njeno poboljšanje.

Ključne reči:
bolesnik, zadovoljstvo; zub, estetika; ortodoncija; zub, kruna; zub, beljenje.

Introduction

Aesthetics is a primary consideration for patients seeking both orthodontic and prosthodontic treatment [1, 2]. Interest in dental esthetics has increased rapidly during the last few decades among both patients and dentists, and the creation of a natural dental appearance has become an important task in all fields of dentistry, especially in prosthodontics and restorative dentistry [3].

The development of new techniques and dental material has led to a higher number of therapeutic options and consequently to an attractive outcome [1].

Numerous factors are related to dental aesthetic, such as the color, shape and position of teeth and the shape of dental arch. These factors are affected by individual preferences, cultural and sociodemographic factors. The viewer's perception of visual experience could be pleasant and beautiful by one individual and culture, while it could be seen as unpleasant in another [4, 5]. Perception of tooth appearance could be influenced by gender, age and education level. Females are reported to be more sensitive than males to the appearance of teeth and the importance of teeth for quality of life decreases with ageing and higher education levels [6]. Previous dental treatments of anterior teeth also have an impact on dental aesthetic, which is affected by individual preferences and cultures. Unfortunately, in some cases, dentists may develop an aesthetic appearance differing from the patient's concepts, resulting in communication problems and unanticipated difficulties [7].

Nowadays, cosmetic dentistry has become an important aspect of dentistry. Tooth whitening treatments, anterior teeth restoration, labial veneers crowns, and orthodontic treatment are frequently demanded by patients who are interested in improving their dental appearance [8].

Factors that influence patients' decision regarding the choice of a particular type of therapy to improve dental aesthetics are still insufficiently explored. Therefore, the aim of this study was to investigate the predictors influencing a patient's decision to choose prosthetic, orthodontic or bleeching type of treatment to improve dental aesthetics in maxillary anterior region in general population. It was hypothesized that significant predictors are age, gender, educational level, previous dental treatment and self-perceived dental appearance. Older subjects, females, higher educated and less satisfied with their dental appearance could be more prone to seeking crowns in maxillary anterior teeth. We assumed that subjects who want bleaching more often hide teeth during smiling, are dissatisfied with dental appearance and are more often females. Orthodontic therapy will probably choose subjects with self-perceived malpositioned and crowded teeth who are more prone to hide their teeth during smiling.

Methods

A total of 700 Caucasian subjects from Rijeka region, Croatia participated in the cross-sectional study (261 men, 439 women, mean age 46.2 ± 18.6 age, median 45 years). Sampling procedure included a convenient sample – consecutive voluntary blood donors in the Department of Transfusion Medicine, University Hospital Rijeka, subjects at the regular annual check-ups in the Institute for Public Health Rijeka, and patients seeking treatment in the University Dental Clinic Rijeka. All the participants included in the study gave written informed consent to the survey procedures, which were approved by the Ethical Committee of the Rijeka University School of Medicine.

The study included clinical examination and a questionnaire. Inclusion criterion was to have all six anterior teeth present in the upper jaw; while exclusion criteria were the evidence of gingival inflammation or gingival hyperplasia, observable gingival recession, observable occlusal wear, participants without active orthodontic therapy by edgewise appliances, participants with temporary crowns in prosthetic rehabilitation, participants in progressive endodontic therapy, participants with splints for treatment of temporomandibular disorders and participants without craniofacial syndromes. The questionnaire was self-administrated and the included questions were based on: self-assessed satisfaction with dental appearance of their maxillary anterior teeth using a three-point scale with possible answers 'dissatisfied', 'moderately satisfied', or 'completely satisfied'. Data on gender, age, educational level and self-reported previous therapy – orthodontic, bleaching, implants, crowns, root canal therapy, root scaling, professional teeth cleaning (dichotomised 0 = absent, 1 = present) were also included. Self-perceived dental appearance included questions on: crowded, malpositioned, protruded, decayed, fractured teeth and bad fixed teeth prosthesis (dichotomised 0 = absent, 1 = present). Clinical examination included assessment of dental status of six maxillary anterior teeth using classification: natural teeth without dental treatment, composite fillings, metal ceramic crowns and ceramic crowns / veneers.

The data were analyzed using SPSS 10.0 statistical software package (SPSS 10.0; SPSS Inc., Chicago, IL, USA). The Chi-square test, *t*-test and Fischer exact test were used to compare differences between population choosing and refusing prosthetics, orthodontic or bleeching. Eta Squred and Cramer's V were used to estimate the size of the effect, that is, the share of total variability of dependent variable explained by the factor tested. Multiple logistic regression analysis was used to explore the significance of predictors of choosing the type of treatment for improvement of aesthetics in maxillary anterior region with 95% confidence

intervals given for the odds ratios, indicating statistically significant relationships if both values were either greater or lesser than 1. The significance of the effects in the logistic regression model was performed via the Wald statistics and likelihood ratio test with chi-square statistics. A statistical significance was preset at $p < 0.05$.

Results

The results of univariate analysis considering choosing crowns for improvement of dental aesthetics are presented in Table 1. To identify predictors for choosing crowns while controlling for other variables in multivariate analysis, two

Table 1

Differences in variables between choosing and non-choosing crowns population

Variables	Seeking crowns		Significance	Effect size
	No (n = 308)	Yes (n = 392)		
Age ($\bar{x}$ ± SD)*, years	43.63 ± 18.52	48.24±18.35	0.001	0.015
Gender**, n (%)				
m	120 (46%)	141 (54%)		
f	188 (42.8%)	251 (57.2%)	0.432	0.001
Education level**, n (%)				
primary / secondary	232 (42.6%)	313 (57.4%)		
college / university	76 (49%)	79 (51%)	0.153	0.003
Satisfaction with dental appearance*	2.52 ± 0.58	1.92 ± 0.78	< 0.001	0.154
crowded teeth**, n (%)				
no	248 (46.9%)	281 (53.1%)		
yes	57 (34.8%)	107 (65.2%)	0.007	0.011
Malpositioned teeth**, n (%)				
no	259 (49.6%)	263 (50.4%)		
yes	48 (27.1%)	129 (72.9%)	< 0.001	0.039
Protrused teeth**, n (%)				
no	258 (45.5%)	309 (54.5%)		
yes	49 (37.1%)	83 (62.9%)	0.098	0.004
Decayed teeth**, n (%)				
no	297 (45.7%)	353 (54.3%)		
yes	10 (20.4%)	39 (79.6%)	< 0.001	0.017
Bad prosthesis**, n (%)				
no	301 (47.8%)	329 (52.2%)		
yes	6 (8.7%)	63 (91.3%)	< 0.001	0.055
Fractured teeth, n (%)				
no	277 (46.6%)	317 (53.4%)		
yes	31 (29.2%)	75 (70.8%)	< 0.001	0.016
Hide teeth during smile**, n (%)				
no	305 (49.3%)	314 (50.7%)		
yes	3 (3.8%)	76 (96.2%)	< 0.001	0.084
Orthodontic th.**, n (%)				
no	225 (40.3%)	333 (59.7%)		
yes	83 (58.5%)	59 (41.5%)	< 0.001	0.022
Bleaching th.**, n (%)				
no	276 (43.7%)	355 (56.3%)		
yes	32 (46.4%)	37 (53.6%)	0.703	0.000
Crowns**, n (%)				
no	240 (50.3%)	237 (49.7%)		
yes	68 (30.6%)	154 (69.4%)	<0.001	0.034
Implants**, n (%)				
no	297 (43.4%)	388 (56.6%)		
yes	11 (73.3%)	4 (26.7%)	0.032	0.008
Root canal th.**, n (%)				
no	217 (54.3%)	183 (45.8%)		
yes	91 (30.3%)	209 (69.7%)	< 0.001	0.057
Professional teeth cleaning**, n (%)				
ne	86 (49.4%)	88 (50.6%)		
da	222 (42.2%)	304 (57.8%)	0.113	0.004
Root scaling**, n (%)				
no	268 (44.9%)	329 (55.1%)		
yes	40 (38.8%)	63 (61.2%)	0.283	0.002
Status MOD***, n (%)				
without therapy	205 (49.5%)	209 (50.5%)		
composite filling	29 (33.7%)	57 (66.3%)		
metal acrylic crowns	33 (32%)	70 (68%)		
ceramic crowns/veneers	41 (42.3%)	56 (57.7%)	0.002	0.021

***t*-test and partial eta squared for effect size; **Fischer exact test and Cramer's V for effect size;
***χ^2-test Cramer's V for effect size.

logistic regression models were used. First logistic regression model used age, gender, education level and current satisfaction with dental appearance for prediction of seeking prosthetic restoration. Choosing prosthetic solution was significantly related to advanced age and decreased satisfaction with personal dental appearance producing OR 1.02 and 0.29, respectively ($p < 0.001$) (Table 2). This model correctly classified 66.1% of population.

Table 2

Logistic regression models for predicting variables influencing crowns choosing

Variables	B	SE	Wald	Sig.	OR	95% CI
Constant (Model 1)*	2.013	0.364	30.581	< 0.001		
Age	0.019	0.005	16.128	< 0.001	1.019	1.010–1.028
Gender (female)	0.302	0.177	2.918	0.088	1.352	0.956–1.912
Educational level (higher)	-0.291	0.200	2.110	0.146	0.748	0.505–1.107
Satisfaction with dental appearance	-1.235	0.124	99.150	< 0.001	0.291	0.228–0.371
Constant (Model 2)**†	0.961	0.580	2.745	0.098		
Age	0.013	0.006	5.275	0.022	1.013	1.002–1.024
Gender (female)	0.416	0.195	4.521	0.033	1.515	1.033–2.222
Satisfaction with dental appearance	-0.923	0.145	40.351	< 0.001	0.397	0.299–0.528
Previous orthodontic th	-1.028	0.260	15.675	< 0.001	0.358	0.215–0.595
Perceived malposition	0.862	0.284	9.183	0.002	2.367	1.356–4.132
Perceived bad fixed prosthesis	1.066	0.499	4.561	0.033	2.903	1.092–7.718
Hide teeth during smiling	2.209	0.625	12.505	< 0.001	9.104	2.677–30.967

***Negelkerke Pseudo R2 = 0.235; 66.1%; $p < 0.001$. **Negelkerke Pseudo r2 = 0.366, 73.5%, $p < 0.001$.**
†Only statistically significant variables are listed.

In the second model variable concerning previous dental therapy, perceived altered dental aesthetics and current dental status were added. For current dental status on maxillary anterior teeth most common restorative solution characteristics were used (mod value). Controlling all other variables in the model the significant predictors for seeking crowns in the maxillary anterior region are: age, female gender, satisfaction with dental appearance, previous orthodontic therapy, perceived malpositioned teeth, perceived bad fixed prosthesis and hiding teeth during smile. Hiding teeth during smile is the most important predictor producing 9.1 fold higher chance respectively for seeking the crowns (OR = 9.1 (95% CI 2.7 – 31.0)) (Table 2). Self-perceived bad fixed prosthesis, malpositioned teeth and female gender produced 2.9, 2.4, and 1.5 fold higher chance respectively, that participants want prosthetic therapy. Advanced age was statistically significant associated with seeking crowns ($p = 0.022$), but odds ratio was very low (OR = 1.02) (Table 2). The increase in satisfaction with dental appearance and previous orthodontic therapy reduced chances for seeking prosthetic therapy with odds ratios (each OR = 0.4) (Table 2). Addition of current dental status as a predictor in a model of logistic regression did not statistically significantly contribute to explanation of variability. None of current dental status (own natural maxillary anterior teeth, composite fillings, metal acrylic crowns and porcelain-fused-to ceramic crowns / ceramic veneers) was statistically significant predictor for seeking fixed prosthodontic restauration.

The results of univariate analysis considering bleeching are presented in Table 3. In multivariate logistic regression model the significant predictors for seeking bleaching were: hiding teeth during smiling, already done bleaching and female gender who increase the chance for seeking bleaching for 5.8, 2.4 and 1.8 times. Searching for bleaching was associated with lower levels of satisfaction with appearance of the teeth and the absence of the previous orthodontic therapy (OR 0.5 and 0.4, respectively; Table 4). The results of univariate analysis considering orthodontics are presented in Table 5. In multivariate logistic regression model the smallest numbers of factors had predictive value in seeking orthodontic treatment. In the first model, only the lower satisfaction with the appearance of the teeth was associated with seeking orthodontic treatment ($p < 0.001$). In the second model, controlling other factors, lower levels of satisfaction, self-perceived crowding, malposition and hiding teeth during smile were significant predictors, producing 2.2, 2.4 and 3.1 times higher chance, respectively, to seek orthodontic treatment (Table 6).

Discussion

For many years clinicians considered aesthetics to be far less important than function, structure and biology. However, nowdays if a treatment plan do not include a clear view of its aesthetics impact on the patient, the outcome could be disastrous [9]. A patient's satisfaction has become an increasingly important factor in dental treatment. Therefore, clinicians should begin a treatment plan with well-defined aesthetics objectives, and then should consider the impact of the planned treatment on function, structure and biology. Such planning requires the clinician to rely on several dental disciplines (namely prosthodontics, periodontics and orthodontics) to deliver the most comprehensive level of dental care to a patient [8].

Therefore, we investigated factors influencing people's decision to choose the type of treatment to improve dental aesthetics. We hypothesised that older subject would prefer prosthetic restoration and younger ones bleaching and orthodontics and that females would be more prone to every type of dental treatment than males. Searching for dental therapy is probably under strong influence of previous dental therapy

Table 3

Differences in variables between bleaching seeking and non-seeking population

Variables	Seeking bleeching		Significance	Effect size
	No (n = 258)	Yes (n = 442)		
Age ($\bar{x}$ ± SD)*, years	45.28 ± 18.68	46.75 ± 18.48	0.313	0.001
Gender**, n (%)				
m	109 (41.8%)	152 (58.2%)		
f	149 (33.9%)	290 (66.1%)	0.043	0.006
Education level**, n (%)				
primary / secondary	204 (37.4%)	341 (62.6%)		
college / university	54 (34.8%)	101 (65.2%)	0.573	< 0.001
Satisfaction with dental appearance*, $\bar{x}$ ± SD	2.53 ± 0.58	1.98 ± 0.78	< 0.001	0.125
Crowded teeth**, n (%)				
no	206 (38.9%)	323 (61.1%)		
yes	50 (30.5%)	114 (69.5%)	0.052	0.006
Malpositioned teeth**, n (%)				
no	214 (41.0%)	408 (59.0%)		
yes	44 (24.9%)	133 (75.1%)	< 0.001	0.021
Protruded teeth**, n (%)				
no	220 (38.8%)	347 (61.2%)		
yes	38 (28.8%)	94 (71.2%)	0.035	0.007
Decayed teeth**, n (%)				
no	248 (38.2%)	402 (61.8%)		
yes	10 (20.4%)	39 (79.6%)	0.014	0.009
Bad prosthesis**, n (%)				
no	248 (39.4%)	382 (60.6%)		
yes	10 (14.5%)	59 (85.5%)	< 0.001	0.024
Fractured teeth, n (%)				
no	234 (39.4%)	360 (60.6%)		
yes	24 (22.6%)	82 (77.4%)	< 0.001	0.015
Hide teeth during smile**, n (%)				
no	254 (41.0%)	365 (59.0%)		
yes	4 (5.1%)	75 (94.9%)	< 0.001	0.056
Orthodontic th.**, n (%)				
no	195 (34.9%)	363 (65.1%)		
yes	63 (44.4%)	79 (55.6%)	0.041	0.006
Bleaching th.**, n (%)				
no	241 (38.2%)	390 (61.8%)		
yes	17 (24.6%)	52 (75.4%)	0.026	0.007
Crowns**, n (%)				
no	184 (38.6%)	293 (61.4%)		
yes	74 (33.3%)	148 (66.7%)	0.207	0.003
Implants**, n (%)				
no	248 (36.2%)	437 (63.8%)		
yes	10 (66.7%)	5 (33.3%)	0.027	0.008
Root canal th.** , n (%)				
no	171 (42.8%)	229 (57.3%)		
yes	87 (29.0%)	213 (71.0%)	< 0.001	0.020
Professional teeth cleaning**, n (%)				
no	73 (42.0%)	101 (58.0%)		
yes	185 (35.2%)	341 (64.8%)	0.123	0.004
Root scaling**, n (%)				
no	222 (37.2%)	375 (62.8%)		
yes	36 (35.0%)	67 (65.0%)	0.740	< 0.001
Status MOD***, n (%)				
without therapy	155 (37.4%)	259 (62.6%)		
composite filling	26 (30.2%)	60 (69.8%)		
metal acrylic crowns	36 (35.0%)	67 (65.0%)		
ceramic crowns/veneers	41 (42.3%)	56 (57.7%)	0.382	0.004

***t*-test and eta squared for effect size; **Fischer exact test and Cramer's V for effect size; *** χ^2-test Cramer's V for effect size.**

Table 4

Logistic regression models for predicting variables influencing bleaching seeking

Variables	B	SE	Wald	Sig.	OR	95% CI
Constant (Model 1)*	2.566	0.409	39.304	0.000		
Age	0.008	0.005	2.724	0.099	1.008	0.999–1.017
Gender (female)	0.499	0.178	7.864	0.005	1.646	1.162–2.333
Education level (higher)	-0.174	0.206	0.707	0.400	0.841	0.561–1.260
Satisfaction with dental appearance	-1.129	0.125	80.963	0.000	0.323	0.253–0.414
Constant (Model 2)**†	1.603	0.574	7.805	0.005		
Gender (female)	0.560	0.188	8.843	0.003	1.750	1.210–2.531
Satisfaction with dental appearance	-0.937	0.145	41.989	0.000	0.392	0.295–0.520
Previous orthodontic th	-0.681	0.243	7.886	0.005	0.506	0.314–0.814
Previous bleaching	0.878	0.336	6.840	0.009	2.405	1.246–4.643
Hide teeth during smiling	1.755	0.547	10.289	0.001	5.784	1.979–16.901

***Negelkerke Pseudo R2 = 0.189, 64.7%, $p < 0.001$; **Negelkerke Pseudo R2 = 0.265, 68%, $p < 0.001$; †Only statistically significant variables are listed.**

Table 5

Differences in variables between orthodontic therapy seeking and non-seeking population

Variables	Seeking orthodontics		Significance	Effect size
	No (n = 308)	Yes (n = 392)		
Age ($\bar{x}$ ± SD)*, years	46.89 ± 18.42	45.68 ±18.66	0.393	0.001
Gender**, n (%)				
m	120 (46.0%)	141 (54.0%)		
f	188 (42.8%)	251 (57.2%)	0.432	0.001
Education level**, n (%)				
primary / secondary	241 (44.2%)	304 (55.8%)		
college / university	67 (43.2%)	88 (56.8%)	0.855	< 0.001
Satisfaction with dental appearance*, $\bar{x}$ ± SD	2.44 ± 0.65	1.97 ± 0.78	< 0.001	0.094
Crowded teeth**, n (%)				
no	269 (50.9%)	260 (49.1%)		
yes	34 (20.7%)	130 (79.3%)	< 0.001	0.067
Malpositioned teeth**, n (%)				
no	271 (51.9%)	251 (48.1%)		
yes	36 (20.3%)	141 (79.7%)	< 0.001	0.077
Protruded teeth**, n (%)				
no	267 (47.1%)	300 (52.9%)		
yes	40 (30.3%)	92 (69.7%)	< 0.001	0.017
Decayed teeth**, n (%)				
no	293 (45.1%)	357 (54.9%)		
yes	14 (28.6%)	35 (71.4%)	0.026	0.007
Bad prosthesis**, n (%)				
no	285 (45.2%)	345 (54.8%)		
yes	22 (31.9%)	47 (68.1%)	0.040	0.006
Fractured teeth, n (%)				
no	273 (46.0%)	321 (54.0%)		
yes	35 (33.0%)	71 (67.0%)	0.015	0.009
Hide teeth during smile**, n (%)				
no	297 (48.0%)	322 (52.0%)		
yes	9 (11.4%)	70 (88.6%)	< 0.001	0.055
Orthodontic th.** , n (%)				
no	242 (43.4%)	316 (56.6%)		
yes	66 (46.5%)	76 (53.5%)	0.509	0.001
Bleaching th.** , n (%)				
no	278 (44.1%)	353 (55.9%)		
yes	30 (43.5%)	39 (56.5%)	1.000	< 0.001
Crowns**, n (%)				
no	224 (47.0%)	253 (53.0%)		
yes	83 (37.4%)	139 (62.6%)	0.018	0.008
Implants**, n (%)				
no	298 (43.5%)	387 (56.5%)		
yes	10 (66.7%)	5 (33.3%)	0.112	0.005
Root canal th.** , n (%)				
no	203 (50.8%)	197 (49.3%)		
yes	105 (35.0%)	195 (65.0%)	< 0.001	0.025
Professional teeth cleaning**, n (%)				
no	77 (44.3%)	97 (55.7%)		
yes	231 (43.9%)	295 (56.1%)	1.000	< 0.001
Root scaling**, n (%)				
no	257 (43.0%)	340 (57.0%)		
yes	51 (49.5%)	52 (50.5%)	0.238	0.002
Status MOD***, n (%)				
without therapy	185 (44.7%)	229 (55.3%)		
composite filling	32 (37.2%)	54 (62.8%)		
metal acrylic crowns	44 (42.7%)	59 (57.3%)		
ceramic crowns/veneers	47 (48.5%)	50 (51.5%)	0.468	0.004

****t*-test and eta squared for effect size; ** Fischer exact test and Cramer's V for effect size; ***χ^2-test Cramer's V for effect size.**

Table 6

Logistic regression models for predicting variables influencing orthodontic therapy seeking

Variables	B	S.E.	Wald	Sig.	OR	95% CI
Constant (Model 1)*	2.176	0.352	38.327	< 0.001		
Age	-0.003	0.004	0.365	0.546	0.997	0.989–1.006
Gender (female)	0.193	0.167	1.326	0.249	1.213	0.873–1.684
Education level (higher)	0.098	0.193	0.258	0.612	1.103	0.755–1.612
Satisfaction with dental appearance	-0.881	0.112	61.985	< 0.001	0.415	0.333–0.516
Constant (Model 2)**†	0.753	0.550	1.875	0.171		
Satisfaction with dental appearance	-0.587	0.134	19.085	< 0.001	0.556	0.427–0.723
Perceived crowding	0.783	0.264	8.774	0.003	2.188	1.303–3.673
Perceived malposition	0.891	0.275	10.476	0.001	2.437	1.421–4.179
Hide teeth during smiling	1.133	0.404	7.871	0.005	3.106	1.407–6.856

***Negelkerke Pseudo R2 = 0.128, 63.5%, $p < 0.001$; **Negelkerke Pseudo R2 = 0.256, 69.8%, $p < 0.001$; †Only statistically significant variables are listed.**

and psychological elements, namely dissatisfaction with own teeth, hiding teeth during smile and self perceived altered aesthetic.

We expected that in older individuals their interest in dental appearance would be diminished, together with the lower socio-economic status of the older patients and their lower incomes (they are not able any more to afford themselves very expensive aesthetic restorations). It seems that older people are more satisfied with their dental appearance than younger [10, 11]. But this finding is under strong influence of their dental status – properly made porcelain-fused-to-metal crowns or fixed partial dentures on their upper anterior teeth [11]. Still, according to our study none of current dental status (own natural maxillary anterior teeth, composite fillings, metal acrylic crowns and porcelain-fused-to ceramic crowns / ceramic veneers) is a significant predictor for seeking fixed prosthodontic restorations. It is reported that age has an impact on desiring prosthetic restorations [12]. This is consistent with data obtained from this study. This research showed that beside age and female gender significant predictors of searching fixed prosthetic restorations are lower satisfaction with dental appearance, self-perceived malpositioned teeth, bad fixed prosthesis and hiding teeth during smile.

Age and gender are considered significant factors in predicting the color of the central incisors [13]. On the biological point of view it is known that with increasing age central incisors become darker, more reddish and more yellow, which is more pronounced in men than in women. Our study demonstrated that female gender is a significant predictor for choosing bleaching to improve dental aesthetics, but the age is not. It is commonly thought that women are more interested in their appearance than men. Indeed, female patients were found to be more concerned with their dental appearance than males, as well as to be more critical in judging their dental appearance [13]. Our study identified lower level of satisfaction with tooth appearance and hiding teeth during smiling as predictors for choosing bleaching to improve altered dental esthetics. It has been reported that 28% of adults in the UK are unsatisfied with the appearance of their teeth and 34 % of adult population in the USA is unsatisfied with their current tooth color [14]. In contrast to crowing or veneering whitening of teeth is relatively non-invasive and preserves hard dental tissues, therefore it is the most-desired basic treatment for the improvement of dental aesthetics [15]. This could be explained by the fact that most of the patients are dissatisfied with their tooth color and many of them had not made any attempt toward tooth whitening in the past. In addition, a study of 180 female patients in South London [16] showed that whitened teeth were preferred over teeth with original color with the former associated with greater attractiveness. Still, according to our data previous bleaching and the absence of previous orthodontic treatment are significant predictors for choosing bleaching. Probably the patients who underwent the procedure of tooth bleaching want more because they saw that it was relatively easy and painless procedure which is unfortunately reversible.

A variety of factors, including socio-economic background, education level, age, gender, self-esteem, self-perceived dental aesthetic, social and cultural norms have been suggested as factors affecting orthodontic treatment motives [2, 17, 18]. Females are often more dissatisfied with their teeth than males [12, 14, 18], but it is also reported that there was no significant association between the desire for orthodontic treatment and the variables gender and age [16], which is confirmed by our study. Poor self-perceived aesthetics and better socioeconomic position more significantly influence the decision to seek orthodontic treatment producing odds ratios of 16.7 and 39.1, than severe malocclusion (OR = 3.4) [19].

Generally lower satisfaction with dental appearance is the main predictor of desire to undergo orthodontic therapy, according to our research, accompanied with self-perceived crowding, malposition and hiding teeth during smile. It is reported that the main factor associated with orthodontic treatment seeking is self-perception of psychosocial impact of malocclusion, and not to improve altered masticatory function [18]. The desire for treatment, concern about dental appearance and oral health related quality of life (OHRQoL) are often interrelated. Malocclusion has modest influence on quality of life [20] that is more evident in altered emotional well-being than in masticatory function or social contacts [2]. Still worse OHRQoL produces 3.1 times higher chance to seek orthodontic treatment, although severly compromised aesthetics is a better predictor of worse OHRQoL than seeking orthodontic treatment [21]. It appears that satisfaction with personal dental appearance and awareness of malocclusion are better related in persons with no treatment need or minor need than in those with major need [22]. Although our study did not find any previous dental treatment as a predictor of desire for orthodontic treatment, it is reported that perception of orthodontic treatment need is higher in previously orthodontically treated subjects [2]. It must be kept in mind that the majority of studies concerning orthodontic treatment motives are done in children and adolescents, and not in adult population. Therefore, the results of our study could not be properly related to published data.

