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Since 1987, every year, on May 31, the World Health Organization and partners everywhere mark World No Tobacco Day, highlighting the health risks associated with tobacco use and advocating for effective policies to reduce tobacco consumption. Newest data suggest that the global tobacco epidemic kills nearly 6 million people each year, of which more than 600,000 are non-smokers dying from breathing second-hand smoke. Unless we forcefully act against tobbaco smoke, the epidemic will kill more than 8 million people every year by 2030.

Despite the adoption of the Law on the Citizens Protection against Tobacco Smoke in 2010, Serbia still has a prominent place among the countries with a large number of active smokers. Of particular concern are data on the prevalence of smoking among health care professionals in our country, on what it has already been written in the "Vojnosanitetski Pregled". In this issue of the Journal there is again an article on this subject (see p. 481–90).

Pod pokroviteljstvom Svetske zdravstvene organizacije (SZO) svake godine, 31. maja, obeležava se Svetski dan bez duvanskog dima (*World No Tobacco Day*). Ovaj dan ustanovljen je 1987. godine sa ciljem podizanja svesti o zdravstvenim rizicima vezanim za upotrebu duvana i duvanskih proizvoda i potrebi za preduzimanjem sveobuhvatnih mera u borbi protiv pušenja.

Prema najnovijim podacima, svake godine od posledica pušenja umre blizu šest miliona ljudi od kojih više od 600 000 su nepušači koji udišu duvanski dim. Ako se energičnije ne budu sprovodile mere borbe protiv pušenja, procena je da će do 2030. godine epidemija pušenja uzrokovati smrt osam miliona ljudi godišnje.

Uprkos donošenju Zakona o zaštiti stanovništva od izloženosti duvanskom dimu 2010. godine, Srbija i dalje zauzima visoko mesto među zemljama sa velikim brojem aktivnih pušača. Zabrinjava podatak o raširenosti pušenja među zdravstvenim radnicima u našoj zemlji, o čemu je nekoliko puta već pisano na stranicama „Vojnosanitetskog pregleda". I u ovom broju časopisa objavljujemo članak na tu temu (vidi str. 481–90).

E D I T O R I A L / U V O D N I K

The new editors at the Vojnosanitetski pregled

Novi urednici časopisa „Vojnosanitetski pregled“

Silva Dobrić

Institute for Scientific Information, Military Medical Academy, Belgrade, Serbia

Ever since 1961 when Editorial Office of the *Vojnosanitetski pregled* (VSP) was included into the Institute of Scientific Information of the Military Medical Academy (MMA) in Belgrade following its constitution, the Head of the Instutute has also been performing the function of chief editor of the Journal. The editorial board is constituted of the teaching staff of the MMA taking care that their competencies cover various medical branches, stomatology and pharmacy since the VSP is a scientific journal of military physicians, pharmacists and stomatologists. As the VSP turned out to be one of the leading biomedical journals in Serbia with increasing number of articles from the civil sector, however, the need arose to include experts from civil medical and academic institutions, which was done in 2002. In 2006 due to international recognition of the VSP the International Editorial Board was formed. Soon after, these changes, together with the decision to publish articles in English, resulted in quality improving of the Journal, its higher visibility in the international scientific community and, finally, its inclusion in the system of indexing by the famous data base of scholarly publications – the Science Citation Index Expanded (SCIe) in 2008, and, subsequently getting the impact factor for the year 2010.

As a rule, the members of the Editorial Board at the VSP are reviewers of articles related to their specialities and scientific fields of interest, and help the chief editor to chose other reviewers. Namely, articles submitted to the Editorial Office of the VSP go to double proofreading, and in case of opposite evaluations a paper is sent to the third proofreading. Yet, the majority of proofreading is done by the members of the Editorial Board, besides their everyday work obligations, implying additional both time and intelectual burden. Just due to that burden, the Operating Procedure of the Editorial Board of the VSP defines a 4-year mandate for any members of the Editorial Board, allowing one additional mandate (with the exception of academicians and those of a very specific scientific fields). It is our practice that a part of the Board remains for one more mandate and to change the other part with the new

Još od 1961. godine kada je posle osnivanja Instituta za naučne informacije Vojnomedicinske akademije (VMA) u Beogradu Redakcija časopisa „Vojnosanitetski pregled“ (VSP) ušla u njegov sastav, načelnik Instituta vrši i funkciju glavnog i odgovornog urednika časopisa. Članovi uredništva biraju se iz redova nastavnika VMA pri čemu se vodilo računa da njihove kompetencije pokriju različite oblasti medicine, stomatologije i farmacije, s obzirom na to da je VSP stručni časopis vojnih lekara, farmaceuta i stomatologa. Međutim, kako je VSP postao jedan od vodećih biomedicinskih časopisa u zemlji u kome su sve više objavljivali radove i autori tzv. civilnog sektora, ukazala se potreba za uključenjem u rad Uređivačkog odbora časopisa i stručnjaka iz civilnih zdravstvenih i akademskih institucija, što je i učinjeno 2002. godine. U cilju međunarodne afirmacije časopisa 2006. godine formiran je i Međunarodni uređivački odbor. Vrlo brzo, ove izmene, zajedno sa odlukom da se sve veći broj članaka objavljuje na engleskom jeziku, rezultirale su podizanjem kvaliteta časopisa, njegovom većom vidljivošću u međunarodnim naučnim krugovima i, konačno, njegovim uključenjem u sistem praćenja čuvene baze naučne publicistike *Science Citation Index Expanded* (SCIe) 2008. godine i dobijanjem impakt faktora 2010. godine.

Članovi Uređivačkog odbora VSP su, po pravilu, recenzenti radova iz oblasti koja je predmet njihovog užeg naučnog i stručnog interesovanja i pomažu glavnom i odgovornom uredniku u odabiru drugih recenzenata. Naime, radovi koji stignu u redakciju VSP podležu dvostrukoj recenziji, a u slučaju oprečnih recenzija, rad se šalje i na treću recenziju. Ipak, najveći deo recenzentskog posla obavljaju, upravo, članovi Uređivačkog odbora i to, po pravilu, uz svoje redovne radne zadatke, što podrazumeva dodatno i vremensko i intelektualno opterećenje. Upravo zbog ovog opterećenja, Poslovnikom o radu Uređivačkog odbora časopisa “Vojnosanitetski pregled” mandat članova Uređivačkog odbora ograničen je na četiri godine s mogućnošću još jednog uzastopnog mandata (izuzetak su članovi odbora iz redova akademika, odnosno eksperti iz pojedinih uskospecifičnih naučnih oblasti). Dosadašnja praksa bila je da deo Uređivačkog odbora ostane još jedan mandat, a da se drugi deo zameni novim članovima. Početkom ove godine navršilo se četiri godine od formiranja, tada novog, Uređivačkog odbora

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members. So, the very beginning of this year, when a 4-year mandate for the Editorial Board, then new, expired, was the opportunity to replace a number of members, mainly those with double mandate with new members.

The new members from the Medical Faculty at the VMA, the University of Defence, Belgrade are: Prof. Dr. Gordana Dedić (psychiatry), Lt. Col. Prof. Dr. Tihomir Ilić (neurology), Col. Assist. Prof. Dr. Srđan Lazić (epidemiology), Prof. Dr. Slavica Rađen (hygiene and nutrition), Assist. Prof. Leposava Sekulović (radiology), Prof. Dr Maja Šurbatović (anesthesiology and intensive therapy), and Col. Prof. Dr. Dino Tarabar (gastroenterology); from the Faculty of Medicine, University of Belgrade: corresponding member of the Serbian Academy of Science and Arts: Prof. Dr. Zoran Krivokapić (surgery), Prof. Dr. Branka Nikolić (gynecology and obstetrics), and Prof. Dr. Slobodan Slavković (orthopedics and traumatology); from the Faculty of Medicine, University of Novi Sad: Prof. Dr. Slavica Ušaj-Knežević (pathology). These new members will join the old ones in editing articles, namely to: Prof. Dr. Bela Balint (transfusiology), Col. Prof. Dr. Dušan Stefanović (rheumatology), Col. Prof. Dr. Aleksandar Đurović (physical therapy and rehabilitation), Col. Prof. Dr. Dragan Mikić (infectology), Lt. Col. Prof. Dr. Slobodan Obradović (cardiology), Prof. Dr. Darko Mirković (surgery), Prof. Dr. Slavica Vučinić (clinical toxicilogy), Prof. Dr. Zlata Brkić (stomatology), Prof. Dr. Zvonko Magić (human genetics), Prof. Dr. Lidija Kandolf-Sekulić (dermatology), corresponding member of the Serbian Academy of Science and Arts, Prof. Dr. Đorđe Radak (vascular hygiene), Prof. Dr. Borislav Janković (pediatrics), Brig. Gen. Academician Prof Dr Miodrag Čolić, Academician Prof. Dr. Radoje Čolović (surgery), Academician Prof. Dr. Vladimir Kanjuh (cardiovascular pathology), Academician Prof. Dr. Vladimir Kostić (neurology), Academician Prof. Dr. Miodrag Ostojić (cardiology), corresponding member of the Serbian Academy of Science and Arts Prof. Dr. Predrag Peško (surgery), and Prof. Dr. Ljubomir Todorović (stomatology/oral surgery).

The new members of the International Editorial Board are: Prof. Dr. Jovan Antonović from the Karolinska Institutet, Stockholm, Sweden (internal medicine – hematology), Prof. Dr. Gerhke Thorsten, from the Medical School, Hamburg, Germany (orthopedics), Prof. Dr.Thomas John from the Layola University, Chicago, USA (ophthalmology), Prof. Dr. Mirjana Pavlović from the Florida Atlantic University, Boca Raton, USA (biochemistry and bioengineering), and Prof. Dr. Sadber Lale Tokgozoglu from the Faculty of Medicine, Hacettepe University, Ankara, Turkey (cardiology).

On behalf of myself and the Editorial Office I want to very many thank to the previous members of the Editorial Board whose mandate expired: Prof. Dr. Snežana Cerović, Prof. Dr. Branka Đurović, Prof. Dr. Gordana Mandić-Gajić, Prof. Dr. Dara Stefanović, Col. Prof. Dr. Đoko Maksić, Col. Prof. Dr. Ranko Raičević, Col. Prof. Dr. Vojkan Stanić, Prof. Dr. Vesna Šuljagić, and Prof. Dr. Milan Višnjić especially to their efforts put to increasing recognition of the Journal and its inclusion in the base SCIe, hoping that our

VSP, što je bila prilika da se deo članova, mahom sa ispunjenim dvostrukim mandatom u radu ovog tela, zameni novim članovima. Iz redova nastavnika Medicinskog fakulteta VMA Univerziteta odbrane u Beogradu, novi urednici VSP postali su: prof. dr Gordana Dedić (oblast psihijatrija), potpukovnik prof. dr Tihomir Ilić (oblast neurologija), pukovnik doc. dr Srđan Lazić (oblast epidemiologija), prof. dr Slavica Rađen (oblast higijena i ishrana), doc. dr Leposava Sekulović (oblast radiologija), prof. dr Maja Šurbatović (oblast anesteziologija i intenzivna terapija) i pukovnik prof. dr Dino Tarabar (oblast gastroenterologija); od nastavnika Medicinskog fakulteta Univerziteta u Beogradu: dopisni član Srpske akademije nauka i umetnosti prof. dr Zoran Krivokapić (oblast hirurgija), prof. dr Branka Nikolić (oblast ginekologija i akušerstvo) i prof. dr Slobodan Slavković (oblast ortopedija i traumatologija) i od nastavnka Medicinskog fakulteta Univerziteta u Novom Sadu – prof. dr Slavica Ušaj-Knežević (oblast patologija). Oni će u narednom periodu, u obavljanju uredničkih poslova pridružiti „starim“ članovima Uređivačkog odbora kojima tek počinje drugi mandat, odnosno urednicima iz redova SANU. To su: prof. dr Bela Balint (oblast transfuziologija); pukovnik, prof. dr Dušan Stefanović (oblast reumatologija); pukovnik, prof. dr Aleksandar Đurović (oblast fizikalna terapija i rehabilitacija); pukovnik, prof. dr Dragan Mikić (oblast infektologija); potpukovnik, prof. dr Slobodan Obradović (oblast kardiologija); prof. dr Darko Mirković (oblast hirurgija); prof. dr Slavica Vučinić (oblast klinička toksikologija); prof. dr Zlata Brkić (oblast stomatologija); prof. dr Zvonko Magić (oblast humana genetika); prof. dr Lidija Kandolf-Sekulović (oblast dermatologija); dopisni član SANU, prof. dr Đorđe Radak (oblast vaskularna hirurgija); prof. dr Borisav Janković (oblast pedijatrija); brigadni general, redovni član SANU, prof. dr Miodrag Čolić (oblast imunologija); redovni član SANU, prof. dr Radoje Čolović (oblast hirurgija); redovni član SANU, prof. dr Vladimir Kanjuh (oblast kardiovaskularna patologija); redovni član SANU, prof. dr Vladimir Kostić (oblast neurologija); redovni član SANU, prof. dr Miodrag Ostojić (oblast kardiologija); dopisni član SANU, prof. dr Predrag Peško (oblast hirurgija); prof. dr Ljubomir Todorović (oblast stomatologija/oralna hirurgija).

Međunarodni uređivački odbor takođe je dobio nove članove. To su: prof. dr Jovan Antonović (Karolinska Institutet, Stockholm, Sweden; oblast interna medicina – hematologija), prof. dr Gerhke Thorsten-a (Medical School, Hamburg, Nemačka; oblast ortopedija), prof. dr Thomas John (Layola University, Chicago, USA; oblast oftalmologija), prof. dr Mirjana Pavlović (Florida Atlantic University, Boca Raton, USA; oblast biohemija i bioinženjering) i prof. dr Sadber Lale Tokgozoglu (Faculty of Medicine, Hacettepe University, Ankara, Turkey; oblast kardiologija).

U svoje ime, kao i u ime Redakcije zahvaljujem dosadašnjim članovima Uređivačkog odbora VSP kojima je prestao mandat (prof. dr Snežana Cerović, prof. dr Branka Đurović, prof. dr Gordana Mandić-Gajić, prof. dr Dara Stefanović, pukovnik prof. dr Đoko Maksić, pukovnik prof. dr Ranko Raičević, pukovnik prof. dr Predrag Romić, pukovnik prof. dr Vojkan Stanić, prof. dr Vesna Šuljagić i , prof. dr Milan Višnjić) na uloženom trudu u podizanju ugleda časopisa i njegovom uključenju u bazu SCIe, uz nadu da ćemo uspešno sarađivati i ubuduće.

cooperation will go on. To the new members I want to very many thank to their being ready to accept this highly responsible job and willingly contribute to further improvement of the Journal and its even better ranking in international scholarly publishing. Hoping that in the next 4-year period we will cooperate successfully and fruitfully, I wish many success in the editing process to all the members of the Editorial Board at the VSP, espacially to the new ones!

Novim članovima Uređivačkog odbora zahvaljujem na spremnosti da prihvate ovaj odgovoran posao i daju svoj puni doprinos daljem unapređenju časopisa i njegovom što boljem pozicioniranju u međunarodnoj naučnoj publicistici. U nadi da ćemo u sledećem četvorogodišnjem periodu ostvariti uspešnu i plodotvornu saradnju, svim članovima Uređivačkog odbora VSP, posebno novoimenovanima, želim mnogo uspeha u uredničkom radu!

Dobrić Silva. Vojnosanit Pregl 2014; 71(5): 429–431.

ORIGINAL ARATICLES

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A single incision transaxillary thoracoscopic sympathectomy

Transaksilarna torakoskopska simpatektomija jednom incizijom

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Abstract

Background/Aim. Primary hyperhidrosis causes are unknown. The disorder begins in early childhood. It intensifies in puberty and maturity. It is equally present in both sexes. The symptoms exacerbate when the body temperature rises and due to emotional stimuli affecting the sympathetic nerve system. The aim of this study was to demonstrate that video-assisted thoracoscopic surgery (VATS) sympathectomy is a method for primary focal hyperhidrosis permanent treatment. The single incision method in properly selected patients maximizes the intervention effectiveness and minimizes aesthetic side effects. **Methods.** This prospective study analysed the findings in patients who had been operated on due to primary focal hyperhidrosis (face, palms, and armpits) using a single small transaxilarry incision in the third inter-rib space at the level of the anterior axillary line with two 5 mm flexible ports. All the patients, with T2–T5 thoracoscopic sympathectomy of the sympathetic chain using a single small incision in the third inter-rib space in the anterior axillary line, were analysed in the period from September 2009 to November 2010 regarding the postoperative morbidity and outcomes of the operation (clinical evaluation and visual analogue scale) with a view to assessing the effectiveness of the surgery conducted in this manner. **Results.** A total of 47 patients (18 men, 29 women), 18 to 48 years old (29 on average) had underwent 94 bilateral video-assisted thoracoscopic sympathectomies. The sympathectomy was indicated in cases of facial blushing and sweating (6.38%), palmary sweating (34.04%), axillary sweating (14.89%) or both palmary and axillary sweating (44.68%). The largest percentage of patients (98.6%) had left the hospital the following day. The postoperative 30 day's mortality was 0 and the conversion into open surgery was not necessary. As for complications, there had been an occurrence of partial pneumothorax in two patients treated by means of exuflation and chest drain, and one case of unilateral transitory Horner's syndrome. Quarterly and annual postoperative monitoring showed excellent aesthetic effects of the surgery without any residual pain. The complete withdrawal of hyperhidrosis symptoms was noted in 44 (93.62%) of the patients. The recurrence of symptoms following the initial regression was seen in 3 (6.38%) of the patients 12 months after the surgery, whereas the patients surgically treated as a result of facial hyperhidrosis saw a significantly increased sweating of feet. The quality of life improved in 45 (95.6%) of the patients. **Conclusion.** Single incision transaxillary thoracoscopic sympathectomy generates excellent aesthetic and functional results in patients with primary focal hyperhidrosis.

Key words:
hyperhidrosis; sympathectomy; treatment outcome.

Apstrakt

Uvod/Cilj. Uzroci primarne hiperhidroze nisu poznati. Ovaj poremećaj javlja se u ranom detinjstvu, a pogoršava se u pubertetu i zrelom dobu. Zastupljen je podjednako kod oba pola. Simptomi se pojačavaju sa porastom temperature, kao i zbog emocionalne napetosti koja deluje na simpatički nervni sistem. Cilj rada bio je prikaz *video-assisted thoracoscopic surgery* (VATS) simpatektomije kao metode trajnog lečenja problema prekomernog znojenja. Metoda jednom incizijom pruža minimalne estetske defekte, a maksimalan efekat operacije. **Metode.** Svi bolesnici koji su podvrgnuti torakoskopskoj simpatektomiji od T2–T5 simpatičkog gangliona primenom jedne male incizije u trećem međurebarnom prostoru, u nivou prednje aksilarne linije, analizirani su u vremenskom intervalu od septembra 2009. do novembra 2010. prema postoperativnom morbiditetu i ishodu operacije (klinička evaluacija i vizuelna analogna skala) radi procene efekta operacije izvedene na ovaj način. **Rezultati.** Analizirani su podaci 47 bolesnika (18 muškaraca, 29 žena), starih od 18 do 48 godina (prosečno 29 godina) kod kojih su urađene 94 bilateralne VATS simpatektomije. Indikacije za simpatektomiju uključivale su znojenje lica (6,38%), znojenje dlanova (34,04%), znojenje pazušnih jama (14,89%) i/ili dlanova i

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pazušnih jama (44,68%). Najveći procenat (98,6%) bolesnika napustio je bolnicu sledećeg dana, postopeativni 30-dnevni mortalitet bio je 0 i nije bilo potrebe za konverzijom u otvorenu proceduru. Od komplikacija zabeležena je pojava parcijalnog pneumotoraksa kod dva bolesnika koji su lečeni eksuflacijom i torakalnom drenažom i pojava unilateralnog tranzitornog Hornerovog sindroma kod jednog bolesnika. Tromesečno i jednogodišnje praćenje nakon operacije ukazalo je na odlične funkcionalne i kozmetske efekte operacije bez rezidualnog bola. Kompletno povlačenje simptoma hiperhidroze zabeleženo je kod 44 (93,62%) bolesnika. Ponavljanje simptoma nakon početne regresije zabeleženo je kod tri (6,38%) bolesnika 12 meseci nakon operacije, dok je kod bolesnika koji su operisani zbog hiperhidroze lica značajno povećano znojenje stopala. Poboljšanje kvaliteta života primećeno je kod 45 (95,6%) bolesnika. **Zaključak.** Transaksilarna torakoskopska simpatektomija jednom incizijom daje odlične kozmetske i funkcionalne rezultate kod bolesnika sa primarnom fokalnom hiperhidrozom.

Ključne reči:
hiperhidroza; simpatektomija; lečenje, ishod.

Introduction

Hyperhidrosis is a phenomenon indicating excessive sweating. In normal circumstances the sweating glands are stimulated by a physical activity, environment factors, as well as emotional factors. In the primary focal hyperhidrosis, sweating is intensified regardless of the climate impact, leading to psycho-social dysfunction.

Primary hyperhidrosis causes are unknown. The disorder begins in early childhood. It intensifies in puberty and maturity. It is equally present in both sexes. The symptoms exacerbate when the body temperature rises and due to emotional stimuli affecting the sympathetic nerve system.