Since aesthetics has become an important issue in modern society and the number of elective aesthetic procedures increases, it seems important to have a good communication between a patient and the dentist, incorporating individual patients' and professional differences when planning the treatment and try to visualize treatments results before finalization.

Conclusion

This research indicates that in clinical works we must always consider the following clinical guidelines: females more often want dental treatments, the current dental status does not necessarily affect the choice of desirable dental treatments, but previous dental treatment experience does. Dental treatment to improve dental aesthetics is under strong influence of self-perceived altered aesthetics and the level of dissatisfaction. There are, unfortunately, a very small number of published papers on this issue, therefore further research should be encouraged.

REFERENCES

1. *Hasanreisoglu U, Berksun S, Aras K, Arslan I.* An analysis of maxillary anterior teeth: facial and dental proportions. J Prosthet Dent 2005; 94(6): 530−8.
2. Spalj S, Slaj M, Varga S, Strujic M, Slaj M. Perception of orthodontic treatment need in children and adolescents. Eur J Orthod. 2010 Aug;32(4):387-94.
3. *Carlsson GE, Johansson A, Johansson AK, Ordell S, Ekbäck G, Unell L.* Attitudes toward dental appearance in 50- and 60-Year-old subjects living in Sweden. J Esthet Restor Dent 2008; 20(1): 46−55; discussion 56.
4. *Lombardi RE.* The principles of visual perception and their clinical application to denture esthetics. J Prosthet Dent 1973; 29(4): 358−82.
5. *Marunick MT, Chamberlain BB, Robinson CA.* Denture aesthetics: an evaluation of laymen's preferences. J Oral Rehabil 1983; 10(5): 399−406.
6. *Vallittu PK, Vallittu AS, Lassila VP.* Dental aesthetics-a survey of attitudes in different groups of patients. J Dent 1996; 24(5): 335−8.
7. *Brisman AS.* Esthetics: a comparison of dentists' and patients' concepts. J Am Dent Assoc 1980; 100(3): 345−52.
8. *Samorodnitzky-Naveh GR, Geiger SB, Levin L.* Patients' satisfaction with dental esthetics. J Am Dent Assoc 2007; 138(6): 805−8.
9. *Spear FM, Kokich VG, Mathews DP.* Interdisciplinary management of anterior dental esthetics. J Am Dent Assoc 2006; 137(2): 160−9.
10. *Alkhatib MN, Holt R, Bedi R.* Age and perception of dental appearance and tooth colour. Gerodontology 2005; 22(1): 32−6.
11. *Lajnert V, Pavičić DK, Gržić R, Kovač Z, Pahor D, Kuiš ̌D*, et al. Influences of age and maxillary anterior teeth status on patient's satisfaction with dental appearance and tooth colour. Gerodontology 2012; 29(2): e674−9.
12. *Akarslan ZZ, Sadik B, Erten H, Karabulut E.* Dental esthetic satisfaction, received and desired dental treatments for improvement of esthetics. Indian J Dent Res 2009; 20(2): 195−200.
13. *Gozalo-Diaz D, Johnston WM, Wee AG.* Estimating the color of maxillary central incisors based on age and gender. J Prosthet Dent 2008; 100(2): 93−8.
14. *Joiner A.* The bleaching of teeth: a review of the literature. J Dent 2006; 34(7): 412−9.
15. *Tin-Oo MM, Saddki N, Hassan N.* Factors influencing patient satisfaction with dental appearance and treatments they desire to improve aesthetics. BMC Oral Health 2011; 11: 6.
16. *Kershaw S, Newton JT, Williams DM.* The influence of tooth colour on the perceptions of personal characteristics among female dental patients: comparisons of unmodified, decayed and 'whitened' teeth. Br Dent J 2008; 204(5): E9; discussion 256−7.
17. *Marques LS, Pordeus IA, Ramos-Jorge ML, Filogônio CA, Filogônio CB, Pereira LJ,* et al. Factors associated with the desire for orthodontic treatment among Brazilian adolescents and their parents. BMC Oral Health 2009; 9: 34.
18. *Xiao-Ting L, Tang Y, Huang XL, Wan H, Chen YX.* Factors influencing subjective orthodontic treatment need and culture-related differences among Chinese natives and foreign inhabitants. Int J Oral Sci 2010; 2(3): 149−57.
19. *Miguel JA, Sales HX, Quintão CC, Oliveira BH, Feu D.* Factors associated with orthodontic treatment seeking by 12-15-year-old children at a state university-funded clinic. J Orthod 2010; 37(2): 100−6.
20. *Liu Z, McGrath C, Hägg U.* The impact of malocclusion/orthodontic treatment need on the quality of life. A systematic review. Angle Orthod 2009; 79(3): 585−91.
21. *Feu D, de Oliveira BH, de Oliveira Almeida MA, Kiyak HA, Miguel JA.* Oral health-related quality of life and orthodontic treatment seeking. Am J Orthod Dentofacial Orthop 2010; 138(2): 152−9.
22. *Spalj S, Slaj M, Athanasiou AE, Simunovic D, Slaj M.* The unmet orthodontic treatment need of adolescents and influencing factors for not seeking orthodontic therapy. Coll Antropol. In press 2012.

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Primena fluorescentne *in situ* hibridizacije u hematologiji

Fluorescence *in situ* hybridization in hematology

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Ključne reči:
hromosomi, aberacije; hromosomi, anomalije; citogenetika; hibridizacija in situ, fluorescentna; hematološke neoplazme.

Key words:
chromosome aberrations; chromosome disorders; cytogenetics; in situ hybridization, fluorescence; hematologic neoplasms.

Uvod

Kod većine malignih hematoloških bolesti detektuju se numeričke i/ili strukturne aberacije hromozoma koje zahvataju gene važne za kontrolu ćelijskog ciklusa. Ovakve promene imaju za posledicu ili izmenjenu gensku ekspresiju ili stvaranje novog, abnormalnog proteina, što dovodi do deregulacije pre svega deobe i apoptoze ćelija [1]. Izmene u kodiranju proteina najčešće nastaju premeštanjem onkogena u blizinu gena koji ima visoku transkripcionu aktivnost. Neke genetske aberacije karakteristične su za određeno oboljenje, te se njihovim dokazivanjem potvrđuje dijagnoza bolesti i detektuje postojanje minimalne rezidualne bolesti tokom i nakon lečenja. Druge genetske promene imaju prognozni značaj, pa je njihovo utvrđivanje važno za primenu odgovarajuće terapije. Detaljnijom analizom genetskih promena omogućen je razvoj ciljane terapije, odnosno stvaranje lekova koji deluju na nivou genskog rearanžmana (npr. imatinib u hroničnoj granulocitnoj leukemiji, transretinoična kiselina u akutnoj promijelocitnoj leukemiji).

Klasična citogenetska analiza

Standardni pregled genetskog materijala ćelije – kariotipizacija vrši se klasičnom citogenetskom analizom. Za ovu analizu koristi se svež ćelijski materijal: aspirat koštane srži (najbolji izvor za analizu citogenetskih poremećaja u hematološkim oboljenjima), periferna krv, efuzioni sadržaj pleure, perikarda, peritoneuma, likvor, sveže (nefiksirano) tkivo limfnog čvora ili solidnog tumora dobijeno biopsijom ili aspiracijom.

Analiza kariotipa može biti direktna ili indirektna. Direktnom citogenetskom analizom dobijeni materijal se fiksira, boji (najčešće prema Giemsa-i) i mikroskopski analizira. Indirektna metoda, koja je češća u praksi, uključuje prethodnu kulturu dobijenog ćelijskog materijala jedan do tri dana, sa ili bez dodatka mitogena. Detektovane hromozomske abnormalnosti opisuju se prema preporukama Internacionalnog sistema za humanu citogenetsku nomenklaturu. Prednost klasične citogenetske analize je u tome što se pregleda celokupan genetski materijal ćelije pa se mogu uočiti i aberacije koje ne očekujemo u datom oboljenju, kao i kompleksne promene. Nedostaci ove analize su brojni i vezani, pre svega, za samu metodologiju rada: pošto je neophodan svež ćelijski uzorak analizu nije moguće uraditi na bioptiranom materijalu koji je fiksiran formalinom i koji se čuva u parafinskim kalupima; pregled hromozoma moguć je samo u ćelijama koje su u metafazi, tako da ne daje adekvantne rezultate u oboljenjima sa niskim mitotskim indeksom, kada je većina ćelija u interfazi (hronična limfocitna leukemija, multipli mijelom); nije moguće uočiti balansirane translokacije i delecije malog dela hromozoma; prilikom kulture ćelija može doći do gubitka vijabilnosti ćelija ili bakterijske kontaminacije; analiza zbog kultivacije ćelija traje dugo i rezultate je moguće dobiti tek nakon sedam dana; tehnički je zahtevna pošto nijedan deo procedure nije automatizovan, pa je neophodno dobro obučeno osoblje.

Fluorescentna *in situ* hibridizacija

Fluorescentna *in situ* hibridizacija (FISH) je molekularna citogenetska tehnika koja se zasniva na hibridizaciji određene genske sekvence ispitivanog hromozoma i odgovarajućeg niza nukleotida vezanog za fluorescentni marker (proba). Prema načinu vezivanja fluorescentnog markera FISH može biti direktna i indirektna. U direktnoj analizi sama proba vezana je za fluorohrom, dok se u indirektnoj analizi nukleotidi probe najpre vezuju za biotin ili digoksigenin, a potom vizuelizuju pomoću antitela na biotin ili digoksigenin vezanih za fluorohrome [2].

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Fluorescentnom *in situ* hibridizacijom može se analizirati svež ćelijski uzorak, prethodno fiksiran smešom metanola i sirćetne kiseline, ali i bioptirani materijal čuvan u parafinskim kalupima koji je najčešće fiksiran formalinom. Materijal za analizu može se dobiti iz uzoraka periferne krvi, koštane srži, efuzije perikarda, pleure, peritoneuma, likvora, briseva sluznica ili biopsija limfnih čvorova i solidnih tumora. Nije potrebna kultivacija ćelija, pa se rezultati analize mogu dobiti za 24–48 sati. Značajno je da se metodom FISH mogu vizualizovati promene na hromozomimna i kada su ćelije u interfazi, tako da se mogu analizirati genetske promene u oboljenjima sa niskim mitotskim indeksom. Fluorescentnom *in situ* hibridizacijom mogu se otkriti numeričke i strukturne promene na hromozomima (hipo- ili hiperdiploidija, delecije, mikrodelecije, inverzije i translokacije hromozoma), identifikovati genetska promena kod marker hromozoma i uraditi gensko mapiranje. Analiza je visokospecifična, pošto se proba vezuje isključivo za odgovarajući niz nukleotida analizirane dezoksiribonukleinske kiseline (DNK). Senzitivnost ove metode je velika zbog mogućnosti analize velikog broja ćelija. Lažno pozitivni rezultati nastaju kao posledica loše obrade preparata i neadekvatne mikroskopske analize (artefakt fluorohroma tumačen kao signal probe vezane za hromozom). Lažno negativni rezultati mogu se javiti ako postoji aneuploidija, ukoliko je jedro ćelije oštećeno ili dolazi do preklapanja ćelija i prekrivanje dela genoma jedne ćelije. Za razliku od konvencionalne citogenetike i molekularnih metoda, FISH omogućava korelaciju između genetske promene i morfologije ćelija.

Protokol fluorescentne *in situ* hibridizacije sastoji se iz „dva koraka“: u prvom se vrši denaturacija proteina visokom temperaturom, pri čemu dolazi do razdvajanja dvostrukih lanaca DNK analiziranih hromozoma, a u drugom dolazi do hibridizacije, tj. vezivanja probe za odgovarajući segment hromozoma koji „označava“ traženu genetsku aberaciju. Ukoliko se za FISH koristi materijal iz parafinskih kalupa, neophodno je prethodno uraditi deparafinizaciju i proteolizu uzoraka radi izolacije jedara ćelija. Pre denaturacije suspenzija ćelija nanosi se na predmetne mikroskopske pločice, fiksira i pere da bi se odstranila prljavština koja bi mogla da ometa analizu. Denaturacija uzorka i probe vrši se na 73^{o}C, a hibridizacija u vlažnoj komori na 37^{o}C tokom 12–16 sati. Rezultat hibridizacije analizira se pomoću fluorescentnog mikroskopa sa odgovarajućim filterima [3].

Postoji nekoliko tipova tehnike FISH koje se razlikuju po broju analiziranih genetskih sekvenci, mestu vezivanja probe i broju proba koje se koriste u analizi. To su: centromera specifične probe, analiza celog hromozoma (*whole chromosome painting probes*), lokus-specifične probe i spektralni kariotiping (SKY, multikolor FISH).

Centromera specifične probe

Centromera specifične probe su bazirane na vezivanju probe za repetitivnu alfa satelitsku sekvencu centromerne DNK. Pošto se alfa satelitske sekvence hromozoma razlikuju, ova vrsta analize koristi se za dokazivanje promene broja hromozoma. Zbog sličnosti alfa satelitske sekvence 13. i 21, odnosno 14. i 22. hromozoma, ovaj tip tehnika FISH ne može se koristiti za analizu aneuploidije ovih hromozoma.

Analiza celog hromozoma

Analiza celog hromozoma tehnikom FISH (*whole chromosome painting probes*) sastoji se od mnogo proba koje se vezuju za specifične sentence čitavom dužinom odabranog hromozoma. Koristi se za identifikaciju marker hromozoma (izmenjenog hromozoma nepoznatog porekla), ili za otkrivanje translokacija koje nisu mogle adekvatno da se interpretiraju pomoću klasične citogenetske analize.

Lokus specifične probe

Lokus specifične probe se najčešće koriste u hematologiji. Odgovarajuće probe vezuju se za nerepetitivnu gensku sekvencu koju želimo da analiziramo. Koriste se za otkrivanje genskih rearanžmana, amplifikacije ili gubitka genske sekvence koje nije bilo moguće uočiti klasičnom citogenetskom analizom. U hematologiji se najčešće koriste za otkrivanje translokacija gena. Postoji nekoliko tipova lokus specifičnih proba. Fluorescentna *in situ* hibridizacija sa jednom probom sastoji se u obeležavanju odgovarajuće genske sekvence jednom fluorescentnom bojom, tako da se u normalnoj ćeliji očitavaju dva signala. Više signala ukazuje na amplifikaciju sekvence, a samo jedan signal na deleciju gena. FISH sa dve probe može biti fuziona (*dual-fusion*) i analiza odvajanja (*break-apart*). Kod fuzione analize koriste se dva fluorohroma različite boje (najčešće crvena i zelena) za obeležavanje dva udaljena gena koji učestvuju u recipročnoj translokaciji. U normalnoj ćeliji ima četiri signala (dva crvena i dva zelena), dok se u ćeliji sa translokacijom gena vidi jedan crveni i jedan zeleni signal koji se nalaze na normalnim hromozomima i jedan žuti signal koji se nalazi na hromozomu na kome je došlo do translokacije i fuzije gena (žuta boja nastaje usled preklapanja crvenog i zelenog signala). Princip analize odvajanja suprotan je fuzionoj: tu se pomoću dva različita fluorohroma obeležavaju suprotni krajevi genske sekvence u kojoj dolazi do prekida i translokacije genetskog materijala na druge hromozome. U normalnoj ćeliji očitavaju se dva žuta signala, a u ćeliji sa translokacijom gena vidi se jedan žuti signal (normalan hromozom), ali i po jedan crveni i zeleni signal (hromozomi na kojima je došlo do translokacije gena).

Spektralni kariotiping

Spektralni kariotiping je tehnika kojom se pomoću dvadeset četiri fluorescentne boje istovremeno obeležavaju svi hromozomi ćelije u metafazi, pa je moguća identifikacija kompletnog kariotipa. Ovakva analiza najčešće se koristi za identifikaciju marker hromozoma i kompleksnih hromozomskih aberacija koje se ne mogu adekvatno interpretirati konvencionalnom citogenetikom.

Ograničenja kliničke primene metoda FISH proističu iz činjenice da se ovom metodom mogu utvrditi samo pretpostavljene genetske aberacije, pošto se ne analizira kompletan kariotip, tako da se hromozomske promene koje ne očekujemo ne mogu detektovati. Zato je najbolje kombinovati ovu

metodu sa klasičnom citogenetskom analizom. Takođe, potreban je veliki broj specifičnih proba za postavljanje adekvatne dijagnoze i prognoze bolesti. Probe su veličine 20–200 kb tako da se ne mogu detektovati veoma male promene genetskog materijala, kao što su male intragenske mutacije, pa je ponekad potrebno dopuniti metodu FISH osetljivijim molekularnim tehnikama ispitivanja DNK.

Primena fluorescentne *in situ* hibridizacije u pojedinim hematološkim oboljenjima

Akutna mijeloblastna leukemija

Citogenetski status izuzetno je značajan za prognozu akutne mijeloblastne leukemije (AML) u vreme postavljanja dijagnoze bolesti (*de novo* AML). Prema broju i vrsti hromozomskih aberacija AML je razvrstana u tri prognostičke grupe koje imaju različit tok bolesti, odgovor na terapiju i dužinu preživljavanja. U grupi sa povoljnom prognozom nalaze se bolesnici sa $t(8;21)^{RUNX1/ETO}$, inv(16) ili $t(16;16)$ i $t(15;17)^{PML/RAR\alpha}$. Bolesnici sa normalnim kariotipom ili izolovanim aberacijama: t(9;11), -Y, del(11q), +13, del(20q) i +21 imaju intermedijarnu prognozu, a oni sa kompleksnim kariotipom ili sa promenama na trećem, petom, šestom, sedmom, osmom i devetom hromozomu [(inv (3), t(3;3), t(6;9), t(6;11), -5, -7, t(9;22), +8)] čine grupu sa nepovoljnom prognozom [4]. U sekundarnim AML, koje nastaju evolucijom mijelodisplastičnog sindroma i hronične granulocitne leukemije, nakon primene citotoksične i/ili radioterapije drugih bolesti, najčešće hromozomske aberacije razlikuju se od onih koji se viđaju u primarnoj AML: -5, del(5q), -7, del(7q), del(11q), del(12q), del(20q), -18, +8, +9, +11, +19, +21 i t(3;21). Pošto se kod oko 45% bolesnika standardnom citogenetskom analizom dobija normalan nalaz kariotipa poželjno je prilikom postavljanja dijagnoze zamrznuti ćelijsku suspenziju ili razmaze periferne krvi i koštane srži, da bi se kasnije mogla uraditi FISH radi detektovanja genskih rearanžmana RUNX1-RUNX1T1, CBFB-MYH11, fuzija EVI1 gena, malih delecija hromozoma 5q i 7q i radi prepoznavanja fuzionog partnera MLL gena u translokaciji 11q23 (preporuke *European LeukemiaNet*) [5, 6].

Za neke tipove AML karakteristične su određene hromozomske aberacije na osnovu kojih se postavlja dijagnoza i određuje prognoza bolesti. Najbolji primer postojanja karakterističnog genetskog markera je akutna promijelocitna leukemija (APL) koja nastaje balansiranom translokacijom gena između 15. i 17. hromozoma i stvaranjem fuzionog onkogena PML/RARα. Konvencionalnom citogenetskom analizom ova translokacija otkriva se u 70–90% slučajeva, ali ako postoje submikroskopske insercije gena dobija se lažno negativan rezultat. Ove kriptične varijante translokacija mogu se otkriti metodom FISH [7]. Uvek je potrebno uraditi i klasičnu citogenetsku analizu, ne samo zbog postojanja varijantnih translokacija t(11;17) i t(5;17) sa stvaranjem novih fuzionih proteina i rezistencijom na lečenje transretinoičnom kiselinom, nego i zbog toga što 25–40% bolesnika ima sekundarne hromozomske promene (+8, +6, +7, +9, +12, +16, +17, +21) [8].

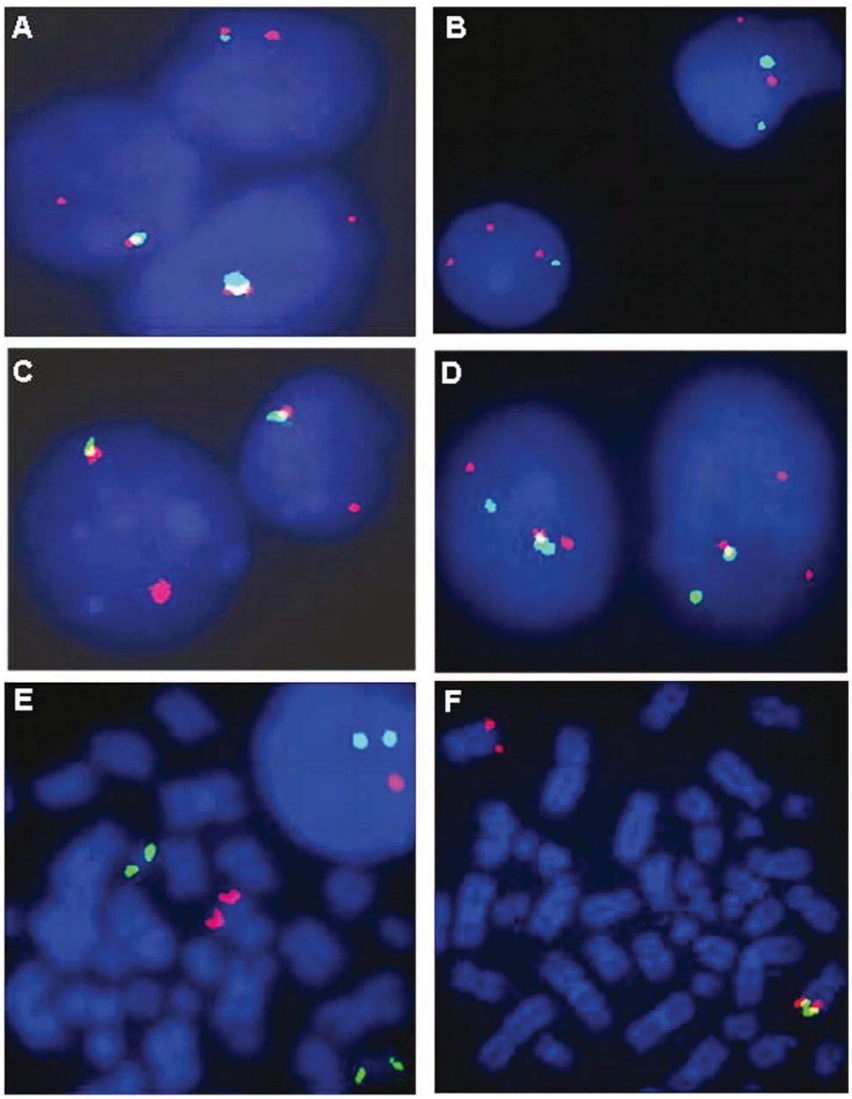
Akutna limfoblastna leukemija

Bolesnici sa akutnom B-limfoblastnom leukemijom (B-ALL) često imaju poremećaj broja i strukture hromozoma. Oni sa hiperdiploidijom (broj hromozoma veći od 50) imaju povoljnu prognozu, pogotovo ako su prisutne trisomije 4, 10. ili 17. hromozoma. Slučajevi sa hipodiploidijom (broj hromozoma manji od 45, pogotovo 24–28 hromozoma) imaju nepovoljnu prognozu, a kada je broj hromozoma veći od 92 postoji loš odgovor na terapiju. Strukturne anomalije hromozoma mogu biti različite, a ukoliko se detektuju, uglavnom su prognostički nepovoljne, na primer: t(9;22) sa stvaranjem bcr/abl gena, t(1;19) sa stvaranjem E2A/PBX1 gena, del(11q23) i t(4;11) koje dovode do izmenjene ekspresije MLL gena, intrahromozomska amplifikacija 21. hromozoma (iamp21) sa izmenjenom ekspresijom RUNX1 gena, t(8;14), t(2;8) i t(8;22) koje dovode do izmena u lokusu c-myc gena, i translokacije vezane za 14. hromozom i CEBP gen (t(8;14), t(14;19), inv(14), t(14;14), t(14;20)) uz stvaranje različitih fuzionih produkata [9]. Zbog velike senzitivnosti metode FISH u detekciji fuzionih onkogena (bcr/abl1, fuzije MLL gena) poželjno je ovu analizu uraditi uz standarnu citogenetsku analizu [10].

U akutnoj T-limfoblastnoj leukemiji (T-ALL) hromozomske promene postoje kod 25–50% slučajeva. Povoljnu prognozu imaju bolesnici sa normalnim kariotipom i t(10;14). Numerički poremećaji su brojni i nisu karakteristični samo za ovu vrstu leukemije (-9, -13, +4, +8, +17, +20, +22, +marker hromozom) [11]. Česte su i translokacije dela 14. hromozoma na kome se nalazi gen za kodiranje T-ćelijskog receptora (TCR) [t(14;20), t(11;14), t(1;14), t(8;14)]. Ove translokacije vezane za TCR lokus mogu biti kriptičke pa je za njihovu identifikaciju neophodno uraditi FISH [12] (slika 1).

Hronična granulocitna leukemija

Hronična granulocitna leukemija (HGL) primer je oboljenja u kome je citogenetski nalaz neophodan za postavljanje dijagnoze, uvođenje ciljane terapije inhibitorima tirozin kinaze i praćenje odgovora na lečenje. Karakteristična hromozomska promena je translokacija genetskog materijala sa devetog na dvadeset drugi hromozom i stvaranje fuzionog gena bcr/abl, koji preko tirozin kinaze c-abl utiče na ćelijski ciklus podstičući proliferaciju ćelija [13]. Kod oko 90% bolesnika detektuje se t(9;22), kod oko 95% bcr/abl gen, a kod oko 5% se dostupnim metodama ne detektuje ni bcr/abl gen. Oko 5–10% bolesnika imaju varijantne ili kompleksne translokacije. Kod većine bolesnika može se dokazati t(9;22) klasičnom citogenetskom analizom, ali su FISH ili „*real-time*“ *polymerase chain reaction* (RQ-PCR) analize koje se koriste za praćenje minimalne rezidualne bolesti, pošto se klasičnom citogenetikom analizira mali broj ćelija. Tehnikom FISH moguće je otkriti velike delecije devetog hromozoma (der9) što nema uticaja na prognozu bolesti [14]. Tokom akceleracije ili blastne transformacije HGL može doći do klonske evolucije i stvaranja dodatnih hromozomskih aberacija: del(17), +8, dupliranje Ph hromozoma, i(17q), +19, -Y (u 20–40% slučajeva). Sa progresijom bolesti često dolazi do amplifikacije bcr/abl gena u ćeliji što se može utvrditi tehnikom FISH [15].



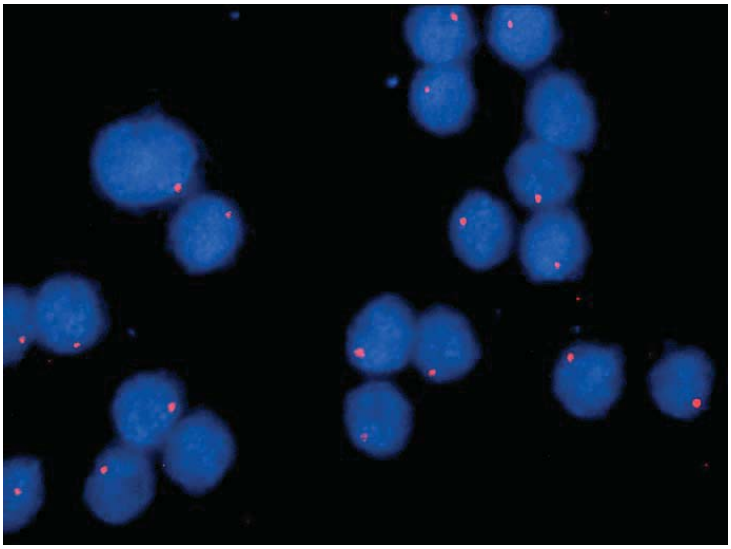
Sl. 1 – Hromozomske aberacije u T-akutnoj limfoblastnoj leukemiji (T-ALL: A) Translokacija (5;14)(TCRA/TLX3), B) Trisomija 6q; C, D) Translokacija (7;10) (TCRB/HOX11); E, F) Delecija(9)(q34)

Mijelodisplastični sindrom

Kod bolesnika sa primarnim mijelodisplastičnim sindromom (MDS) detektuju se hromozomske aberacije kod 40–70% slučajeva, a kod sekundarnog (postterapijskog) MDS-a procenat genetskih anomalija veći je i dostiže 90% [16]. Značajan prognostički faktor čine promene u broju i strukturi hromozoma, na osnovu koga se bolesnici dele u tri grupe u odnosu na tok i ishod bolesti. Grupu sa povoljnom prognozom čine bolesnici sa normalnim kariotipom, -Y, izolovan -5q (5q- sindrom) i izolovan -20q. Na nepovoljnu prognozu ukazuje postojanje kompleksnog kariotipa ili aberacija vezanih za peti i sedmi hromozom (-5, del(5), -7, del(7q)), a na intermedijarnu prognozu trisomija 8 i del(11q)[17]. Određene hromozomske aberacije karakteristične su za tip MDS-a, npr. u refrakternoj anemiji (RA) najčešća je del(5q), u refrakternoj anemiji sa ekscesom blasta (RAEB) i hroničnoj mijelomonocitnoj leukemiji -5, -7 i der(12p), a u refrakternoj anemiji sa *ring* sideroblastima (RARS) trisomija 8. hromozoma i der(11q)[18]. Najčešću hromozomsku abnormalnost u MDS-u čine delecije hromozoma i, ukoliko su to mikrodelecije, one se ne mogu uočiti standardnom citogenetskom analizom, pa je poželjno uraditi FISH, posebno za detekciju malih delecija 5. i 7. hromozoma [19]. Kompleksne promene kariotipa bolje se uočavaju pomoću spektralne kariotipizacije u kombinaciji sa lokus-specifičnom FISH nego klasičnom citogenetikom [20].

Hronična limfocitna leukemija

Hromozomske aberacije su nezavisan prediktor progresije bolesti i dužine preživljavanja bolesnika sa hroničnom limfocitnom leukemijom (HLL) [21, 22]. Standardnom citogenetskom analizom promene na hromozomima otkrivaju se kod 36,7% bolesnika zbog malog broja ćelija u metafazi i slabog odgovora na mitogene u kulturi ćelija [23]. Pošto se interfaznom FISH metodom hromozomske aberacije otkrivaju kod preko 80% analiziranih bolesnika, poželjno je uraditi ovu analizu prilikom postavljanja dijagnoze HLL. Najčešća genetska promena, del(13q14), otkriva se kod 40–60% bolesnika i marker je povoljne prognoze (slika 2). Delecija 11.



Sl. 2 – Delecija 13q u hroničnoj limfocitnoj leukemiji (HLL)

hromozoma u delu gde se nalazi ATM gen (11q22-23) znak je nepovoljne prognoze i detektuje se kod 12–23%. Delecija 17. hromozoma (17p13) koja dovodi do poremećaja funkcije p53 gena dovodi do rezistencije na terapiju fludarabinom, pa bolesnici sa ovom aberacijom imaju izrazito nepovoljnu prognozu [24]. Ovu genetsku promenu poželjno je utvrditi na početku bolesti radi pravovremene odgovarajuće terapije [25]. Ako se pomoću metode FISH testira postojanje delecije 17. hromozoma na početku i u odmaklim fazama bolesti, uočava se da ovu aberaciju ima 7% bolesnika na početku lečenja, a 50% sa refrakternom bolešću [26]. Trisomija 12. hromozoma često se nalazi u atipičnim varijantama bolesti, kada ćelije imaju nekarakterističnu morfologiju ili aberantni imunofenotip i znak je povoljne prognoze [27]. *International Workshop on Chronic Lymphocytic Leukemia* (IWCLL) preporučuje da se FISH obavezno uradi pre započinjanja lečenja, naročito ako će bolesnici biti uključeni u kliničke studije, ali i da se ova analiza ponovi pre druge, treće i narednih linija terapije zbog velike verovatnoće nastanka dodatnih genetskih aberacija tokom progresije bolesti [28].

Multipli mijelom

Numeričke, strukturne ili kompleksne promene kariotipa detektuju se kod oko 90% bolesnika sa multiplim mijelomom (MM) i postojanje ovih aberacija nezavisan je prognostički marker [29, 30]. Genetske aberacije u ovoj bolesti retko se otkrivaju konvencionalom citogenetskom analizom zbog malog broja ćelija u mitozi, pa je neophodno uraditi FISH ili molekularne analize radi primene adekvatne terapije [31, 32]. Hiperdiploidiju ima 60% bolesnika, najčešće trisomiju 3, 5, 7, 9, 11, 15, 19. i 21. hromozoma. Česte su i monosomije 8, 13, 14, 17. i X hromozoma. Nepovoljnu prognozu imaju bolesnici sa hipodiploidijom i delecijom 13. hromozoma, kao i sa rearanžmanom IGH gena na 14. hromozomu (14q32): t(4;14), t(14;16), t(14;20). Delecija 17p13 detektuje se kod 11–15% obolelih od MM i prognostički je nepovoljna, kao i promene na prvom hromozomu (1q21) [33, 34]. Postojanje translokacija t(11;14) i t(6;14) ne utiče na dužinu preživljavanja ako su prisutne kao izolovane anomalije. Tokom progresije MM dolazi do stvaranja novih genetskih aberacija i nastajanja kompleksnog kariotipa [35]. S obzirom na to da su bolesnici sa MM na osnovu postojanja citogenetskih abnormalnosti svrstani u grupe sa visokim i niskim rizikom i da se terapija ove dve grupe bolesnika razlikuje, preporuke *International Myeloma Working Group* (IMWG) je da se pre početka lečenja uradi FISH sa probama za del(13q14), del(17p), t(4;14), t(11;14), t(14;16) i t(6;14) [36].

Limfomi

Limfomske ćelije obično imaju genetske aberacije čije otkrivanje doprinosi postavljanju dijagnoze i određivanju prognoze bolesti. To su najčešće translokacije hromozoma koje uzrokujući stvaranje fuzionih onkogena utiču na nastanak i/ili progresiju bolesti. Ukoliko su ove translokacije kriptične, često se jedan od gena koji učestvuju u stvaranju fuzionog onkogena ne može detektovati standardnom citogenetskom analizom, pa je poželjno na početku bolesti uraditi FISH. U daljem tekstu navedene su promene na hromozomima koje su karakteristične za određene tipove limfoma [37].

Folikularni limfom

Bolesnici sa folikularnim limfomom u 80-90% slučajeva imaju karakterističnu translokaciju t(14;18)(q32;q21) tokom koje se bcl2 gen premešta u blizinu gena za teške imunoglobulinske lance, stvarajući fuzioni gen bcl2/IGH sa posledičnom prekomernom ekspresijom bcl2 [38]. Najsenzitivnija metoda za otkrivanje ovog fuzionog onkogena (IgH/bcl2) je FISH [39]. Pri postavljanju dijagnoze citogenetskim analizama često se otkrivaju i dodatne hromozomske promene koje su obično prognostički nepovoljne: del(1q), del(6q), del(10q), poliploidija (češća transformacija u difuzni krupnoćelijski limfom), +12, del(1p), dup(18q) – kraće preživljavanje [40].

Mantle cell limfom

Za ovaj tip limfoma karakteristična je translokacija sa 11. na 14. hromozom (t(11;14)(q13;q32)) koja dovodi bcl1 gen pored gena za teške imunoglobulinske lance. Umesto

ove karakteristične može biti prisutna varijantna translokacija sa 9. na 14. hromozom (t(9;14)(p13;q32)) kojom se pored IGH gena smešta PAX5 gen [41]. Metodom FISH može se tačno detektovati fuzioni partner IGH gena [42]. Bolesnici sa MCL često imaju i dodatne hromozomske promene ili kompleksan kariotip, a među njima su najčeće: del(13q), del(17p) i trisomija 12 koji su markeri loše prognoze [43].

MALT limfom

Bolesnici sa MALT (*Mucosa-Assosiated Lymphoid Tissue*) limfomom mogu imati brojne izolovane ili udružene hromozomske aberacije. Najčešća izolovana genetska promena je t(11;18) kojom se stvara fuzioni API2/MALT gen [44]. Ukoliko ova translokacija nije prisutna česte su numeričke promene kariotipa: +3, +7, +18, +12, del (16), kao i sledeće translokacije: t(11;18), t(1;14), t(14;18), t(3;14) [45]. Učestalost određenih genetskih aberacija je različita i zavisi od primarne lokalizacije limfoma, t(11;18) najčešće se javlja u gastričnom i pulmonalnom MALT limfomu, t(14;18) kada su primarno zahvaćene orbita, koža ili pljuvačne žlezde, a trisomija 3. ili 18. hromozoma kada je mesto nastanka tumora intestinalna sluznica.

Difuzni krupnoćelijski limfom

Za difuzni krupnoćelijski limfom karakteristična je fuzija bcl6 gena koji se nalazi na dugom kraku trećeg hromozoma (3q27) sa različitim partnerskim genima, najčešće sa genima za teške imunoglobulinske lance (14q32), lake imunoglobulinske lance tipa kappa (2p12) ili lake imunoglobulinske lance tipa lambda (22q11) i posledične prekomerne ekspresije bcl6 [46]. Čak i postojanje malih delecija ili *point* mutacija u regionu ovog gena ukazuje na nepovoljnu prognozu [47]. Poželjno je uraditi FISH prilikom postavljanja dijagnoze pošto se standardnom citogenetskom analizom često ne mogu detektovati fuzioni partneri bcl6 gena. Oko 25% bolesnika ima druge, nekarakteristične genske aberacije, kao što su t(14;18) ili t(8;14) [48].

Burkitt-ov limfom

Za ovaj tip limfoma karakteristična je translokacija c-MYC onkogena sa hromozoma osam na 14, 8. ili 22. hromozomu gde se nalaze geni za kodiranje imunoglobulinskih lanaca i stvaranje fuzionih gena c-MYC/IGH, c-MYC/IGK i c-MYC/IGL [49]. Tačno mesto translokacije c-MYC gena može se otkriti tehnikom FISH [50]. Oko 50% bolesnika ima dodatne hromozomske aberacije koje su najčešće vezane za prvi, šesti, trinaesti, sedamnaesti i dvadeset drugi hromozom (+1, +1q, del6, +6, t(1;6), rearanžman (13q), t(17; 22) [51].

Anaplastični krupnoćelijski limfom

Za ovaj limfom porekla T limfocita karakteristično je stvaranje fuzionog gena NPM/ALK translokacijom (2;5)(p23;q35) koji kodira protein p80 [52]. Identifikovani su i drugi geni koji se spajaju sa ALK genom kao posledica translokacija na prvom, trećem i sedamnaestom hromozomu: TPM3/ALK [(t(1;2)(q21;p23)), TFG/ALK (t(2;3)(p23;q21))] i CLTC/ALK [(t(2;17)(p23;q23))] [53].

Zaključak

Većina hematoloških maligniteta ima karakteristične hromozomske aberacije koje se ne mogu uvek uočiti standarnom citogenetskom analizom, npr. balansirane translokacije, delecije malog dela hromozoma ili kriptične rearanžmane gena, a mogu se detektovati fluorescentnom *in situ* hibridizacijom. Prednosti ove metode su mogućnost otkrivanja genetskih aberacija u ćelijama koje su u interfazi, primena i na svežem i na ranije fiksiranom materijalu i kratko vreme trajanja analize. Sve ovo omogućava brzo postavljanje dijagnoze, određivanje prognoze bolesti i započinjanje adekvatne terapije.

L I T E R A T U R A

1. *Jaffe ES, Harris NL, Stein H, Vardiman JW*. World Health Organization Classification of Tumors. Pathology and Genetics of Tumors of Hematopoietic and Lymphoid Tissues. Lyon: IARC Press, 2001.
2. *Tibiletti MG, Bernasconi B, Dionigi A, Riva C*. The applications of FISH in tumor pathology. Adv Clin Path 1999; 3(4): 111−8.
3. *Trask B, Pinkel D*. Fluorescence in situ hybridization with DNA probes. Methods Cell Biol 1990; 33: 383−400.
4. *Arber DA, Stein AS, Carter NH, Ikle D, Forman SJ, Slovak ML*. Prognostic impact of acute myeloid leukemia classification. Importance of detection of recurring cytogenetic abnormalities and multilineage dysplasia on survival. Am J Clin Pathol 2003; 119(5): 672−80.
5. *Mrózek K, Marcucci G, Paschka P, Whitman SP, Bloomfield CD*. Clinical relevance of mutations and gene-expression changes in adult acute myeloid leukemia with normal cytogenetics: are we ready for a prognostically prioritized molecular classification? Blood 2007; 109(2): 431−48.
6. *Döhner H, Estey EH, Amadori S, Appelbaum FR, Büchner T, Burnett AK,* et al. Diagnosis and management of acute myeloid leukemia in adults: recommendations from an international expert panel, on behalf of the European LeukemiaNet. Blood 2010; 115(3): 453−74.
7. *Brockman SR, Paternoster SF, Ketterling RP, Dewald GW*. New highly sensitive fluorescence in situ hybridization method to detect PML/RARA fusion in acute promyelocytic leukemia. Cancer Genet Cytogenet 2003; 145(2): 144−51.
8. *Grimwade D, Biondi A, Mozziconacci MJ, Hagemeijer A, Berger R, Neat M*, et al. Characterization of acute promyelocytic leukemia cases lacking the classic t(15;17): results of the European Working Party. Groupe Français de Cytogénétique Hématologique, Groupe de Français d'Hematologie Cellulaire, UK Cancer Cytogenetics Group and BIOMED 1 European Community-Concerted Action "Molecular Cytogenetic Diagnosis in Haematological Malignancies". Blood 2000; 96(4): 1297−308.
9. *Forestier E, Johansson B, Gustafsson G, Borgström G, Kerndrup G, Johannsson J*, et al. Prognostic impact of karyotypic findings in childhood acute lymphoblastic leukaemia: a Nordic series comparing two treatment periods. For the Nordic Society of Paediatric Haematology and Oncology (NOPHO) Leukaemia Cytogenetic Study Group. Br J Haematol 2000; 110(1): 147−53.
10. *Moorman VA, Chilton L, Wilkinson J, Ensor MH, Brown N, Proctor JP,* et al. A population – based cytogenetic study of adults with acute lymphoblastic leukemia. Blood 2010; 115(2): 206−14.

11. *van Grotel M, Meijerink JP, Beverloo HB, Langerak AW, Buys-Gladdines JG, Schneider P,* et al. The outcome of molecular-cytogenetic subgroups in pediatric T-cell acute lymphoblastic leukemia: a retrospective study of patients treated according to DCOG or COALL protocols. Haematologica 2006; 91(9): 1212−21.
12. *Graux C, Cools J, Michaux L, Vandenberghe P, Hagemeijer A.* Cytogenetics and molecular genetics of T-cell acute lymphoblastic leukemia: from thymocyte to lymphoblast. Leukemia 2006; 20(9): 1496−510.
13. *Ren R.* Mechanisms of BCR-ABL in the pathogenesis of chronic myelogenous leukaemia. Nat Rev Cancer 2005; 5(3): 172−83.
14. *Amiel A, Yarkoni S, Fejgin M, Gaber E, Nagler A, Manor Y,* et al. Clinical detection of BCR-abl fusion by in situ hybridization in chronic myelogenous leukemia. Cancer Genet Cytogenet 1993; 65(1): 32−4.
15. *Cortes JE, Talpaz M, Giles F, O'Brien S, Rios MB, Shan J,* et al. Prognostic significance of cytogenetic clonal evolution in patients with chronic myelogenous leukemia on imatinib mesylate therapy. Blood 2003; 101(10): 3794−800.
16. *Heim S.* Cytogenetic findings in primary and secondary MDS. Leuk Res 1992; 16(1): 43−6.
17. *Bernasconi P, Klersy C, Boni M, Cavigliano PM, Calatroni S, Giardini I,* et al. Incidence and prognostic significance of karyotype abnormalities in de novo primary myelodysplastic syndromes: a study on 331 patients from a single institution. Leukemia 2005; 19(8): 1424−31.
18. *Bennett JM, Kouides PA, Forman SJ.* The myelodysplastic syndromes: morphology, risk assessment and clinical management (2002). Int J Hematol 2002; 76(Suppl 2): 228–38.
19. *Haase D.* Cytogenetic features in myelodysplastic syndromes. Ann Hematol 2008; 87(7): 515−26.
20. *Olney HJ, Le Beau MM.* Evaluation of recurring cytogenetic abnormalities in the treatment of myelodysplastic syndromes. Leuk Res 2007; 31(4): 427−34.
21. *Dierlamm J, Michaux L, Criel A, Wlodarska I, Van den Berghe H, Hossfeld DK.* Genetic abnormalities in chronic lymphocytic leukemia and their clinical and prognostic implications. Cancer Genet Cytogenet 1997; 94(1): 27−35.
22. *Pettitt AR.* Genetic markers of prognosis in chronic lymphocytic leukemia. Education program for the 15th Congress of the European Hematology Association Barcelona, Spain; 2010June 10−13; Hematology Education: the education program for theannual congress of the European Hematology Association 2010; 4: 85−92.
23. *Ripollés L, Ortega M, Ortuño F, González A, Losada J, Ojanguren J,* et al. Genetic abnormalities and clinical outcome in chronic lymphocytic leukemia. Cancer Genet Cytogenet 2006; 171(1): 57−64.
24. *Dohner H, Stilgenbauer S, Fischer K, Bentz M, Lichter P.* Cytogenetic and molecular cytogenetic analysis of B cell chronic lymphocytic leukemia: specific chromosome aberrations identify prognostic subgroups of patients and point to loci of candidate genes. Leukemia 1997; 11(Suppl 2): S19–24.
25. *Grever MR, Lucas DM, Dewald GW, Neuberg DS, Reed JC, Kitada S,* et al. Comprehensive assessment of genetic and molecular features predicting outcome in patients with chronic lymphocytic leukemia: results from the US Intergroup Phase III Trial E2997. J Clin Oncol 2007; 25(7): 799−804.
26. *Shanafelt TD, Witzig TE, Fink SR, Jenkins RB, Paternoster SF, Smoley SA,* et al. Prospective evaluation of clonal evolution during long-term follow-up of patients with untreated early-stage chronic lymphocytic leukemia. J Clin Oncol 2006; 24(28): 4634−41.
27. *Döhner H, Stilgenbauer S, Benner A, Leupolt E, Kröber A, Bullinger L,* et al. Genomic aberrations and survival in chronic lymphocytic leukemia. N Engl J Med 2000; 343(26): 1910−6.
28. *Hallek M, Cheson BD, Catovsky D, Caligaris-Cappio F, Dighiero G, Döhner H,* et al. Guidelines for the diagnosis and treatment of chronic lymphocytic leukemia: a report from the International Workshop on Chronic Lymphocytic Leukemia updating the National Cancer Institute-Working Group 1996 guidelines. Blood 2008; 111(12): 5446−56.
29. *Seong C, Delasalle K, Hayes K, Weber D, Dimopoulos M, Swantkowski J,* et al. Prognostic value of cytogenetics in multiple myeloma. Br J Haematol 1998; 101(1): 189−94.
30. *Stewart AK, Fonseca R.* Prognostic and therapeutic significance of myeloma genetics and gene expression profiling. J Clin Oncol 2005; 23(26): 6339−44.
31. *Avet-Loiseau H, Attal M, Moreau P, Charbonnel C, Garban F, Hulin C,* et al. Genetic abnormalities and survival in multiple myeloma: the experience of the Intergroupe Francophone du Myélome. Blood 2007; 109(8): 3489−95.
32. *Harousseau JL, Dreyling M.* Multiple myeloma: ESMO Clinical recommendations for diagnosis, treatment and follow-up. Ann Oncol 2008; 19(suppl 2): ii55–7.
33. *Laï JL, Zandecki M, Mary JY, Bernardi F, Izydorczyk V, Flactif M,* et al. Improved cytogenetics in multiple myeloma: a study of 151 patients including 117 patients at diagnosis. Blood 1995; 85(9): 2490−7.
34. *Stewart AK, Bergsagel PL, Greipp PR, Dispenzieri A, Gertz MA, Hayman SR,* et al. A practical guide to defining high-risk myeloma for clinical trials, patient counseling and choice of therapy. Leukemia 2007; 21(3): 529−34.
35. *Schmidt-Wolf IG, Glasmacher A, Hahn-Ast C, Jüttner A, Schnurr T, Cremer F,* et al. Chromosomal aberrations in 130 patients with multiple myeloma studied by interphase FISH: diagnostic and prognostic relevance. Cancer Genet Cytogenet 2006; 167(1): 20−5.
36. *Fonseca R, Bergsagel PL, Drach J, Shaughnessy J, Gutierrez N, Stewart AK,* et al. International Myeloma Working Group molecular classification of multiple myeloma: spotlight review. Leukemia 2009; 23(12): 2210−21.
37. *Jaffe ES.* The 2008 WHO classification of lymphomas: implications for clinical practice and translational research. Hematology Am Soc Hematol Educ Program 2009; 523−31.
38. *Tilly H, Rossi A, Stamatoullas A, Lenormand B, Bigorgne C, Kunlin A,* et al. Prognostic value of chromosomal abnormalities in follicular lymphoma. Blood 1994; 84(4): 1043−9.
39. *Einerson RR, Kurtin PJ, Dayharsh GA, Kimlinger TK, Remstein ED.* FISH is superior to PCR in detecting t(14;18)(q32;q21)-IgH/bcl-2 in follicular lymphoma using paraffin-embedded tissue samples. Am J Clin Pathol 2005; 124(3): 421−9
40. *Horsman DE, Connors JM, Pantzar T, Gascoyne RD.* Analysis of secondary chromosomal alterations in 165 cases of follicular lymphoma with t(14;18). Genes Chromosomes Cancer 2001; 30(4): 375−82.
41. *Espinet B, Solé F, Woessner S, Bosch F, Florensa L, Campo E,* et al. Translocation (11;14)(q13;q32) and preferential involvement of chromosomes 1, 2, 9, 13, and 17 in mantle cell lymphoma. Cancer Genet Cytogenet 1999; 111(1): 92−8.
42. *Pileri SA, Falini B.* Mantle cell lymphoma. Haematologica 2009; 94(11): 1488−92.
43. *Schraders M, Pfundt R, Straatman HM, Janssen IM, van Kessel AG, Schoenmakers EF,* et al. Novel chromosomal imbalances in mantle cell lymphoma detected by genome-wide array-based comparative genomic hybridization. Blood 2005; 105(4): 1686−93.
44. *Auer IA, Gascoyne RD, Connors JM, Cotter FE, Greiner TC, Sanger WG,* et al. t(11;18)(q21;q21) is the most common translocation in MALT lymphomas. Ann Oncol 1997; 8(10): 979−85.
45. *Streubel B, Simonitsch-Klupp I, Müllauer L, Lamprecht A, Huber D, Siebert R,* et al. Variable frequencies of MALT lymphoma-associated genetic aberrations in MALT lymphomas of different sites. Leukemia 2004; 18(10): 1722−6.

46. *Lo Coco F, Ye BH, Lista F, Corradini P, Offit K, Knowles DM,* et al. Rearrangements of the BCL6 gene in diffuse large cell non-Hodgkin's lymphoma. Blood 1994; 83(7): 1757−9.
47. *Ruminy P, Etancelin P, Couronné L, Parmentier F, Rainville V, Mareschal S*, et al. The isotype of the BCR as a surrogate for the GCB and ABC molecular subtypes in diffuse large B-cell lymphoma. Leukemia 2011; 25(4): 681−8.
48. *Alizadeh AA, Eisen MB, Davis RE, Ma C, Lossos IS, Rosenwald A,* et al. Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling. Nature 2000; 403(6769): 503−11.
49. *Boxer LM, Dang CV*. Translocations involving c-myc and c-myc function. Oncogene 2001; 20(40): 5595−610.
50. *Archibald S, Perkins S, Friedberg JW*. Burkitt lymphoma in adults. Hematology 2008; 1: 341−8.
51. *Kornblau SM, Goodacre A, Cabanillas F.* Chromosomal abnormalities in adult non-endemic Burkitt's lymphoma and leukemia: 22 new reports and a review of 148 cases from the literature. Hematol Oncol 1991; 9(2): 63−78.
52. *Shiota M, Mori S*. Anaplastic large cell lymphomas expressing the novel chimeric protein p80NPM/ALK: a distinct clinicopathologic entity. Leukemia 1997; 11 Suppl 3: 538−40.
53. *Falini B.* Anaplastic large cell lymphoma: pathological, molecular and clinical features. Br J Haematol 2001; 114(4): 741−60.