Hans Christian Jacobeus, the Swedish internist, who is called the 'Father of Thoracoscopy', performed the first thoracoscopy in 1865 jointly with Francis Richard Cruise, the Irish physicist [1]. The first published surgical sympathectomy was presented by Alexander [2] and it was performed at the level of the neck because of epilepsy.

In 1911 in London Meachen [3] described profuse sweating localized in feet, on the face and palms. He used peroral drugs, as well as X-ray to treat the disorder.

In 1927 Kuntz [4] described aberrant branching of the sympathetic chain inferior to the stellate ganglion at the level of the first and the second thoracic nerve. Such anatomical variations are responsible for the failure of sympathectomy after the sympathetic trunk is appropriately disrupted.

The most common nonsurgical modern treatments for hyperhidrosis, which fall broadly into the categories of topical treatments are iontophoresis, oral medications, and botulinum toxin (BTX) [5]. For those who fail such treatment, are surgery is typically recommended for palmar and axillary hyperhidrosis [6].

These were rare reports in regard to hyperhidrosis-based sympathectomy until the 90s of the last century when video-assisted thoracoscopic surgery (VATS) became more accessible owing to the improved technical scope. The beginnings of the minimally invasive thoracic surgery are associated with the year of 1992 and Chandler [7] from Alabama who presented a case study of dorsal sympathectomy which he performed at the T2-T3 level of ganglionectomy due to the post-traumatic pain syndrome. In 1993 the first international symposium on thoracoscopic sympathectomy was held in Boras, Sweden. The procedure immediately saw an increasing popularity, particularly in Sweden [8] to become a regular procedure nowadays in all larger thoracic surgery centers worldwide [9].

The aim of this study was to assess long-term outcomes and efficiency of bilateral sympathectomy using single incision in the skin with two flexible 5 mm ports inserted for a camera and an electric knife for transection of the sympathetic chain at an appropriate level.

Methods

Between September 2009 and November 2010 all the patients with VATS sympathectomy for primary focal hyperhidrosis (face, palms, armpits) were included in this study. They were carefully selected following a detailed anamnesis and confirmed symptoms leading to a condition which hindered their work and social activities and gave rise to a social phobia. The pre-conducted clinical check-up and lab analyses excluded the possibility of the secondary hyperhidrosis as a result of a possible disorder of the thyroid gland, diabetes or cancer [10]. X-ray of the lungs was being performed preoperatively on a regular basis to exclude possible pleural adhesions which would compromise the intervention or make it impossible.

The surgery was performed during a single lung ventilation by using a double lumen tube. The position of a patient on the operating table was semi-sitting, with arms in abduction under the angle of 90°. After excluding the lung from ventilation, the pleural area was accessed by making an incision of around 15 mm in length in the anterior axillary line behind the course of fibres of the grand thoracic muscle, through which two flexible 5 mm ports are inserted (Figure 1).

After the optical system was distributed, the sympathetic chain could be seen extending usually posterior from the head of the ribs. The first rib was identified through palpation by means of instruments, whereafter the coagulation of an appropriate ganglion was performed by means of low voltage electrocauterization, along with disconnection of the sympathetic chain at the appropriate level. Regarding facial blushing and sweating, the level was directly under the first thoracic vertebra T1, for excessive palmary sweating it was T2, whereas for profuse axillary sweating the levels were T3 and T4. Communicant branches [4] at the distance of 3 cm from the point of transection were also coagulated with the maximum protection of intercostal neurovascular structures. The surgery was completed following the haemostasis control tests and a complete video assisted reexpansion of lungs (Figure 2).

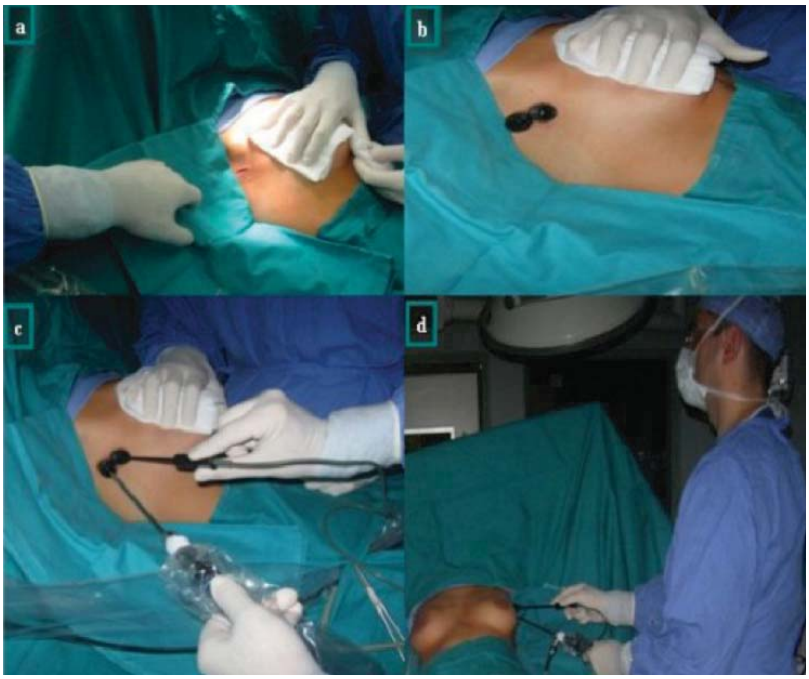

Fig. 1 – a) The incision on the wall of the thorax at the level of the third inter-rib space in the anterior axillary line; b) The flexible 5 mm ports inserted in the pleural area; c, d) The camera and the employed instrument distributed through the ports.

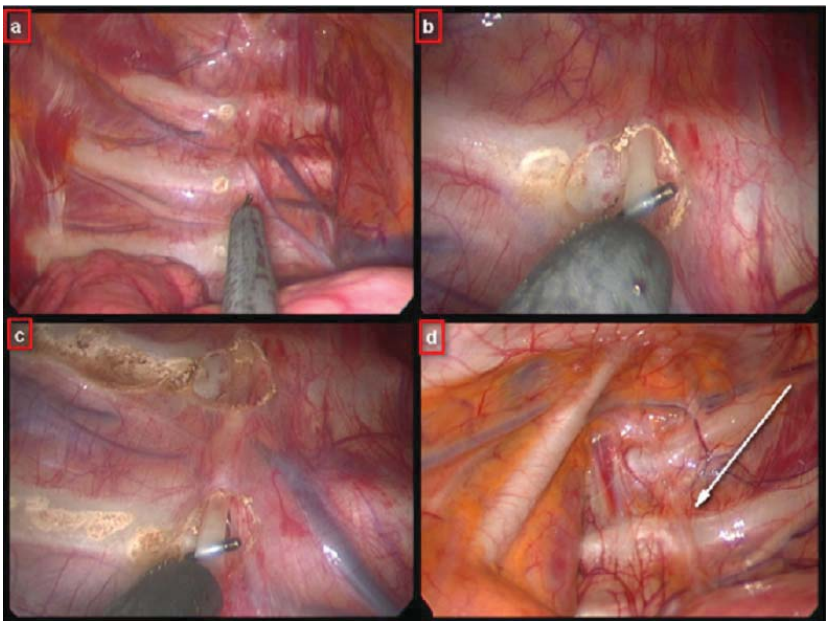

Fig. 2 – Intrapleural video assisted display of the sympathetic chain.
a) The ribs 2, 3 and 4 marked; b) The sympathetic chain at the level of the second rib, lifted by the instrument; c) The sympathetic chain at the level of the second rib cut off, whereas it is lifted by the instrument at the level of the third rib; d) Intrapleural figure of the sympathetic chain to the left (white arrow).

The 24 Fr lumen chest drain was inserted through the same incision, and connected to the water container system with the underwater drain. The same procedure was repeated on the other side insofar as the patient remained in the same position on the operating table.

The duration of the surgery, the time spent in hospital, complications and recidives were recorded. The results were evaluated during medical controls a year after the operation, based on received answers to questions on symptoms. The answers were graded in the ten degree visual analogue scale (VAS) where the score 0 was the best, and it implied the absence of symptoms, whereas the score 10 was the worst implying the same or the worse intensity of problems than the intensity found preoperatively. The statistics was erform using the nonparametric test (Wilcoxon). The *p*-value smaller than 0.05 was considered statistically significant.

Results

A total of 47 consecutive patients, 18 men and 29 women, average age 29 years (18–48 years), underwent bilateral transaxillary thoracoscopic sympathectomy at the Military Medical Academy (MMA), Clinic for Thoracic Surgery, between September 2009 and November 2010. Out of the total number, 68% of the patients were on beta-blockers therapy, sedatives or psychotherapy due to intensive hyperhidrosis symptoms, but without any significant subjective improvement. The sympathectomy was indicated in cases of facial blushing and sweating in 3 (6.38%) patients, palmary sweating in 16 (34.04%) patients, axillary sweating in 7 (14.89%) patients or both palmary and axillary sweating in 21 (44.68%) patients. The average duration of the bilateral surgical procedure was 45 minutes. After the surgery, the palms of all the operated patients were warm and dry. Pleural adhesions in one patient were so striking that the operation took 90 minutes, but the conversion into the open surgery was not necessary in any patient. Pleural drains were removed 4 hours after the surgery on average. The sum of 98.6% (44/47) of the patients left the hospital the following day.

Complications observed postoperatively were recorded in 3 patients, in two (0.94%) cases partial pneumothorax after the removal of chest drains, and in one (0.47%) case unilateral transitory Horner's syndrome. One of the patients suffering from partial pneumotorax was released two days after the surgery as the result of exuflation was satisfactory. His control X-ray after two days indicates a complete reexpansion of the lungs, whereas the other patient was drained and remained in hospital for five days in total due to persisting problems. A complete remission was accomplished after 16 weeks in the patient with signs of Horner's syndrome. Severe complications were not noted.

All the patients (100%) were monitored after a year. Regular healing of wounds was ascertained along with excellent aestetic results. The shoulder area was symmetrical and functional in all the patients without any residual pain syndrome. All the 3 patients operated on for the reasons of facial blushing and sweating were openly satisfied with the effectiveness of the surgery, but intensified sweating of feet was noted.

The reduction in or absence of palmar sweating was noted in all the patients, (100%), who underwent surgery due to hyperhidrosis. The reduction in axillary sweating was found in 18/28 (64.28%) patients. Compensatory sweating of the trunk and lower limbs was stated in 32/44 (72.27%) patients. The improvement of life style and the absence of social phoebia was recorded in 45/47 (95.6%) patients whereas 2 (4.4%) patients were not satisfied with the effectiveness of the procedure due to excessive compensatory sweating in the lower part of the body after the 12 month's monitoring. All the patients returned to work after a medium period of sick leave of 7 days (ranging from 1 to 15 days). Peroral use of analgetics up to 7 days following the surgical procedure was required in 90% of the patients (Tables 1 and 2).

Table 1

Symptoms overview in the patients operated on due to excessive facial blushing; prior to the operation and after a 12-month monitoring on the basis of the visual analogue scale (0 means the absence of symptoms and 10 means the worst possible symptom).

Patients with excessive facial blushing	Prior to the surgery	Monitoring after 12 months	*p*
Facial blushing	9.0 ± 1.6	3.0 ± 2.1	< 0.0005
Cardiac palpitations	4.7 ± 1.6	1.3 ± 0.9	< 0.008
Anxiety	4.3 ± 2.5	2.3 ± 1.1	< 0.004
Compensatory body sweating	3.0 ± 0.5	4.5 ± 1.1	= 0.3
Compensatory feet sweating	2.7 ± 0.7	6.0 ± 1.3	< 0.004

Note: results are given as $\bar{x}$ ± SD.

Table 2

Symptoms overview in the patients operated on due to excessive sweating of the palms and axilae, prior to the operation and after monitoring on the basis of the visual analogue scale (0 means the absence of symptoms and 10 means the worst possible symptom).

Hyperhidrosis (axilla, palms)	Prior to the surgery	Monitoring after 12 months	*p*
Palmar sweating	8.1 ± 2.5	0.3 ± 0.8	< 0.0001
Axillary sweating	5.4 ± 2.8	3.4 ± 1.4	< 0.02
Compensatory body sweating	3.6 ± 1.1	5.3 ± 2.2	< 0.004
Compensatory feet sweating	3.8 ± 1.5	6.0 ± 2.5	< 0.008

Note: results are given as $\bar{x}$ ± SD.

Discussion

Thoracoscopic sympathectomy is an optimal procedure for surgical treatment of excessive facial, palmar and axillary blushing and sweating. Unlike many non-surgical treatments where results are slow and insufficient, the effect of this procedure is felt by patients immediately after waking up from anaesthesia.

Hyperchidrosis is a chronic disease that can have a significant negative impact on a patient's quality of life. Primarily involving the axillae, palms, soles, and face, patients who have primary focal hyperhidrosis seek medical advice often long after they have been living with functional and psychosocial disability for some time. For every affected area of hyperhidrosis and at every stage of disease severity, conservative measures should be exhausted before progressing to surgical options. Generally, the most conservative op-

tions with the fewest adverse effects are attempted first. These include iontophoresis. A minimally invasive procedure, intradermal botox (BTX) injections have been shown to improve the disease significantly in these patients [5]. Serving as an intermediate between conservative therapy and invasive surgery, BTX has revolutionized the treatment of focal hyperhidrosis. Oral medications such as glycopyrrolate can be tried alone at any point in treatment, or as a useful adjunct, but side effects are common and caution should be taken with highrisk patients. For all the forms of hyperhidrosis, the desired outcome for treatment should be qualitative and quantitative and it is critical for physicians to assess the patient's improvement periodically and adjust therapy, based on these theraupetic landmarks.

Thoracoscopic sympathectomy for the excessive facial blushing and sweating was described for the first time in 1985 [11]. Still, the first comprehensive study on excessive facial blushing was published by the Swedish 'Boras Group' in 1998 [12, 13]. Several papers addressing isolated treatment of excessive facial blushing and sweating by means of thoracoscopic sympathectomy [14–22] have been published since then, but the number of papers on this subject is far smaller than the number of those dealing with the primary focal hyperhidrosis (axillae, palms), and because a small number of surgeons perform thoracoscopic sympathectomy in patients with the isolated problem of excessive facial blushing and sweating [23].

Different surgical techniques are applied depending on the following: the number of incisions in the skin, usually two; the point where the ports are inserted; the manner of accessing the sympathetic chain; whether the chain is transected or the ganglia are electrocoagulated and disconnected at the head of the rib or metal clips are inserted [10].

This paper contains a series of 47 patients who underwent video-assisted thoracoscopic bilateral sympathectomy in a manner where ports were inserted through a single incision in the skin in the anterior axillary line at the level of the third inter-rib space posteriorly from the course of fibres of the large thoracic muscle. The lumen of the ports is 5 mm, one port serving for the insertion of the camera, the other for distributing instruments. This technique was described for the first time by Lardinois and Ris [24] ten years ago who used the paedriatic cysto-resectoscope to transect the sympathetic chain. Benefits of this mechanism for performing the procedure are primarely reflected in a good aesthetic effect as the incision is small, and transaxillary localized. They are also reflected in the fact that the pain is lesser. In addition, the sympathetic chain can be clearly seen from the point of the first rib. The flexible 5 mm ports inserted through the same incision do not compromise one another, and the tissue is not significantly traumatized. Clinical check-up after 3 months from surgery showed regular healing of wounds and excellent aesthetic and functional results in all the patients. The complications found in 3 (1.4%) of the patients were smaller than normally [25] and the conversion into open surgery was not necessary. Partial pneumothorax after endoscopic or open chest surgery is a known complication, and it is not specific when this intervention is concerned. Horner's syndrome found in one (0.47%) patient is specific for this procedure, the incidence of which is more rare than the one normally found in the literature (2.7%) [26]. Symptomatology withdrew completely in patients after 16 weeks. The complication is most likely the result of the thermic injury of the stellate ganglion during identification of the sympathetic chain between the first rib and the second rib. In order to achieve a maximum reduction of this incidence, i.e. complication, it is necessary to identify precisely the first rib [27].

Optimal understanding of denervation of the sympathetic chain due to excessive sweating is still a subject of a number of controversies, primarely due to a significant anatomic variability of the sympathetic chain. Some authors advocate for a limited sympathetic chain (T2–T3) transection in hyperhidrosis palmaris and additional transection T4 in axillary hyperhidrosis [28]. When facial blushing and sweating is concerned, Yilmaz et al. [18] recommend, as an additional step, excision of the lower third of the stellate ganglion. Most authors perform transection of the chain from T2 to T4 for optimal sympathetic denervation.

Severe side effects, particularly compensatory sweating (in this paper compensatory sweating of the trunk and lower limbs was stated in 32/44 (72.27%) of the patients, develop after multiple ganglionectomy. Some of these patients might be managed with medication. If that fails, then reversal is the only remaining option [29]. If an extensive resection of the chain is performed, then a difficult nerve interposition is the only option.

In this paper we used the technique to perform transection of the sympathetic chain at a certain level depending on predominant symptoms and problems, and after precise anatomic identification of the first rib, and the separation of the sympathicus in layers thereafter. In cases of excessive facial blushing, the transection was performed inferior to the first rib T1, in the isolated excessive palmar sweating inferior to the second and the third ribs T2 and T3, in excessive axillary sweating under T4, and from the second through the fifth rib T2–T5, a complete transection was performed in cases of joint medical problems.

After a 12-month monitoring, the recurrence of symptoms following the initial regression was noted in 3 (6.38%) of the patients which is in conformity with the standards referred to in the world books where the recidive rate ranges from 4% to 8% [17, 29].

Conclusion

A single incision transaxillary thoracoscopic sympathectomy offers great aesthetic and functional results. Potential sequellae and painful sensations on the chest wall, which can be a consequence of a surgical technique with 2 to 3 incisions for inserting ports, are avoided by means of this procedure. Sympathetic chain branching and its transection depending on discomfort generate the best results in patients suffering from hyperhidrosis palmaris.

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Lung tumors: early and delayed ratio of ^{99m}Tc-methoxy-2-isobutylisonitrile accumulation

Tumori pluća: rana i odložena akumulacija ^{99m}Tc-metoksi-2-izobutilizonitrila

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Abstract

Background/Aim. Currently used radiopharmaceuticals are nonspecific and most of them are accumulated by benign tumors as well as inflammatory lesions, abscess or granulomatous lesions. Some factors such as the choice of radiopharmaceutical applied, histopathologic type of tumor, its size, location or previous tumor treatment could influence tumor imaging sensitivity. The aim of this study was to investigate accumulation of ^{99m}Tc-methoxy-2-isobutylisonitrile (^{99m}Tc-MIBI) by counting early/delayed uptake and release of this radiopharmaceutical inside lung tumors and evaluating possible factors which could be involved in its accumulation. **Methods.** Two-phase ^{99m}Tc-methoxy-2-isobutylisonitrile single photon emission computed tomography scan (early and delayed scan) was performed in 60 patients with lung tumors (the group 1 – 30 benign, and the group 2 – 30 malignant tumors). We calculated the uptake ratio on early (early ratio – ER), delayed images (delayed ratio – DR) and retention index (RI). Individual influence of etiology, diameter, localization, and histological type on uptake/release values was evaluated with regression analysis. **Results.** The values of ER and DR were significantly different in both groups ($p < 0.01$), showing lower values in benign *vs* malignant lung tumors (ER 1.36 ± 0.094 and DR 1.25 ± 0.089 *vs* ER = 1.93 ± 0.106 and DR = 1.7 ± 0.095 respectively). Tumor size showed a significant influence on the change of ER and DR values ($p < 0.01$), with greater uptake in tumors > 3 cm. RI values showed no significance between the two groups ($p > 0.05$). **Conclusion.** The uptake ratio of ^{99m}Tc-methoxy-2-isobutylisonitrile could be a useful index in differentiating lung tumors, while RI has no influence on this. Among the evaluated factors, ER and DR values are significantly influenced only by the diameter of lung tumor, while localization or different histological types between the groups has no influence on this.

Key words:
radiopharmaceuticals; tomography, emission-computed; lung neoplasms; diagnosis, differential; pathology; sensitivity and specificity.

Apstrakt

Uvod/Cilj. Novi radiofarmaceutici su nespecifični i većina njih se akumuliše u benignim tumorima, ali i u zapaljenskim lezijama, apscesima ili granulomatoznim lezijama. Na senzitivnost snimaka tumora utiču razni faktori kao što su izbor radiofarmaceutika, patohistološki tip tumora, veličina, lokalizacija ili način lečenja. Cilj ove studije bio je da se proceni nakupljanje ^{99m}Tc-metoksi-2-izobutilizonitrila (^{99m}Tc-MIBI) unutar tumora pluća određivanjem rane/odložene akumulacije, odnosno eliminacije ^{99m}Tc-MIBI, kao i da se utvrde mogući faktori koji bi mogli uticati na akumulaciju ovog radiofarmaceutika. **Metode.** Dvofazna ^{99m}Tc-metoksi-2-izobutilizonitril jednofotonska emisiona kompjuterizovana tomografija (rana i kasna) urađena je kod 60 bolesnika sa tumorom pluća (grupa 1 – 30 benignih i grupa 2 – 30 malignih tumora). Izračunavali smo odnos nakupljanja na ranim (*early ratio* – ER) i odloženim snimcima (*delayed ratio* – DR), kao i indeks retencije (*retention index* – RI). Regresiona analiza korišćena je u cilju utvrđivanja pojedinačnog uticaja etiologije, veličine, lokalizacije i patohistološkog tipa tumora na vrednosti akumulacije/eliminacije radiofarmaka. **Rezultati.** Vrednosti ER i DR bile su statistički značajno različite u obe ispitivane grupe ($p < 0,01$), sa manjim izmerenim vrednostima kod benignih u poređenju sa malignim tumorima (ER 1,36 ± 0,094 i DR 1,25 ± 0,089 *vs* ER = 1,93 ± 0,106 i DR = 1,7 ± 0,095, respektivno). Utvrđeno je da veličina tumora pluća značajno utiče na promene vrednosti ER i DR ($p < 0,01$), sa intenzivnijom akumulacijom unutar tumora dimenzija preko 3 cm. **Zaključak.** Rana i odložena akumulacija ^{99m}Tc-metoksi-2-izobutilizonitrila u tumorima pluća je koristan parametar za njihovu diferencijaciju, dok vrednost indeksa retencije nema uticaja na diferenciranje benignih i malignih tumora pluća. Veličina tumora pluća statistički značajno utiče na vrednosti ER i DR. Ostali ispitivani faktori, kao što su lokalizacija ili patohistološki tip unutar grupe 1 ili grupe 2, ne utiču na vrednosti ER i DR.