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Lajm borelioza i trudnoća

Lyme borreliosis and pregnancy

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lyme disease; pregnancy; diagnosis; therapeutics; treatment outcome; congenital abnormalities; risk assessment.

Uvod

Lajmska bolest (*Lyme borreliosis – L. borreliosis*) je infektivno, multisistemsko oboljenje izazvano spirohetom *Borrelia burgdorferi* (*B. burgdorferi*) koju na čoveka ubodom prenose krpelji roda *Ixodes*. Bolest može zahvatiti različite organe (kožu, nervni sistem, zglobove) prema kojima *B. burgdorferi* ima tropizam, pa klinički izgled i tok bolesti mogu biti različiti [1–13]. *L. borreliosis* je tipična prirodnožarišna infekcija. To je zoonoza koju karakteriše endemičnost i sezonsko javljanje. *L. borreliosis* je usko povezana sa periodom aktivnosti krpelja *Ixodes* i registruje se najčešće od ranog proleća do kasne jeseni. Uglavnom se javljaju sporadična oboljenja, a epidemije su zabeležene u grupi ljudi koji borave u sezoni aktivnosti krpelja na endemskom području [14–16].

Nakon izolacije uzročnika *L. borreliosis* (Burgdorfer, 1981) koja je zbog toga nazvana *Borrelia burgdorferi*, ona je zbog strukturalnih i fenotipskih sličnosti sa leptospirom i treponemom svrstana u rod *Borrelia* i red spiroheta, familiju eubakterijskih *spirohetacea*.

Između izolata B. *burgdorferi* u Evropi i SAD postoje razlike u morfologiji, površinskim proteinima, plazmidima i homologiji DNA, što može uticati na regionalne različitosti u ispoljavanju kliničke slike *L. borreliosis* [17–21].

Lajm borelioza i transplacentalna transmisija

Klasifikacija novootkrivenog uzročnika, koji ima sličnosti sa uzročnikom sifilisa, dovela je do razmatranja mogućnosti transplacentalnog prenosa borelije i mogućnosti oštećenja ploda. Iako je transplacentalna transmisija borelije dokazana, postoje neslaganja oko mogućnosti njenog uticaja na ishod trudnoće. Najveći rizik od infekcije *B. burgdorferi* za plod je u prvom tromesečju, te ona predstavlja značajan perinatalni problem. U drugom i trećem tromesečju trudnoće neželjene pojave se ređe javljaju [22–29].

Kod nelečenih trudnica sa infekcijom borelijom dolazilo je do prevremenog porođaja, abnormalnosti srca i velikih krvnih sudova, smrti fetusa, mrtvorođenosti i raša na rođenju. Nepovoljan ishod trudnoće registrovan je i kod trudnica sa *L. borreliosis* koje su lečene oralnom primenom antibiotika [30, 31].

Kongenitalne infekcije

Klinički oblici infekcije ploda izazvane *B. burgdorferi* podsećaju na one viđene u toku sifilisa. Najčešće nastaju: prevremeni porođaj, intrauterina smrt fetusa i malformacije [32, 33].

U drugom stadijumu bolesti *B. burgdorferi* prolazi fetoplacentarnu barijeru. Osim smrti ploda najčešće nastaju: sindaktilija, gubitak vida, prevremeno rođenje, neonatalna ospa, oštećenja srca, jetre, bubrega i centralnog nervnog sistema. Bojić [34] navodi da su opisani i pojedinačni slučajevi gubitka ploda u toku akutne infekcije izazvane *B. burgdorferi* nastale za vreme trudnoće. Kako je do sada utvrđeno, za smrt fetusa odgovorni su oslobođeni citokini interleukin 1 (IL-1), faktor nekroze tumora (TNF)-alfa, azot monoksid, interferon (IFN)-gama i drugi zapaljenski medijatori, koji izazivaju nespecifičnu destrukciju fetalnog tkiva.

Dijagnoza

Osnovni parametri za dijagnozu *L. borreliosis* su simptomi i znaci oboljenja, epidemiološki podaci, povoljan odgovor na antibiotsku terapiju i prisustvo specifičnih antitela na *B. burgdorferi*, što se odnosi i na dijagnostiku *L. borreliosis* u toku trudnoće [26].

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Za mikrobiološku dijagnostiku *L. borreliosis* date su preporuke za dvostepeni dijagnostički algoritam: inicijalni (skrining) test – indirektna imunofluorescencija (IIF), *Enzyme-linked immunosorbent assay* (ELISA) i dopunski (potvrdni) test – imunoblot [35, 36].

Terapija

Ukoliko je ispoljen *erythema migrans* (EM) trudnicu treba lečiti primenom penicilina G 20 miliona/dnevno, u toku 3–4 nedelje ili amoksicilinom 500 mg, 3 × dnevno, u toku 3 nedelje [34].

Tetraciklini su kontraindikovani u trudnoći i periodu dojenja. Ceftriakson i cefuroksim se mogu primeniti sa oprezom u prvom trimestru trudnoće, jer njihov uticaj na plod nije dovoljno ispitan [37, 38].

Uticaj lajmske bolesti trudnice na tok i ishod trudnoće

Zbog kliničkog sindroma koji nastaje kongenitalnom infekcijom drugim spirohetama (uzročnik sifilisa), postoji mogućnost prenosa *B. burgdorferi* sa inficirane trudnice na plod [37]. Interhumani prenos nije dokazan, ali je moguć transplacentarni prenos borelije [39].

Mehanizam oštećenja tkiva fetusa i njegove smrti se razlikuju od mehanizma oštećenja tkiva u artritisu. U tkivu fetusa nije nađen infiltrat polimorfonukleara. Objavljeni su slučajevi smrti prevremenog rođenja deteta zbog kongenitalne srčane bolesti, kao i neonatalne smrti na vreme rođenog deteta zbog oštećenja mozga, u čijem su tkivu nađene spirohete. Uočene su infekcije neonatusa čije su majke imale *L. borreliosis* u prva tri meseca trudnoće. Opisane su i kongenitalne malformacije udružene sa infekcijom *B. burgdorferi*, bez definitivne potvrde da *B. burgdorferi* izaziva kongenitalnu bolest ploda. Postojanje takvog sindroma nije potvrđeno, a, i ako postoji, mora da je veoma retko. Dokumentovana je transplacentarna infekcija ploda na animalnim modelima. Povećanje inokuluma uzročnika i/ili njegova primena intrauterino ili intraamnotično povećava učestalost infekcije ploda. Ovaj model može da posluži za izučavanje *L. borreliosis* u toku trudnoće. Nije utvrđeno postojanje značajne razlike u učestalosti kongenitalnih malformacija novorođenčadi majki koje su bile 5–20 puta izloženije infekciji *B. burgdorferi* nego majke u kontrolnoj grupi. Učestalost malformacija srca bila je veća u grupi novorođenčadi čije su majke iz područja sa endemskom *L. borreliosis* [34].

Objavljena je i opisana identifikacije *B. burgdorferi* u tkivima ploda posle abortusa, kao i kod nekoliko živorođene dece sa kongenitalnim anomalijama, ali bez histološke evidencije zapaljenja i sigurne etiološke potvrde [40, 41].

Humana transplacentarna transmisija spirohete, vrste *Borrelia*, može imati za posledicu fetalnu infekciju u svakom trimestru trudnoće, a ponekad čak i fetalnu smrt. Najčešća su oštećenja kardiovaskularnog sistema fetusa, ali je uzročnik izolovan i iz drugih fetalnih tkiva, kao što su: jetra, srce, nadbubrežna žlezda, bubreg, moždanice, likvor. Infekcija u toku trudnoće može izazvati abortus, fetalnu smrt, prevremeni porođaj, intrauterini zastoj rasta ili akutno oboljenje [34].

Poznato je da se u vreme pojave EM, rane lokalizovane faze borelija infekcije, spirohete mogu diseminovati hematogenim ili limfogenim putem iz kože u različite organe i organske sisteme. Poslednjih godina potvrđeno je da tokom spirohetemije *B. burgdorferi sensu lato* može proći fetoplacentalnu barijeru i dovesti do štetnih posledica po trudnoću [34].

Za dijagnozu *L. borreliosis* kod trudnica odlučujuću ulogu ima klinička procena. Nije potrebno utvrđivanje seropozitivnosti pre graviditeta, jer asimptomatska seropozitivnost trudnice nije udružena sa rizikom od *B. burgdorferi* infekcije za plod. Ukoliko se trudnica leči antibioticima duže od dve nedelje, nije opisan prenos na plod [34].

Radi sprečavanja nastanka infekcije borelijom u trudnoći potrebno je što pre ukloniti krpelja iz kože. Ne preporučuje se profilaktička primena antibiotika kod trudnica, već upoznavanje trudnice sa praćenjem promena na koži oko mesta uboda krpelja (strategija „čekati i gledati“), kao i rana primena antibiotika u slučaju pojave simptoma *L. borreliosis* Preporučeno je lečenje antibioticima samo trudnica sa pouzdanom kliničkom dijagnozom *L. borreliosis*, pre svega intravenska primena penicilina ili 2,0 g ceftriaksona dnevno tokom 14 dana, ne samo za trudnice sa ranom diseminovanom *L. borreliosis* već i za one sa solitarnim EM [42].

Opisana je pojava intrauterine smrti ploda, neonatalne smrti, sindaktilije, kortikalnog slepila i hidrocefalusa sa spinom bifidom, a takođe i pojava *hemangioma cavernosus*, *cheilognathopalatoschysis, dysplasia coxae i hypospadiae* koje mogu biti udružene sa borelija infekcijom tokom trudnoće. Nije otkrivena uzročna veza između infekcije *B. burgdorferi* i pojave anomalija. Kod nekih nelečenih, ali i antibioticima lečenih trudnica, iako su spirohete identifikovane mikroskopski ili kulturelno – iz brojnih organa na autopsiji novorođenčadi i/ili iz placente, nije bilo znakova inflamacije, nekroze ili granulomatoznih formacija u afektiranim tkivima. Spirohetemija fetusa nastaje verovatno u toku postojanja EM lezije [32, 33].

Prospektivnom studijom praćenja 105 trudnica sa tipičnim EM, utvrđeno je da je 88,6% trudnoća završena rođenjem zdravih beba u terminu, a kod 11,4% je došlo do nepovoljnog ishoda, bilo da se razvio *missed abortion*, spontani pobačaj ili prevremeni porođaj. Jedno prevremeno rođeno novorođenče je imalo srčanu anomaliju, a dva su ubrzo umrla nakon rođenja. Kod četiri novorođenčeta (3,8%), rođenih u terminu, otkrivene su kongenitalne anomalije. Nije dokazana uzročna veza sa borelija infekcijom ni u jednom od ovih slučajeva nepovoljnog ishoda trudnoće. Promptni tretman *iv* primenom antibiotika tokom celog gestacijskog perioda daje efektivnost i sigurnost u rešavanju problema *L. borreliosis* u trudnoći [33].

Infekcija fetusa nastaje verovatno u toku postojanja EM, iako klinički još uvek nisu utvrđeni karakteristični znaci diseminacije borelije. Infekcija majke *B. burgdorferi sensu lato* je retka, kao i iskustva u njihovom lečenju, a tok bolesti predmet je brojnih ispitivanja. Još nema preciznih i uniformnih modela lečenja trudnica. Dosadašnja iskustva su bazirana na pojedinačnim slučajevima nepovoljnog ishoda trudnoće [33].

Što je starija trudnoća, manja je verovatnoća fetalne smrti. Najveći rizik za plod je u prvom tromesečju trudnoće. Fetalnu smrt pre izaziva odgovor majke na akutnu infekciju, nego sama infekcija fetusa. Hronična infekcija majke ne izaziva smrt ploda [34].

Za sada nema dovoljno podataka o prenatalnoj infekciji sa *B. burgdorferi* jer postoje samo dva objavljena slučaja sa dokazanim prisustvom IgG antitela novorođenčadi majki sa *L. borreliosis* [39].

Prvi slučaj EM u trudnoći i prvo iskustvo u lečenju EM posle uboda krpelja kod trudnice u našoj zemlji objavljen je 1993.godine [31]. Ubod krpelja bio je u šestoj nedelji trudnoće, a EM se razvio u osmoj nedelji, u glutealnoj regiji, promera 10 cm. Primenjena je terapija analogna terapiji ranog sifilisa, 800.000 penicilina I.J. *im* u toku 20 dana. Osim groznice i nagona na povraćanje, drugih subjektivnih tegoba nije bilo. Serološke reakcije na *B. burgdorferi* u uzorcima seruma pre početka terapije bile su negativne, kao i kasnije uzeti uzorci, jednako kao i testovi na sifilis (TPHA i VDRL). Redovni ginekološki pregledi nisu pokazivali nikakve patološke promene i trudnoća se završila rođenjem zdravog novorođenčeta [44, 45].

Rezultati ispitivanja ukazuju na to da se infekcija *B. burgdorferi* ne može smatrati jednim od relevantnih uzroka ranih spontanih pobačaja, iako nije rađena izolacija *B. burgdorferi* iz fetalnih tkiva. Histopatološki pregled posteljice bio je bez ikakvih zapaljenskih infiltrata [43]. Zbog mogućeg transplacentarnog prenosa borelije i potencijalnih ozbiljnih sekvela na plod, trudnicama se posle uboda krpelja daje profilaktički oralno penicillin [44].

Mali broj ispitivanih trudnica sa pozitivnim epidemiološkim podatkom i urednim tokom trudnoće ne daje osnovu za definitivne zaključke, posebno stoga što nije bilo trudnica sa klinički manifestnom *L. borreliosis.* Kod novorođenčadi sa prisutnim antitelima konstatovana je niža porođajna masa i jače izražena žutica. Mrtvorođenost i teža neonatalna oštećenja (kongenitalne anomalije srca i velikih krvnih sudova) opisani su u slučajevima gde su majke imale manifestnu *L. borreliosis* u toku trudnoće i oko 26% perinatalnih komplikacija (prematuritet, kožni osip novorođenčeta, sindaktilija) [45].

Rizik od transplacentalne transmisije *B. burgdorferi* verovatno je minimalan kada se primene antibiotici u lečenju trudnica sa *L.borreliosis*. Do danas još uvek nije publikovan kongenitalni *L. borreliosis* sindrom [46].

U toku pet godina, od 1990. do 1994. u prospektivnoj studiji praćeno je 58 trudnica, sa EM. Kod 87,9% trudnica trudnoća se završila normalno, rođenjem klinički zdravih beba u terminu. Od preostalih 7 trudnica, jedna je imala *missed abortion*, a pet prevremeni porođaj, dok je jedna trudnica rodila bebu sa srčanom manom. Trudnice sa EM treba lečiti primenom ceftriaksona (2 g *iv*/14 dana) [32].

Transplacentalna infekcija *B. burgdorferi*, uzročnika *L. borreliosis* nedavno je dokumentovana. Fetalna infekcija potvrđena je kulturom u visokoendemskim regijama (Long Island, NY). Od devetnaest trudnica, pet je imalo nepovoljan ishod trudnoće, uključujući: *syndactylia, cortical blindness,* intrauterinu fetalnu smrt, prematuritet i usporen rast novorođenčeta. Frekvencija ovih nepovoljnih ishoda opravdava studije praćenja i lečenja trudnica sa *L. borreliosis* [47].

Studija praćenja 49 žena sa spontanim pobačajima u endemskom području severne Italije pokazala je neznatni porast seropozitivnosti (12%) u poređenju sa kontrolnom grupom žena sa trudnoćom donešenom do termina (6%) [48]. Kasnije sprovedene studije kod 143 žene nisu potvdile porast spontanih pobačaja kod seropozitivnih, asimptomaskih žena. Studija sprovedena 1994. godine, ukazala je na utvrđene neurološke sekvele dece žena sa gestacijskom *L. borreliosis* [49].

Velika raznovrsnost kliničkih manifestacija gestacijske *L. borreliosis* je slična raznovrsnim manifestacijama kongenitalnog sifilisa, jer je moguća transplacentalna transmisija spirohete sa majke na fetus. Dalja ispitivanja su neophodna radi utvrđivanja mogućeg teratogenog efekta spiroheta i spirohetemije u toku organogeneze [25].

Iz mozga i jetre novorođenčeta izolovana je *B. burgdorferi,* iako je majka lečena oralno penicilinom zbog *L. borreliosis* u ranoj trudnoći. Smatra se da je smrtni ishod nastupio zbog moždanih oštećenja i nemogućnosti disanja. Trudnica nije imala nikakve druge simptome niti znakove aktivnosti bolesti tokom lečenja i tokom dvogodišnjeg perioda. U zaključku autori naglašavaju da je oralno dat penicilin bio dovoljan da se EM povuče, ali nije sprečio nastanak infekcije ploda. Parenteralno lečenje trudnica je potrebno radi sprečavanja neuroloških komplikacija ploda. U nekoliko objavljenih radova opisane su komplikacije kao što su meningitis, artritis i karditis kod osoba koje su oralno lečene penicilinom u toku rane *L. borreliosis* [50].

U USA svake godine registruje se 16 000–17 000 slučajeva sa *L. borreliosis*, a oko polovina, tj. 8 000, su žene. Oko 1 200–3 400 su žene u reproduktivnom periodu (životno doba 20–49 godina). Četvrtina ovih žena su trudnice, od kojih sigurno 10% uopšte nisu lečene ili su neadekvatno lečene, a u jednoj petini slučajeva moglo je doći do transmisije mikroorganizma u fetus ili novorođenče. U zaključku se ističe da je rana, brza dijagnoza i adekvatna antibiotska terapija esencijalna za lečenje kongenitalne *L.borreliosis* i rođenje zdravog novorođenčeta. Prognoza gestacijske *L. borreliosis* je dobra ukoliko se dijagnostikuje i leči adekvatno. Prognoza novorođenčeta sa ranom kongenitalnom *L. borreliosis* zavisi od brze dijagnoze. Dugotrajno praćenje je neophodno radi detekcije mogućeg pogoršanja bolesti [51].

Iako je poznat uzročnik bolesti i njegova osetljivost na antibiotike, ne postoje jasni kriterijumi procene efekta terapije, tako da je lečenje obolelih od lajmske bolesti praćeno mnogobrojnim teškoćama. Izolacija *B. burgdorferi* kao metoda nije pogodna ni za dijagnostiku, niti za procenu efekta primenjene terapije. Takođe, ni do sada poznati serološki, mikrobiološki i imunski testovi ne omogućavaju validnu procenu efekata antibiotika. Jedini kriterijumi koji se mogu koristiti su klinički nalaz i subjektivne tegobe bolesnika. Uz napred navedeno, složena patogeneza bolesti, mehanizmi pokretanja autoimunosti i reakcija uzročnika u organizmu na delovanje antibiotika i efektora imunskog sistema su teškoće sa lečenjem ove bolesti, pa su i razumljivi različiti stavovi u tom pogledu [52].

B. burgdorferi može biti potencijalni etiološki agens gubitka fetusa nepoznatog uzroka, zatim kongenitalnih srčanih defekata i pobačaja, ali uz analizu kulture fetalnog tkiva. Rezultati seroloških testova za *L. borreliosis* često su negativni prvih nedelja infekcije, pa bi dijagnozu trebalo postaviti na osnovu poznatih kliničkih kriterijuma i lečenje započeti odmah, kako bi se sprečila transplacentarna infekcija ploda [43].

Kao mogući uzrok mrtvorođenosti navodi se nekoliko mehanizama: direktna infekcija ploda, oštećenja placente ili bolesti majke, pa se ovde navodi i *L. borreliosis* kao mogući uzrok mrtvorođenosti, sve češće u celom svetu [53].

Objavljeni su slučajevi endometrioze, steriliteta i *L. borreliosis* u šestogodišnjoj studiji praćenja žena sa endometriozom. Kod tri žene je dokumentovana *L. borreliosis,* a sve tri su imale tipičan EM, groznicu, zamor; serološki je potvrđena infekcija *B. burgdorferi*. Autori su citirali literaturu koja potvrđuje jedinstvenost koegzistencije *L. borreliosis* kod žena sa endometriozom u ovim slučajevima [54].

Rezultati eksperimentalne studije na miševima inficiranim *B. burgdorferi* dovode do zaključka da su smrtni ishodi gravidnih ženki i fetusa posledica akutne infekcije, odnosno odgovora gravidne ženke na akutnu infekciju, a ne fetalne infekcije. Ovi rezultati mogu objasniti sporadične slučajeve fetalne smrti gravidnih žena inficiranih *B. burgdorferi* u toku trudnoće [55].

U poslednjoj dekadi postoje razlike u preporučenoj antibiotskoj terapiji *L. borreliosis* u trudnoći, uključujući i parenteralnu antibiotsku terapiju trudnica. Evaluacijom najnovije literature zaključuje se da se oralna terapija smatra dovoljnom terapijom nekomplikovanog EM. Autori, međutim, smatraju da se za lečenje oba stadijuma *L. borreliosis,* i ranog i kasnog preporučuje kontinuirana primena parenteralne terapije [48]. Tetraciklini se naravno moraju izbegavati u lečenju trudnica.

Prema nekim autorima, u slučaju *L. borreliosis* majke (trudnice), adekvatna antibiotska terapija amoksicilinom sprečava oštećenja fetusa [38].

Zaključak

Analiza domaće i strane stručne literature o infekciji *B. burgdorferi* i ispoljavanju simptoma *L. borreliosis* u trudnoći pokazuje nekada i dijametralno različita shvatanja i stavove u pogledu ove problematike. Akcenat je na prevenciji infekcije, odnosno ranom uočavanju i adekvatnom uklanjanju krpelja, upoznavanju trudnica sa praćenjem promene na koži oko mesta uboda krpelja i eventualne pojave kožne i/ili druge simptomatologije *L. borreliosis*. Ukoliko do infekcije, odnosno pojave kliničkih manifestacija dođe, potrebna je primena odgovarajućih antibiotika po odgovarajućoj šemi i dovoljno dugo, kao i dugotrajno praćenje trudnica i toka trudnoće radi uočavanja eventualnih komplikacija.

Za rasvetljavanje mnogobrojnih problema, pa i mogućnosti transplacentalne transmisije *B. burgdorferi*, kao i mogućnosti nastanka kongenitalnih infekcija potrebna su, svakako, dalja istraživanja.