Ključne reči:
radiofarmaci; tomografija, kompjuterizovana, emisiona; pluća, neoplazme; dijagnoza, diferencijalna; patologija; osetljivost i specifičnost.

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Introduction

Tumor imaging with various radiopharmaceuticals has been a focal point for nuclear medicine researchers. Currently used radiopharmaceuticals are nonspecific and most of them are also accumulated by benign tumors and infectious lesions, such as inflammatory lesions, abscess or granulomatous lesions. Some factors such as the choice of radiopharmaceutical applied, histopathologic type of tumor, its size, location or previous tumor treatment could influence sensitivity of tumor imaging.

Commonly used radiopharmaceuticals in lung cancer imaging are ^{201}Tl-chloride (talium-201 chloride) and ^{99m}Tc-MIBI (technetium-99m labelled hexakis-2-methoxyisobutylisonitrile) [1–3]. New studies introduce simultaneous double-tracer single photon emission tomography (SPECT) with 99m-Tc-technetril and 67-Ga-citrate for follow-up of patients with non-small cell lung cancer [4]. Studies *in vitro* in cultured tumor cells found out that the uptake of ^{201}Tl is almost 3-fold greater than ^{99m} Tc-MIBI, while the cellular release or the washout rate is almost identical for both radiopharmaceuticals [5]. Kinetics of some radiopharmaceuticals can be changed by adding certain drugs such as actinomycin D resulting in increased cellular release of ^{201}Tl while it has no change in washout rate of ^{99m}Tc-MIBI [6]. Slower washout of ^{201}Tl in high growth cells enables this tracer to act as an indicator of tumor malignancy. Some papers have reported the benefit of ^{201}Tl-chloride in detecting tumor malignancies which is determined by the grade of washout rate to normal tissue especially in lung cancer lesions and mediastinal lymph node metastases [6]. Among these two radiopharmaceuticals, ^{99m}Tc-MIBI has been emphasized because of its superior physical characteristics such as shorter half-life, better dosimetry, and optimal photon energy peak. Moreover, it is continuously available for use, permits freedom in scheduling patients and the injected concentration is higher.

^{99m}Tc-MIBI which is used in everyday practice for myocardial perfusion imaging, also gains its roll in evaluating various malignant tumors such as breast carcinoma, thyroid cancer, central nervous system malignancies and lung cancer [7–9]. Recently, many papers have reported accumulation of this radiopharmaceutical within tumor lesion considering its role in differentiating benign from malignant lesions [10–12] but only few have evaluated factors related to kinetics, uptake and washout of radiopharmaceuticals [13–15]. One of the earliest studies considering the uptake and kinetics of ^{99m}Tc-MIBI in malignant lung lesions was reported by Hassan et al [13].

This study is designed to investigate kinetics of ^{99m}Tc-MIBI by investigating its early and delayed uptake, release of ^{99m}Tc-MIBI in benign and malignant lung lesions and evaluating possible factors which could be involved in the kinetics of this radiopharmaceutical.

Methods

A total of 60 patients with lung lesions (LLs) was evaluated from 2006 to 2009 (45 males and 15 females, age 37–76 years, mean age ± SD: 56.70 ± 9.527 years). All the patients were divided into 2 groups: the group 1 included 30 patients with benign LLs, and the group 2 included 30 patients with malignant solitary pulmonary nodule (SPN). This study included only the patients who had been evaluated by chest computed tomography (CT) (reporting diameter and localization of LLs) prior to ^{99m}Tc-MIBI scanning and with the final diagnosis. The diagnosis was made by pathohistology findings after surgery (49/60 or 81.7%), cytological findings of sputum or by positive TBC culture (4/60 or 6.7%) or by clinical course of the disease (7/60 or 11.6%).

Two-phase (early and delayed) ^{99m}Tc-MIBI SPECT imaging was performed prior to definitive diagnosis. Early ^{99m}Tc-MIBI imaging was performed 10 minutes after the intravenous injection of 740 MBq ^{99m}Tc-MIBI with dual-headed gamma-camera equipped with low-energy, high resolution collimator. Delayed imaging was done 60–120 minutes after the intravenous injection of the radiopharmaceutical. ^{99m}Tc-MIBI was prepared according to the instructions of the manufacturer. The images were acquired every 20 seconds, at the angle of 3º, in a circular orbit of 180º per detector array, and stored in 64 × 64 matrix. The equipment was calibrated for a photopeak of 140 keV with a symmetric 20% window. The images were reconstructed in the coronal, transversal and sagital sections and both early and delayed images were evaluated qualitatively considering positive findings if there was an increased accumulation of the radiopharmaceutical in the lung area corresponding to the location of the LLs. Quantification of the images included evaluation of the uptake ratio on early (ER = early ratio) and delayed images (DR = delayed ratio) calculated on transverse slices placing region of interest (ROI) around abnormal uptake of ^{99m}Tc-MIBI (T = tumor) and in an area of contralateral normal (N = normal) lung tissue (ER or DR = T/N). The ROIs of the early images were copied and set on LLs on delayed images. For semiquantitative evaluation of the degree of retention in LLs the retention index (RI) was calculated as:

$$RI = [(\text{delayed ratio} - \text{early ratio}) / \text{early ratio}] \times 100.$$

To test the differences between ER, DR and RI in benign (group 1) and malignant (group 2) LLs, Student's *t*-test was used. The results were considered significant when the *p* value was less than 0.05. Mutual and individual influence of diameter, localization, and histological type of LLs on uptake values (ER, DR, RI) and on ^{99m}Tc-MIBI accumulation was analyzed with regression analysis.

Results

Visual evaluation of ^{99m}Tc-MIBI accumulation included positive and negative findings assessment on early and delayed images in both groups of patients. In the group 1, 23 (76.7 %) of the patients were negative on both early and delayed images: 8 inflammatory pseudonodules, 7 cases of tuberculosis, 6 hamarthoma, 1 primary neuroendocrine cyst and 1 fibrotic nodule. The majority of false positive results in the group 1 (7/30 or 23.3%) occurred in inflammatory pseudonodule and only one was the case of botryomycosis (Figure 1).

Most of lung lesions from the group 2 were positive on both early and delayed images (27/30 or 90%): 9 adenocarcinoma, 13 squamous-cell carcinoma, 3 large-cell carcinoma and 2 small-cell carcinoma (Figure 2).

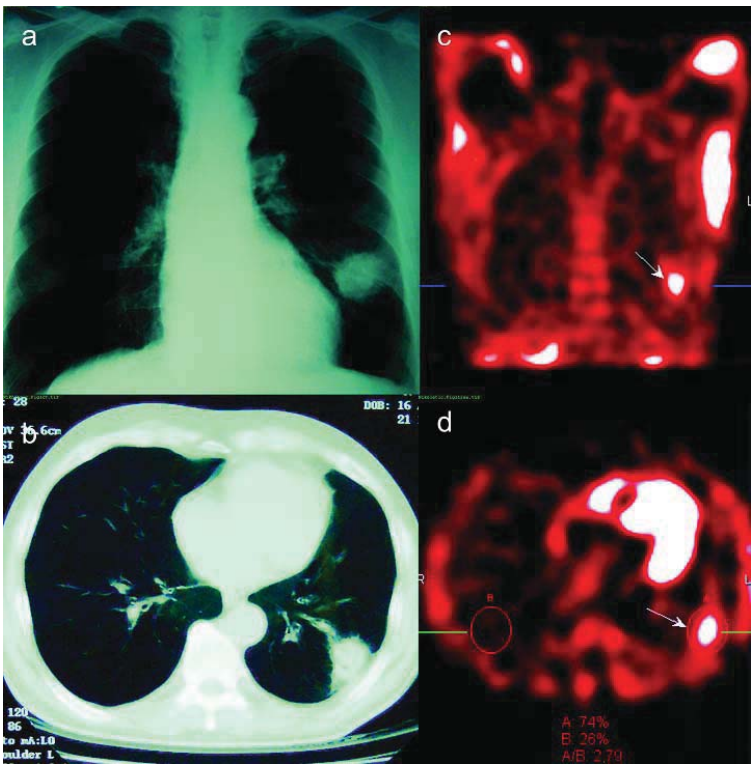

Fig. 1 – A 70-year-old patient with a lung lesion located in the left lower lobe identified on X-ray and computed tomography (CT) scan (a, b). ^{99m}Tc-metoxy-2-isobutylisonitrile single photon emission computed tomography (^{99m}Tc-MIBI SPECT) scan in the same patient shows intense accumulation of ^{99m}Tc-MIBI in the lung region corresponding to the location of the nodule (c, d); the tumor/nodule (T/N) ratio was 2,79 on early scan. Percutaneous transthoracic fine needle aspiration cytology identified lesion as chronic pneumonia – a false positive result.

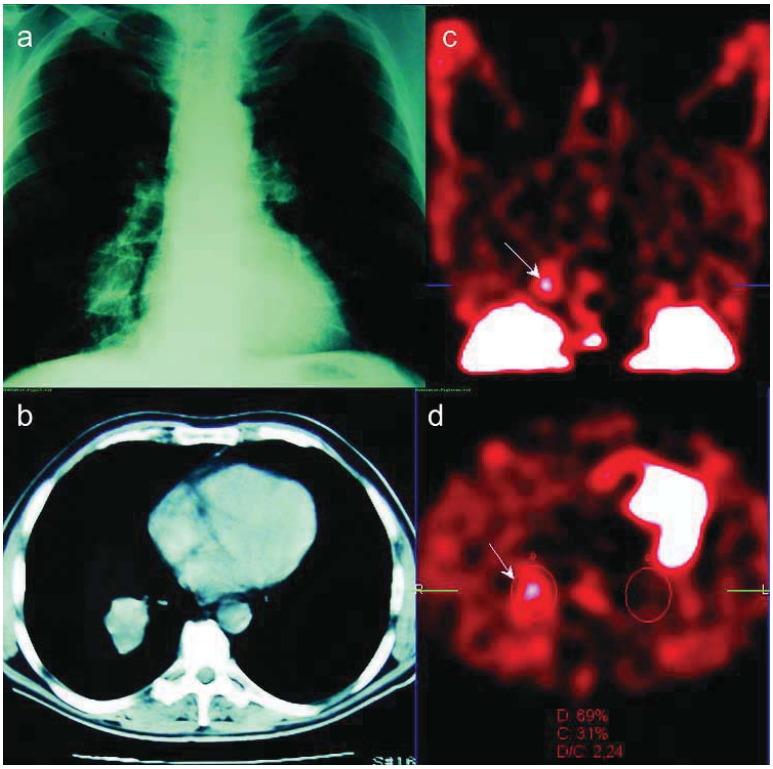

Fig. 2 – A 53-year-old patient with a lung lesion located in the right lung identified on radiography and computed tomography (CT) scan (a, b). ^{99m}Tc-metoxy-2-isobutylisonitrile single photon emission computed tomography (^{99m}Tc-MIBI SPECT) scan in the same patient shows high accumulation of ^{99m}Tc-MIBI in the lung region corresponding to the location of the nodule (c, d); the tumor/nodule (T/N) ratio was 2.24 on early scan. After lobectomy, the lesion was identified as squamous-cell carcinoma – a true positive result.

Only 3 lesions from this group did not accumulate the radiopharmaceutical (false negative): 1 adenocarcinoma (Figure 3), 1 undifferentiated squamous-cell carcinoma and 1 small-cell carcinoma.

The difference in ER values between groups was statistically significant ($t = 3.982$, $p < 0.05$).

Also, the difference in DR values between groups was statistically significant ($t = 3.448$, $p < 0.05$).

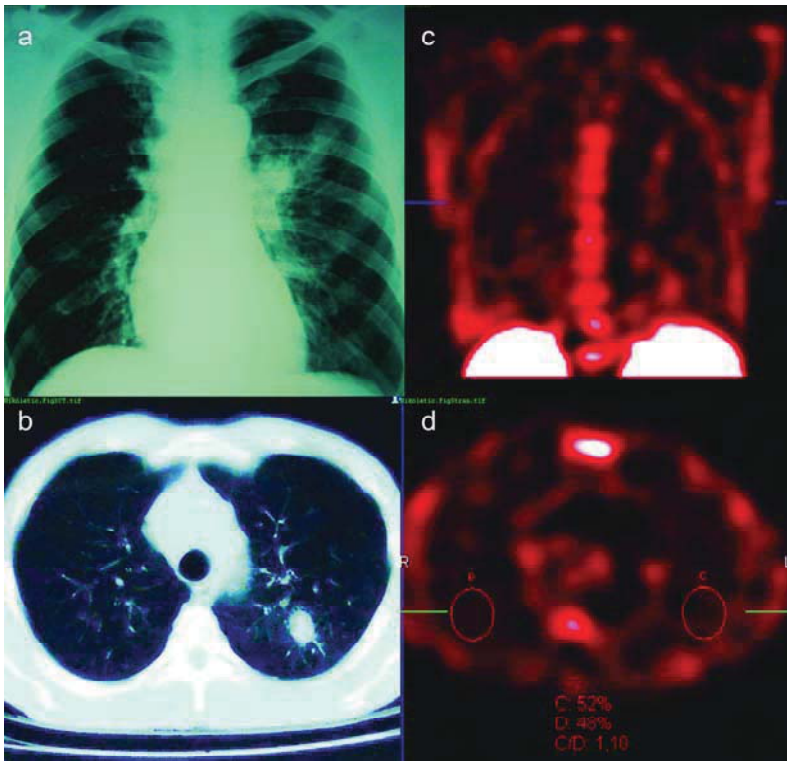

Fig. 3 – A 51-year-old patient with a lung lesion located in the left upper lobe identified on radiography and computed tomography (CT) scan (a, b). ^{99m}Tc-metoxy-2-isobutylisonitrile single photon emission computed tomography (^{99m}Tc-MIBI SPECT) scan in the same patient shows no accumulation of ^{99m}Tc-MIBI in the lung region corresponding to the location of the nodule (c, d); the tumor/nodule (T/N) ratio was 1.10 on early scan. Percutaneous transthoracic fine needle aspiration cytology and sputum cytology identified lesion as adenocarcinoma – a false negative result.

In both groups, the values of ER, DR and RI were evaluated. The mean ER and DR values ± SD are shown in Figures 4 and 5.

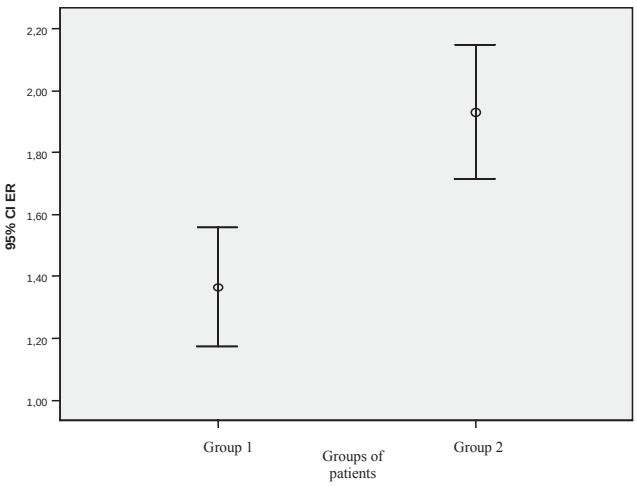

Fig. 4 – Early ratio (ER) values in the patients with benign (the group 1) and malignant (the group 2) lung lesions.
95% CI – 95% confidence interval.

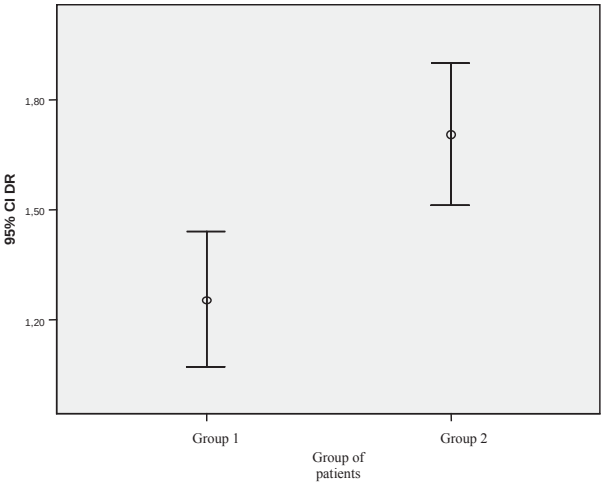

Fig. 5 – Delayed ratio (DR) values in the patients with benign (the group 1) and malignant (the group 2) lung lesion.
95% CI – 95% confidence interval.

There was no significant difference between the RI values in benign and malignant SPN (-7.512 ± 10.44 and -11.394 ± 5.945, respectively) (Figure 6).

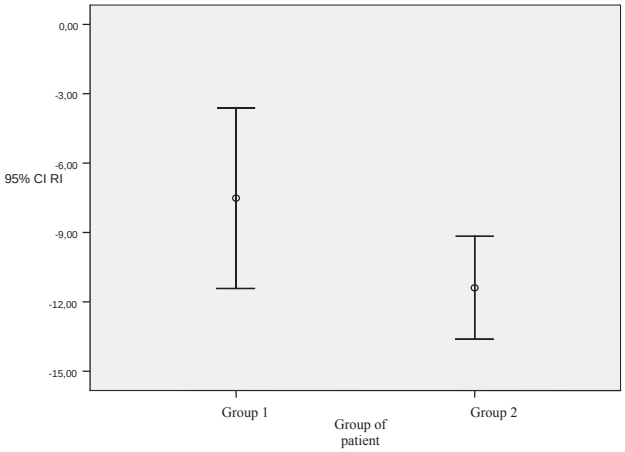

Fig. 6 – Retention index (RI) values in the patients with benign (the group 1) and malignant (the group 2) lung lesion.

95% CI – 95% confidence interval.

Among the false positive results in the group 1 (7 patients with a benign lung lesions), 5 cases of inflammatory pseudonodule showed washout of the tracer on delayed images. No washout of the tracer was noticed in 2 cases of benign lung lesions (1 botryomycosis and 1 inflammatory pseudonodule) with high values of RI (24.6 and 10.8, respectively).

All the true positive malignant lung lesions (27/30) showed washout of the tracer on delayed images. A highest difference between early and delayed ratio was noticed among adenocarcinomas and smallest difference among small-cell carcinomas. Two cases of adenocarcinoma had the highest values of ER in the group 2 (ER = 2.9 and ER = 3.0), but there was no significant difference in the ER values among the different histopathological types in the group ($p < 0.05$).

In the group 1, according to histopathological type, false positive findings were registered in 7 cases of benign LLs (6 pneumonia and 1 botryomycosis) with high values of ER = 2.2 ± 0.4 and DR = 2.0 ± 0.5. Among the two different histopathological types of benign lung lesions leading to false positive findings, there was no significant difference in ER, DR and RI values. In the group 2, the lowest ER and DR values were found in large-cell carcinoma (1.7 ± 0.2 and 1.5 ± 0.2, respectively) followed by small-cell and squamous-cell carcinoma with almost identical results (Table 1).

Adenocarcinoma had the highest ER and DR values of all histopathological types (2.2 ± 0.7 and 1.9 ± 0.7, respectively). Among the 4 different histopathological types of lung carcinoma, there was no significant difference in ER, DR and RI values.

Considering the size, malignant lung lesions were divided into 3 groups (small: 0–1.9 cm; medium: 2.0–3.9 cm and large lesions: over 4 cm). The results showed that the values of both early and delayed ratio increased with the larger size (ER: small 1.5; medium: 1.9; large: 2.1 and DR: small 1.3; medium 1.7, and large 1.8). There was a significant difference in ER and DR values between smallest and medium or large lung lesions ($p < 0.05$). There was no significant difference in retention index values compared to size of lung lesion (Table 2).

Table 1

Values of early (ER), delayed ratio (DR) and retention index (RI) according to histopathology (HP) type of lung lesion

HP type	mean ER ± SD	mean DR ± SD	mean RI ± SD
Adenocarcinoma	2.2 ± 0.7	1.9 ± 0.7	-10.1 ± 4.7
Squamous-cell carcinoma	1.8 ± 0.5	1.6 ± 0.4	-12.1 ± 6.8
Large-cell carcinoma	1.7 ± 0.2	1.5 ± 0.2	-12.9 ± 3.9
Small-cell carcinoma	1.8 ± 0.6	1.6 ± 0.4	-10.8 ± 4.7
Benign lung lesions (false positive)	2.2 ± 0.4	2.0 ± 0.5	-8.5 ± 21.3

Table 2

Values of early (ER), delayed ratio (DR) and retention index (RI) according to the size of malignant lung lesion

Size (mm)	mean ER ± SD	mean DR ± SD	mean RI ± SD
0–19	1.5 ± 0.3	1.3 ± 0.3	-12.1 ± 4.6
20–39	1.9 ± 0.6	1.7 ± 0.5	-9.6 ± 5.6
> 40	2.1 ± 0.6	1.8 ± 0.5	-13.9 ± 6.7

The values of ER, DR and RI were analyzed for lung lesions in both groups considering its localization in the right or the left lung and its lobes. In the group 1, 17 lesions were found in the left (10 were located in the lower lobe, 7 in the upper lobe) and 13 in the right lung (7 in the lower lobe, 5 in the upper lobe and 1 in the middle lobe). Among the lung lesions in the group 2, 16 were located in the right (6 in the upper, 5 in the middle and 5 in the lower lobe), and 14 in the left lung (8 in the upper and 6 in the lower lobe). No significant difference was found in ER, DR and RI values between the group 1 and the group 2 considering localization of lung lesions.

Regression analysis was used to evaluate how size, localization, histopathological report, ER and DR values influence ^{99m}Tc-MIBI accumulation (dependent variable – ^{99m}Tc-MIBI accumulation). There was a significant influence of mutual factor such as size, localization, histopathological report, ER and DR values on ^{99m}Tc-MIBI accumulation ($p <$

0.05). ^{99m}Tc-MIBI accumulation findings were explained with 75.4% ($R^2 = 0.754$) change of LLs size, localization, histopathological report, ER and DR values meaning that 75.4% of all ^{99m}Tc-MIBI findings were dependent on these factors.

The values of ER and DR were evaluated by mutual influence of the factors such as size, localization and histopathological report of lung lesions showing a significant influence of these factors on ER and DR values ($p < 0.05$). ER value was explained with 29.5% ($R^2 = 0.295$) change of these factors meaning that 29.5% ER values were dependant on these factors. At the same time, values of ER and DR were evaluated by individual influence of above factors, and the results showed that the size of lung lesions and histopathological report had significant influence on ER and DR values ($p < 0.05$; $R^2 = 0.185$ and $R^2 = 0.137$, respectively), while localization had no influence on ER or DR values (Figure 7).

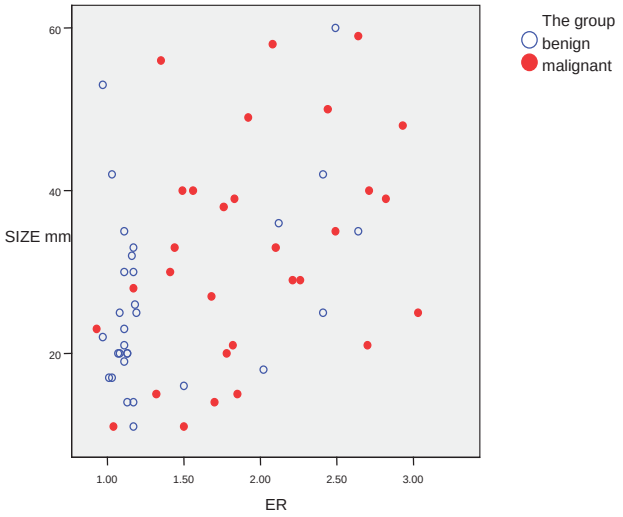

Fig. 7 – Early ratio (ER) values and size of lung lesion in the patients with benign (the group 1) and malignant (the group 2) lung lesion.

Discussion

The uptake mechanism of ^{99m}Tc-MIBI by tumor cells is not yet known but there are some possible factors influencing the uptake such as the amount of mitochondria in the cell, cell membrane potential, increased tumor blood flow and capillary permeability.[16] It is known that accumulation of ^{99m} Tc-MIBI in the tumor cell is reversely proportional to the level of P-glycoprotein (Pgp), the protein responsible for the transport of many chemotherapeutic drugs, thus increased Pgp level in the tumor is related to the resistance of malignant tumor to chemiotherapy [17]. Increased levels of Pgp were found in tumor biopsies from relapsing cancer patients. Accumulation of ^{99m} Tc-MIBI in cells is inversely proportional to the level of Pgp. Functional imaging of tumors with ^{99m} Tc-MIBI may provide important information about the Pgp status of tumors [18].

Our study on 60 patients with lung lesions resulted in a significant difference between ER and DR values of ^{99m} Tc-MIBI accumulation in benign and malignant lesions showing increased values in carcinomas which could be explained with differences in mitochondrial cell amount between malignant and healthy cells, as it was reported that passive ^{99m} Tc-MIBI uptake is dependent on negative potential of the cytoplasmatic and mitochondrial membrane of neoplastic cells, showing increased accumulation in cells with higher number of mitochondria [19].

We found no significant difference in retention index values between the group 1 and the group 2. Therefore, the result suggests that the ^{99m} Tc-MIBI uptake ratio is more useful as a parameter for either benign or malignant lung lesion than values of RI. Our results are confirmed with previously reported *in vitro* studies with Hela cells where washout rate of ^{99m} Tc-MIBI from cultured cells was not related to their malignant potential [5]. Nishiyama et al. [20] agree with the conclusion that there is no significant tumor washout of ^{99m} Tc-MIBI from the tumor mass according to RI values. Concurrently, some papers report no significant difference in ER and DR values or RI of ^{99m} Tc-MIBI between benign and malignant lung lesions [21].

In our study, ER and DR values were greater in malignant than in benign lesions of the lung (1.4-fold greater and 1.36-fold greater, respectively) which is the case in numerous studies [13, 22, 23]. Hassan et al. [13] investigated accumulation of ^{99m} Tc-MIBI in 19 patients on planar images, and reported even higher DR values: 1.58-fold greater in tumor tissue than in normal lung tissue at 30 minutes. Compared to these results, our lower DR values could be explained with the methodology of our study, calculating DR on images after 60 to 120 minutes.

Our study came up with the result that the size of lung lesion significantly affects ER and DR values tending to increase values of ER and DR in larger lesions which is in concordance with the results of other papers [20, 22]. Nishiyama et al. [20] investigated 45 patients with malignant lung lesions divided by size (≤ 3 cm, ≤ 6 and ≥ 6 cm) and reported higher ER and DR values of ^{99m} Tc-MIBI (ranged from 2.1–3.3 and 1.9–3.0, respectively) than in our study but with the same growing tendency as the size of lung lesion increases. Minai et al. [11] reported a correlation of quantitative uptake of ^{99m}Tc-MIBI with the diameter of the nodule with a correlation coefficient of 0.61 ($p = 0.02$). However, several studies reported no influence of sex, age, size of tumor and histological type on ^{99m}Tc-MIBI accumulation results [22–24].

In benign group of patients (the group 1) false positive results occurred in 7 out of 30 benign lesions, 6 in inflammatory pseudonodule and 1 in botryomycosis. It is well-known that chronic inflammation and active pulmonary tuberculosis could lead to high ^{99m}Tc-MIBI uptake due to tissue factors such as high degree of tissue vascularization and capillary permeability. Alterations in cell metabolism that affect membrane potential, as might be the case in inflammatory lung lesions, could influence accumulation of ^{99m}Tc-MIBI. Furthermore, a rich mitochondrial content of epitheloid cells in granulomas might be a key point for ^{99m}Tc-MIBI uptake in tuberculosis [25]. Onsel et al. [26] investigated ^{99m}Tc-MIBI accumulation in extensive pulmonary dis-

ease with bilateral infiltrates and gained ^{99m}Tc-MIBI scan positive results in 92% cases. These results show that chronic inflammatory diseases, inflammatory pseudonodules and active tuberculosis limit the value of ^{99m}Tc-MIBI in differentiation benign from malignant lung lesions and have similar limitations as are known for ^{18}FDG-PET/CT [27].

In our series of malignant lung lesions, there was no significant difference in ER, DR and RI values among four different histopathological types. Of the 3 malignant lung lesions with ER values less than 1.20, one was adenocarcinoma (ER = 1.04), one was squamous-cell carcinoma (ER = 0.93) and one small-cell carcinoma (ER 1.17). Nishiyama et al. [20] report that squamous-cell carcinoma has lower ER and DR values compared to adenocarcinoma (concordant to our results) and small-cell carcinoma (discrepant to our results). Hassan et al. [13] found that adenocarcinoma and small-cell carcinoma have higher T/N values than squamous-cell carcinoma, but the results of our study show equal accumulation of ^{99m}Tc-MIBI in both squamous-cell and small-cell carcinoma. These authors also highlight that undifferentiated squamous-cell carcinoma can show no accumulation of ^{99m}Tc-MIBI, a finding that was confirmed in our study by the one case of this pathohistological cancer type which was negative on ^{99m}Tc-MIBI scan. In our study the finding that small-cell carcinoma has similar ER values as squamous-cell is in discrepancy with the Sahin et al. [28] results showing lower uptake of ^{99m}Tc-MIBI in squamous-cell than small-cell carcinoma (1.22 ± 0.14 and 1.39 ± 0.1, respectively) with a significant difference in ER and DR values between these two histopathological types of lung cancer.

There are certain limitations of our patients population preventing us to give final conclusion about the correlation between histological type and ^{99m}Tc-MIBI scan results. Firstly, in our study on 30 patients with malignant lung lesions, there were only 2 cases of small-cell carcinoma with respect to 27.2% of cases reported by Hassan et al. [13]. Secondly, the majority of malignant lung lesions in our patients were squamous-cell carcinoma, with a low prevalence of large-cell carcinoma.

Conclusion

This study suggests that the uptake ratio of ^{99m} Tc-MIBI (early and delayed ratio) could be a useful index in evaluating benign and malignant lung lesions, while retention index has no influence on this. Considering the factors related to uptake and release of the radiopharmaceutical, there was a significant difference in ER and DR values between smallest and medium or large lung lesions, while there was no significant difference in retention index values. Other investigated factors, such as localization of the lesion or different pathohistological types among benign or malignant lesions, showed no influence on the values of ER, DR or RI.

Declaration of interest

There was no financial support received for the work and the authors had no financial involvement, or affiliation nowith any organization whose financial interests may be affected by the material in the manuscript, or which might potentially bias it.

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Testing of urodynamic dysfunctions in patients with multiple sclerosis

Ispitivanja urodinamskih disfunkcija kod bolesnika sa multiplom sklerozom

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Abstract

Background/Aim. Multiple sclerosis (MS) is a chronic autoimmune inflammatory disorder of the unknown origin leading to multifocal demyelization, axonal damage and the loss of the nervous tissue in various parts of the central nervous system. Most MS patients have decreased functionality of the bladder leading to various dysuria disorders during the course of the illness. However, in 2% of the cases dysuric problems are the first symptoms of the disease. Urodynamic testing could help to diagnose functional disorders of the lower urinary tract, which might not be otherwise possible by performing the standard invasive procedures or noninvasive scans, such us ultrasound, computed tomography or functional magnetic resonance imaging (fMRI). **Methods.** Urodynamic testing – cystometry with electromyographic (EMG) potentials from the external anal sphincter (EAS), was performed in 34 patients (25 female and 9 male patients). Those patients fulfilled Mc Donald's multiple sclerosis criteria. The urodynamic values were compared to neurological signs and the present disease symptoms. **Results.** The MS patients with (27) and without (7) miction problems were tested. Detrusor hyperreflexia is the most common finding, present in 58.8% of the cases. More than a half of the patients have detrusor sphincter dissynergia. **Conclusions.** Urodynamic testing helps us to determine neurological disorders characteristics and to prepare an appropriate treatment plan. During the course of the disease different urodynamic disfunctions may occur as well as changes in the urinating functionality. The rationale for urodynamic testing in patients suffering from MS before any other treatment procedure is to confirm the diagnosis of dysuric disorders and to secure appropriate treatment.

Key words:
multiple sclerosis; urination disorders; urodynamics; electromyography.

Apstrakt

Uvod/Cilj. Multipla skleroza (MS) je hronično zapaljensko autoimuno oboljenje nepoznate etiologije koje dovodi do multifokalne demijelinizacije, oštećenja aksona i gubitka nervnog tkiva u različitim delovima centralnog nervnog sistema. Većina bolesnika sa multiplom sklerozom ima i poremećenu funkciju mokraćne bešike koja dovodi do različitih dizuričnih smetnji tokom trajanja bolesti. Samo kod 2% bolesnika ove smetnje su prvi simptom bolesti. Urodinamsko ispitivanje omogućava nam da postavimo dijagnozu funkcionalnih poremećaja donjeg urinarnog trakta, što uobičajenim invazivnim procedurama ili neinvazivnim snimanjima (ultrazvuk, kompjuterizovana tomografija ili funkcionalna magnetna rezonanca) često nije moguće ustanoviti. **Metode.** Urodinamsko ispitivanje – cistometrija i registrovanje elektromiografskih (EMG) potencijala sa spoljašnjeg analnog sfinktera (SAS) urađeno je kod 34 bolesnika (25 žena i 9 muškaraca), koji ispunjavaju Mc Donaldove dijagnostičke kriterijume za multiplu sklerozu. Dobijene vrednosti su upoređivane sa neurološkom simptomatologijom i znacima bolesti. **Rezultati.** Ispitivani su bolesnici sa (n = 27) i bez (n = 7) mikcionih tegoba. Hiperrefleksija detrusor bila je najčešći nalaz, prisutan čak kod 58,8% bolesnika. Više od polovine ovih bolesnika imalo je detrusor-sfinkter disinergiju. **Zaključak.** Urodinamsko ispitivanje može pomoći da se utvrde postojeći neurourološki poremećaji i na osnovu njih planira sprovođenje odgovarajućeg terapijskog plana. Tokom trajanja bolesti mogu se ustanoviti različiti oblici urodinamskih nalaza disfunkcije, kao i promena funkcije mokrenja. Razlog za sprovođenje urodinamskog ispitivanja kod bolesnika sa MS pre svake terapije bio bi postavljanje jasne dijagnoze dizuričnih poremećaja koja bliže određuje pravilnu i adekvatnu terapiju.

Ključne reči:
multipla skleroza; mokrenje, poremećaji; urodinamika; elektromiografija.

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Introduction

Multiple sclerosis (MS) is a chronic disease of the central nervous system (CNS) characterized by the widespread multifocal lesions in the brain and spinal cord, leading to visual, sensory, motor and urogenital impairments. The first attack of the disease usually occurs between the second and third decade of life, affecting working and living activity of the patients, depending on the severity and the diversity in the clinical course of the disease [1, 2].

MS is the disease with extremely varying clinical expression, with remissions and exacerbations of different symptoms, usually starting with visual impairments, weakness of extremities, diplopia, sensory disturbances and gait difficulties and disturbances, and urinary and anal sphincter dysfunctions [3]. Those dysfunctions comprise of frequent urinating, urgency, incontinency, retention or hesitance. According to several studies, the incidence of dysuric disorders in MS is 50–97% [3–5]. The form of the present dysuric disorder depends on the size and the position of demyelinated plaques. Therefore, any type and combination of neurogenic bladder and sphincter dysfunctions is possible during the course of the disease [6].

Urodynamic testing is a useful tool in lesion localization, determination of neurogenic bladder type and might help to apply the appropriate therapy protocol, based on findings during the disease progression.

Urinary disturbances are caused by the lesion of the neural systems controlling the act of miction, and the consequences of these disturbances have to be monitored during rehabilitation of the disease [4]. Therefore, the aim of this study was to choose adequate functional diagnostic tests which could enable us to distinguish the causes of disturbed urodinamics. Performing rehabilitation of MS patients with neurogenic dysfunctions of urination enables preservation of the anatomic integrity and functionality of the structures involved in the act of urination. Depending on the phase of the illness, and the type of dysuric disfunctions, proper therapies and procedures should been applied.

Methods

At the Urodynamic Department, Clinic for Rehabilitation "Dr Miroslav Zotović", Belgrade, Serbia, patients with urinary disturbances were tested. The testing protocol comprises of reviewing patients medical documentation, as well as urodynamic testing. A total of 34 patients, 25 female and 9 male patients, previously diagnosed with MS were examined.

Medical data were obtained from anamnesis data, the history of the disease, the order of the symptoms and signs of the disease appearance, so the phases of exacerbation and remission of the symptoms were noted. Obtaining data on urination function is important, as well as the problem onset and the type of dysuric disorders and the duration of the previous predicaments.

Prior to start of the urodynamic testing, post mictional residual urine was determined. Also, laboratory analysis of urine, urinoculture, blood analysis, sedimentation, and the serum urea, creatinine, uric acid, bilirubin and glucose levels were determined.

In order to choose adequate functional tests which could enable distinguishing disturbances in urodynamics, cystometry was used, combined with description of neurogenic dysfunction of urination. Measurement gives important data concerning the act of urination – function of the bladder and preserved sphincter mechanisms [7].

Urodynamic studies were performed using a Dantec Logic (Dantec Inc, Copenhagen, Denmark). A double lumen 6–8 F urethral catheter was introduced and normal saline solution (0.9% sodium chloride) was used at the rate of 10–20 mL/min to fill the bladder. Bladder volume, maximum bladder capacity, bladder compliance, vesical (Pves), abdominal (Pabd) and detrusor pressures (Pdet) were monitored simultaneously during the filling and voiding phases [8–10]. Surface electromyography of the external sphincter activity was performed.

On the basis of urodynamic studies according to International Continence Society standards [7], bladder dysfunction was classified into 3 groups: detrusor areflexia (*DA*), defined as acontractility caused by abnormality of nervous control, and detrusor hyporeflexia, defined as detrusor contraction of inadequate magnitude and/or duration to effect bladder emptying in a normal time span. The patients with detrusor areflexia and detrusor hyporeflexia were grouped together for analyses due to the small sample size in our study and similar procedure management; detrusor external sphincter dyssynergia (*DSD*), defined as detrusor contraction concurrent with an involuntary contraction of the urethral and/or periurethral striated muscle; detrusor hyperreflexia (*DH*), defined as involuntary detrusor contraction during the filling phase which may be spontaneous or provoked, and cannot be completely suppressed due to disturbances of nervous control mechanisms.

The presence of urinary infection changes the severity of the disease symptoms. Therefore, infection must be treated early with the appropriate therapy before urodynamic investigation takes place. At the time of urodynamic investigation, patients were without urinary tract infection and no drugs that influence detrusor and striated sphincter behavior.

The numerical data are described by mean and their standard deviations ($\bar{x} \pm$ SD), and the categorical data are expressed as counts and percentages. The numerical data were compared with the *t*-test, and χ^2-square or Fisher's exact test were used to compare the categorical data. Two-sided tests are used, and p-value < 0.05 was considered to indicate statistical significance. All the data in the present study were analyzed with the commercial statistical software (SPSS version 13.0 for Windows; SPSS Inc, Chicago, Il, USA).

Results

The study included 34 MS patients – 25 (73.5%) females and 9 (26.5%) males with urinary symptoms and disturbances (Table 1).

Table 1

Demographic and clinical characteristics of the patients with urodynamic dysfunction (n = 34)

Demographic and clinical characteristics	Female	Male	*p*
Number (%)	25 (73.5)	9 (26.5)	< 0.01
Age of the moment of testing (years), $\bar{x} \pm SD$	32.6 ± 9.7	35.5 ± 10.4	n.s.
Age at the disease onset (years), $\bar{x} \pm SD$	30.1 ± 10.2	27.6 ± 9.1	n.s.
Years elapsed from the disease onset. $\bar{x} \pm SD$	5.3 ± 3.9	4.9 ± 3.4	n.s.
Years elapsed from the urodynamic dysfunction onset, $\bar{x} \pm SD$	4.3 ± 2.1	4.1 ± 1.9	n.s.

n.s. – no significant difference

Comparing the mean age of the patients at the moment of testing, no statistically significant difference between the male and female patients was found. The youngest participant was a male, aged 15, and the oldest was a female patient, aged 57. The average age at the onset of the disease was 30.1 years for the female and 27.6 years for the male patients, and no statistically significant difference between the male and female patients was found concerning this parameter. Also, no significant difference between the patients with or without bladder symptoms was found ($t = 0.62$, $p > 0.05$) (Table 1).

The duration of the disease varies from 1 to 15 years; however, most patients had urinary dysfunctions in the range from 1 to 6 years. There was no statistically significant difference in the duration of the disease between the females and males also (Table 1). Most patients developed bladder dysfunction several years after the first neurological symptoms but in 4 of the patients only urinary symptoms were present at the time of the disease onset. However, urinary dysfunction was not the sole presenting symptom of MS in any of our patients.

A total of 27 (79.4%) patients suffered from dysuric disturbances and in 7 (20.6%) patients no irritation or obstruction was found. However, there was a statistically significant difference in the duration of the disease between the patients with and without the symptoms concerning urinary system (Table 2).

The symptoms of irritation (urgency, frequency, urge incontinency) were present in 59% of the symptomatic patients, contrary to obstructive symptoms (hesitancy, retention, interrupted stream, sensation of incomplete bladder emptying) present in 41% of the symptomatic patients (Table 3). The most common urinary symptoms were irritative. Difficulty in initiating voluntary voiding was a concurrent symptom in approximately half of the patients. The patients with more severe bladder dysfunction became unable to void voluntarily and could empty the bladder only when they had spontaneous hyperreflexic detrusor contractions.