L I T A R A T U R A

1. *van Burgel ND, Kraiczy P, Schuijt TJ, Zipfel PF, van Dam AP.* Identification and functional characterisation of Complement Regulator Acquiring Surface Protein-1 of serum resistant Borrelia garinii OspA serotype 4. BMC Microbiol 2010; 10: 43.
2. *Relić M.* LYME DISEASE-The Clinical-epidemiological examinations, diagnostics procedures and terapical modalities compounds [dissertation]. Kosovska Mitrovica: Faculty of Medicine; 2005 (Serbian).
3. *Aberer E.* What should one do in case of a tick bite? Curr Probl Dermatol 2009; 37: 155−66.
4. *Ljøstad U, Mygland A.* Lyme borreliosis in adults. Tidsskr Nor Laegeforen 2008; 128(10): 1175−8. (Norwegian)
5. *Centers for Disease Control and Prevention (CDC).* Lyme disease-United States, 2003-2005. MMWR Morb Mortal Wkly Rep 2007; 56(23): 573−6.
6. *Guy N.* Lyme disease: basis for treatment strategy, primary preventive care and secondary preventive care. Med Mal Infect 2007; 37(7−8): 381−93. (French)
7. *Tsao JI.* Reviewing molecular adaptations of Lyme borreliosis spirochetes in the context of reproductive fitness in natural transmission cycles. Vet Res 2009; 40(2): 36.
8. *Stübs G, Fingerle V, Wilske B, Göbel UB, Zähringer U, Schumann RR,* et al. Acylated cholesteryl galactosides are specific antigens of borrelia causing lyme disease and frequently induce antibodies in late stages of disease. J Biol Chem 2009; 284(20): 13326−34.
9. *Relić M, Krstić N.* The therapy of early Lyme borreliosis – different recommendations. Pharmaca Serbica 2009; 1(3−4): 23−6. (Serbian)
10. *Relić M.* ERYTHEMA MIGRANS-diagnostic and therapeutic challenge. XIII Congress of the Serbian association of Dermatovenereologists with international participation. Belgrade; 2009 June 4−6. (Serbian)
11. *Relić M, Vesić S, Vujičić C.* Lyme borreliosis - epidemiological and clinical characteristics. Belgrade: XIII Belgrade Dermatology Deys; 2006. (Serbian)
12. *Relić M, Krstić N, Radomirović D, Đurđević M.* Erythema migrans mammae dextri – case report. The First Congress of Infectiologiest of Srbia. Tara; 2007 October 4−6. (Serbian)
13. *Baljošević S, Relić M, Mitić N, Stajković N.* A possible approach to the pathogenesis of Lyme borreliosis. The First Congress of Infectiologiest of Srbia. Tara; 2007 October 4−6. (Serbian).
14. *Relić M.* The Lyme borresliosis - clinical and epidemiological studies. Belgrade: Zadužbina Andrejević; 2007. (Serbian)
15. *Hercogova J, Vanousova D.* Syphilis and borreliosis during pregnancy. Dermatol Ther 2008; 21(3): 205−9.
16. *Margos G, Vollmer SA, Cornet M, Garnier M, Fingerle V, Wilske B,* et al. A new Borrelia species defined by multilocus sequence analysis of housekeeping genes. Appl Environ Microbiol 2009; 75(16): 5410−6. .
17. *Gern L.* Borrelia burgdorferi sensu lato, the agent of lyme borreliosis: life in the wilds. Parasite 2008; 15(3): 244−7.
18. *Veljković M, Isailović G, Stanojev D.* Lyme disease in dermatology practice. Glas Srp Akad Nauka Med 1993; 43: 177−82. (Serbian)
19. *Dmitrović R.* Lyme borreliosis in Yugoslavia. Belgrade: Velarta; 1996. (Serbian)
20. *Pokorni D, Lako B.* Etiology and pathogenesis of Lyme borreliosis. Glas Srp Akad Nauka Med 1993; (43): 123−32. (Serbian)

21. *Dimitrijević V*. Lyme borreliosis. In: *Žigić D*, *Lapčević M, Popović J, Ivanković D*, editors. General medicine. Belgrade: Section of General Medicine Serbian Medical Society; 2000. p. 690−97. (Serbian)
22. *Peltomaa M, Saxen H, Seppälä I, Viljanen M, Pyykkö I*. Paediatric facial paralysis caused by Lyme borreliosis: a prospective and retrospective analysis. Scand J Infect Dis 1998; 30(3): 269−75.
23. *Forshner K*. Canadian Lyme Disease Foundation. Available from: http://www.canlyme.com. [cited 2006 May 13].
24. *Markowitz LE, Steere AC, Benach JL, Slade JD, Broome CV*. Lyme disease during pregnancy. JAMA 1986; 255(24): 3394−6.
25. *MacDonald AB*. Gestational Lyme borreliosis. Implications for the fetus. Rheum Dis Clin North Am 1989; 15(4): 657−77.
26. *Jovičić V*. Diagnosis of Lyme borreliosis using strains of B. burgdorferi from our geographical area as the modern immunodiagnostic antigen tests [dissertation]. Belgrade: Military Medical Academy; 1999. (Serbian)
27. *Lakos A, Solymosi N*. Maternal Lyme borreliosis and pregnancy outcome. Int J Infect Dis 2010; 14(6): e494−8.
28. *Mylonas I*. Borreliosis during pregnancy: a risk for the unborn child? Vector Borne Zoonotic Dis 2011; 11(7): 891−8.
29. *Walsh CA, Mayer EW, Baxi LV*. Lyme disease in pregnancy: case report and review of the literature. Obstet Gynecol Surv 2007; 62(1): 41−50.
30. *Isailović G, Veljković M, Soć N, Krstić B, Bjekić M*. Erythema migrans after a tick bite in a pregnant woman. Glas Srp Akad Nauka Med 1993; (43): 173−5. (Serbian)
31. *Bojić I, Mijusković P*. Modern therapy of Lyme disease. Glas Srp Akad Nauka Med 1993; (43): 257−62. (Serbian)
32. *Maraspin V, Cimperman J, Lotric-Furlan S, Pleterski-Rigler D, Strle F*. Treatment of erythema migrans in pregnancy. Clin Infect Dis 1996; 22(5): 788−93.
33. *Maraspin V, Cimperman J, Lotric-Furlan S, Pleterski-Rigler D, Strle F*. Erythema migrans in pregnancy. Wien Klin Wochenschr 1999; 111(22−23): 933−40.
34. *Bojić I*. European borreliosis (Lyme disease) and other diseases whose causative agents transmitted by tick bite.. Loznica: Naš dom; 2000. (Serbian)
35. *Centers for Disease Control and Prevention (CDC)*. Recommendations for test performance and interpretation from the Second National Conference on Serologic Diagnosis of Lyme Disease. MMWR Morb Mortal Wkly Rep 1995; 44(31): 590−1.
36. *Wilske B, Fingerle V, Schulte-Spechtel U*. Microbiological and serological diagnosis of Lyme borreliosis. FEMS Immunol Med Microbiol 2007; 49(1): 13−21.
37. *EUCALAB-European Union Concerted Action on Lyme borreliosis*. International Masters Course on Molecular Recognition of Tick-borne Pathogens [updated 2012 April 22]. Available from: www.eucalb.com
38. *Müllegger RR, Glatz M*. Skin infections in pregnancy. Hautarzt 2010; 61(12): 1034−9. (German)
39. *Pavlović DM, Dmitrović R*. Lyme neuroboreliosis. Beograd: Elit; 1996. (Serbian)
40. *Edlow JA*. Erythema migrans. Med Clin North Am 2002; 86(2): 239−60.
41. *Shapiro ED*. Tick - Borne Infections. In: *Katz SL, Gershon AA, Hotez PJ,* editors. Krugman's: Infectious Diseases of Children. 10th ed. St. Louis, Missouri: Mosby-Year Book Inc; 1988. p. 508−15.
42. *Hotez PJ,* editors. Krugman's: Infectious Diseases of Chil-dren. 10th ed. St. Louis, Missouri: Mosby-Year Book Inc; 1988.p. 508−15.
43. *Maraspin V, Strle F*. How do I manage tick bites and Lyme borreliosis in pregnant women? Curr Probl Dermatol 2009; 37: 183−90.
44. *Jovanović R, Hajrić A, Cirković A, Miković Z, Dmitrović R*. Lyme disease and pregnancy. Glas Srp Akad Nauka Med 1993; (43): 169−72. (Serbian)
45. *Bojić I, Mijusković P, Dokić M, Nožić D, Lako B, Kapulica I,* et al. Clinical characteristics of Lyme disease. Vojnosanit Pregl 1993; 50(4): 359−64. (Serbian)
46. *Nadelman RB, Wormser GP*. Lyme borreliosis. Lancet 1998; 352(9127): 557−65.
47. *Lavoie PE, Lattner BP, Duray PH, Barbour AG, Johnson HC*. Death 8-Day Old Californian Baby Boy. Arthritis Rheum 1987; 30(4 Suppl 3): S5.
48. *Carlomagno G, Luksa V, Candussi G, Rizzi GM, Trevisan G*. Lyme Borrelia positive serology associated with spontaneous abortion in an endemic Italian area. Acta Eur Fertil 1988; 19(5): 279−81.
49. *Elliott DJ, Eppes SC, Klein JD*. Teratogen update: Lyme disease. Teratology 2001; 64(5): 276−81.
50. *Weber K, Bratzke HJ, Neubert U, Wilske B, Duray PH*. Borrelia burgdorferi in a newborn despite oral penicillin for Lyme borreliosis during pregnancy. Pediatr Infect Dis J 1988; 7(4): 286−9.
51. *Gardner T*. Lyme disease. In: Remington J, Klein JO, eds. *Infectious Diseases of the Fetus and Newborn Infant*. Philadelphia, Pa: WB Saunders; 2001: 519-641.
52. *Bojić I, Karadaglić Đ*. Lyme borreliosis. In: *Karadaglić Đ,* editor. Dermatology. Beograd: Vojnoizdavački zavod; 2000. p. 1897−904.
53. *McClure EM, Goldenberg RL*. Infection and stillbirth. Semin Fetal Neonatal Med 2009; 14(4): 182−9.
54. *Matalliotakis IM, Cakmak H, Ziogos MD, Kalogeraki A, Kappou D, Arici A*. Endometriosis-associated Lyme disease. J Obstet Gynaecol 2010; 30(2): 184−6.
55. *Silver RM, Yang L, Daynes RA, Branch DW, Salafia CM, Weis JJ*. Fetal outcome in murine Lyme disease. Infect Immun 1995; 63(1): 66−72.

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CURRENT TOPIC

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The position of Chinese massage (Tuina) in clinical medicine

Mesto kineske masaže (*tuina*) u kliničkoj medicini

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medicine, chinese traditional; clinical medicine; massage; acupuncture therapy; combined modality therapy; health status.

Ključne reči:
medicina, kineska tradicionalna; medicina, klinička; masaža; lečenje akupunkturom; lečenje, kombinovano; zdravstveno stanje.

Introduction

Traditional Chinese Medicine (TCM) in its narrow sense refers to acupuncture, Tuina (Chinese massage), moxibustion, herbalism and diagnostics, tongue treatment and pulse palpation. Tuina is defined as a therapeutic treatment utilizing hands and/or instruments locally, at the place of pain, or distantly, *via* meridians for the treatment and management of pain and injuries [1]. *Tui* means to "push" and *Na* means to "grasp" [2]. In the period between the year 221 BC and 265 AD the first book on Tuina was written, and its principles have been utilized ever since. In the period 265–960 AD special departments for Tuina were established in the National Hospital in China intended only for imperators, ministers and officers. Tuina was being developed really fast in the period 960–1368, and the second book was written with complete instructions, techniques, first instruments and immobilization treatments. The period from 1368–1911 is considered to be the peak in the improvement of Tuina. Today, Tuina has a wide spectrum of applications worldwide, and especially in China where it is applied in almost every family [3].

Tuina is an important segment of TCM, which independently or in a combination with other TCM techniques provides better results in treatment of certain diseases in comparison to Western medicine. Having knowledge of symptoms and diseases for which TCM provides a better treatment, one should rather consider Tuina, acupuncture, or moxibustion. Since 1974, World Health Organization (WHO) has considered TCM as an separate and equal branch of medicine. New textbooks on physical therapy describe techniques of classical manual massage and Tuina treatment, so in reflexology massage there are texts on periosteal massage, connective tissue massage, segment massage, acupressure, feet reflexology massage, *Shiatsu* massage (Japanese interpretation of Chinese acupressure) and manual lymphatic drainage [4].

Manual massage is the most applied manner of passive kinesiotherapy. Therapeutic massage is utilized in the treatment of a number of diseases and injuries. It is divided into classical therapeutic massage, lymphatic drainage and reflexology massage. The massage principle is based on the mechanical irritation on exter-, proprio- and interoreceptors, followed by the reflex reactions of tissue and organs. Furthermore, massage also has a direct mechanical action on tissue, increasing the flow of lymphatic and venous blood, initiating the appearance of active hyperemia of arterioles and capillaries in tissue, increasing local metabolism and elimination of disintegrated metabolic products, increasing swelling resorption and diverse pathological deposits in the tissue, improving the trophic tissue, etc. Massage is analgesic and spasmolytic, it increases secretory activity of sweat and sebaceous glands, increases the turgor and elasticity of skin, reduces fatigue and improves the contractile muscle ability [4].

If one compares classical manual massage and Tuina, one can observe great similarities regarding techniques, indications and contraindications. The names of the pain points to be treated are diverse. In classical manual massage these are maximal "painful" points, in Tuina these are acupunctural and *Ashi* points (*ashi* = painful). The main differences are in the concept of treatment, where Western medicine treats the disorder of organs or organ systems, while TCM treats energy balance disorder in the entire body. Tuina is practiced in Serbia at the Military Medical Academy, Belgrade (and in just a

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several private clinics), although there are no literary information on its clinical efficiency in this region.

The aim of the paper was to present the physiological and therapeutic action of Tuina with a the special insight in its clinical efficiency.

Tuina: traditional concept of physiological basic and therapeutic effects

Physiological approach

The main principles of Tuina are regulation of *Zang* and *Fu* organs, regulation of *Yin/Yang* balance, and meridian and collateral activation. Tuina promotes the circulation of *Qi* and activates blood, normalizes blood flow and repairs synovial structures and ligaments [1, 3].

In the oldest known medical book in China, the Inner Canon of the Yellow Emperor (475-221 BC) the following is written: "*Yin* is inside the person and it is the material basis for *Yang*. *Yang* is on the outside as a function, a manifestation of *Yin*". This was said 4 500 years ago, and written 2 500 years ago. The disease appears when there is the excess of *Yin* or *Yang*, or the deficit of *Yin* or *Yang*, *ie*, when the circular flow of energy is in any kind of disarrangement. *Qi* is commonly translated as bioenergy, a special vital energy. *Qi* circulates through acupuncture channels, and has its transformations in acupuncture points: *Qi* is *Yang*. *Xue* is its *Yin*, whose best translation is blood, though it is not blood in the classical sense of the word. Even though it also circulates through acupuncture channels, it is actually a body fluid [5].

sympathetic. The consequence is the relaxation of the heart and muscles. Opposite to gentle touch, strong massage differs action, since it inhibits the parasympathetic and stimulates the sympathetic, which is then reflected in the stimulation of the central nervous system and in the inhibition of the peripheral nervous system, as well as in the increase of muscle tension. After 10–15 minutes, relaxation and sedation occur. Tuina acts on the circular system in such a manner as to dilate capillaries and promote reconstruction of the blood network, increase elasticity of blood vessels, activate bloodstream and reduce blood viscosity. Tuina acts on the digestive system in such a manner as to regulate muscle movements, their work and tension by abdomen massage. It results in better digestion. Tuina acts on the urinary system by inducing sphincter contraction and promoting urination. Tuina acts on the immune system by stimulating it. Thus, Tuina is utilized in the prevention of diverse diseases, especially by foot massage. It has been proved that the action of Tuina on the endocrine system promotes insulin secretion in patients with diabetes. Tuina also regulates menstrual problems.

Tuina acts on the locomotion system by activating blood flow after muscle movement. Tuina repairs traumatic lesions and removes tendon adhesion. It also corrects the anatomic dislocations and influences the resorption of edemas and hematomas. The essence of Tuina is the action of an external force. Force is a stimulating factor that activates the channel system and modifies their function [8]. Hence, this is the manner in which the rehabilitation is explained (Figure 1).

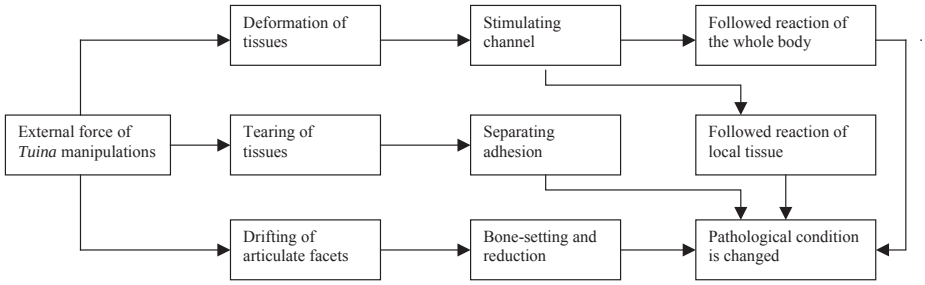


Fig. 1 – Illustrations of Tuina Manipulations [8]

The fundamental physical and therapeutic approach of Tuina is to treat primary causes, and then the consequences. In exceptional cases the *vice versa* principle is applied (for example, if the cause is coma, first the points *Du* 26 and *Ki* 1 are stimulated). Tuina stimulates vital *Qi* and eliminates a pathological factor; it regulates *Yin* and *Yang* by reducing excess syndromes and stimulating the deficit ones. The therapeutic treatment of Tuina is also in the fact that the treatment is planned in accordance with the season, as well as mental and individual abilities [6, 7].

Therapeutic effect

Therapeutic effect of Tuina has been an issue of investigation of modern medicine, mostly in France, and the Chinese are very proud of that fact. The most investigated technique is rubbing. Tuina acts on the nervous system by gentle skin touch, activating the parasympathetic and inhibiting the

Tuina induces both, central and local analgesia. Central analgesia occurs by reducing the brain stimulus, while local analgesia occurs due to better regulation and *via* the trigger point (points where the pain "disperses").

Application techniques

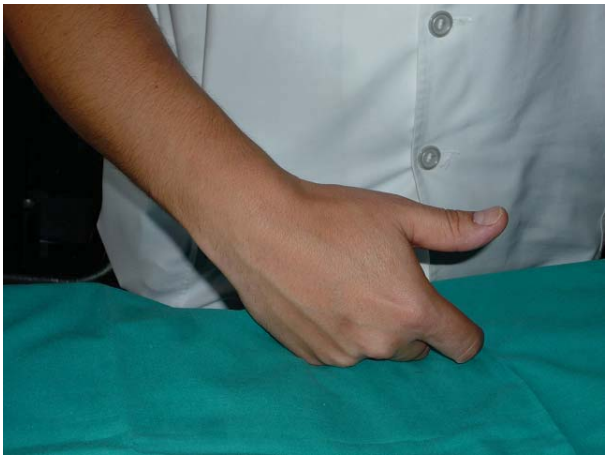
The main Tuina techniques are as follows: scraping, pressure, patting, shaking, rolling (Figure 2 a, b and c), rotating, and wave techniques, as well as compound techniques which are the combinations of the listed or those specially designed for children. They are introduced as surface and deep manipulation; they act along the meridian movement or opposite to it, locally or on acupunctural points, or alternating pressure – relaxation.

The doctor performing treatment by Tuina has to have clean hands, no jewelry, has to heat the hands before the

treatment during the winter season and has to cover the patient with a towel (Figure 2 c), which is opposite to classical massage in Western medicine where the therapist has a direct contact with a patient's skin or *via* a medium in the form of oil or the like. It is necessary for the doctor to follow a patient's reaction, and if needed, to make alterations in the therapy. Chinese massage should not be applied if the patient is too hungry or to full, nor when they are too tired (*eg* after practicing sports). The entire body is divided into six zones: face and head, neck and shoulders, chest and abdomen, lumbar spine, hands, legs, and almost every point utilized in acupuncture can be utilized here [9].

lateral position. Medical examination is based on inspection and deduction, following the teaching of Daoists [10]. The diagnosis is set both based on the pulse palpation [11] and tongue check [12]. Applying the fundamental manipulations, the doctor persistently and constantly utilizes fingers, thumb, hands, elbows, and feet, acts on the body surface and stimulates the points. When fine pressure is achieved by placing a finger on an acupunctural point (AP), a completely the same effect is obtained as in classical acupuncture. In clinical practice, individual manipulations of Tuina are rare; rather, combined manipulations are applied. There are more that 110 diverse manipulations, though only 20–30


a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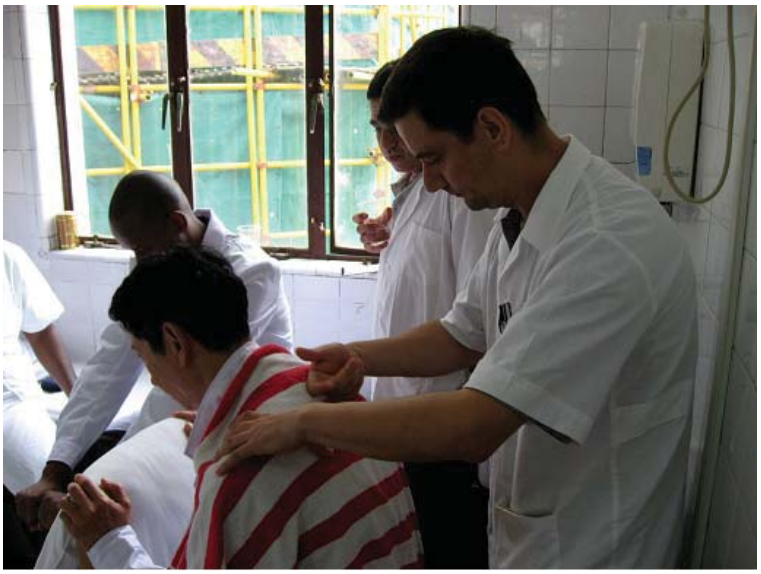
c

Fig. 2 – One of the main Tuina techniques – rolling
a) beginning position, b) ending position, c) rolling treatment using the towel

For chronic diseases, Tuina is applied every second day, and for acute diseases every day or even twice a day. The therapy usually lasts for seven to ten, or even fourteen days. There is a possibility of the occurrence of side-effects to the therapy in the form of pain or a complete body weariness. In that case, the patient is given a rest for a few days. If side-effects are repeated, the therapy should be altered. The main patients' positions are supination, lying and sitting, and pronation, as well lying and sitting, and the are the most common ones in practice [13]. Warm compresses and ventuses are also included in Tuina as supplementary mediums and supplementary methods. Various mediums like oils, powder or fat reduce skin irritation. If a medicament is the medium, it increases the therapeutic effect. Mediums like garlic, ginger and medicament in the form of fat heat up the channel and remove cold. Cold water is also a good medium for Tuina since it decreases temperature, the same as sesame oil and egg white [14].

Indications and contraindications

Main indications of Tuina are: injuries; rheumatic, cardiac, gynecologic, otolaryngologic, ophthalmic, pediatric diseases; special entities such as insomnia, neurasthenia, headache, epigastric pain, diarrhea, constipation, hemiplegia, facial nerve palsy, stiff neck, shoulder pain (frozen shoulder), general obesity, muscular torticollis, etc. [15] Contraindication are: acute infective diseases, fractures in early stages, malignant tumors, strong intern diseases, mental diseases, pregnancy and menstruation, hemorrage and inclination to hemorrage, etc. With the stated diseases, one can apply traditional Chinese medicine and proscription one [16].

Tuina: clinical efficiency

The paper utilized databases as follows: PubMed, Medline, and eCAM with the key words: Tuina, massage, acupuncture, Tuina for infants, Tuina for adults. Data on application of Tuina as one of the basic TCM methods, the representation of application within diverse pathological states, and the degree of rehabilitation efficiency have been obtained on the basis of data published in the world-known periodicals on traditional Chinese medicine (Journal of Acupuncture and Tuina Science and Journal of Traditional Chinese Medicine) in the period from August 2003 – December 2009.

There are studies stating that massage utilizing acupressure reduces pain better than the classical massage, though it provides worse results in comparison to transcutaneous electrical nerve stimulation [17]. When considering soft tissue injuries, Tuina in combination with acupuncture provides much better results than Western medicine [18]. The channels are the ones to connect the body surface with the organs inside. Tuina acts in a manner as to regulate the inner organs via meridians [19] *eg* successful treatment of multiple sclerosis (MS) and rigidity within Parkinson's disease utilizing Tuina, without medicaments, where the author, the neurologist, has also been the patient with MS [20, 21].

A large number of papers present that acupuncture provides better results in the treatment of neurological, rheumatic and traumatic diseases than Western medicine. Acupuncture and Tuina together offer better results in comparison to monotherapy with acupuncture or monotherapy with Tuina. Likewise, results are better if Tuina is combined with other traditional Chinese medicine methods, like *Qi Gong*, moxibustion, Chinese herbal medicine, etc.

As an example, four studies compare a combined therapy of acupuncture and other methods of traditional Chinese medicine, including injections into the acupuncture points [22], intravenous injections of purified Chinese herbs [23] and Tuina [24, 25] in comparison to Western medicine methods. Lu and Yan [25] combine acupuncture with Chinese Tuina, providing a comparison with indometacin (25 mg twice a day for 30 days), a standard drug for migraine, and obtaining better results. Acupuncture and adequate traditional Chinese medicine methods have presented significantly better results in the treatment of migraine in relation to the control group treated with Western medicine [26]. With inflammatory diseases (arthritis, colitis), the acupuncture is more successful in the early stage of diseases; however, in medium and late disease stadium, Tuina provides better results [27] (Table 1).

Table 1

Gansu Chinese Medicine (Gan Su Zhong Yi) [27]

Response	Treatment			
	Acupuncture		Tuina	
	early stage (n = 24)	late & middle stage (n = 66)	early stage (n = 15)	late & middle stage (n = 35)
Excellent (n)	20	8	2	8
Good (n)	2	21	3	20
Satisfactory (n)	2	2	7	5
Poor (n)	0	9	3	2
Total (%)	100	86	80	94
Excellent & good (%)	91	43	33	80

In the treatment of 18 cases with chronic prostatitis, the efficiency of acupuncture and Tuina was 94.4% [28]. In the therapy of primary dysmenorrhea (30 cases), acupuncture in combination with Tuina presented the efficiency of 93.3% in relation to the control group treated only with acupuncture, where the efficiency was 73.3% [29]. The insomnia occurring due to joint deficit of the heart and spleen, treated with acupuncture, moxibustion and Chinese Tuina (92 cases), provided significantly better results than in the patients treated only with acupuncture and moxibustion [30]. The effects of acupuncture and Tuina on the patients having stroke, presented better results in comparison to Western medicine treatment, especially in the rehabilitation of hemiplegia, facial nerve palsy and disphagia [31].

The treatment of 120 cases with rigidity syndrome ligament Nuchae with acupuncture and Tuina was successful in 95.8% of cases [32]. With 42 cases with stiff neck, after acupuncture, Tuina was applied in the zone of pain and muscle spasm. After 1–3 treatments, all patients were cured [33]. Acupuncture plus Tuina with cervical spondylosis provides better results (92.1%) than monotherapy with acupuncture (68.4%) or with Tuina (65.8%) [34]. With the protrusion of cervical intervertebral disk (8 cases), all symptoms disappeared and the full mobility in this segment returned after the treatment of Tuina and acupuncture [35]. The therapy of musculus supraspinatus tendinitis (100 patients) was 96% successful in the group

treated with acupuncture and Tuina in comparison to the control group (74% efficiency) treated only with acupuncture [36]. Clinical study utilizing warm needles, moxibustion and Tuina with periarthritis humero-scapular joint (80 cases) was efficient in 95% of cases in comparison to the control group treated only with Tuina (85% efficiency) in removing pain [37]. Headache induced by cervical syndrome (80 cases) was significantly better treated with acupuncture and Tuina together than with individual therapies [38].

Therapeutic effects of acupuncture and Tuina in the treatment of sprains and strains of joints in fists and feet (metacarpophalangeal and metatarsophalangeal joints) provided better results (93.4% efficiency) in comparison to monotherapy with acupuncture (70%) and Tuina (73.3%) (90 patients were treated) [39].

Tuina manipulation is successfully applied in lumbal intervertebral disk herniation [40]. Cervical spondylosis inducing compression on artery vertebralis treated only with Tuina (157 cases) presented excellent results in 86.6% cases [41]. Application of Tuina in clinical observation study with 47 patients with vertigo treated them with massage of the triple heater meridian (sanjiao) and gallbladder meridian on the head. The results presented a complete healing in 36 cases and significant improvement in 11 cases. The efficiency of the therapy was 100% [42].

Tuina applied in 37 cases with postoperative urinary retention led to the healing of 36 patients [43]. Research has proven that Tuina acts on postpartum milk secretion, considering the initial lactation time, serum prolactin level, and lactation volume [44] and is recommended for the treatment of postpartum hypolactation [45].

Tuina in pediatrics is an important component of traditional Tuina therapy, whose unique manner of treatment was formed during the Ming and Qing dynasty. The aim of Tuina in pediatrics is to unblock meridians and collaterals, to start *Qi* and activate blood, to balance *Yin* and *Yang*, to harmonize *Zang* and *Fu* organs and to increase the organism resistance to diseases [46].

Traditionally, in the therapy with Tuina there is a difference in treatment according to the infantile sex (*Yin* and *Yang* presentation). In the therapy prescription, the points and channels of the right arm are stimulated for girls and of the left arm for boys, while other points and channels are stimulated bilaterally [47]. Even though Tuina as an independent method provides good results in infantile treatments, especially when considering injuries brachial plexus (99.3% [48]), anorexia (96.1% [49]), and constipation (96.5% [50]), a combination of acupuncture and Tuina has proven to be more efficient with infantile torticollis (95.8% [51]), the application of moxibustion, ginger and Tuina presented the efficiency of 100% with diarrhea [52], while the application of acupuncture injections (injections of diverse medicine in acupunctural points), functional exercises, Tuina and acupuncture provide best results in the therapy of cerebral paralysis [53].