The relation between dysuric disturbances and the neurological findings did not meet the criteria for applying proper statistical tests, but the tendencies pointed to the occurrence of irritative disturbances combined with pyramidal symptomatology. The majority of patients with MS and lower limbs pyramidal signs (13 patients) had dysuric disturbances of both types (Figure 1).

Table 3

Distribution of urinary symptoms in the multiple sclerosis (MS) patients

Symptoms	Patients, n (%)
Irritative	
urgency	10 (29)
frequency	2 (6)
urge incontinency	4 (12)
Obstructive	
hesitancy	7 (21)
retention	4 (12)
Without simptoms	7 (21)

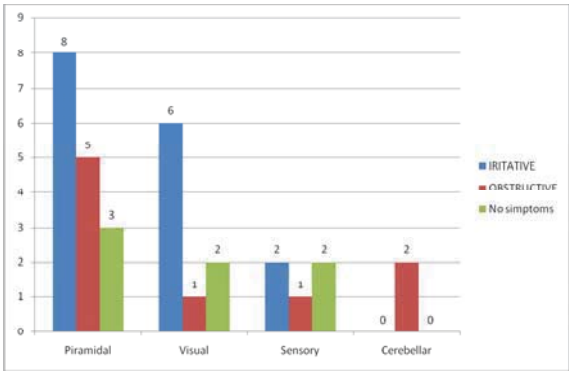

Fig. 1 – Number of the patients with (irritative and obstructive) and without urinary symptoms related to various nervous system dysfunctions.

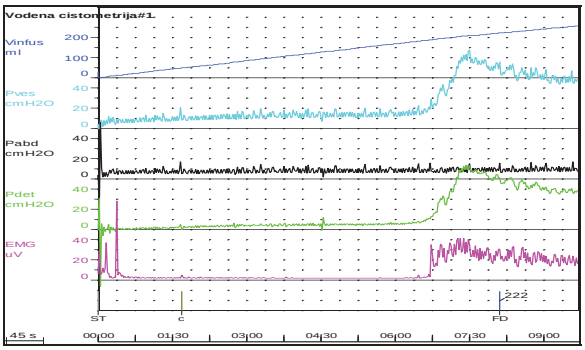

Fig. 2 – An example of cystometry with electromyography (EMG) of external anal sphincter in a patient with multiple sclerosis (MS) (findings: small capacity, high detrusor pressure, detrusor sfincter dyssinergia).

Vinfus = infusion fluid volume; Pves = vessical pressure; Pabd = abdominal pressure, Pdet = detrusor pressure; FD = first desire to void.

Table 2

Correlation between the duration of the disease and the patients with and without urinary simptoms

Parameters	Symptomatic	Asymptomatic	*p*
Number (%) of patients	27 (79.4)	7 (20.6)	< 0.01
Disease duration (years), $\bar{x} \pm SD$	6.1 ± 3.7	1.7 ± 1.3	< 0.01

Cystometry showed detrusor hyperreflexia in 20 (59%) of the patients, detrusor areflexia in 10 (29%), normal detrusor activity in 4 (12%) of the patients, implying 3 more MS patients (comparing to subjective report) with subclinical urinary dysfunction who did not recognize symptoms.

Comparative analysis of EMG potential from the external urethral sphincter (EUS) registered indirectly with cystometrography *via* the external anal sphincter (EAS) (Figure 3), demonstrated a high occurrence of detrusor-sphincter dissynergia in more than 40% of the patients (Figure 3).

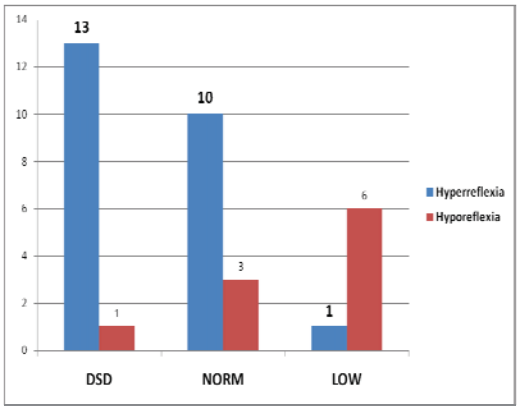

Fig. 3 – Number of the patients with detrusor hyper- and hyporeflexia related to external anal sphincter (EAS) electromyographic (EMG) activity
(DSD = detrusor sphincter dyssynergia; NORM = normal EMG activity; LOW = decreased EMG activity).

Discussion

The bladder, sphincter mechanisms and urethra forming the terminal parts of the urinary tract can be seen as a functional unit. Their basic function, filling the bladder, continence and miction make a group of very complex mechanisms and activities of antagonistic groups of smooth and skeletal muscles combined with complex innervations. The interruption of the nervous pathways with occurrence of demyelinating plaques in multiple sclerosis causes different forms of neurogenous bladder, with different disturbances and outcomes during the disease progress.

Our study included 34 MS patients – 25 (73.5%) females and 9 (26.5%) males with urinary symptoms and disturbances. It was in accordance with earlier studies, confirming predomination of female MS patients [3, 6, 11]. Symptom severity ranged from mild to very severe.

The most common urinary symptom of patients with MS is urgency. Series of urodynamic studies have shown that this is due to underlying detrusor hyperreflexia [3, 6, 12]. Urge incontinence is likely to be a problem if the patient also had impaired mobility and difficulty to access a toilet [10, 13]. The symptoms of impaired voiding are usually less prominent in testing and may be disclosed only during anamnesis or self reports.

The most common urodynamic finding was detrusor hyperreflexia suggesting that MS patients with irritative bladder symptoms and with lower limb pyramidal involvement are highly likely to have detrusor hyperreflexia. However, the explanation for detrusor areflexia in MS remains uncertain. Several investigators have reported that in some patients with MS initial urodynamic tests showed detrusor areflexia but subsequent studies demonstrated also hyperreflexia [5, 6, 12]. The neurological basis for this complete change in detrusor activity remains unclear [11].

Similar findings related to DSD in our study were also reported in previous studies [14, 15], although with significant differences in DSD appearance between patients with hiperreflexic and hyporeflexic bladder. The urodynamic finding of hyperreflexia correlated well with the symptoms of urgency, frequency and urge incontinence [16].

A modern concept of neural control of the bladder is based on long loop reflexes *via* the pontine tegmentum [3, 10, 16]. Pathways interruption between the sacral cord and pons may result in detrusor hyperreflexia and the loss of a coordinated action of a detrusor and the external striated urethral sphincter during voiding, the condition known as DSD. The reported incidence of DSD in MS patients varies from 18% to 66% [16]. DSD is important in the treatment of MS patients due to the tendency for incomplete bladder emptying, accompanied by poorly sustained detrusor contractions [10, 17]. Hesitancy of micturition, interrupted urinary stream and the finding of a high postmicturition residual volume in a patient with spinal cord disease therefore suggest DSD.

Applying classical urological diagnostic procedures could help to describe changes in parts of the lower urinary tract. Functional diagnostics provides monitoring and registering parameters of urination during filling and emptying of the bladder at approximately physiological conditions. Collected data could describe the degree of damage of bladder function as a consequence of the present CNS lesions [11, 18]. It is evident that in MS patients urodynamic findings can be changed through the disease duration, as well as the neurological signs and symptoms [10]. Different forms of dysfunctions are the result of the form of the disease, and the character and localization of demyelinating plaques in MS. Some of those signs and symptoms may develop before neurological changes, therefore urodynamic testing should be a part of routine testing of the disease, especially in patients developing changes during the course of the disease.

Appropriate therapeutic planning should be based on urodynamic findings. Some authors state that there is a poor correlation between subjective discomfort and objective neurological parameters [1, 2, 11, 19]. Therefore, neurological testing has important role not only in patients with dysuric disturbances, but also in patients without urinary discomfort, which was shown in this study.

By combining cystometry and EMG of EAS it is possible to register the occurrence of detrusor sphincter dissinergy which is an important urodynamic indicator of the progression of the disease disturbances (Figure 2). Those findings combined with hyperreflexia of m. detrusor appear to be most important urodynamic finding in patients during progressing MS. The data described here as neurogenous dysfunction of the bladder are in agreement with the criteria according to meta-analyses of urodynamic findings of 22 studies with 1882 patients diagnosed with MS [4].

Analysis of the urodynamic findings of this study emphasizes significant changes in the function of the bladder in MS patients, which further deteriorate their clinical picture and symptoms during the course and progression of the disease. During the progression of the disease, bladder dysfunction may become more and more difficult to treat. Main reasons could be described as worsening detrusor hyperreflexia, decreased efficiency in emptying due to worsening of the paraparesis neurological condition, appearance of recurrent urinary infections, spasticity and worsening of patients general immobility, and possibly their cognitive impairment [10, 20].

Conclusion

Based on the findings of this study we can conclude that: urodynamic tests of dysuric disturbances enable us to properly describe and monitor changes in factors of urination; the results of testing show the severity of urinary tract disturbances in patients with MS; the results of urodynamic test allow as to make appropriate urinary bladder training in patients with MS; an early therapeutic approach to preservation of physiological features of the bladder (elasticity and contractility) is very important due to the fact that satisfactory effect of the newly reorganized nervous control can be valid only in case of preserved physiological functions and structures. However, higher number of patients and broader variety of applied tests might give clearer perspective of urinary dysfunctions treatment in MS patients with different MS subtypes and forms.

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ORIGINAL ARTICLE

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Implant stability and marginal bone level of microgrooved zirconia dental implants: A 3-month experimental study on dogs

Implantatna stabilnost i nivo marginalne kosti kod cirkonijum endoosealnih implantata sa mikrostrukturiranom površinom: tromesečna eksperimentalna studija na psima

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Abstract

Background/Aim. The modification of implant surfaces could affect mechanical implant stability as well as dynamics and quality of peri-implant bone healing. The aim of this 3-month experimental study in dogs was to investigate implant stability, marginal bone levels and bone tissue response to zirconia dental implants with two laser-micro-grooved intraosseous surfaces in comparison with nongrooved sandblasted zirconia and sandblasted, high-temperature etched titanium implants. **Methods.** Implant surface characterization was performed using optical interferometric profilometry and energy dispersive X-ray spectroscopy. A total of 96 implants (4 mm in diameter and 10 mm in length) were inserted randomly in both sides of the lower jaw of 12 Fox Hound dogs divided into groups of 24 each: the control (titanium), the group A (sandblasted zirconia), the group B (sandblasted zirconia plus microgrooved neck) and the group C (sandblasted zirconia plus all microgrooved). All the implants were immediately loaded. Insertion torque, periotest values, radiographic crestal bone level and removal torque were recorded during the 3-month follow-up. Qualitative scanning electon microscope (SEM) analysis of the bone-implant interfaces of each group was performed. **Results.** Insertion torque values were higher in the group C and control implants ($p < 0.05$). Periotest values increased in all the periods in proportion to the extent of microgrooving as follows: the group C > the control > the group B > the group A ($p < 0.05$). Radiographic measurements showed minimal crestal bone loss at 3 months for microgrooved zirconia implants (groups C and B) and control implants compared with the group A implants ($p < 0.05$). The removal torque values increased with time for all the groups as follows: the group C > the control > the group B > the group A ($p < 0.05$). SEM showed that implant surfaces of the groups B and C had an extra bone growth inside the microgrooves that corresponded to the shape and direction of the microgrooves. **Conclusion.** The addition of microgrooves to the entire intraosseous surface of zirconia dental implants enhances primary and secondary implant stability, promotes bone tissue ingrowth and preserves crestal bone levels.

Key words:
dental implants; surface properties; biomechanics; microscopy, electron, scanning; alveolar bone loss; zirconium; titanium; dogs.

Apstrakt

Uvod/Cilj. Modifikacija površine implantata može uticati na njegovu mehaničku stabilnost kao i na dinamiku i kvalitet periimplantatnog koštanog zarastanja. Cilj ove tromesečne eksperimentalne studije na psima bio je da se ispita stabilnost implantata, nivo marginalne kosti i odgovor koštanog tkiva na cirkonijum endoosealne implantate sa dve intraosealne površine mikrostrukturirane laserom u poređenju sa peskiranim cirkonijum implantatima čija površina nije mikrostrukturirana kao i sa titanijum implantatima čije su površine peskirane i nagrižene visokom temperaturom. **Metode.** Karakterizacija površine implantata učinjena je optičkom interferometrijskom profilometrijom i analizom energetskog spektra pri difrakciji X-zračenja. Ukupno 96 implantata (prečnika 4 mm i dužine 10 mm) ugrađeno je nasumično i obostrano u donju vilicu

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kod 12 pasa (lisičara) i podeljeno u četiri grupe po 24: kontrolna (titanijum implantati); grupa A (peskirani cirkonijum implantati); grupa B (peskirani cirkonijum implantati sa mikrokanalima u koronarnoj trećini); grupa C (peskirani cirkonijum implantati sa mikrokanalima duž cele površine). Svi implantati su odmah opterećeni. Meren je obrtni momenat pri ugradnji implantata, vrednosti periotesta, radiografski nivo marginalne kosti i obrtni moment za uklanjanje implantata tokom tromesečnog perioda praćenja. Međuspoj kosti i implantata iz svake grupe ispitivan je kvalitativnom skenirajućom elektronskom mikroskopijom (SEM). **Rezultati.** Veći obrtni momenat zabeležen je pri ugradnji implantata kod grupe C i kontrolne grupe ($p < 0,05$). U ispitivanom vremenskom periodu, vrednosti periotesta uvećavale su se srazmerno obimu mikrostrukturiranja površine i to: grupa C > kontrolna grupa > grupa B > grupa A ($p < 0,05$). Radiografskom analizom utvrđen je minimalni gubitak marginalne kosti u trećem mesecu praćenja oko cirkonijum implantata sa mikrokanalima (grupa B i C) i kontrola u poređenju sa implantatima grupe A ($p < 0,05$). Vrednosti obrtnog momenta za uklanjanje implantata vremenom su se uvećavale u svim grupama na sledeći način: grupa C > kontrolna grupa > grupa B > grupa A ($p < 0,05$). Kod implantatnih površina grupa B i C, SEM je pokazala dodatni rast koštanog tkiva unutar mikrokanala koji odgovara njihovom obliku i pravcu. **Zaključak.** Formiranje mikrokanala duž cele intraosealne površine cirkonijum endoosealnih implantata povećava primarnu i sekundarnu implantatnu stabilnost, podstiče urastanje koštanog tkiva i održava nivo marginalne kosti.

Ključne reči:
implantati, stomatološki; površina, svojstva; biomehanika; mikroskopija, elektronska, skenirajuća; kost, resorpcija; cirkonijum; titanijum; psi.

Introduction

Although titanium can still be considered the reference standard material for dental implants, recent advances in the development of high mechanical strength ceramics have made them a viable alternative [1]. Yttrium partially stabilized tetragonal zirconia (Y-TZP) offers several advantages due to its flexural strength and high resistance to fracture [2, 3], favorable esthetics as well as excellent osseointegration observed in animal studies [4, 5].

Regardless of the type of implant material, rough surface achieves a great contact area with adjacent bone tissue, providing better mechanical stability of the implant which is the basic prerequisite for successful osseointegration. The implant surface modification promotes contact osteogenesis which results in accelerated and enhanced healing [6]. The topography of the coronal aspect of the implant could affect maintenance of marginal bone level [7].

However, roughening the surface of the zirconia implant is a challenge mainly due to its resistance to chemical or physical modifications. Several approaches have been proposed: chemical and pharmacological surface modification [8], sand-blasting and acid etching [9], the use of nanotechnology [10], or biomimetic coatings [11], and addition of micro and macro-retentions [12]. As a result, various degrees of surface roughness and traces of contaminants compromising implants` biocompatibility have been observed. Recently, technique for microstructuring cylindrical zirconia implants by femtosecond laser ablation has been introduced. Initial findings have shown increased surface roughness, a decrease in the presence of contaminants such as aluminum and carbon, an increase in oxygen presence and a decrease in monoclinic phase zirconia on the processed surfaces [13].

Previous studies have shown that the application of microgrooves to the implant surface can direct cellular morphology and cell migration [14, 15], improve cell adhesion [14, 16, 17] and also improve cell differentiation and mineralized matrix deposition [17, 18]. On titanium dental implant surfaces, the incorporation of microthreads [19] and microgrooves of different sizes in the neck area can guide specific cellular lines including osteoblasts (12 μm microgrooves) and fibroblasts (8 μm microgrooves), resulting in better quality connective tissue insertion and reduced crestal bone loss [20–22]. To date no research has been carried on stability variations and alterations to crestal bone for zirconia implants with the intraosseous portion microgrooved in different areas.

The aim of this 3-month experimental study in dogs was to examine zirconia dental implants with two laser-micro-grooved surfaces in terms of their stability, changes in marginal bone level and bone tissue response in comparison with nongrooved sandblasted zirconia and sandblasted, high-temperature etched titanium implants.

Methods

Twelve Fox Hound dogs of approximately one year of age, each weighting between 14 to 15 kg were used in the experiment. The Ethics Committee for Animal Research at the University of Murcia, Spain, approved the study (Murcia-November-2010 and August-2011), which followed a guidelines established by the European Union Council Directive of November 24th, 1986 (86/609/EEC).

Implants and surface characterization

A total of 104 commercially manufactured implants of 4 mm diameter and 10 mm length were used for the study. Four groups were studied: the control group – 26 titanium BlueSKY® implants (Bredent medical® GMBH & Co. KG, Senden, Germany); the group A – 26 sandblasted WhiteSKY® zirconia implants (Bredent medical® GMBH & Co. KG, Senden, Germany); the group B – 26 WhiteSKY® sandblasted zirconia implants (Bredent medical® GMBH & Co. KG, Senden, Germany) treated with femtosecond laser pulses to create 30 μm wide, 70 μm pitch length microgrooves over 2 mm of the neck area; the group C – 26 WhiteSKY® sandblasted zirconia implants (Bredent medical® GMBH & Co. KG, Senden, Germany) treated with femtosecond laser pulses to create 30 μm wide, 70 μm pitch length microgrooves over the entire intraosseous surface (Figures 1–3).

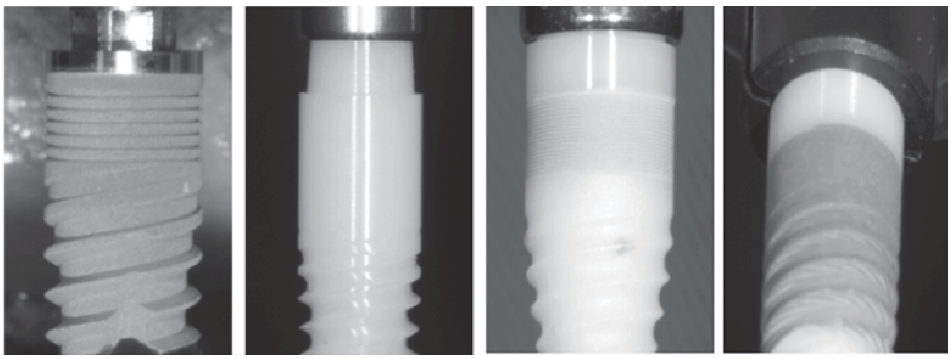

Fig. 1 – Clinical view of groups of implants used in this study. From left to right, the control (titanium implant), the group A (zirconia implant with sandblasted surface), the group B (zirconia implant with microgrooved neck), and the group C (zirconia implant all microgrooved). The zirconia laser treated implants showed a characteristic darkness area corresponding to laser microgrooved surfaces.

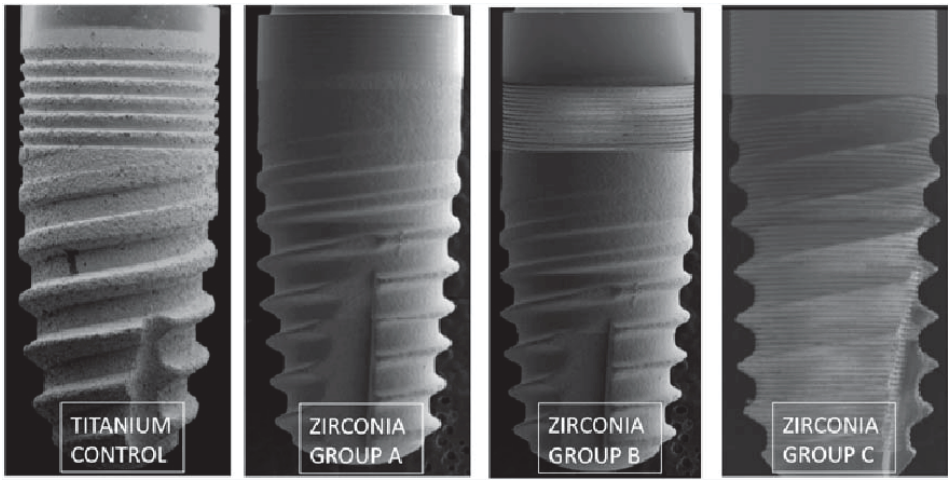

Fig. 2 – Scanning electron microscope (SEM) image composition of implants used in this study. The control implants have microthreads at neck level. All the implants have the same geometry. The laser processed surfaces of the group B and the group C showed the microgrooves at the neck level or in all surface, respectively.

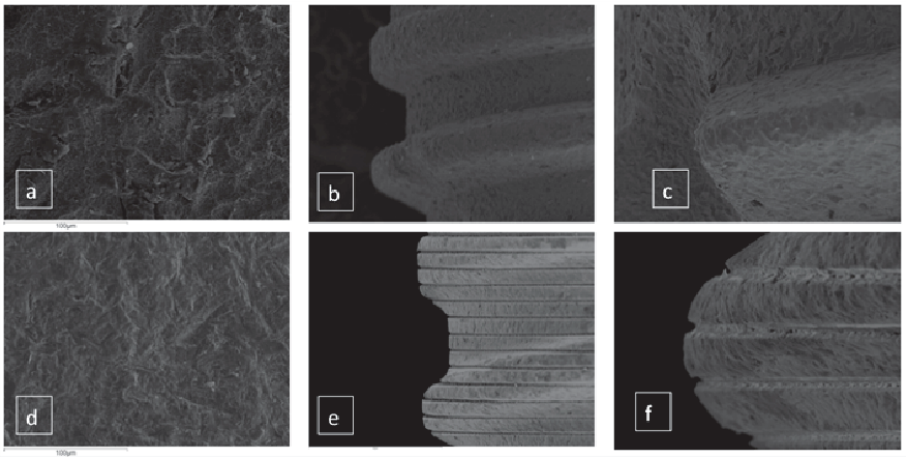

Fig. 3 – Scanning electron microscope (SEM) higher magnification reveals: a) typical image of titanium implant of the control group; b) view of threads zone in the control; c) close view of thread with typical roughness in the control; d) the group A surface with lower roughness; e) threads with microgrooves in symmetric and parallel position; f) close view of a thread with microgrooves and increased roughness inside the microgrooves and between them.

Two implants per group were used to analyze surface roughness and chemical composition of the surfaces. A Veeko NT 1100® non-contact interferometric microscope (Wyco Systems, New York, USA) was used to quantify following surface roughness parameters: Ra (average surface roughness), root mean square roughness (Rq), average maximum height of the surface (Rz), maximum height of the surface (Rt). Ten random measurements with 20.7 X magni-

fication in VSI mode were performed within the intraosseous portion of the implant surfaces. The sampling areas were 227.2 μm × 298.7 μm. Elemental chemical composition analysis was carried out by Energy Dispersive X-ray spectroscopy (EDX) using an OXFORD INCA 300 system (Oxford Instruments, UK). All specimens were coated with a thin layer of conductive carbon in a sputter-coating unit (SCD 004 Sputter-Coater with OCD 30 attachment, Bal-Tec, Vaduz, Liechtenstein). Chemical composition analysis was performed in ten sampling areas on the surfaces of the intraosseous portions.

Surgical procedure

The animals were pre-anesthetized with acepromazine (0.2–1.5% mg/kg) 10 min. before administering butorphanol (0.2 mg/kg) and medetomidine (7 mg/kg). The mixture was injected intramuscularly in the femoral quadriceps. An intravenous catheter was inserted in the cephalic vein and propophol was infused at a slow, constant rate of 0.4 mg/kg/min. Local infiltrative anesthesia was administered at the surgical sites. An intrasulcular incision was performed from distal aspect of the first mandibular premolar (P_1) to a point mesial of the second mandibular molar (M_2), bilaterally and a full thickness flaps were raised. Following tooth section the second mandibular premolars (P_2), the third mandibular premolars (P_3), the fourth mandibular premolars (P_4) and the first mandibular molars (M_1) were extracted bilaterally, using a periotome and forceps, without damaging the bony walls. Wound closure was carried out using single resorbable sutures.

During the first week after the surgery, the animals received antibiotics and analgesics: amoxicillin (500 mg, twice daily) and ibuprofen (600 mg, three times a day) *via* the systemic route. After 14 days of soft diet, a normal pellet diet was established.

After a 2-month healing period, a total of 96 implants were placed. Implant positions and implant type were determined using a random allocation software, so that each hemimandible received four implants from any group inserted randomly at P_2, P_3, P_4 and M_1 positions.

After crestal incision, a full thickness flap from distal aspect of P1 to mesial aspect of M2 medially was reflected and implant sites of 4 mm diameter and 10 mm length were prepared with strict adherence to manufacturer`s protocol (Figures 4 a-d). Each mandible received 8 cylindrical screw implants, all with the same dimensions at the intraosseous portion. Implants from the groups A, B and C were inserted with shoulders 2 mm above the osseous crest while in the control group implant shoulders were at the crestal level.

On the day of implant placement, provisional splints were made and all implants were immediately loaded (Figures 4 e-i).

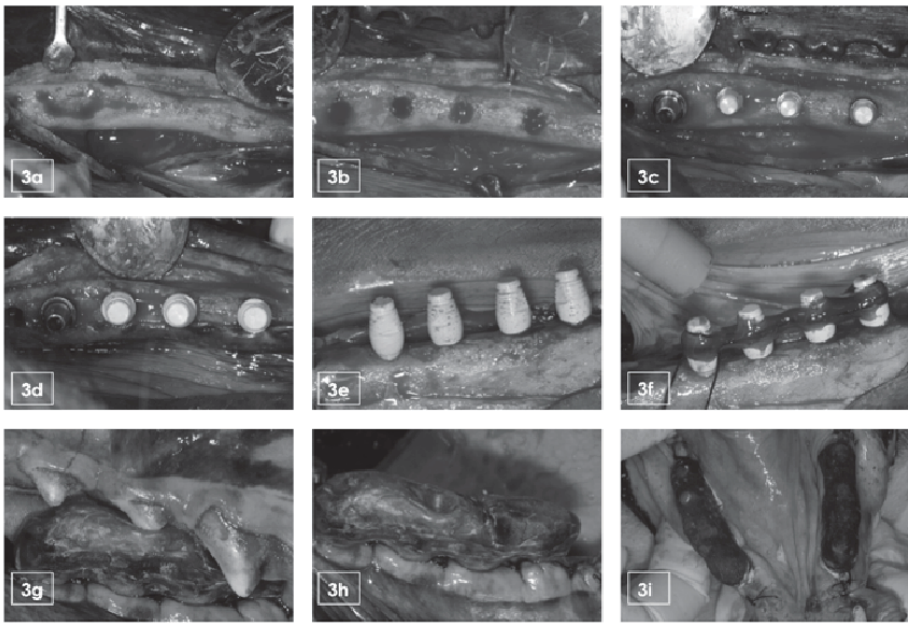

Fig. 4 – Surgical and prosthetic step-by-step procedures: a) complete open mucoperiosteal flap; b) prepared implant beds, c) randomly inserted implants: titanium implant with prosthetic abutment and zirconia implants; d) occlusal view; e) special plastic cover placed over the implants; f) implants splinted by orthodontic wires and acrylic resin; g) occlusion test; h) occlusal adjustment and gingival finishing; i) provisionalization completed, occlusal view of bilateral temporary acrylic splint.

Four animals were sacrificed for each evaluation time after the first, second and third months. The animals were pre-anesthetized following the protocol described earlier, followed by a perfusion of sodium pentothal (Abbot Laboratories, Chicago, IL, USA) through the carotid artery.

Insertion torque

Insertion torque (IT) values were measured at day 0 on 96 implants by means of an electronic instrument (FRIOS® Unit E, W&H Dental Werk GmbH, Buermoos, Austria) during low-speed insertion, registering the maximum peak (Ncm) reached at the crestal implant level.

Periotest values

The Periotest values (PTV) were registered following the animals' sacrifice at 1st, 2nd and 3rd postoperative months. For that purpose, acrylic splints that link the implant posts were removed and each extracted mandible was stabilized in a metallic support to ensure immobility. Since the zirconia implants used in this study were one-piece implants, the titanium implants of the control group received a straight titanium abutment SKY-EM00® (Bredent medical® GMBH & Co. KG, Senden, Germany) tightened to the implant with a torque of 25 Ncm in order to provide uniform conditions in terms of immediate loading and PTV testing for all experimental groups.

When the titanium abutments had been attached, the secondary stability of all implants was evaluated with a Periotest® device (Siemens, Bensheim, Germany), calibrated from -7 (maximum stability) to +7 (minimum stability) for each zirconia or titanium implant. The point of the instrument was placed perpendicularly to the middle third of the abutments' vestibular face and three evaluations were registered by a single operator, who recorded the mean value.

Radiographic crestal bone level

Radiographic crestal bone level (RCBL) interpretation was performed from digital retroalveolar radiographs at one month, 2-month and 3-month observation (Kodak Ultra-speed size II double film, Eastman Kodak, Rochester, NY). The radiographs were taken using a customized acrylic support in order to ensure reproducibility. Standardized exposure parameters and processing procedures were used. Each radiograph was then digitalized, magnified at 7 × and analyzed for changes to crestal bone levels using Image J® software (National Institutes of Health, Bethesda, Maryland, USA).

The obtained images were processed using edge-location techniques followed by color inversion and lastly a thresholding procedure was performed. Implant shoulders and the first point of crestal bone contact were localized on both the mesial and distal aspects of each implant. For zirconia implants, two points were located, one distal and one mesial, situated 2 mm from the abutment platform, coinciding with the bone crest; for titanium implants, two points were located at the implant platform, one distal, one mesial, located 1mm from the machined neck (Figures 5).

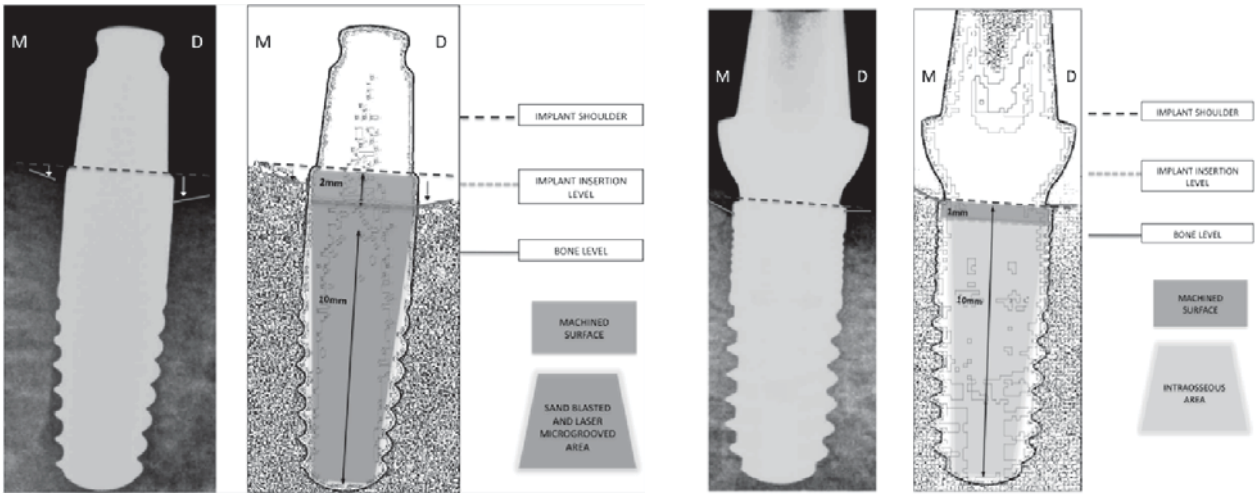

Fig. 5 – Radiographic reference points after image processing to increase detail: left) zirconia implant with landmarks; right) titanium implant with landmarks.

Removal torque

Removal torque (RT) evaluation was performed at the 1st, 2nd and 3rd postoperative months. After the meticulous elimination of all soft tissues, the mandible was fixed in a special support adding acrylic resin until the bone was covered to 1 mm below the bone crest. A Sky-WTK6 driver was used for evaluationng of titanium implants and a SKYC-WM6 driver for zirconia implants. Radiographic testing (RT) was performe by a counterclockwise rotation at a rate of 0.1°/sec using a reverse torque testing machine (Instron, Bucks, UK), recording the peak when implant movement occurred. RT was defined as the maximum torque necessary to start rotational movement of the implants. Eventual implant fracture was recorded as well.

Qualitative SEM analysis

To obtain additional information about the characteristics of the broken interfaces, qualitative SEM analysis was performed in one sample of each group after reverse torque test. A block containing the implant and surrounding bone was extracted, fixed by immersion in a 4% formalin solution, dehydrated in a graded ethanol series and embedded in light-curing resin (Technovit® 7210; Kulzer & Co, Hanau, Germany). Then, the blocks were sectioned sagittally in two halves. One was polished using a manual grinder with 800 grit silicon carbide paper, mounted on an aluminum stub and carbon coated (Polaron sputter coater, East Grinstead, Sussex UK). Samples were examined using backscattering and EDX at a working distance of 19 mm and an acceleration voltage of 20 kV under 15X, 80 X, 100 X magnification. The second was used to observe the separated implant and bone surfaces at same parameters.

Data obtained from 96 implants were analyzed using descriptive and inferential statistics. The difference in mean values of single outcome variable (IT, PTV, RCBL and RTV) between the study groups at a given time of observation was analyzed using one way ANOVA followed by Tukey`s multiple comparison test or Kruskal-Wallis test followed by Dunn's post test, depending on the nature of data distribution. Pearson correlation coefficients were calculated in order to reveal the strength of the relationship between PTV and RT, as well as RCBL and RT. *P*-values < 0.05 were considered to indicate statistically significant differences.

Results

All the animals were available for evaluation. The healing period was uneventful. The placed implants were primarily stable and subsequently osseointegrated. No implant fracture nor implant loss were detected during the study.

Surface characterization

The implants from the group C exhibited the highest values of roughness parameters (Table 1) and reduced presence of contaminants (Table 2).

Insertion torque

None of the zirconia implants has been fractured as a result of the insertion torque applied. The insertion torque values of the implants from the group C were significantly higher ascompared with each of the remaining groups ($p < 0.05$). In the group A and the group B significantly lower insertion torque values were recorded in relation to the control group ($p < 0.05$). Furthermore, the difference in mean insertion torque values between the group A and the group B was also statistically significant ($p < 0.05$) (Table 3).

Periotest results

At the first month of observation, the implants from the group C demonstrated the highest stability (ie. the lowest PT value). Dunn's multiple comparison showed a significant difference in the mean PTV between the group C and the group B ($p < 0.01$). The lowest stability was recorded in the group A and compared with the group C as well as with the controls the difference was statistically significant ($p < 0.01$), (Table 4). During a 3-month observation period, the stability of the implants in all the study groups was increasing whereas the pattern of statistical sig-

Table 1

Topographic characteristics of implants used in the study

Roughness parameters	Experimental groups			Control
	group A	group B	group C	
R_a(μm)	1.28 ± 0.2	2.43 ± 0.6*	9.50 ± 0.25*	1.78 ± 0.6
R_q(μm)	1.82 ± 0.51	3.48 ± 0.30*	11.51 ± 0.31*	2.02 ± 0.43
R_z(μm)	11.4 ± 0.6	40.42 ± 0.25*	40.74 ± 0.28*	15.8 ± 0.5
R_t(μm)	18.46 ± 0.82	52.68 ± 0.9*	60.36 ± 0.22*	23.63 ± 0.32

The results expressed as $\bar{x}$ ± SD; *$p < 0.05$.
R_a – avarage surface roughness; R_q – root mean square roughness; R_z – avarage maximum height of the surface; R_t – maximum width of the surface.

Table 2

Elements present in surface chemical composition

EDX surface analysis	Experimental groups			Control
	group A	group B	group C	
C	19.7 ± 0.8	1.6 ± 0.35*	0.3 ± 0.12*	2.3 ± 1.7
Al	4.3 ± 0.9	1.16 ± 0.2*	0.18 ± 0.1*	1.7 ± 0.3
O	12.6 ± 0.5	22.7 ± 0.2*	23.1 ± 0.12*	15 ± 0,6
Zr	60.2 ± 0.7	73.7 ± 0.15*	76.3 ± 0.2*	0
Ti	0	0	0	81 ± 1.3

The results expressed in percentages as $\bar{x}$ ± SD; *$p < 0.05$.
Other elements traces sometimes present in zirconia samples like Hf, were not detected by this probe. EDX – energy dispersive X-ray spectroscopy.

Table 3

Descriptive statistics of Insertion Torque (IT) values recorded at implant placement

Experimental group	IT(Ncm)			
	$\bar{x}$	SD	SE	Median
Control	57.10	1.80	0.51	55.76
Group A	46.08	0.70	0.20	44.87
Group B	53.20	1.30	0.37	50.98
Group C	69.60	1.20	0.34	67.82

$\bar{x}$ – mean; SD – standard deviation; SE – standard error.

Table 4

Results from the removal torque test (RT) performed at three evaluation time points

Experimental groups	RT (Ncm)		
	month 1	month 2	month 3
Group A	64.08 ± 0.42 (64.07)	78.24 ± 0.35(78.38)	199.19 ± 0.99 (199.47)
Group B	69.19 ± 0.37 (69.17)	88.82 ± 0.41 (88.86)	215.13 ± 0.99 (215.06)
Group C	84.95 ± 0.25 (85.03)	126.96 ± 0.81 (126.65)	240.15 ± 1.04 (239.90)
Control	71.25 ± 0.43 (71.28)	99.85 ± 0.44 (99.98)	226.98 ± 1.06 (226.72)

Values are expressed as $\bar{x}$ ± SD (median).

nificance of differences among the groups was the same as at the first month (Table 5).

Pearson correlation coefficient revealed a strong, highly significant and negative relation between PTV and RT (r = - 0.726; p = 0.000), whereas RCBL and RT had a moderate and positive correlation (r = 0.506; p = 0.000).

Table 5

Changes in Periotests values (PTV) during three-month follow-up

Experimental groups	PTV		
	month 1	month 2	month 3
Group A	-1.52 ± 0.01 (-1.52)	-2.17 ± 0.01 (-2.17)	-2.41 ± 0.02 (-2.41)
Group B	-1.85 ± 0.02 (-1.85)	-2.42 ± 0.01 (-2.42)	-3.11 ± 0.01 (-3.11)
Group C	-2.49 ± 0.02 (-2.5)	-4.16 ± 0.01 (-4.16)	-5.69 ± 0.03 (-5.7)
Control	-2.11 ± 0.35 (-2.00)	-2.70 ± 0.01 (-2.70)	-3.59 ± 0.05 (-3.60)

The values expressed as $\bar{x}$ ± SD (median).

Radiographic crestal bone level

No peri-implant radiolucency was observed.

The differences in crestal bone loss among the study groups were statistically insignificant at the first month of observations (p > 0.05) (Table 6). Peri-implant bone loss was increasing with time. In the second month of follow-up, the highest crestal bone loss was observed around implants in the group A and comparison of the mean RCBL values with those obtained in the group B and the group C revealed a statistically significant difference (p < 0.05, respectively). The lowest crestal bone loss was recorded in the group B and comparison of these RCBL values with the values from the controls showed a statistically significant difference (p < 0.05). At this evaluation time, a higher bone loss was recorded around implants from the group C as compared with the group B, but the observed difference was statistically insignificant (p > 0.05) (Table 6). However, in the third month of observations the lowest bone loss was recorded in group C and comparison with either the group B or the group A revealed a statistically significant difference (p < 0.05). The difference in mean crestal bone loss values between the group A and controls was also statistically significant (Table 6).

Table 6

Radiographic crestal bone loss (RCBL) recorded during the first three months of loading

Experimental groups	RCBL (mm)		
	month 1	month 2	month 3
Group A	0.27 ± 0.03 (0.26)	0.32 ± 0.01 (0.32)	0.56 ± 0.01 (0.56)
Group B	0.25 ± 0.03 (0.23)	0.22 ± 0.02 (0.23)	0.36 ± 0.01 (0.36)
Group C	0.24 ± 0.02 (0.22)	0.24 ± 0.01 (0.24)	0.26 ± 0.01 (0.26)
Control	0.27 ± 0.04 (0.28)	0.30 ± 0.02 (0.30)	0.36 ± 0.01 (0.36)

The values expressed as $\bar{x}$ ± SD (median).

Removal torque

The removal torque values recorded in all the examined groups were constantly increasing during a 3-month observation period, but the statistical significance of differences between the groups followed the same pattern at each evaluation time. The highest RT values were observed in the group C and they were significantly higher than those in the group A, as well as compared with the group B, (p < 0.05, respectively). The difference in mean RT values was statistically significant between the group A and controls (p < 0.05). The lowest RT values were recorded in the group A (Table 4).

SEM analysis of broken interfaces

Observation of broken interfaces, showed the bone fragments attached to implant surfaces in all the groups. In the control group and the group A, the border of the threads had bone at different extensions (Figures 6 a and b), while, the groups B and C showed additional bone fragments inside the microgrooves.

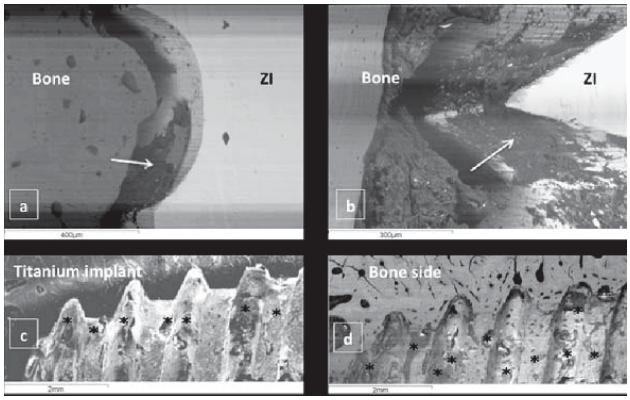

Fig. 6 – Scanning electron microscope (SEM) observation of fractured interfaces: a) backscattered image of group A, white arrow indicates fractured bone fragments adhered to bottom of interloop area of zirconia implant; b) backscattered image of the group A, top of the thread with adhered bone fragments; c) isolated control implant surfaces, black marks signaling bone fragments adhered to surface; d) isolated bone side, black marks signaling fractured bone.