Conclusion

Tuina is a type of massage. It is used as an independent method and as an additional method to traditional and Western methods of treatment. Review of the literature shows that it has been successfully applied to the states after injuries, rheumatic, neurological and other diseases.

In published studies, the synergistic effect of Tuina with acupuncture and other methods of TCM is emphasized. Effects alone and in combination with TCM therapy are better compared to Western medicine. Future studies, planned in the Department of Physical Medicine and Rehabilitation, will compare the effect of Tuina and acupuncture with conventional massage and ultrasound massage, as well as with other agents in physical and rehabilitative medicine.

Classical massage is similar to Tuina, and following the historical line, it emerged from it. If they are applied together, the indication area is expanded. In the novel literature, it is justifiable to utilize the term "integrative medicine" instead of the term "alternative medicine". It is all one medicine.

REFERENCES

1. *Yanfu Z*. Chinese Tuina (massage). Shanghai: Publishing House of Shanghai of Traditional Chinese Medicine; 2002.
2. *Bullock RR*. Essential Traditional Chinese Medicine. London: Caxton Editions; 2002.
3. *Yanfu Z*. Basic Theory of Traditional Chinese Medicine. Shanghai: Publishing House of Shanghai of Traditional Chinese Medicine; 2002.
4. *Mihajlović V*. Physiotherapy Rijeka Crnojevića: Obodsko slovo; 2002. (Serbian)
5. Essentials of Chinese Acupuncture. Shanghai: Beijing College of Traditional Chinese Medicine; 2002.
6. The Miracle of Acupuncture. Beijing: Foreign Languages Press, 1993.
7. *Gongwang L*. Clinical Acupuncture and Moxibustion. China, Shanghai: Huaxia *Publishing House. 2006*.
8. *Guoquan S, Juntao Y*. Illustrations of Tuina Manipulations. Shanghai: Shanghai Scientific and Technical Publishers; 2003.
9. *Yanfu Z*. Chinese Acupuncture and Moxibustion. Shanghai: Publishing House of Shanghai University of Traditional Chinese Medicine; 2002.
10. *Gongwang L*. Fundamentals of Acupuncture and Moxibustion. China, Shanghai: Huaxia Publishing House; 2006.
11. *Yanfu Z*. Diagnostics of Traditional Chinese Medicine. . Shanghai: Publishing House of Shanghai University of Traditional Chinese Medicine; 2002.
12. *Ying X, Xiazhen G*. Tongue Diagnosis (Chinese-English). Shanghai: Traditional Chinese Medicine; 2006.
13. *Xiangcai X*. Chinese Tui Na Massage. Boston, MA, USA: YMMA Publication Center; 2002.
14. *Yanfu Z*. Science of Chinese Material Medica. Shanghai: Publishing House of Shanghai University of Traditional Chinese Medicine; 2002.
15. *Wen ZX*. Atlas of Chinese Massage Therapy. Shanghai: Shanghai University of T.C.M. Press; 2006.
16. *Yanfu Z*. Science of Prescriptions. Shanghai: Publishing House of Shanghai University of Traditional Chinese Medicine; 2002.
17. *Tsao JC*. Effectiveness of massage therapy for chronic, nonmalignant pain: a review. Evid Based Complement Alternat Med 2007; 4(2): 165−79.

18. *Li YM, Sheng ZX*. Treatment of Soft Tissue Injury with Chinese Tuina Therapy. Beijing: China. Publishing House of Traditional Chinese Medicine and Pharmacology; 1993.
19. *Yu DF*. Science of Chinese Massage. Shanghai: Shanghai Publishing House of Science and Technology; 1996.
20. *Chen M, Logan K, Zhang L*. Tuina Therapy as Novel Therapeutic Strategy for Neurodegenerative Diseases. Sporadic Neurodegeneration Symposium. 2008 July 31-August 01; Boston, Massachusetts, USA. 2008.
21. *Brown P*. Pathophysiology of spasticity. J Neurol Neurosurg Psychiatry.1994; 57(7): 773−7.
22. *Liu y, Zhang xP, Chen JL*. Observation on curative effect of acupuncture on migraine. Shanghai J Acupunct Moxibustion 2002; 21(2): 24−5.
23. *Wang JL, Gao XL*. Clinical trial of combined acupuncture and medicine in treating migraine. Shanxi J Tradit Chin Med 2004; 25(6): 549−50.
24. *Shao Y,Yan B, Zhang QW, Peng XM*. Clinical Observation of Combined Acupuncture and Tuina in Treating Migraine. New J Tradit Chin Med 2005; 37(9): 56−7.
25. *Lu ZQ, Yan SG*. Clinical Trial of Combined Acupuncture and Medicine in Treating Migraine. Chin J Curr Tradit West Med 2004; 2(6): 538−9.
26. *Wang YY, Zheng Z, Xue C*CL. Acupuncture for migraine. Austral J Acupunc Chin Med 2008; 3(1): 1−16.
27. *Hai-Ying MA*. A Report on the Treatment of 20 Cases of Chronic Colitis with Spirit Damp Illumination Combined with Electro-acupuncture. Gan Su Zhong Yi 2004; 9: 37.
28. *An-fu Y, Jing LV, Fen-Ying LI*. Treatment of 18 Cases of Chronic Prostatitis by Acupuncture Plus Tuina Therapy. J Acupunc Tuina Sci 2005; 3(6): 11−2.
29. *Guo A, Meng Q*. Acupuncture combined with spinal tui na for treatment of primary dysmenorrhea in 30 cases. J Tradit Chin Med 2008; 28(1): 7−9.
30. *Guo A, Meng Q*. Acupuncture combined with spinal tui na for treatment of primary dysmenorrhea in 30 cases. J Tradit Chin Med 2008; 28(1): 10−2.
31. *Jin-su W*. Effect of acupuncture and tuina on stroke. J Acupunc Tuina Sci 2005; 3(1): 31−2.
32. *Yun L, Xiao-li K*. Treatment of 120 Cases of Nuchal Ligament Strain by Acupuncture and Tuina. J Acupunc Tuina Sci 2007; 5(4): 250−1.
33. *Yong C*. Treatment of 42 Cases of Stiff Neck by Relaxing Needling plus Tuina. J Acupunc Tuina Sci 2005; 3(6): 32.
34. *Yang C*. Therapeutic Effect of Acupuncture plus Tuina on cervical Spondilosis. J Acupunc Tuina Sci 2008; 6(6): 344−6.
35. *Xiao C*. Treatment of Protrusion of Cervical Intervertebral Disc with Tuina Therapy plus Acupuncture. J Acupunc Tuina Sci 2003; 1(4): 41−2.
36. *Gao Y*. Observations of Efficacy of Acupuncture plus Tuina in Treating Supraspinatus Tendinitis. J Acupunc Tuina Sci 2009; 7(2): 94−7.
37. *Shui-rong H*. Clinical Study of Warming Needle Moxibustion plus Tuina for Shoulder Periarthritis. J Acupunc Tuina Sci 2008; 6(1): 39−41.
38. *Wei-qiong H, Si-wei X, Li-wei Z*. Observations on the Effect of Tuina plus Acupuncture for Cervical Migraine J Acupunc Tuina Sci 2004; 2(4): 50−2.
39. *Cui M, Jian-you J*. Observation on Therapeutic Effects of Acupuncture and Tuina in Treatment of Sprains and Strains of Minor Joints at our Extremities J Acupunc Tuina Sci 2009; 7(4): 228−30.
40. *Huang SR, Shi YY, Shi GT*. Pathogenic Factors of Blood Circulation Disturbance in Lumbar Intervertebral Disc Herniation and Mechanism of Tuina Manipulation in Promoting Circulation. J Chin Integrat Med 2003; 1(4): 255−8.
41. *Yan-min G*. Treatment of 157 Cases of Cervical Spondylosis of Vertebral Artery Type by Tuina Therapy. J Acupunc Tuina Sci 2009; 7(2): 113−5.
42. *Wen-zhong G*. Clinical Observation of Tuina Therapy in Treating 47 Cases of Vertigo. J Acupunc Tuina Sci 2008; 6(1): 49−51.
43. *Li-di K*. Tuina Treatment for 37 Cases of Postoperative Urinary Retention. J Acupunc Tuina Sci 2009; 7(2): 116−7.
44. *Ping L, Juan-Juan Z, Yi Z, Jia-qi C*. Research Advance on Tuina and Postpartum Milk Secretion. J Acupunc Tuina Sci 2009; 7(6): 375−8.
45. *Zheng JJ, Lu P, Zhao Y*. Research on Tuina treatment for postpartum hypolactation. Zhongguo Zhen Jiu 2009; 29(6): 501−3. (Chinese)
46. *Wei Z*. Introduction to Infantile Tuina. J Acupunc Tuina Sci 2004; 2(5): 8−10
47. *Rossi E*. Pediatric Tuina and Acupuncture: The Xiaoxiao Clinic in Milan. J Chin Med 2007; 85: 59−60.
48. *Shi-chun H*. Treatment of 150 Cases of Infantile Brachial Plexus Injury by Tuina. J Acupunc Tuina Sci 2005; 3(2): 39−40.
49. *Xiao-yu Z*. Clinical Observation of Tuina in Treating Anorexia in 78 Children. J Acupunc Tuina Sci 2004; 2(6): 43−4.
50. *Yong-mei W, Chun-lan S*. Observation on Therapeutic Results in Tuina Treatment of 56 cases of Infantile Constipation. J Acupunc Tuina Sci 2004; 2(5): 22−3.
51. *Jian-zhong L*. Tuina Manipulation on Infantile Myogenic Torticollis. J Acupunc Tuina Sci 2004; 2(5): 24−5.
52. *Xiao-feng L*. Treatment of Child Diarrhea by Ginger-Partitioned Moxibustion plus Tuina. J Acupunc Tuina Sci 2003; 1(6): 20−1.
53. *Wei-guon Z*. Treatment of Infantile Cerebral Palsy Mainly by Acupoint-Pressing and Tuina. J Acupunc Tuina Sci 2005; 3(1): 48−50.

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CASE REPORTS

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Primary pulmonary alveolar proteinosis

Primarna plućna alveolarna proteinoza

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Abstract

Introduction. Pulmonary alveolar proteinosis is an uncommon disease characterized by the accumulation of surfactant proteins and phospholipids within the alveolar spaces. Acquired disease can be idiopathic (primary) and secondary. The prevalence of acquired pulmonary alveolar proteinosis is about 0.37 per 100,000 persons. Common symptoms are dyspnea and cough. Chest X-ray shows bilateral perihilar infiltrates. Open-lung biopsy is the gold standard for the diagnosis. Treatment includes whole-lung lavage, application of granulocyte-macrophage colony-stimulating factor and lung transplantation. **Case report.** We reported a 51 year-old man with primary form of the disease. It was the second case of this extremely rare disease in the past 30 years in our clinic. The symptoms were long-lasting dry cough, fever and physical deterioration. Chest X-ray revealed bilateral pulmonary infiltrates; computed tomography showed patchy ground-glass opacification with interlobular thickening. The diagnosis was established by open lung biopsy. Additional tests were performed to exclude secondary form of the disease. **Conclusion**. We presented a rare clinical entity with typical clinical features and clinical and radiological course of the disease, in order to improve differential diagnostic approach to patients with bilateral lung infiltrations. In patients with pulmonary alveolar proteinosis timely diagnosis and adequate treatment can improve a prognosis.

Key words:
pulmonary alveolar proteinosis; diagnosis, differential; radiography; tomography, x-ray computed; biopsy.

Apstrakt

Uvod. Plućna alveolarna proteinoza je retka bolest koja se karakteriše akumulacijom proteina surfaktanta i fosfolipida u alveolarnim prostorima. Stečena forma bolesti može biti idiopatska (primarna) i sekundarna. Učestalost primarnog oblika bolesti je oko 0,37 na 100 000 osoba. Simptomi bolesti su otežano disanje i kašalj. Radiografija pluća pokazuje bilateralne perihilarne infiltrate. Otvorena biopsija pluća je zlatni standard za postavljanje dijagnoze. U lečenju se primenjuju ponavljane bronhioalveolarne lavaže čitavih pluća, primena faktora stimulacije kolonija granulocita i makrofaga i transplantacija pluća. **Prikaz bolesnika.** Prikazali smo 51-godišnjeg bolesnika sa primarnim oblikom bolesti. Radi se o drugom slučaju ove izuzetno retke bolesti u prethodnih 30 godina u našoj klinici. Bolest se prezentovala dugotrajnim suvim kašljem, povišenom telesnom temperaturom i telesnim propadanjem. Radiografski, viđeni su obostrani plućni infiltrati, a kompjuterizovana tomografija pokazala je mestimične infitracije izgleda mlečnog stakla sa interlobularnim zadebljanjima. Dijagnoza je postavljena otvorenom biopsijom pluća. Učinjena su i dodatna ispitivanja kako bismo isključili sekundarnu formu bolesti. **Zaključak.** Prikazan je redak klinički entitet sa tipičnom kliničkom slikom, kliničkim i radiološkim tokom bolesti da se poboljša diferencijalno dijagnostički pristup bolesnicima sa obostranim promenama u plućima. Kod bolesnika sa plućnom alveolarnom proteinozom pravovremena dijagnoza i adekvatno lečenje mogu popraviti prognozu.

Ključne reči:
pluća, alveolna proteinoza; dijagnoza, diferencijalna; radiografija; tomografija, kompjuterizovana, rendgenska; biopsija.

Introduction

Pulmonary alveolar proteinosis (PAP) is an uncommon disease characterized by the accumulation of surfactant proteins and phospholipids within the alveolar spaces [1].

Acquired PAP can be idiopathic (primary) PAP and secondary PAP. Secondary PAP is associated with hematological malignancies, *Pneumocystis carinii* pneumonia and inhalation of silica or titanium [2, 3].

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The prevalence of acquired pulmonary alveolar proteinosis has been estimated to be 0.37 per 100,000 persons [4]. It is a primary acquired disorder in more than 90 percent of cases [4, 5]. It is thought that impairment of surfactant clearance by alveolar macrophages, by autoantibody inhibition of the action of granulocyte-macrophage colony-stimulating factor (GM-CSF) may underlie many acquired cases, whereas congenital disease is most commonly attributable to mutations in surfactant protein genes, but may also be caused by GM-CSF receptor defects [2, 3].

Symptoms are persistent dry cough, progressive dyspnea, fatigue and malaise, weight loss, intermittent low-grade fever and/or night sweats and pleuritic chest pain. Signs are usually non-specific and include: fine end-inspiratory crackles, digital clubbing and cyanosis [2, 4].

In acquired pulmonary alveolar proteinosis, routine blood counts and the results of routine blood chemical analysis and urine analysis are usually normal [5, 6].

Pulmonary function tests (PFTs) can be normal, but typically there are restrictive ventilatory defect with slight impairments in the forced vital capacity and total lung capacity and a disproportionate, severe reduction of the carbon monoxide diffusing capacity [7]. Hypoxemia is caused by ventilation–perfusion inequality and intrapulmonary shunting, resulting in a widened alveolar–arteriolar diffusion gradient [5, 8].

Chest X-ray (CXR) shows bilateral perihilar consolidation. Changes progress into a diffuse reticular pattern [1]. High-resolution computed tomography scan of the chest shows patchy ground-glass opacification with interlobular thickening. Similar appearance can be seen in lipoid pneumonia, sarcoidosis and acute respiratory distress syndrome [3].

In most cases, the diagnosis is confirmed by bronchoalveolar lavage (BAL) and transbronchial biopsy. Macroscopically, BAL fluid shows milky appearance and microscopically characteristic acellular globules. Histopathological finding of lung biopsy shows periodic acid schift (PAS)-positive material within the alveoli but contains no organisms or any excessive cellular response [3, 7]. Surgical lung biopsy is rarely necessary [5, 8].

Treatment includes whole-lung lavage which often produces a dramatic response [9]. Subcutaneous application GM-CSF and lung transplantation are the therapeutic options too, [5].

Case report

A 51-year-old male was admitted to the Clinic for Lung Diseases due to fever and radiological changes in the lungs bilaterally (Figure 1). Symptoms were as follows fever (up to 37,8°C), dry cough, weakness, fatigue, physical decline lasted for several months. The patient denied any possible exposure to occupational hazards or toxic fumes. He had no risk factors for human immunodeficiency virus (HIV) or other infections. He denied any previous medical illnesses and was not taking any medications. He was a smoker for 30 years, smoking 40 cigarettes per day.

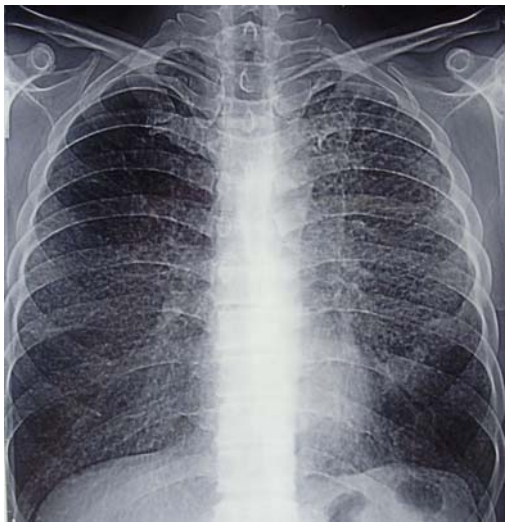

Fig. 1 – Frontal chest radiography reveals bilateral air-space opacity without evidence of pleural effusion or mediastinal widening. A faintly reticular pattern is present, representing thickened, interlobular septa

On two occasions, he was examined and treated in the hospital, with different antibiotic therapy and low doses of corticosteroids with minimal improvement: he became afebrile, but symptoms and radiographic changes continued to progress.

Physical examination showed a dysphonic patient with mild peripheral cyanosis. Auscultation of the lungs revealed weakened respiratory sound, prolonged expirium bilaterally and diffuse fine end-inspiratory crackles. Other physical examination findings were within normal limits.

Initial laboratory tests revealed erythrocyte sedimentation rate of 40 mm/h and normal complete blood count. Parameters of blood biochemistry, transaminases and tumor markers were in normal ranges except lactate dehidro genase (LDH) 484 IU/L (normal range 200–378 IU/L).

Virus analyses such as hepatitis B surface antigen (HbsAg), hepatitis C virus (HCV) and HIV antibodies were negative. Sputum and blood cultures were bacteriologically and cytologically negative.

PFTs showed a severe restrictive ventilatory changes (forced vital capacity was 35 percent predicted). The respiratory arterial blood gases analysis at rest showed severe hypoxemia with pO2 6,4 KPa (9.1 is normal for his age), oxygen saturation at 86% (normal > 94 %) and mild hypocapnia with pCO2 4.5 KPa (normal range 4,6–6).

Multislice computed tomography scan of the chest revealed patchy ground-glass opacification with interlobular thickening bilaterally, without fibrotic changes (Figure 2). Bronchoscopic finding was normal. Histological findings of transbronchial biopsy specimen were nonspecific. The examination of bronchial aspirates did not reveal any biological agents. CXR changes continued to progress. The open-lung biopsy was performed because there was no diagnosis. Five days after the intervention, tachypnea, tachycardia and cyanosis occurred with severe impairment of consciousness and a progression of radiographic changes (Figure 3).

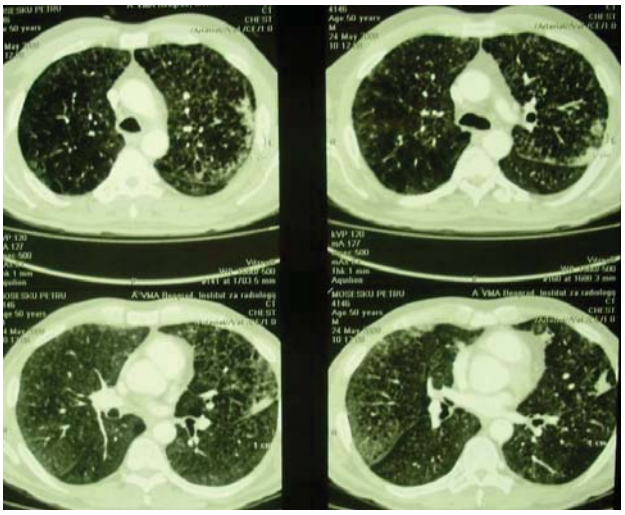

Fig. 2 – Multislice computed tomography scan of the chest reveals patchy ground-glass opacification with interlobular thickening bilaterally, without fibrotic changes

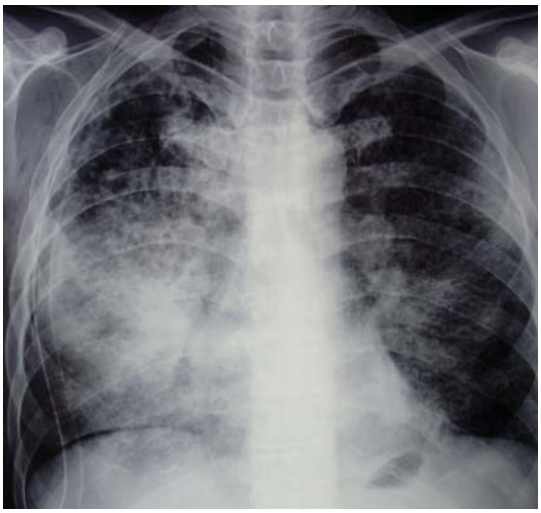

Fig. 3 – The fourth postoperative day: progression of radiographic changes

The arterial blood gases were as follows pO = 6.0 KPa, pCO = 2 4.4 KPa; sO2 = 82%.

Additional tests were performed for suspected pulmonary thromboembolism. Electrocardiography showed sinus tachycardia, with the frequency of 150/min and incomplete right bundle branch block. D-dimmer (fibrin degradation fragment) was 747 ng/mL (normal < 500 ng/mL). Echocardiography revealed normal left ventricle systolic and diastolic function, mild elevation of right ventricle systolic pressure (5 KPa) with normal dimensions of heart cavities.

The patient developed respiratory failure. Mechanical ventilation was applied, but despite the intensive treatment there was a fatal outcome on the same day.

Immediate cause of the patient's death was not determined because the autopsy was not performed.

Histopathological findings from an open lung biopsy, arriving three days after the patient's death, showed PAP (Figures 4, 5 and 6).

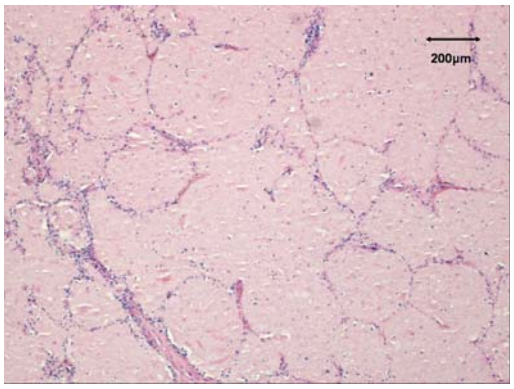

Fig. 4 – Alveoli are filled with eosinophilic lipoproteinaceous material with otherwise relatively preserved lung architecture; clefts of cholesterol crystals are marked (HE, ×5)

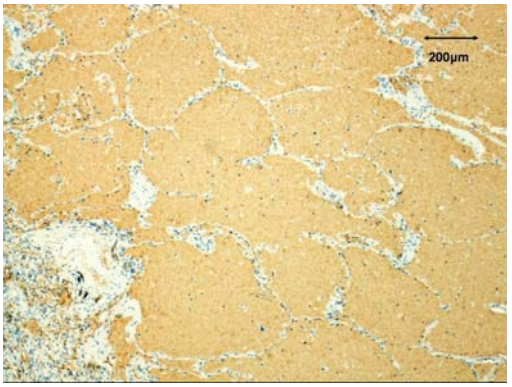

Fig. 5 – Diffuse immunohistochemical reaction in the alveolar content (Surfactant A, ×5)

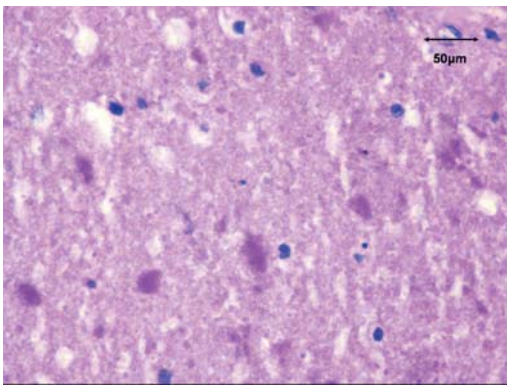

Fig. 6 – Alveoli are filled with granular material (PAS, ×40)

Discussion

Primary PAP is a rare syndrome that was first described by Rosen et al. in 1958 [4]. This disorder is characterized by abnormal intra-alveolar surfactant accumulation with a variable clinical course, ranging from respiratory failure to spontaneous resolution [6]. Three distinct clinical forms of PAP can be distinguished: congenital, secondary, and primary (idiopathic) [4].

This rare lung disorder generally occurs in persons of 30 to 50 years of age [6]. The median age at the time of diag-

nosis is 39 years [4]. Most patients are men, and 72% have a history of smoking [5].

Congenital PAP is a heterogeneous group of disorders caused by mutations in surfactant proteins B or C, or the receptor for GM-CSF [2, 3, 4]. Secondary PAP can develop in association with various conditions, such as immunodeficiency states, acute silicosis and other inhalational syndromes, hematologic malignancies and myelodysplastic syndromes [5, 6]. In all of these conditions there is a reduction in the number and/or functional impairment of alveolar macrophages [6, 10]. More than 90% of all cases of PAP occur as the primary (idiopathic) form [5]. Recent studies have led to the current concept that primary PAP is an autoimmune disease, which produces neutralizing immunoglobulin G (IgG) antibodies against GM-CSF [11]. Surfactant is normally cleared by uptake into alveolar macrophages and GM-CSF is critical for this process, as it is a cytokine stimulating the production of alveolar macrophages by the bone marrow. Therefore, all the three forms of PAP share the feature of an impairment in the number and/or activity of alveolar macrophages leading to the alveolar accumulation of surfactant [1, 3].

The major complication of PAP is an infection with unusual organisms such as *Aspergillus* species, *Nocardia* species, *Mycobacterium* species, *Cryptococcus neoformans*, *Histoplasma capsulatum*, *Pneumocystis carinii*, and viruses [9].