Observation of the isolated bone surface showed the fractured areas of bone related to the bone fragments adhered to implant surfaces in titanium and zirconia implants (Figures 6c and 6d). Several bony growth extensions with the

same shape and dimensions of microgrooves, were observed at vertex of micro-grooved zirconia implants (Figure 7).

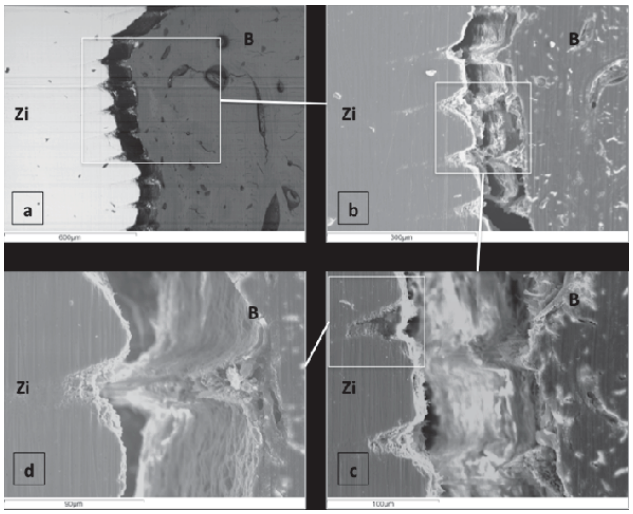

Fig. 7 – Scanning electron microscope (SEM) observation of fractured interfaces of microgrooved implants: a) backscattered image of the group C, zirconia side showing the microgrooves with bone fragments inside and bone side showing micro bone extensions with fractured tops; b) detail of microgrooves and micro bone extensions like a gear; c) and d) bone fragments inside microgroves and fractured top of bone extensions with the same shape of microgrooves, little bone residues adhered to plain zirconia surfaces could be observed.

Elemental analysis of microgrooves content revealed the presence of calcium (Figure 8).

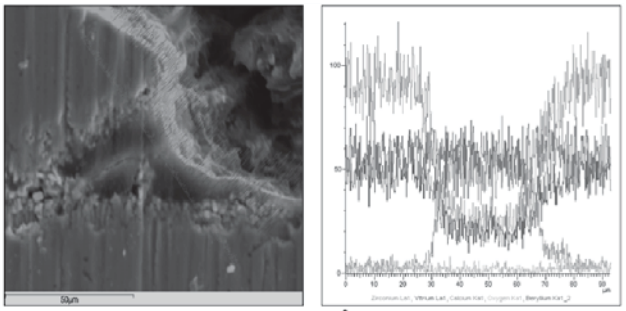

Fig. 8 – Energy dispersive X-ray spectroscopy (EDX) line scan of microgrooves showed increased calcium content inside at different levels.

Discussion

Since surface roughness could affect both mechanical and biological aspects of implant therapy, several roughness approaches have been studied [8–12, 23]. This 3-month study using a dog`s model focused on the 2 femtosecond laser-treated zirconia implants and their influence on implant stability and marginal bone level preservation. The implant collar was selected as a microstructuring target since this is the region subjected to the strongest mechanical stress once the implant is operative [24, 25]. But the intraosseous implant surface is also under stress in the apical region [26] and for this reason another study group was created in which laser processing was applied to the entire intraosseous surface. A 30 μm microstructure size was selected *a priori* in order to guide and optimize osteoblast cellular growth and to act as a cell and bone reservoir [27–29], also providing an additional increase to surface area and so possible positive effects on implant stability. The current results indicate good primary and secondary implant stability, enhanced bone tissue ingrowth as well as marginal bone preservation associated with femtosecond laser-treated zirconia implants, particularly when entire intraosseous surface has been modified.

Primary implant stability and the avoidance of micromotion are obvious necessities for undisturbed healing and successful implant treatment. It is affected not only by surgical technique and bone density at recipient site, but also degree of bone-implant contact surface determined by implant macro and micro design [30].

The implants used in the present study had the same macro geometry, with only slight differences between the (control group) titanium implant neck area (which had microthreads) and the rest. Nevertheless, insertion torque peak values registered for zirconia implants treated with laser micro-grooves over the entire surface indicate that the surface treatment produced an increase in IT due to the implants' surface roughness and micro geometry.

In the present study, the addition of microgrooves increased surface roughness by 6.5 × in the neck-processed zirconia implants and almost 12 × in the zirconia implants processed over the entire intraosseous surface and this resulted in the increase in insertion torque and decrease of PTV values. This has two possible explanations: firstly, a sufficient increase in surface roughness increases mechanical friction, and secondly, as pointed out Gedrange et al. [31], a greater bone-to-implant contact will lead to greater stability as microgrooves will produce more retentive areas and greater bone-to-implant contact.

The Periotest® was used to evaluate variations in the secondary implant stability as since resonance frequency analysis requires an abutment attachment type which zirconia implants do not have. A succession of measurements supplied information about stability behavior at different points in time. Given the animal head position, anatomical differences and the requirements of reproducible conditions the PTV values at day 0 were excluded. Thus, only after the sacrifice the extracted jaw was fixed in a holder under the same conditions and the PTV values were registered. The lower initial implant stability observed during the first month coincides with resorption and bone neoformation processes [32]. Nevertheless, the zirconia implants with the entire surface microgrooved achieved the highest stability throughout the entire study period compared with the remaining investigated implants, which may be due to the increased surface area available and increased roughness. During the second and third months implant stability rose, possibly coinciding with the calcification of the neoformed matrix. Peak occurred in the third month when mature calcified bone began to predominate.

It is believed that implant stability depends on cortical bone density and thickness [33]. In the present study, implants were placed in the molar and premolar areas of the lower jaw, in healed bone of similar intra-animal cortical thickness at all study sites. The increase in stability after 2 and 3 months may therefore be attributable, not only to stability provided by cortical bone, but also to an increase in bone-to-implant contact in the trabecular zone guided by the microgrooves.

The current study confirmed the previously described strong inverse relationship between PTV and RT, a more negative PTV, the greater value of reverse torque, and the usefulness of both to test stability.

The high removal torque values shown by all microgooved zirconia implants and titanium controls may be attributed to different factors: firstly, the controls had microthreads at the neck which increase mechanical retention, and the zirconia implants had microgrooves in all the surface, in addition an increased roughness surface, this micro geometric features could produce larger friction areas and greater bone contact along the whole length of the implant. The lack of statistical significance in the difference in PTV between the zirconia implants with microgrooves on the neck area, and the titanium controls could be related to the extension of the microgrooved area, meaning that the effects of microgrooves are reflected only with more processed surface like 10 mm processed surface of group C implants.

This is similar to the results obtained by Sennerby et al. [34] who used rabbit femurs and tibiae to evaluate RT, comparing oxidized titanium implants with surface modified coated 3.75 mm diameter zirconia implants after 6 weeks of healing. They found higher RT values with the titanium implants (59 Ncm) and the surface-modified zirconia implants (73–75 Ncm) compared to noncoated zirconia controls (18 Ncm). The authors concluded that surface modification of zirconia implants increased surface roughness and resistance to removal torque, achieving a good level of stability.

Opposed to our results, Hoffmann et al. [35] in the study on rabbits, recorded similar RT values for laser-modified zirconia implants as for the sandblasted zirconia, sintered zirconia and acid-etched titanium implants at either 6 or 12 weeks of healing. It remains unclear whether or not this result is a consequence of similar surface roughness of investigated implants because the surface topography was neither measured nor described. The lack of more distinct difference the authors explained by the fact that at the time of observation, the bone had already healed, regardless of the type of implant surface, providing similar implant stability.

Various studies carried out to date involving RT with mechanical testing of zirconia implants should be carefully compared due to the interspecies differences in the dynamics of bone healing, as well as different removal torque apparatus, implant diameters and lengths used.

SEM observation of fractured interfaces give us additional qualitative information related to bone and implant surfaces and revealed the presence of bone fragments attached to the implant surfaces demonstrating the union of titanium and zirconia with hosting bone. Higher magnification of microgrooved zirconia surfaces showed bone penetration into microgrooves. This indicates that bone can grow in small areas of 30 μm width and defined diameters. The elemental analysis within microgrooves in the sagittal section showed calcium presence in deeper zones, likely indicating the secretion of bone matrix and calcified tissue inside microgrooves.

Isolated bone surface observation showed fractured areas of bone related to the bone fragments adhered to implant surfaces in titanium and zirconia implants, in addition showed bony growth extensions with the same shape and dimensions of microgrooves, several of this fractured at vertex in microgrooved zirconia implants. All these findings, bone prolongations that form additional surface areas at microgrooved implants, the interdigitation between micro bone extensions and microgrooves, and the presence of bone fragments inside microgrooves could explain the increased stability of zirconia implants with entire microgrooved surface compared with the remaining implants.

Radiographic analysis in this study used different reference points depending on whether implants were of titanium or zirconia. Whilst titanium implants have a clearly visible platform, monobloc zirconia implants do not and so the shoulder was taken as a reference point. One difficulty of radiographic analysis of bone height around zirconia implants is the material's high radiopacity, which can make the identification of crestal bone margins difficult.

Although some clinical studies in humans have used periapical retroalveolar radiographs for evaluating crestal bone around zirconia implants [36], the measurement method used was not described. Other study on minipig maxillae has used contact microradiography to evaluate osseointegration or its lack, a technique that suffers the same difficulties as radiographic study of zirconia [37]. The image processing technique we used was chosen in order to overcome this problem, allowing us to define the uppermost part of the crestal bone as well as the implant edges, with increased image clarity, eliminating the chance of superimposed images.

The results of this 3-month study revealed improved maintenance of crestal bone level around microgrooved implants in comparison with microthreaded implants (titanium controls) and particularly with rough neck implants without microthreading (sand blasted zirconia). Although microthreads at implant neck transform the shear force between the implants and crestal bone into the compressive force to which bone is the most resistant allowing preservation of bone tissue, addition of microgrooves that interlock the adjacent bone seems to be more efficient [38]. However, there are several limitations of the present study. Differences in implant-abutment junction between the investigated implants (all zirconia implants were one-piece whereas titanium controls were two-piece implants but placed in one-stage manner) could possibly affect crestal bone level. Therefore, the greater bone loss noted around the titanium implants could be due to the presence of a microgap that allows accumulation of debris and bacteria that cause inflammation and could not be attributed only to the lack of microgrooves [39]. The

other limitation is the short-term follow up period (3 months after functional loading) that is insufficient for marginal bone stabilization because the most critical period of the bone level changes occurs 1 year after loading [40].

Conclusion

Within the limitations of the present study on dogs´ mandibles it may be concluded that addition of microgrooves on the surface of zirconia dental implants by means of laser ablation enhances primary and secondary implant stability, promotes bone tissue ingrowth and preserves crestal bone level after a 3-month follow-up. This could be attributed to the increased implant surface roughness and reduced presence of contaminants following laser microtexturing. Mechanical and biological advantages of this surface modification are even more pronounced when applied to the entire intraosseous surface of zirconia implants.

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ORIGINAL ARTICLE

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The efficacy of hydrothermally obtained carbonated hydroxyapatite in healing alveolar bone defects in rats with or without corticosteroid treatment

Uticaj hidrotermalno sintetisanog hidroksiapatita na zarastanje koštanih defekatakod pasa sa ili bez tretmana kortikosteroidima

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Abstract

Background/Aim. Autogenous bone grafting has been the gold standard in clinical cases when bone grafts are required for bone defects in dentistry. The study was undertaken to evaluate multilevel designed carbonated hydroxyapatite (CHA) obtained by hydrothermal method**,** as a bone substitute in healing bone defects with or without corticosteroid treatment in rats as assessed by histopathologic methods. **Methods.** Bone defects were created in the alveolar bone by teeth extraction in 12 rats. The animals were initially divided into two groups. The experimental group was pretreated with corticosteroids: methylprednisolone and dexamethasone, intramuscularly, while the control group was without therapy. Posterior teeth extraction had been performed after the corticosteroid therapy. The extraction defects were fulfilled with hydroxyapatite with bimodal particle sizes in the range of 50–250 μm and the sample from postextocactional defect of the alveolar bone was analyzed pathohystologically**.** **Results.** The histopatological investigations confirmed the biologic properties of the applied material. The evident growth of new bone in the alveolar ridge was clearly noticed in both groups of rats. Carbonated HA obtained by hydrothermal method promoted bone formation in the preformed defects, confirming its efficacy for usage in bone defects. Complete resorption of the material's particles took place after 25 weeks. **Conclusion.** Hydroxyapatite completely meets the clinical requirements for a bone substitute material. Due to its microstructure, complete resorption took place during the observation period of the study. Corticosteroid treatment did not significantly affect new bone formation in the region of postextractional defects.

Key words:
tooth extraction; alveolar bone loss; transplants; rats; durapatite; adrenal cortex hormones.

Apstrakt

Uvod/Cilj. Autogeni koštani graftovi predstavljaju zlatni standard u stomatologiji za popunjavanje koštanih defekata. Studija je sprovedena kako bi se ispitala efikasnost višefaznog karbonatnog hidroksiapatita (HA), dobijenog hidrotermalnam metodom, kao zamene za kost kod *in vivo* zarastanja koštanih defekata. Procena efikasnosti izvršena je patohistološkom analizom na pacovima (Sprague Dawley). **Metode.** Koštani defekti načinjeni su u alveolarnoj kosti ekstrakcijom bočnih zuba kod 12 pacova. Eksperimentalne životinje prvo su bile podeljene u dve grupe. Prva, kontrolna grupa, bila je bez terapije, dok je druga, eksperimentalna grupa intramuskularno dobijala kortikosteroidnu terapiju i to metilprednizolon i deksametazon. Ekstrakcija bočnih zuba izvršena je nakon resorpcije izazvane terapijom kortikosteroidima. Ekstrakcione rane ispunjene su hidroksiapatitom čestica veličine 50–250 μm, a uzorci uzeti iz postekstrakcionih defekata alveolarne kosti analizirane su patohistološki. **Rezultati.** Patohistološkom analizom potvrđena su biološka osteokonduktivna svojstva primenjenog materijala. Intenzivni rast nove kosti unutar alveolarnog grebena jasno je uočen u obe grupe eksperimentalnih životinja. Karbonatni HA dobijen hidrotermal-

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nim metodom inicirao je formiranje kosti preko površine defekata, potvrđujući efikasnost njegove primene kod koštanih defekata. Do potpune resorpcije materijala došlo je posle 25 nedelja. **Zaključak.** Ispitivani hidroksiapatit u potpunosti zadovoljava kliničke zahteve kao zamena za kost, poštujući ograničenja eksperimentalne namene studije. Zbog mikrostrukture materijala došlo je do kompletne resorpcije tokom perioda posmatranja. Lečenje kortikosteroidima nije značajno uticalo na stvaranje nove kosti u predelu potekstrakcionih defekata.

Ključne reči:
zub, ekstrakcija; alveolna kost, gubitak; graftovi; pacovi; hidroksiapatiti; kortikosteroidni hormoni.

Introduction

Autogenous bone grafting has been the gold standard in clinical cases when bone grafts are required for bone defects in dentistry. The proven advantages of autogenous bone grafting are: osteogenic potential, satisfactory mechanical properties and the absence of adverse immunological response [1], but there are also some limitations, such as: requirement of additional surgery for harvest, reduced availability of adequate quantity and quality of graft material and the risk of patient morbidity [2–4]. In order to overcome these disadvantages, many kinds of synthetic biomaterials have been developed as bone substitutes, such as hydroxyapatite (HA), alumina, zirconia, bioglass, polymers, metal, and organic or inorganic bone substitutes [5–7].

For biomedical indications, HA has been used extensively as a substitute in bone grafts [5], because the natural bone is similar to HA. From the 1980s to nowadays, various forms of HA have been used in orthopaedic, dental or maxillofacial surgery [5, 6]. For specific form of HA, carbonated calcium hydroxyapatite [CHA; $Ca_{10}(PO_4)_{6-x}CO_{3x}(OH)_2$], osteoconductive properties have been proven [8]. Osteoconductive properties are significantly dependent on the porous structure of CHA, surface area, morphology and size of its particles. Furthermore, it has been shown that the CHA is bioresorbable and more bioactive than stoichiometric HA [4, 5, 9, 10].

The mechanism of glucocorticoid effect on bone metabolism is rather complex, and its role in arresting wound healing is not clearly described. Glucocorticoids modify osteoblastic cell differentiation, their number and function, thus inhibiting bone formation [11]. Inhibition of bone formation is simultaneously followed by bone resorption and subsequent bone loss. When administered for prolonged periods, glucocorticoid therapy is inevitably associated with bone loss, arrested osteoblast activity and suppressed bone formation *via* the osteoclasts [12]. Data regarding the influence of corticosteroid therapy on the teeth extraction wound healing are scarce.

After surgical procedure of tooth extraction, a coagulum fulfils the alveolar socket and a process of wound healing occurs. The healing never allows *ad integrum* restitution of the alveolar bone ridge, resulting in decreased bone volume and physiological resorption. Bone resorption leads to a decrease of height and width of the alveolar ridge which is a significant clinical problem [13]. Socket preservation is a procedure in which graft material is placed into the alveolar socket of an extracted tooth at the time of extraction in order to maintain the volume of the alveolar ridge [14]. For this purpose, various techniques and materials have been employed, such as alloplast materials, autogenous bone, allograft bone, atraumatic extraction, immediate placement of dental implants or immediate socket filling with osteoconductive material. In recent years synthetic bone substitutes based on HA are frequently used for this particular clinical indication and are considered as promising materials due to their physical properties and similarity to natural bone.

The aim of the study was to assess the efficacy of hydrothermally obtained CHA in healing alveolar bone defects in rats with or without corticosteroid therapy.

Methods

Precursors for CHA synthesis were prepared as follows: chicken egg shells were calcined at 900 °C till complete carbon removal and dissociation of $CaCO_3$ to CaO. The second precursor was Merk's *pro analysis* quality $(NH_4)_2HPO_4$. A total of 500 mL of 2.32 cmol $(NH_4)_2HPO_4$ solution was poured into 500 mL of 3.02 cmol $Ca(OH)_2$ solution and thoroughly mixed. In the end, 0.1 N HCl and $(NH_4)OH$ were added to buffer the pH value of the solution to 7.4 according to the methodology previously described by Jokanović et al. [15, 16]. This mixture was covered using a glass plane and put into the autoclave at 150° C and pressure up to 10 bar for 8 h.

After hydrothermal treatment in the autoclave, precipitates were decanted from glasses and dried at 80° C during the period of 48 h, disintegrated, rinsed with deionized water, and centrifuged with the purpose to get the purest possible CHA. After the process of characterization, hydrothermally obtained CHA particles were put into the glass pipettes and sterilized using gamma rays with the dose of 25 Gy.

Animal model and surgical procedure

The experiment was conducted according to the Good Laboratory Practice (GLP) at the Faculty of Dentistry, University of Belgrade, with tried and tested experimental facilities.

A total of 12, 6–8-week-old, 250–275 g weighty, syngeneic female Sprague Dawley rats who had attained sexual maturity, were used in the study. Rats were housed 4 *per* cage with water and food at will. The animals were put in quarantine for at least 10 days prior to intervention. The animals were initially divided into 2 groups. The experimental group was treated with intramuscular glucocorticoids: methylprednisolone (Lemond-Solu®, Hemofarm, Vrsac, Serbia) and dexametosane (Dexason®, Galenika, Belgrade, Serbia). The control group was without corticosteroid therapy. The dose of glucocorticoids was 2 μg/g of body mass. Both medicines were given every second day. The teeth extraction from posterior region was performed after the corticosteroid therapy. The extraction defects were fulfilled with the CHA

with bimodal particle sizes in the range of 50–100 μm and 200–250 μm**.** The surgical procedure was performed under general anaesthesia using a halogenous compound, oxygen/isoflurane (Forane R®, Abbott Laboratories, Abbott Park, Illinois, USA).

About 0.3 g on average of CHA was put into created wounds with approximately equal diameters and depth of about 0.5 mm. No antibiotic treatment was administrated after the surgical procedure. The animals were sacrificed 5, 15 and 25 weeks after surgery using mechanism of an intracardiac overdose with sodium pentobarbital (Dolethal®, Vetoquinol, Lure, France).

The sample for histopathological analysis was the alveolar bone from the region of the artificial postextractional defect of the jaw. The bone was rinsed with a physiological solution, fixed with 10% formalin and decalcified by electrolysis in a solution of concentrated formic acid during the period of 10–12 h. After decalcination, dehydration of the tissue was performed using ethanol solution. Finally, all samples were formed in paraplast, cut using microtome and colored by hemotoxylin-eosin (HE) method.

The histological preparations were histopathologically analyzed by an image analysis software, Lucia 32G (Laboratory Imaging, Prague, Czech Republic) on a microscope (LEICA DMR) with 10× magnification (NA = 0.5) and a digital camera (with 640 × 480 pixels).