The serum level of LDH is frequently elevated, but this finding is non-specific [5]. It may be a useful marker of the severity of the disease [3]. Elevations in the serum levels of carcinoembryonic antigen, cytokeratin 19, mucin KL-6, and surfactant proteins A, B, and D are described but with limited significance [6].

Open-lung biopsy is the gold standard for the diagnosis and reveals alveoli filled with granular, eosinophilic material that is stained with PAS with preservation of the alveolar architecture [3, 4]. This procedure is not always required and can be complicated by false negative results due to sampling error [4]. Transbronchial biopsy can generally provide a sufficient tissue sample [7].

In one third of the patients, no appreciable disability develops and the disease remits spontaneously or fails to progress. The natural history depends on the underlying etiology. Estimates of a 5-year mortality rates vary between 10% and 30% [9].

Successful lung transplantation has been reported in cases of congenital PAP [6]. The whole-lung lavage remains the standard of care for primary PAP, although some patients may respond to subcutaneous application of GM-CSF11. In secondary PAP, the treatment depends on the underlying cause [6].

Our patient had a typical clinical and radiological presentation of PAP. It was a second case of this extremely rare disease in the past 30 years in our clinic.

The results of imaging methods and PFTs supported the diagnosis, which was confirmed by open lung biopsy. Other causes of PAP were not found, and we estimated the existence of a primary form of the disease which had a progressive course, with severe ventilatory changes and manifested partial pulmonary failure.

The surgical procedure additionally impaired the lung function, so there was a lethal outcome of the disease before obtaining the histopathologic confirmation and applying a specific treatment.

Conclusion

We presented a case of rare clinical entity, primary pulmonary alveolar proteinosis, with typical clinical features and clinical and radiological course of the disease, in order to improve a differential diagnostic approach to patients with bilateral lung infiltrations. In patients with pulmonary alveolar proteinosis, the timely diagnosis and adequate treatment can improve a prognosis.

REFERENCES

1. *Levine SM.* Alveolar filling disorders. In: Goldman L, Ausiello D. Cecil Medicine. 23rd ed. Philadelphia, Pa: Saunders Elsevier; 2007. p. 91.
2. *Shah PL, Hansell D, Lawson PR, Reid KB, Morgan C.* Pulmonary alveolar proteinosis: clinical aspects and current concepts on pathogenesis. Thorax 2000; 55(1): 67−77.
3. *Nogee LM, de Mello DE, Dehner LP, Colten HR.* Brief report: deficiency of pulmonary surfactant protein B in congenital alveolar proteinosis. N Engl J Med 1993; 328(6): 406−10.
4. *Rosen SH, Castleman B, Liebow AA.* Pulmonary alveolar proteinosis. N Engl J Med 1958; 258(23): 1123−42.
5. *Seymour JF, Presneill JJ,* Schoch OD, Downie GH, Moore PE, Doyle IR, et al. Pulmonary alveolar proteinosis: progress in the first 44 years. Am J Respir Crit Care Med 2002; 166(2): 215−35.
6. *Trapnell BC, Whitsett JA, Nakata K. Pulmonary* alveolar proteinosis. N Engl J Med 2003; 349(26): 2527−39.
7. *Teja K, Cooper PH, Squires JE, Schnatterly PT.* Pulmonary alveolar proteinosis in four siblings. N Engl J Med 1981; 305(23): 1390−2.
8. *Kitamura T, Uchida K, Tanaka N, Tsuchiya T, Watanabe J, Yamada Y,* et al. Serological diagnosis of idiopathic pulmonary alveolar proteinosis. Am J Respir Crit Care Med 2000; 162(2 Pt 1): 658−62.
9. *Mason RJ, Broaddus VC, Murray JF, Nadel JA.* Murray and Nadel's Textbook of Respiratory Medicine. 4th ed. Philadelphia, Pa: WB Saunders; 2005.
10. *Uchida K, Beck DC, Yamamoto T, Berclaz PY, Abe S, Staudt MK,* et al. GM-CSF autoantibodies and neutrophil dysfunction in pulmonary alveolar proteinosis. N Engl J Med 2007; 356(6): 567−79.
11. *Robinson TE, Trapnell BC, Goris ML, Quittell LM, Cornfield DN.* Quantitative analysis of longitudinal response to aerosolized granulocyte-macrophage colony-stimulating factor in two adolescents with autoimmune pulmonary alveolar proteinosis. Chest 2009; 135(3): 842−8.

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CASE REPORT

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Ossifying chondrolipoma of the tongue

Osifikujući hondrolipom jezika

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Abstract

Introduction. Chondrolipomas and osteolipomas are uncommon variants of lipomatous tumors. **Case report.** We presented a 60-year-old woman with ossifying chondrolipoma of the tongue. Clinical examination revealed a firm nodular mass, located in the midline of the posterior region on the dorsal surface of the tongue. Histologically, the lesion was well-delimited showing areas of mature adipocytes arranged in lobules and separated by fibrous connective tissue septa, islands of mature cartilaginous tissue and osseous metaplasia. Trabeculae of lamellar bone within a fibro-fatty background were visible throughout the tumor. The cartilaginous areas merging centrally with bone formation and fatty marrow tissue were present, as well as the hematopoietic elements in the fatty marrow. The bone forming was found to be through both membranous and enchondral mechanisms. **Conclusion.** Ossifying chrondrolipoma with hematopoietic elements is extremely unusual lesion. This interesting entity should be kept in mind in the differential diagnosis of lingual lesions.

Key words:
tongue neoplasms; neoplasms, complex and mixed; chondroma; osteoma; lipoma; mesenchymoma; diagnosis; diagnosis differential.

Apstrakt

Uvod. Hondrolipomi i osteolipomi predstavljaju retke varijante lipoma. **Prikaz bolesnika.** Prikazana je bolesnica, stara 60 godina, sa osifikujućim hondrolipomom jezika. Kliničkim pregledom otkrivena je nodularna masa čvrste konzistencije, koja je lokalizovana medijalno u zadnjem regionu dorzuma jezika. Histološki, lezija je bila jasno ograničena i sastojala se od polja zrelih lipocita lobularnog rasporeda sa vezivnotkivnim septama, ostrva zrelog hrskavičavog tkiva i polja koštane metaplazije. U tumoru su nađene rasute trabekule kosti uklopljene u fibromasnu osnovu. Uočena su polja hrskavice povezana formiranjem kosti i masnom koštanom srži u centru, u kojoj su, takođe, bili prisutni hematopoetski elementi. Utvrđeno je da se formiranje kosti odvijalo prema oba mehanizma, membranoznim i enhondralnim. **Zaključak.** Osifikujući hondrolipom sa hematopoetskim elementima je izuzetno retka lezija. U diferencijalnoj dijagnozi lezija jezika treba misliti i na ovaj interesantan entitet.

Ključne reči:
jezik, neoplazme; neoplazme, kompleksne i mešovite; hondrom; osteom; lipom; mezenhimom; dijagnoza; dijagnoza, diferencijalna.

Introduction

Lipomas are very common benign soft tissue tumors. They may occur anywhere in the body, but usually present as slow-growing, solitary and asymptomatic subcutaneous or superficial lesions. Histologically, lipomas are composed of mature adipocytes arranged in lobules that are separated by fibrous connective tissue septa, and occasionally associated with one or more secondary mesenchymal elements. Different variants of lipoma have been described, such as fibrolipoma, angiolipoma, myolipoma, spindle cell lipoma, chondroid lipoma, chondrolipoma, and osteolipoma [1, 2].

Although lipomas are one of the most common soft tissue tumors [1–3], with about 20% of cases affecting the head and neck region [4], only 1%–4% of these neoplasms involve the oral cavity [3, 5]. Oral lipomas usually develop in patients over the age of 40 [2, 3, 5]. The buccal mucosa is the most affected site [3–6], and lipomas and fibrolipomas are being the most frequently observed in the oral cavity [5, 6]. Among the other histopathological variants, lipomas with cartilaginous or osseous metaplasia, called chondrolipomas or osteolipomas, respectively, are rare in the oral cavity [2, 6–8]. Only 10 cases of chondrolipoma have been reported in the literature [9], with a total of 15 cases in the international literature (Pub-

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med Database). Oral osteolipomas are less common than chondrolipomas [10]. A review literature revealed 8 cases of this variant located in the oral cavity [2, 6, 8, 10, 11].

Lipomas with cartilaginous and osseous metaplasia have been described in the subcutaneous and deep soft tissues, particularly in the parosteal localization [12–14]. To our knowledge, there is no case of this variant located in the oral cavity reported in the literature. We present an unusual case of ossifying chondrolipoma of the tongue containing hematopoietic elements and discuss the pathogenesis and the differential diagnosis.

Case report

A 60-year-old female was referred to the Department of Otorhinolaryngology with a painless mass on the dorsum of her tongue which caused discomfort during chewing. The lesion was first noticed about 5 years earlier and had not grown remarkably so far. The patient reported no previous trauma to the affected region. Her medical history was noncontributory.

Clinical examination showed a well-defined nodular mass of firm consistency, which was located in the midline of the posterior region on the dorsal surface of the tongue (Figure 1). The remainder of intraoral examination was otherwise unremarkable, with no evidence of submandibular and cervical lymphadenopathy. The lesion was completely excised, under local anesthesia, and sent to the Department of Pathology for examination.

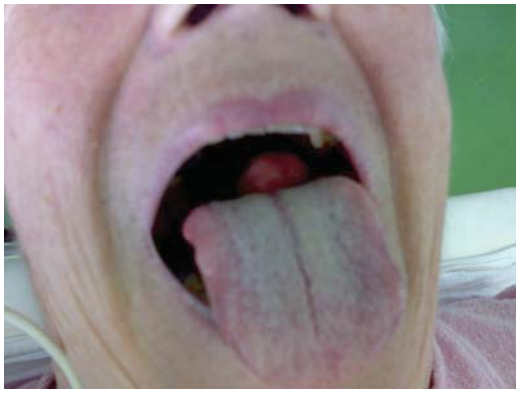

Fig. 1 − Clinical view of nodular lesion in the midline on the dorsum of the tongue

Grossly, the excised specimen consisted of firm, yellowish-white oval nodule, measuring 20 x 17 mm**,** covered by normal mucosa. Histologically, the tumor showed areas of mature adipocytes arranged in lobules and separated by septa of fibrous connective tissue (Figure 2). The amount of fibrous septa was increased. Islands of mature cartilaginous tissue were identified in the proximity to the fibrous connective tissue septa (Figure 3). These cartilage islands containing lacunae filled with chondrocytes were variable sizes and surrounded by adipose tissue. Trabeculae of lamellar bone within a fibro-fatty background were visible throughout the lesion, especially toward the central areas (Figure 4). They were surrounded by spindle-shaped mesenchymal cells, and a small rim of osteoblasts was observed. A limited myxoid change was present but no necrosis was observed. In addition, partly cartilaginous areas merging centrally with bone formation and fatty marrow tissue were found (Figure 5), suggesting that a part of the bone had been formed through the enchondral mechanism. There were hematopoietic elements also present in the fatty marrow tissue (Figure 5). No prominent inflammatory reaction was detected. The tumor showed both enchondral and membranous ossifications. The bone forming di-

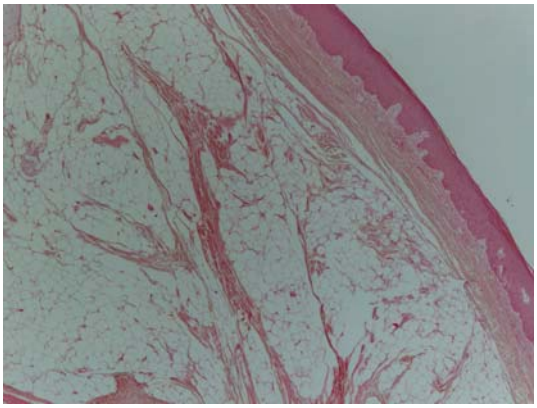

Fig. 2 − Well-demarcated collection of mature adipocytes arranged in lobules separated by septa of fibrous connective tissue (HE, ×40)

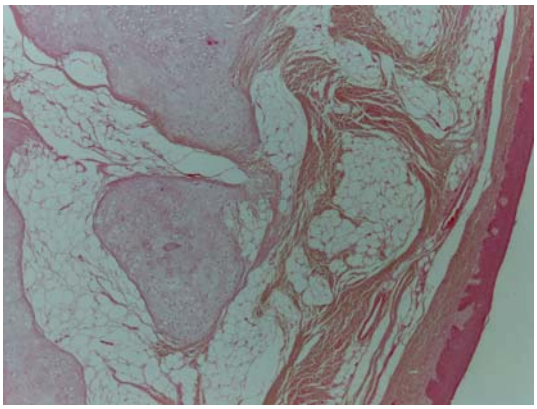

Fig. 3 − Islands of mature cartilage in the proximity to the fibrous connective tissue, surrounded by mature adipocytes (HE, ×40)

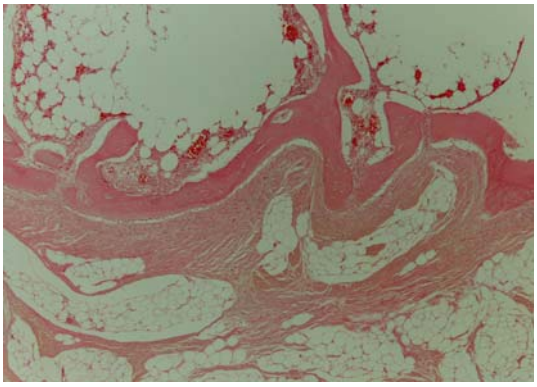

Fig. 4 − Bone trabeculae surrounded by thick fibrous connective tissue septa and areas of mature adipose tissue (HE, ×40)

rectly from the fibrous background (membranous ossification) predominated in this lesion. No nuclear atypia or mitotic figures were observed. The tumor was well-delimited and covered by normal surface mucosa. The lesion was diagnosed as ossifying chondrolipoma with hematopoietic elements. The recovery course was uneventful, and after a 2-year follow-up, the patient showed no signs of recurrence.

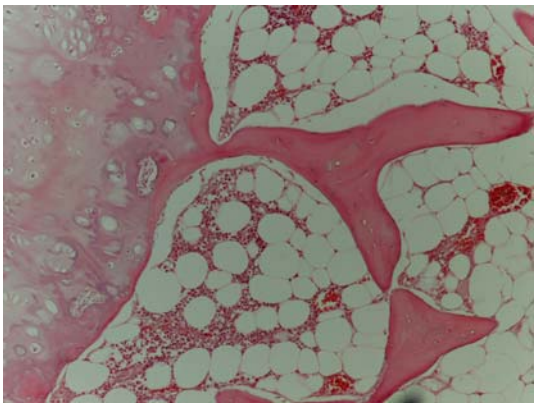

Fig. 5 − Mature cartilage centrally merging with bone formation and fatty marrow tissue (HE, ×100)

Discussion

Lipomas of the oral cavity are uncommon and represent about 1%–5% of all neoplasms of the oral cavity [5]. They are slow-growing tumors and usually presenting a mean diameter of 2.0 cm at the time of diagnosis [3, 5, 6]. Variants of lipoma with cartilaginous or osseous change have been called as chondrolipoma, osteolipoma, lipoma with cartilaginous or osseous metaplasia, or ossifying lipoma [8, 11]. These tumors are rarely observed in the oral cavity [2, 6–11]. Osteolipomas are less common than chrondrolipomas [10], similar to finding in the extraoral soft tissue [1, 12]. There currently are no more than 8 reported cases of osteolipomas in the oral cavity [2, 6, 8, 10, 11]. While oral lipomas are believed to be more common in men [3], it seems that there is no clear sex preponderance in patients with osteo/chondrolipomas in the oral cavity.

Chondrolipomas are characterized by the proliferation of mature adipocytes with additional mature cartilaginous tissue formation [6, 7, 9]. Cases of chondrolipoma of the oral cavity have been diagnosed in patients aged from 2 to 72 years, and most of the tumors are found in the tongue [3, 6, 7, 9] and in the lower lip [10]. Osteolipomas**,** in turn**,** are characterized by formation of bone trabeculae scattered among proliferating mature adipocytes [2, 6, 11]. Most cases of oral osteolipoma are diagnosed after the fifth decade of life [2, 11]. The most affected sites are the buccal mucosa and vestibulum [2, 6], tongue [8], and alveolar mucosa [11]. Osteolipomas have been reported in patients with a long history measured in years [8, 11], as in our patient.

The case presented in this report shows three rare alterations. First, mature cartilaginous tissue was a prominent component of the lesion. Usually the cartilage in lipoma represent a small part of the tumor [12], but lipoma of the tongue with marked cartilaginous metaplasia [3] and chondrolipoma of the tongue with two main components (adipose and cartilage) [7] have been reported. Second, this lesion exhibited osseous metaplasia. The bone was found to be formed through the membranous and the enchondral mechanisms. Lipomas showing cartilaginous and osseous metaplasia were observed in the subcutaneous and deep soft tissues, often in the parosteal localization [12–14]. Rau et al. [14] reported a case of parosteal lipoma with extensive areas of cartilaginous and osseous differentiation, considered as an osteochondrolipoma. These authors identified bone formation through both enchondral and membranous mechanisms. Bone forming directly from the fibrous background (membranous ossification), typically seen in heterotopic bone formation, predominated in our case. Third, in the fatty marrow tissue of the lesion, hematopoietic elements were identified. Extramedullary hematopoiesis is known to be an integral part of myelolipoma arrising in the adrenal glands most commonly, but occasionally in other sites including pelvic soft tissue [1]. This phenomenon is rarely seen in the other lipoma variants, and to our knowledge none of the intraoral cases reported to date contained hematopoietic elements.

The differential diagnosis of osteo/chondrolipomas depends on their location. When osteolipomas affecting alveolar mucosa, lesions such as tori and exostoses have to be taken into consideration [11], but a histological finding of mature ossification with fatty tissue usually can help with diagnosis [11, 13]. In the cases arising in the tongue, such as the presented case, osteo/cartilaginous choristomas should be considered, as they commonly affect the tongue [15–17]. However, these lesions consist almost entirely of cartilage and/or bone and usually contain less fatty tissue [16, 18]. In addition, mature cartilaginous areas in a lipoma should be distinguished from chondroid lipoma [3, 14, 19]. This lipoma variant is uncommon in the oral cavity, consisting of mature adipocytes and multivacuolated cells in a myxohyaline matrix that has a chondroid appearance [3, 19]. Chondroid lipoma, a newly described lesion, having an immature aspect may be mistaken for malignancy [1, 3], while chondrolipoma can easily be identified as benign tumor [1, 3]. As chondroid lipoma is integrated into the newest classification of lipomatous lesions [1], Rau et al. [14] proposed that chondrolipomas, osteolipomas, and osteochondrolipomas should also be included as one subvariety of lipomas.

The nomenclature of osteo/chondrolipomatous lesions is controversial. Hietanen and Makinen [7] reported an example of chondrolipoma of the tongue and stated that the tumor meets the criteria of benign mesenchymoma, as well. According to the inclusion criteria by Jones et al. [10], benign mesenchymoma is an unencapsulated tumor composed of two or more mature mesenchymal tissues (excluding fibrous connective tissue), with no single mesenchymal element predominating in respect to others. In contrast, in osteo/chondrolipomatous tumors a predominating component is a mature fatty tissue [8, 9, 11–14]. There is, however, no straight border between benign mesenchymomas and chondro- or osteolipomas[12], and some of previously published cases of oral chondrolipoma and osteolipoma may represent examples of benign mesenchymoma [10]. Jones et al. [10] reported 10 well-documented examples of intraoral benign mesenchymoma, one of which arose on the buccal mucosa composed of adipose tissue, cartilage and bone in addition to fibrous connective tissue. In the presented case, lipomatous component with an increased

amount of fibrous septa predominated, although areas of cartilage were prominent and osseous metaplasia was notable.

The pathogenesis of these lipomatous tumors is uncertain. Different theories have been proposed to explain the formation of cartilaginous and osseous tissues in lipomas. One theory suggests that adipose, cartilaginous and osseous components originate from multipotent undifferentiated mesenchymal cells [1, 3]. Alternatively, cartilaginous and osseous components may represent a metaplastic process in preexisting lipoma [1, 2, 7, 12]. The pathogenesis of osteo/chondrolipomas as primary mixed tumors seems improbable [12], as ossification is usually found in long-standing tumors [8, 11, 13]. According to Katzer [12], the formation of cartilage and bone within lipomas can be explained by combination of local trauma with a special reactivity of the mesenchyma, which is possibly influenced by localization (i.e. proximity to periosteum). Mesenchymal cells can be modified by local or systemic factors such as permanent mechanical stress, repeated microtraumas, and reduced blood supply [1, 12]; obviously, these factors can act as cartilaginous- and osseous-metaplasia inducing agents within lipoma. The origin of these lesions from multipotent cells seems attractive [14], as these cells have been identified in adult differentiated fat tissue [20]. *In vitro* and animal models showed a multidirectional differentiation capacity of adipose tissue-derived stem cells. This permits formation of bone, cartilage, fat, muscle, blood vessels and fibrous tissue form the same precursor cells [20].

Rau et al. [14] well documented two ways of bone formation in their case of parosteal osteochondrolipoma, suggesting that a more pleomorphic differentiation capacity of deeply located lipomatous tumors might reflect the existence of more multipotent stem cells. In this regard, osteo/chondrolipomas arising from the oral soft tissues presumably reflect the multipotent nature of undifferentiated mesenchymal cells of this region. The presented case had no history of trauma preceding the appearance of the lesion. Thus, it might be speculated that the chronic irritation of the lesion combined with the local microenviroment was a significant inductor of osseous metaplasia with hematopoiesis.

Conclusion

Despite controversies on nomenclature and pathogenesis of osteo/chondrolipomas of the oral cavity, complete surgical excision is the treatment of choice. There have been no reports on recurrence. These rare tumors must be kept in mind both clinically and pathologically when evaluating lingual lesions. The presented ossifying chrondrolipoma with hematopoietic elements is extremely unusual lesion. Further studies are necessary for more detailed characterization of this interesting entity.

REFERENCES

1. *Weiss SW, Goldblum J*R. Benign lipomatous tumors. In: *Enzinger FM, Weiss SW,* editors. Enzinger and Weiss's soft tissue tumors. St Louis: Mosby; 2001. p. 571−639.
2. *Castilho RM, Squarize CH, Nunes FD, Pinto Junior DS*. Osteolipoma: a rare lesion in the oral cavity. Br J Oral Maxillofac Surg 2004; 42(4): 363−4.
3. *Furlong MA, Fanburg-Smith JC, Childers ELB.* Lipoma of the oral and maxillofacial region: site and subclassification of 125 cases. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2004; 98(4): 441−50.
4. *Bandeca MC, Padua JM, Nadalin MR, Ozorio JEV, Silva-Sousa YTC, Perez DEC*. Oral soft tissue lipomas: a case series. J Can Dent Assoc 2007; 73(5): 431−4.
5. *Fregnani ER, Pires FR, Falzoni R, Lopes MA, Vargas PA*. Lipomas of the oral cavity: clinical findings, histological classification and proliferative activity of 46 cases. Int J Oral Maxillofac Surg 2003; 32(1): 49−53.
6. *Juliasse LER, Nonaka CFW, Pinto LP, Freitas RA, Miguel MCC*. Lipomas of the oral cavity: clinical and histopathological study of 41 cases in a Brazilian population. Eur Arch Otorhinolaryngol 2010; 267(3): 459−65.
7. *Hietanen J, Makinen J*. Chondrolipoma of the tongue. A case report. Int J Oral Maxillofac Surg 1997; 26(2): 127−8.
8. *Piattelli A, Fioroni M, Iezzi G, Rubini C*. Osteolipoma of the tongue. Oral Oncol 2001; 37(5): 468–70.
9. *Nonaka CFW, Miguel MCC, Souza LB, Pinto LP*. Chondrolipoma of the tongue: a case report. J Oral Sci 2009; 51(2): 313−6.
10. *Jones AC, Trochesset D, Freedman PD*. Intraoral benign mesenchymoma: A report of 10 cases and review of the literature. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2003; 95(1): 67−76.
11. *Saghafi S, Mellati E, Sohrabi M, Raahpeyma A, Salehinejad J, Zare-Mahmoodabadi R*. Osteolipoma of the oral and pharyngeal region: report of a case and review of the literature. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2008; 105(6): e30−4.
12. *Katzer B*. Histopathology of rare chondroosteoblastic metaplasia in benign lipomas. Path Res Pract 1989; 184(4): 437−43.
13. *Obermann EC, Bele S, Brawanski A, Knuechel R, Hofstaedter F*. Ossifiyng lipoma. Virchows Arch 1999; 434(2): 181−3.
14. *Rau T, Soeder S, Olk A, Aigner T*. Parosteal lipoma of the thigh with cartilaginous and osseous differentiation: an osteochondrolipoma. Ann Diagn Pathol 2006; 10(5): 279−82.
15. *Mosqueda-Taylor A, Gonzalez-Guevara M, de la Piedra-Garza JM, Diaz-Franco MA, Toscano-Garcia I, Cruz-Leon A*. Cartilaginous choristomas of the tongue: review of the literature and report of three cases. J Oral Pathol Med 1998; 27(6): 283−6.
16. *Lee FP*. Whitish lobulated tumor of the tongue. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2001; 92(4): 367−9.
17. *Demirseren ME, Aydin NE*. A lingual osteoid mass originating from hyaline cartilage. J Cranio-Maxillofac Surg 2007; 35(2): 132−4.
18. *Sera H, Shimoda T, Ozeki S, Honda T*. A case of chondroma of the tongue. Int J Oral Maxillofac Surg 2005; 34(1): 99−100.
19. *Darling MR, Daley TD*. Intraoral chondroid lipoma: a case report and immunohistochemical investigation. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2005; 99(3): 331−3.
20. *Strem BM, Hicok KC, Zhu M, Wulur I, Alfonso Z, Schreiber RE, et al.* Multipotential differentiation of adipose tissue-derived stem cells. Keio J Med 2005; 54(3): 132–41.

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CASE REPORT

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Acute small bowel obstruction due to ileal endometriosis: a case report and review of the most recent literature

Akutna opstrukcija tankog creva izazvana endometriozom ileuma: prikaz bolesnice i pregled najnovije literature

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Abstract

Introduction. Endometriosis is defined as the presence of benign endometrial glands and stroma outside the normal anatomical location. Endometriosis of the small bowel, especially symptomatic small bowel involvement, is very unusual. **Case report.** We presented a 45-year-old woman with acute intestinal obstruction due to ileal endometriosis The patient complained of severe abdominal pain, nausea and vomitting. Immediate laparotomy was carried out. Above the ileocecal valve there was an ulcerated, edematous and fragile segmental lesion that caused intestinal obstruction. Histology of this ileal segment revealed endometriosis and an annular stricture that again showed foci of endometriosis. **Conclusion.** In reproductive-age women with the symptoms of intestinal obstruction, intestinal endometriosis should be kept in mind.

Key words:
ileum; intestinal obstruction; endometriosis; abdomen, acute; diagnosis; surgical procedures, operative; treatment outcome.

Apstrakt

Uvod. Endometrioza je prisustvo benignih endometrijumskih žlezda i strome van normalne anatomske lokacije. Endometrioza tankog creva, posebno simptomatsko uklještenje tankog creva, veoma je retko. **Prikaz bolesnika.** Prikazali smo 45-godišnju bolesnicu sa akutnom opstrukcijom tankog creva usled ilealne endometrioze. Bolesnica je primljena sa tegobama – jakim bolom u trbuhu, mučninom i povraćanjem. Urađena je hitna laparotomija. Iznad ileocekalne valvule utvrđeno je postojanje ulcerisane, edematozne i fragilne lezije koja je izazvala opstrukciju creva. Histološkim pregledom segmenta ileuma otkrivena je endometrioza i anularno suženje u kojem su opet utvrđena žarišta endometoze. **Zaključak.** Kod žena u reproduktivnom dobu koje imaju simptome opstrukcije creva treba imati na umu mogućnost intestinalne endometrioze.

Ključne reči:
ileum; creva, opstrukcija; endometrioza; abdomen, akutni; dijagnoza; hirurgija, operativne procedure; lečenje, ishod.

Introduction

Endometriosis is defined as the presence of benign endometrial glands and stroma outside the normal anatomical location. It is a painful chronic disease occurring in 5%–15% of menstruating women [1]. Endometriosis can be divided into intra- and extraperitoneal sites. In decreasing order of frequency, intraperitoneal locations are ovaries (30%), uterosacral and large ligaments (18%–24%), fallopian tubes (20%), pelvic peritoneum, pouch of Douglas, and gastrointestinal (GI) tract. Extraperitoneal locations include cervical portio (0.5%), vagina and rectovaginal septum, round ligament and inguinal hernia sac (0.3%–0.6%), navel (1%), abdominal scars after gynaecological surgery (1.5%) and caesarian section (0.5%). Endometriosis rarely affects extraabdominal organs such as the lungs, urinary system, skin and the central nervous system [2].

While endometriosis in the gastrointestinal tract is relatively common (3%–37%), it is most often encountered in the rectum and sigmoid colon [3]. On the other hand, endometriosis of the small bowel, especially symptomatic small bowel involvement, is very unusual. A clinical picture of obstruction may be caused by stenosis or kinking as a result of adhesions or fibrosis, while intestinal intussusception and volvulus have also been described [4].

Severe acute abdomen due to small bowel involvoment is a rare phenomenon. In this paper, we presented a case of acute intestinal obstruction due to ileal endometriosis, in a 45-year-old multiparous woman.

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Case report

A 45-year-old multiparous woman presented with severe abdominal pain, nausea and vomiting for last past four days. Her last menstrual period was normal and began 8 days ago. She was a non-smoker, non-alcoholic. There was no family history of bowel cancer or inflammatory bowel disease. On physical examination. the patient appeared uncomfortable. The patient was hemodynamically stable. Rectal examination was normal. The abdomen was distended and on palpation there was general and rebound tenderness. Bimanual pelvic examination revealed left adnexal tenderness. The leukocyte count was 16,000/mm^3 and urinary pregnancy test was negative. Plain abdominal radiograph revealed bowel obstruction showing dilated loops with different air-fluid levels (Figure 1). Sonography revealed thickened, aperistaltic and distended loops of the small bowel. Also, the uterus was normal and both ovaries had multiple follicles.

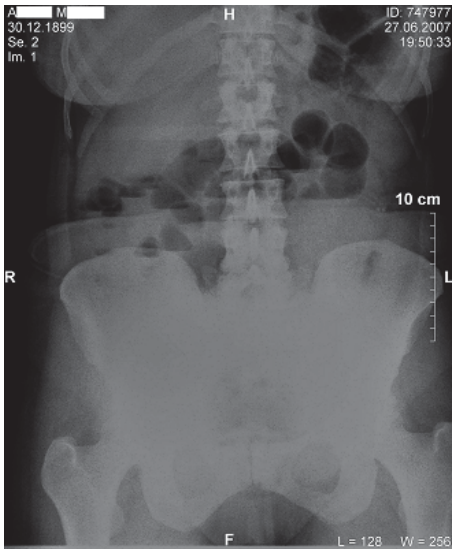

Fig. 1 – Preoperative plain abdominal radiography

The patient was hospitalized in the 4th Surgery Clinic, Izmir (Turkey), and an emergency laparotomy was performed with the diagnosis of complicated acute abdomen. The appendix was normal, but there was a tumoral mass of 5 cm above the ileocecal valve. Loops of ileum and jejunum, which were dilated, were seen proximal of the lesion. Bilateral ovaries were attached to the uterus and a left hydrosalphinx was revealed. There were follicle like lesions at the bilateral ovaries ranged between 3.0–3.5 centimeters in diameter. There was no any other intraabdominal lesion. Right hemicolectomy and ileotransverostomy were carried out for the tumoral mass causing intestinal obstruction. There was no any informed consent form for total abdominal hysterectomy with bilateral salpingo-oophorectomy. Left total salpingectomy was carried out for hydrosalpinx. Ovarian cysts were drained.

An ulcerated, odematous and fragile segmental lesion was seen 5 cm above the ileocecal valve causing annular stricture on macroscopic postoperative examination. The histopathological examination of this ileal segment revealed endometriotic implants (Figure 2). The resected specimen consisted of endometrial gland and stroma, especially in the *muscularis propria* and the submucosa. Cytological examination of cyst contents revealed hemosiderin-laden macrophages. Also, hemosiderin-laden macrophages were dedected in the tubal mucosa (Figure 3). These findings confirmed endometriosis.

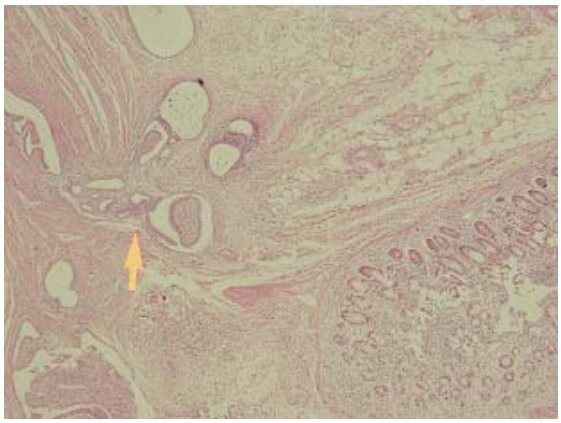

Fig. 2 – The focuses which consited of endometrial gland and stroma in the ileal wall (HE, ×60)

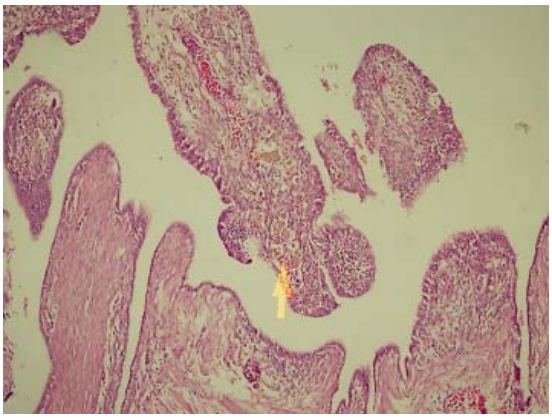

Fig. 3 – Hemosiderine-loaded macrophages in the tubal mucosa (HE, ×60)

The patient's postoperative course was uneventful, and she was discharged on the sixth day after the surgery. Also, the patient had gonadotropin-releasing hormone (GnRh) suppression therapy with leuprolide acetate for 12 months. The patient was disease-free for 36 months since operative treatment with normal ultrasonographic findings and Ca-125 levels within normal limits.

Discussion

Endometriosis is a common disease of unknown etiology. Many theories have been proposed to explain this condition. The most widely accepted is Sampson's retrograde menstruation theory: during menstruation, endometrial tissue

refluxes through the fallopian tubes, implanting and growing on the serosal surface of abdominal and pelvic organs. Coelomic metaplasia, lymphatic and/or vascular spread of vital endometrial tissues should be also taken into consideration [5]. Activated macrophages and lymphocytes can exist in endometriotic implants. The role of cytokines and growth factors in the pathophysiology of endometriosis has been investigated extensively in the past decade. The existence of cytokines and growth factors are also explain the ability of neovascularisation, pseudoinvasion and infiltration of endometrial implants [6].

Intestinal endometriosis consists of 7%–37% of all endometriosis cases [3]. In intestinal endometriosis the endometriotic involvoment is in the recto-sigmoid colon, cecum, and appendix, 86%, 4% and, 3% respectively. Small bowel involvement is only 7%. Small bowel involvement is less than 1% in all patients with endometriosis [6, 7].

Ileal endometriosis usually involves termimal ileum. Similar to our case, the endometriotic implants usually locate within 10 cm nearby the ileocecal valve [8]. This involvement may be the result of more retrograde menstruel flow towards the terminal ileum than other intestinal loops. There is a close relationship between terminal ileum and left fimbrial ending anatomically.

The symptomatology of intestinal endometriosis often characterized by nonspecific abdominal symptoms such as chronic abdominal pain, sometimes in relation to the menstrual cycle, and intestinal disorders. Dysmenorrhea, dyspareunia and infertility may occur in up to 50% of patients [9]. Constipation is seen in the distal colonic lesions. Diarrhea, nausea and vomitting are the cardinal symtoms in small intestinal lesions. Symptoms are usually cyclic. If the disease progresses, patients may complain persistently [10]. Intestinal endometriosis may mimic common gastrointetinal diseases such as appendicitis, diverticulitis, irritable bowel syndrome, intraabdominal adhesions, acute cholecystitis or Chron′s disease, and also gastrointestinal/gynecologic malignancies. Also, diffuse serosal/peritoneal involvement may results in carcinomatosis-like condition by causing exudative ascites and hemoperitoneum [11].

Preoperative confirmation of intestinal endometriosis is uncommon. Kaufman et al. [12] showed that colonoscopy with biopsy confirmed the diagnosis in 29.6% of patients tested and only 15% of patients with intestinal endometriosis had histologic lesions involving mucosa. In five patients who underwent endoscopic ultrasound, the diagnosis of intestinal endometriosis was established in all cases (n = 4) where histology or cytology was obtained. Malignancy was considered nearly as frequently as intestinal endometriosis preoperatively, and 90.4% of patients underwent laparotomy as the initial surgical approach [12]. Also Li Destri G et al. [9] claim that the diagnosis of intestinal endometriosis is very difficult and can be made by radiological methods (computed tomography or magnetic resonance imaging) or by endoscopic ultrasound only for the rectal localization. On the other hand, the diagnosis often nowadays is due to laparoscopic techniques.

Medical management of intestinal endometriosis is currently speculative; expectant management should be carefully balanced with the severity of symptoms and the feasibility of prolonged follow-up. Several studies demonstrated an improvement in quality of life after extensive surgical excision of the disease. Bowel endometriotic nodules can be removed by various techniques: mucosal skinning, nodulectomy, full thickness disc resection, and segmental resection. Although indications for colorectal resection are controversial, recent data suggest that aggressive surgery improves symptoms and quality of life [13]. But the treatment of acute small bowel obstructions due to ileal endometriosis should be segmental resection, as we carried out. End-to-end anastomosis may be done in patients with acute small bowel obstructions. Although the residual disease and the other endometriotic foci must be treated at the postoperative period too, for preventing a new advanced stage disease.

In Table 1 the presentation and first line treatment of recently reported cases of acute intestinal obstruction caused by endometriosis are given.

Table 1

The presentation, and treatment of some of recently reported cases of acute intestinal obstruction due to endometriosis

Author (year)	Presentation	First line treatment
Wickramasekera et al. (1999) [14]	distal ileum	segmental resection
Grodziński et al. (2003) [15]	ileocecal valve	right hemicolectomy
Ridha et al. (2003) [16]	terminal ileum	segmental resection
Beltrán et al. (2006) [17]	terminal ileum	segmental resection
Vanrell Garau et al. (2007) [18]	terminal ileum	right hemicolectomy
Preziosi et al. (2007) [19]	ileocaecal junction and rectum	hemicolectomy
De Ceglie et al. (2008) [20]	ileum	right hemicolectomy
Kalu et al. (2008) [21]	ileocecal valve	laparascopic colectomy
Ruiz et al. (2008) [22]	ileocecal valve	segmental resection
Chaâbouni et al. (2009) [23]	ileum	partial resection
Alatise et al. (2010) [24]	adhesive ileal obstruction	intestinal resection
Slesser et al. (2010) [25]	appendix and ileocaecal junction	right hemicolectomy

Regression and atrophy of endometrial glands is the aim of therapy. Danazol and GnRH analogs cause regression of endometriotic implants by inhibiting luteinizing hormone/follicle-stimulating hormone secretion and ovulation. It is shown that

these medications are effective for inhibiting symptomatic regression and reccurrence of intestinal endometriosis [26].

Conclusion

Clinical findings and diagnostic tools are defective for preoperative diagnosis of intestinal endometriosis causing acute abdomen. The physician must carefully examine the presence of premenstrual pain, dysmenorrhea, dyspareunia or other common symptoms of endometriosis in reproductive-age women with the intestinal obstruction symptoms. Careful preoperative evaluation of these patients is very important for providing the most convenient surgical therapy.

REFERENCES

1. *Olive DL, Schwartz LB. Endometriosis*. N Engl J Med 1993; 328(24): 1759–69.
2. *Bergqvist A*. Different types of extragenital endometriosis: a review. Gynecol Endocrinol 1993; 7(3): 207–21.
3. *MacAffee CHG, Greer HLH*. Intestinal endometriosis: a report of 29 cases and a survey of the literature. J Obstet Gynaecol Br Empire 1960; 67: 539–55.
4. *Kimura H, Konishi K, Yabushita K, Maeda K, Tsuji M, Miwa A*. Intussusception of a mucocele of the appendix secondary to an obstruction by endometriosis: report of a case. Surg Today 1999; 29(7): 629–32.
5. *Witz CA*. Current concepts in the pathogenesis of endometriosis. Clin Obstet Gynecol 1999; 42(3) : 566–85.
6. *Taylor RN, Lebovic DI, Mueller MD*. Angiogenic factors in endometriosis. Ann NY Acad Sci 2002; 955: 89–100. discussion 118, 396–406.
7. *Martimbeau PW, Pratt JH, Gaffey TA*. Small bowel obstruction secondary to endometriosis. Mayo Clin Proc 1975; 50(5): 239–43.
8. *Melody GF*. Endometriosis causing obstruction of the ileum. Obstet Gynaecol 1956; 8(4): 468–72.
9. *Li Destri G, Iraci M, Latino R, Carastro D, Li Destri M, Di Cataldo A*. Intestinal obstruction from undiagnosed rectal and ileal endometriosis. Two clinical cases and review of the most recent literature. Ann Ital Chir 2010; 81(5): 383–8. (Italian)
10. *Yantiss RK, Clement PB, Young RH*. Endometriosis of the intestinal tract: a study of 44 cases of a disease that may cause diverse challenges in clinical and pathologic evaluation. Am J Surg Pathol 2001; 25(4): 445–54.
11. *Decker D, König J, Wardelmann E, Richter O, Popat S, Wolff M*, et al. Terminal ileitis with sealed perforation—a rare complication of intestinal endometriosis: a case report and short review of the literature. Arch Gynecol Obstet 2004; 269(4): 294–8.
12. *Kaufman LC, Smyrk TC, Levy MJ, Enders FT, Oxentenko AS*. Symptomatic Intestinal Endometriosis Requiring Surgical Resection: Clinical Presentation and Preoperative Diagnosis. Am J Gastroenterol 2011; 106(7): 1325–32.
13. *Remorgida V, Ferrero S, Fulcheri E, Ragni N, Martin DC*. Bowel endometriosis: presentation, diagnosis, and treatment. Obstet Gynecol Surv 2007; 62(7): 461–70.
14. *Wickramasekera D, Hay DJ, Fayz M*. Acute small bowel obstruction due to ileal endometriosis: a case report and literature review. J R Coll Surg Edinb 1999; 44 (1): 59–60.
15. *Grodziński T, Gackowski W, Radwański P*. Intestinal obstruction caused by endometriosis. Ginekol Pol 2003 ; 74(3): 234–6. (Polish)
16. *Ridha JR, Cassaro S*. Acute small bowel obstruction secondary to ileal endometriosis: report of a case. Surg Today 2003; 33(12): 944–7.
17. *Beltrán MA, Tapia Q TF, Araos HF, Martínez GH, Cruces KS*. Ileal endometriosis as a cause of intestinal obstruction. Report of two cases. Rev Med Chil 2006; 134(84): 485–90. (Spanish)
18. *Vanrell Garau M, Ginard Vicens D, Mariño Méndez Z, Bosque López MJ, Reyes Moreno J, Escarda Gelabert A*, et al. Ileal perforation secondary to intestinal endometriosis. Gastroenterol Hepatol 2007; 30(5): 274–6. (Spanish)
19. *Preziosi G, Cristaldi M, Angelini L*. Intestinal obstruction secondary to endometriosis: a rare case of synchronous bowel localization. Surg Oncol 2007; 16 Suppl 1: S161–3.
20. *De Ceglie A, Bilardi C, Blanchi S, Picasso M, Di Muzio M, Trimarchi A*, et al. Acute small bowel obstruction caused by endometriosis: a case report and review of the literature. World J Gastroenterol 2008; 14(21) :3430–4.
21. *Kalu E, Richardson R, Sellu D, Kubba F*. Endometriosis-associated ileo-cecal perforation in a woman on the pseudopregnancy regimen. J Minim Invasive Gynecol 2008; 15(6): 764–6.
22. *Ruiz R, Pacheco M, Oliden O*. Ileal endometriosis as cause of intestinal obstruction. A case presentation. An Med Interna 2008 ; 25(6): 307–8. (Spanish)
23. *Chaâbouni S, Makni SK, Kallel RI, Gouiaa N, Bahri I, Mnif L*, et al. Unusual cause of intestinal obstruction: ileal endometriosis. Pathologica 2009; 101(3): 130–2.
24. *Alatise OI, Sabageh D, Ogunniyi SO, Olaofe OO*. Ileal endometriosis presenting as acute small intestinal obstruction: a case report. West Afr J Med 2010; 29(5): 352–5.
25. *Slesser AA, Sultan S, Kubba F, Sellu DP*. Acute small bowel obstruction secondary to intestinal endometriosis, an elusive condition: a case report. World J Emerg Surg 2010; 5: 27.
26. *Mussa FF, Younes Z, Tihan T, Lacy BE*. Anasarca and small bowel obstruction secondary to endometriosis. J Clin Gastroenterol 2001; 32(2): 167–71.

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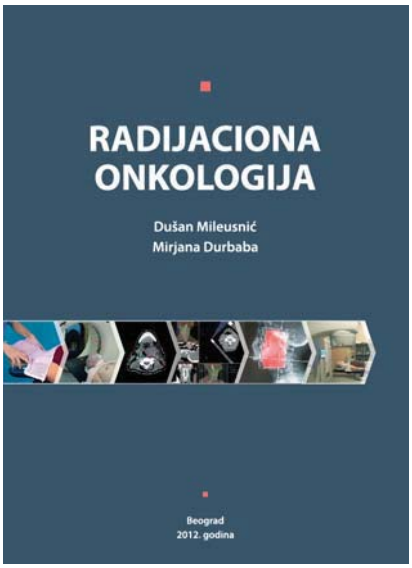
P R I K A Z K N J I G E

Radijaciona onkologija



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U udžbeniku „Radijaciona onkologija“ autori Dušan Mileusnić i Mirjana Durbaba na sveobuhvatan i pristupačan način, veoma studiozno obradili su ovu spečifičnu i vrlo aktuelnu oblast onkologije.

Knjiga ima 24 poglavlja, koja su funkcionalno razvrstana u dva dela. U prvom, opštem delu, koji se sastoji od 10 poglavlja, obrađene su teme vezane za jonizujuće zračenje i mehanizme njegovog delovanja, odnosno radiofiziku, radiobiologiju, radioterapijske tehnologije, radiološku dijagnostiku, radioterapijske aparate, tehnike zračenja, 3D planiranje i konformalnu radioterapiju, kao i komplikacije. Takođe, sažeto je prikazana istorija razvoja radijacione onkologije.

Drugi, specijalni deo sastoji se od 14 poglavlja. U njemu je prikazana klinička primena radijacione terapije, svrstana prema lokalizaciji tumora. Ovde su prikazane i palijativna radioterapija i specijalne/kompleksne tehnike zračenja. Svako poglavlje je sistematično i celovito obrađeno, jer počinje od anatomskih karakteristika tumora, epidemiologije, histopatologije, stepenovanje stadijuma i kliničke slike bolesti. Posebno detaljno je obrađena tehnika radioterapije tumora određene lokalizacije a, takođe, i njena neželjena rana i kasna dejstva i komplikacije, koje je ponekad teško izbeći. Ovo poglavlje može pomoći i lekarima drugih specijalniosti da bolje sagledaju načine rešavanja ovog problema.

Ističem da je udžbenik „Radijaciona onkologija“ autora Dušana Mileusnića i Mirjane Durbaba prva knjiga ove vrste napisana na srpskom jeziku. Time se popunjava jedan ozbiljna praznina u domaćoj medicinskoj literaturi. Kako je knjiga napisana pristupačnim stilom, ona je od koristi ne samo lekarima, tehničarima i ostalim profilima koji se bave zračenjem, već i lekarima drugih specijalnosti, koji se svakodnevno sreću sa obolelima od malignih bolesti. Stručnost, sveobuhvatnost teme i lako razumljiv stil kojim je knjiga napisana nesumljivo će preporučiti ovu knjigu medicinskoj javnosti, koja će znati da ceni izvanredan trud autora da sagledaju i ovako stručno obrade ovu, veoma aktuelnu oblast medicine.

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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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All measurements should be reported in the metric system in terms of the International System of Units (SI). Standard, internationally accepted terms should be used. Papers should be submitted on a diskette (CD) in triplicate (original and two copies).

MS Word for Windows (97, 2000, XP, 2003) is recommended for word processing; other programs are to be used only exceptionally. Illustrations should be made using standard **Windows** programs**.** Avoid the use of colors in graphs.

Papers are reviewed anonymously by at least two editors and/or invited reviewers. Remarks and suggestions are sent to the author for final composition. Galley proofs are sent to the first author for corrections that should be returned within 3 days. Manuscripts accepted for publication are not being returned.

Preparation of manuscript

Parts of the manuscript are: **Title page; Abstract with key words; Text; References**.

1. ***Title page***

a) The title should be concise but informative. Subheadings should be avoided;

b) full name of each author;

c) name and place of department(s) and institution(s) of affiliation, clearly marked by standard footnote signs.

2. ***Abstract and key words***

The second page should carry a structured abstract with the title for original articles, metanalyses and case reports. The abstract should state the purposes of the study or investigation, basic procedures (selection of study subjects or laboratory animals; observational and analytical methods), main findings (giving specific data and their statistical significance, if possible), and the principal conclusions. It should emphasize new and important aspects of the study or observations. S t r u c t u r e d abstract should contain typical subtitles: *background/aim, methods, results* and *conclusion*. The abstract for metaanalyses and obrginal papers should have up to 450 words, and up to 150 words for case reports (with subtitles *background, case report, conclusion*). Below the abstract authors should provide, and identify as such, 3–10 key words or short phrases that will assist indexers in cross-indexing the article and will be published with the abstract.

3. ***Text***

The text of original articles is divided into sections with the headings: **Introduction**, **Methods**, **Results**, and **Discussion**. Long articles may need subheadings within some sections to clarify their content.

In the **Introduction** repeat the title of the article, excluding the names of authors. State the purpose of the article and summarize the rationale for the study or observation. Give only strictly pertinent references and do not include data or conclusions from the work being reported.

Methods. Describe your selection of the observational or experimental subjects (patients or experimental animals, including controls) clearly. Identify the methods, apparatus (manufacturer's name and address in parentheses), and procedures in sufficient detail to allow other workers to reproduce the results. Give references to established methods, including statistical methods. Identify precisely all drugs and chemicals used, with generic name(s), dose(s), and route(s) of administration. State the approvement of the Ethnics Committe for the tests in humans and enimals.

Results should be presented in logical sequence in the text, tables and illustrations. Emphasize or summarize only important observations.

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.

References

References should be superscripted and numbered consecutively in the order in which they are first mentioned in the text. **The references must be verified by the author(s) against the original document.** List all authors, but if the number exceeds 6, give 6 followed by et al. Do not use abstracts, secondary publications, oral communications, unpublished papers, official and classified documents. References to papers accepted but not yet published should be designated as ”in press“. Information from manuscripts not yet accepted should be cited in the text as ”unpublished observations“. References are cited according to the ***International Committee of Medical Journal Editors.*** *Uniform Requirements for Manuscripts Submitted to Biomedical Journals. Ann Intern Med 1997; 126: 36–47. Updated October 2001.*

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

Tables

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.

Illustrations

Figures are submitted in triplicate, and for the final version also on diskette/CD. Photos should be sharp, glossy black and white photographic prints, not larger than 203 × 254 mm. Letters, numbers, and symbols should be clear and even throughout and of sufficient size that when reduced for publication, each item will still be legible. Each figure should have a label on its back indicating the number of the figure, author's name, and top of the figure. If a figure has been published, acknowledge the original source.

Legends for illustrations are typed on a separate page, with arabic numerals corresponding to the illustrations. Identify and explain each one clearly in the legend symbols, arrows, numbers, or letters used to identify parts of the illustrations. Explain the internal scale and identify the method of staining in photomicrographs.

Abbreviations and symbols

Use only standard abbreviations. Avoid abbreviations in the title and abstracts. The full term for which an abbreviation stands should precede its first use in the text.

Detailed Instructions are available at the web site:
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