Results

The signs of the initial osteogenesis were clearly observed in both groups. For animals treated with intramuscular glucocorticoids, the remnants of the implanted materials sized between 200 and 250 μm were visible 5 weeks after implantation of CHA. The interposed capillars, vascular structures and cells typical for a young bone were clearly observed (Figure 1a). Similarly, for the group without therapy, intergrowth of the capillary within the implanted material was evident 5 weeks after the implantation of CHA (Figure 1b). The average particles size of the implanted material in the control group was less than 200 μm.

Fifteen weeks after implantation, infiltration of the implanted CHA with blood vessels and osteoblasts migrating from the surrounding bone was slightly more intensive in the control group. It was evident that the new bone more intensively fulfilled the defects, transforming the CHA granule into a new bone. Remodulation processes in the alveolar defects were observed (Figure 1c). For the control group, the implanted material was saturated by blood capillary and osteoblasts from the surrounding bone as well. Defects were partially fulfilled with newly formed bone (Figure 1d).

After 25 week, implanted CHA was substituted completely by new immature bone in the control group. In the center of the defect, bone slowly became mature, while bone structure fulfilled almost complete defect. The complete integration of the new and existing bone tissue at the connective lines was almost completed in numerous spots (Figure 1f). In the experimental group, defects in the alveolar bone were fulfilled almost completely by the new bone tissue. When compared with the control group signs of slightly less intensive bone maturation were noticed, and the bone structure was slightly more irregular. (Figure 1e).

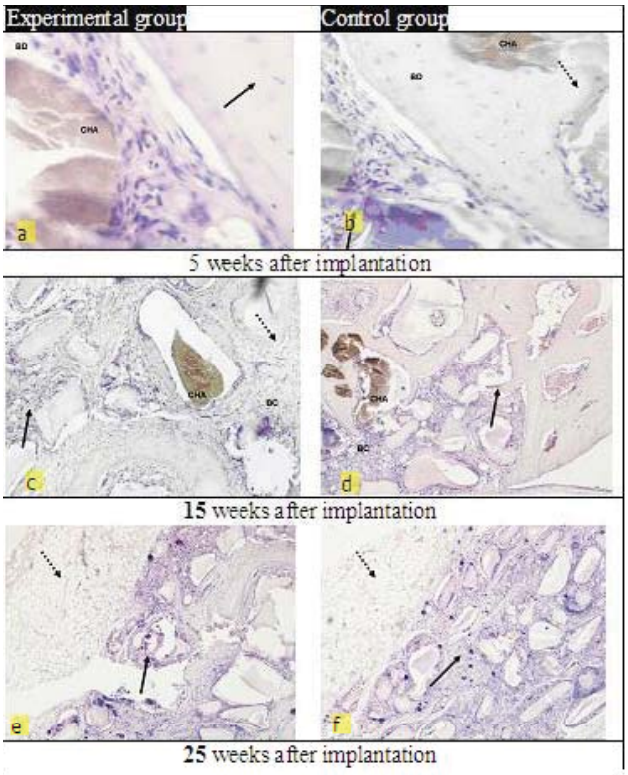

Fig. 1 – a, b) Five weeks after implantation of hydrothermally obtained carbonated hydroxyapatite (CHA), evident intergrowth of the capillary started inside the implanted material. The beginning of the initial osteogenesis was visible in both groups; c, d) Fifteen weeks after implantation, the implanted material (CHA) was saturated by blood capillary and osteoblasts (BC) from the surrounding bone (black arrow). The bony defect (BD) was partially fulfilled with a new bone (dotted arrow); e, f) Twenty five weeks after implantation, alveolar bone defects were fulfilled almost completely by new bone in both groups. Slightly more intensive osteogenesis was noticed in the control group. The integration of new and existing bone tissue was almost completed in the numerous spots.

Discussion

Nanostructured bone substitutes such as CHA particles with a high surface area are desirable in many fields including tissue engineering. The synthesis of nanostructured CHA was mostly based on the precipitation reaction developed by Nelson and Featherstone which was described in details by Barralet et al. [9]. Hasegawa et al. [10] have also successfully produced sintered CHA which can be resorbed by osteoclasts both under *in vitro* and *in vivo* conditions, while classic sintered stoichiometric CHA cannot be resorbed. Apart from particle size and size distribution, the shape of bioactive and reinforcing particles are also important when developing bioactive bone substitute materials based on HA (porous or non-porous) for human tissue repair [10, 17].

Besides the better mechanical properties of sintered materials obtained from nanosized CHA, Evis et al. [18] reported faster osteoblast proliferation and greater osteoclast activity on nano-CHA in comparison with the conventional CHA, which has micron grain sizes. It was proven that in extracellular culture the synthesis of alkaline phosphatase by osteoblast on nano-CHA was faster than on the conventional micron grain size CHA [8]. The nano-CHA integrated in the structure of bone defects also has better osteointegrative properties. Therefore, it could be assumed that CHA with nanosized basic particles integrated in the granule design similar to natural bone would exhibit better biocompatibility [19, 20].

In the present investigation, a longer observation period of 25 weeks following surgery may be a factor of complete CHA reabsorption. In addition, very fine particle size and the small level of impurities in our CHA can act as catalysts of CHA biological activity, and contribute to the significant reabsorption.

A special structure of hydrothermally obtained hydroxyapatite and its potential very high surface activity caused by its nanometric size of apatite crystallite and very small particle size were the basis for the expectation of its substantially improved osteogenic activity on the site of bone defect [19, 20]. The pores within the material, sized about 200 μm, cylindrically shaped, and located inside of a CHA granule, have dimension which may enable the proliferation of young connective tissue and provide the environment for osteoblast cell activity expression. It may be assumed that a very small size of CHA crystallites (several nanometers) strongly promoted boundary activity of osteogenic cells with CHA. In the present study, the result of these processes was the higher rate of CHA disintegration, its transformation and osteointegration into a new bone, even in the group of experimental animals that were submitted to the corticosteroid treatment.

The structure of CHA and its pore distribution is multimodal and follows not only the size of the primary particles, the smallest ones, but also the size of the other particles packed into the clustered powder particles. The pores are distributed from the smallest ones in the range of 1.5–15 μm up to the largest with the range of 50–250 μm. The largest pores correspond to the largest particles approximately 250 μm in size, clustered mostly into agglomerated particles 1–5 μm large, which can be seen in SEM micrographs of synthesized CHA, as previously described in detail in the study by Jokanović et al. [16]. These particles are finally joined in the granules with the diameter between 300 μm and 1000 μm.

In the present investigation, the osteogenic potential of CHA was evaluated with regard to corticosteroid treatment. Therefore, the experiments with the rats previously treated with corticosteroid therapy were made, similarly to the previously conducted research [21]. These animals were compared with 6–8-week-old healthy Sprague Dawley rats that attained sexual maturity in which bone mineralization process had been completed. The morphology of postextractional wound healing of the alveolar bone was investigated. The process of new bone formation inside of the defect area was observed in order to compare it with bone formation in the control group of animals. Newly formed bone with obvious evidences of mature bone characteristics were noticed 25 weeks after implantation in both investigated experimental groups. Inside healthy bone, it was obvious that healing was going on rapidly and very efficiently without any additional stimulation of osteogenesis [21–24].

In the experimental group of animals, it was noticed that osteointegration of bone tissue had begun, but that this process is in the starting phase. The rate of the process of intergrowth of blood capillarity and activation of the osteoblast was more intensive for the control group of animals during the same observation period.

Opposite, in the subgroup where CHA was implanted for a longer time, in both groups of animals the intensive formation of new bone, increased binding, as well as migration and distribution of blood vessels and osteogenic cells within the remnants of implanted material were noticed, which confirmed the CHA osteoconductive effect [25].

A significantly higher rate of osteogenesis in rats without corticosteroid treatment, in comparison to the control group, is probably caused by the activity of osteoblast cells which accelerated not only their proliferation and differentiation, but also the formation of new bone [26, 27].

According to the recently published investigations, the bone morphogenetic protein can be produced as the basis for the recruitment of mesenchymal stem cells in the region of defect by means of hemotaxis, initiating further quick proliferation and differentiation into chondroblasts and chondrocytes [21]. These cells later enter the cartilage-like matrix, which is calcified into bone. The final phase is the bone tissue remodeling and formation of mature lamellar bone that was clearly noticed in the present study.

Generally, the examined hydrothermally obtained CHA exhibited strong osteoconductive effect, enabling formation organization of osteons similar to normal bone. That is clear evidence that hydrothermally obtained CHA has a potential to form new bone and to replace bone tissue due to its osteoconductive properties. Therefore, hydrothermally obtained CHA can be used for the surgical treatment of defects caused by the resorption of alveolar bone ridge.

Conclusion

This histopathological investigation of hydroxyapatite showed that the hydrothermally obtained carbonated hydroxyopatite has a potential to be applied as an osteoconductive material. The intensive growth of new bone tissue in the compact jaw ridge was evidently approved. Corticosteroid treatment did not significantly affect new bone formation in the region of postextractional defects. The hydrothermally obtained carbonated hydroxyapatite shows a significant potential and efficiency for reparation, healing and preservation of alveolar bone defects.

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The prevalence of substance use among adolescents and its correlation with social and demographic factors

Rasprostranjenost upotrebe psihoaktivnih supstanci kod adolescenata i njena povezanost sa sociodemografskim faktorima

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Abstract

Backround/Aim. Adolescence is the period of greatest risk of starting to use substances: cigarette smoking, alcohol and illicit drugs. In the first decade of this millennium substance use among adolescents has increased. The aim of this study was to explore the prevalence of substances use among adolescents and its correlation with social and demographic factors. **Methods.** The study was conducted among adolescents in Novi Sad during 2010–2011 and included 594 conveniently selected adolescents (275 male and 319 female), aged 15–19 years. A special questionnaire was used and statistical analysis performed in SPSS17. The correlation between parameters was evaluated by the Pearson correlation method and frequency differences were analysed using χ^2 test and starting level was $p < 0.05$. **Results.** The prevalence of substance use was statistically higher in males. Cigarettes were smoked daily by 21.45% males and 15.67% females ($p < 0.01$), alcohol was consumed by 81.6% males and 69.11% females ($p < 0.001$) and illicit drugs were used by 13.65% males and 8.30% females ($p < 0.05$). There was a positive correlation between smoking cigarettes and alcohol consumption, but negative between smoking cigarettes and the use of illicit drugs ($p < 0.01$). The prevalence of substance use was statistically higher among adolescents with poor achievement in school ($p < 0.01$), who lived in a broken home (illicit drugs $p < 0.01$) and who had more pocket money (cigarette smoking $p < 0.01$, and alcohol consumption $p < 0.5$). **Conclusion.** Stable family, lower amount of pocket money weekly and good school performance are protective factors in prevention of substances use among adolescents.

Key words:
substance related disorders; adolescent; smoking; alcohol drinking; street drugs; risk factors.

Apstrakt

Uvod/Cilj. Adolescencija je period najvećeg rizika za početak upotrebe psihoaktivnih supstanci: pušenje cigareta, konzumacije alkohola i nezakonitih droga. U prvoj deceniji novog milenijuma zapažen je porast upotrebe psihoaktivnih supstanci kod adolescenata. Cilj rada bio je utvrđivanje prevalencije upotrebe psihoaktivnih supstanci kod adolescenata i povezanost sa sociodemografskim faktorima. **Metode.** Istraživanje je sprovedeno među adolescentima u Novom Sadu, tokom 2010–2011. godine, i uključilo je 594 adolescenta (275 muških i 319 ženskih), uzrasta 15–19 godina. Korišćen je anketni upitnik, specijalno sastavljen za ovo istraživanje. Statistička obrada rađena je u SPSS17. Povezanost parametara procenjivana je metodama korelacije po Pirsonu, a razlike frekvencija ispitane su pomoću χ^2-testa. Početni stepen statističke značajnosti bio je $p < 0,05$. **Rezultati.** Rasprostranjenost upotrebe psihoaktivnih supstanci bila je statistički veća kod muškog pola. Cigarete je pušilo 21,45% muških i 15,67% ženskih ($p < 0,01$), alkohol konzumiralo 81,6% muških i 69,11% ženskih ($p < 0,001$) i nezakonite droge koristilo 13,65% muških i 8,30% ženskih ispitanika ($p < 0,05$). Postojala je pozitivna korelacija između pušenja cigareta i konzumiranja alkohola, a negativna kod korišćenja nezakonitih droga ($p < 0,01$). Učestalost upotrebe psihoaktivnih supstanci bila je statistički veća kod adolescenata koji imaju lošiji uspeh u školi ($p < 0,01$), žive u poremećenim porodicama (pušenje cigareta $p < 0,5$, nezakonite droge $p < 0,01$) i imaju veći nedeljni džeparac (pušenje cigareta $p < 0,5$; konzumiranja alkohola $p < 0,01$). **Zaključak.** Stabilna porodica, mali nedeljni džeparac i odličan uspeh u školi su protektivni faktori u prevenciji upotrebe psihoaktivnih supstanci kod adolescenata.

Ključne reči:
poremećaji izazvani supstancama; adolescenti; pušenje; alkohol, pijenje; ulični lekovi; faktori rizika.

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Introduction

Adolescence is a transition from childhood to adulthood. This is a period of intensive biological growth and sexual, emotional and psychosocial maturation. In this period they want to identify themselves, to experiment, to try out certain behaviours, because of curiosity, desire to imitate someone or self-assertion. Vast majority of adolescents consider starting smoking and drinking alcohol as a reflection of maturity [1–4]. In this period adolescents are prone to risky behaviour. The most common risky behaviour among adolescents is substance use: smoking cigarettes, alcohol consumption and illicit drugs use.

Numerous international studies direct attention to the significant prevalence of substance use: smoking, alcohol consumption and illicit drugs use among adolescents in the first decade of this millennium [5, 6].

Data on the prevalence of substance use among adolescents is very diverse and difficult to follow because of the different research methodologies.

In Europe, the European School Survey Project on Alcohol and Other Drugs (ESPAD) study has been conducted every four years since 1995 in 39 European countries. The ESPAD study controls the frequency of smoking, alcohol and illicit drugs use. Serbia was included in this study in 2005, which is understandable because of political and social events proceeding that period [7, 8]. Monitoring of the incidence of substance use among adolescents has been carried out in the United States of America (USA) since 1975 using the long-term and comprehensive Monitoring the Future (MTF) [9] study, supported by the U.S. National Institutes (NIDA). Another national study Young Risk Behaviour Survey (YRBS) has been conducted since 1991 in the USA [10].

The researches have shown that the trend of prevalence of cigarette smoking is decreasing among adolescents since 1999. It is significantly lower nowadays than in the past. The studies of alcohol use among young people have shown that alcohol use is increasing, especially in developing countries [7, 9, 10]. The prevalence of illicit drug use had an increasing trend from 1991 to 2003 and was followed by a slight decline from 2007 to 2011 in many European countries[7]. However, in the USA the prevalence of illicit drug use is still slightly increasing [9].

Complete and stable families can play a positive role in terms of prevention of risky behaviour among young people [1]. Alienation and non-communication in the family have a great impact on the occurrence of risky behaviour among adolescents. The families are usually unaware of the presence of a problem related to risky behaviour, whether it is connected with alcohol consumption, smoking or use of illicit drugs, until a conflict occurs at school or with the police [2].

Social and economic changes occurring after the collapse of former Yugoslavia resulted in the appearance of social pathology. The appearance of substance use is increasing, especially the consumption of illicit drugs during the last few decades. The researches have shown that the use of illicit drugs increased dramatically over the last decade [1, 8, 11].

Substance abuse moves towards younger ages, and the addiction increases. Because of that, it would be very important to conduct a comprehensive epidemiological study which would provide guidelines for organized and efficient prevention.

The fact that this issue has not been sufficiently explored either in the foreign or in domestic literature encouraged us to explore the prevalence of risky behaviour and its connection with social and demographic conditions. Thus, the aim of this study was to explore the prevalence of substance use among adolescents and its correlation with social and demographic factors.

Methods

This cross-sectional survey was conducted in the period from 2010 to 2011, approved by the Ethical Committee of the University of Medicine in Novi Sad.

The study included adolescents aged 15–19 years, conveniently selected in three secondary schools in Novi Sad during one regular school class period. All the participants were informed about the purpose of the survey (participation was voluntary and anonymous). The survey was administrated through personal contact with respondents and, thus, the occurrence of logic errors was avoided.

The original questionnaire designed for collecting the research data was modelled on a questionnaire about the substance use among adolescents in the Countrywide Integrated Noncommunicable Diseases Intervention Programme (CINDI), which was used in earlier studies in Novi Sad [12, 13].

Each questionnaire had an identification number, ranging from 1 to 600. Improper and under-staffed polls were not taken into account. The response rate to questionnaires distributed was 98.9% (594–275 male and 319 female), with only 1.01% (6) rejected.

The questionnaire contained 15 questions divided into three parts. The first part of the questionnaire contained general questions: year of birth and gender.

The second part contained questions that assessed the social and demographic factors: success in school, place of residence (city, village and suburbs), the family status (living with parents, with single father, with single mother, with relatives or in a boarding school), the economic status (the amount of pocket money weekly). The third part of the questionnaire contained questions related to the assessment of the prevalence of substance use: smoking cigarettes, drinking alcohol and using illicit drugs.

For cigarette smoking, the respondents were asked if they smoke (never, sometimes, every day) and how many cigarettes they smoke daily (0, 1–5, 6–14 or more than 15).

On alcohol, the respondents were also asked two questions. In the first question, they were asked if they drink alcohol, and the answers given were never, sometimes, 3–4 times a month or every day. They were also asked about the type of alcohol they drink the most (wine, beer, etc.).

On drugs, the respondents were asked five questions. Firstly, the respondents were asked whether or not they have tried it at least once during their lifetime. It was examined

how many types of drugs they have tried (the answers given were one, two or three or more). The frequency of illicit drug consumption was measured (once, not more than 7, more than 7). The respondents were asked about first-time use of illicit drug (they were to write the answer on their own). The adolescents were instructed that the illicit drugs include: marijuana or hashish, inhalants (glue), ecstasy, amphetamines (LSD), cocaine, heroin and a combination of pills (sedatives and analgesics, without doctor`s prescription) with alcohol.

The data was computer processed. Statistical analysis was performed in SPSS17. For statistical analyses absolute numbers and percentages, a Pearson χ^2 test and correlation test were used ($p < 0.05$ was statistically significant).

Results

The prevalence of smoking cigarettes among adolescents in Novi Sad is shown in Table 1. There was a statistically significant difference, in terms of daily smoking, between boys and girls ($\chi^2 = 10.55$ $p < 0.01$).

In relation to the number of cigarettes smoked *per* day most girls (41.33%) smoked 6 to 14 cigarettes a day, while the highest percentage of boys (39.47%) smoked over 15 cigarettes, which represents a significant risk for cardiovascular diseases.

Consumption of alcohol was the most common risky behaviour in adolescents, because 74% consumed alcohol occasionally or frequently. Boys consumed alcohol more frequently, and the differences between genders were statistically significant ($\chi^2 = 23.84$, $p = 0.000$) (Table 1).

Girls usually drunk wine (31.69%) and boys usually drunk beer (37.37%). Both genders drunk hard liquor in the same percentage (27.6%).

Male respondents used illicit drugs more often than female and the differences by gender were statistically significant ($\chi^2 = 7.545$, $p = 0.006$) (Table 1).

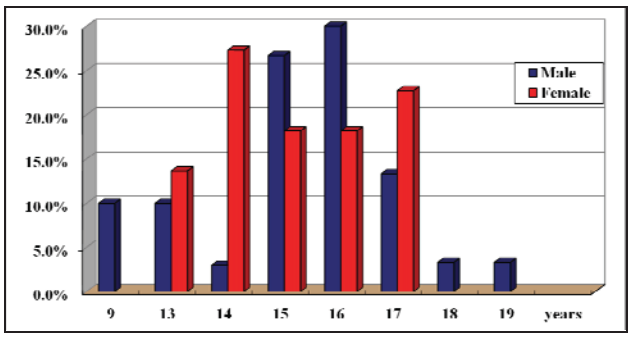

Fig. 1 – Distribution of illicit drugs first-time use according to age and gender.

Observed by the types of illicit drugs, adolescents used mostly marijuana or hashish in 9%, followed by a combination of pills and alcohol, cocaine, LSD and ecstasy. There was no statistically significant difference in types of illegal PAS by gender (Table 2).

Table 2

Distribution of the types of illicit drugs used among adolescents by gender

Substance of abuse	Patients (%)	
	male	female
Marijuana, hashish	12.7	5.9
Pils+alcohol	2.5	1.3
Ectasy	1.5	0.6
LSD	1.5	0.6
Inhalats-glue	1.1	0.0
Heroine	0.7	0.3

Adolescents usually used one type of illicit drugs (59.18%), 18.37% used two types of illicit drugs, but it was a very disturbing fact that 22.45% used more than three illicit drugs.

Table 1

The prevalence of smoking cigarettes, alcohol consumption and lifetime use of illicit drugs

Gender	Male	Female	Total
	n (%)	n (%)	n (%)
Smoking cigarette			
never	200 (72.72)	228 (5.82)	428 (72.05)
sometimes	16 (5.82)	41 (12.85)	57 (9.95)
every day	59 (21.45)**	50 (15.67)	109 (18.35)
Alcohol consumption			
never	46 (18.78)	97 (30.99)	143 (25.63)
sometimes	152 (62.04)	194 (61.98)	345 (62.01)
3–4 times a month	31 (12.65)**	18 (5.75)	49 (8.78)
every day	16 (6.53)**	4 (1.28)	20 (3.58)
Lifetime used illicit drugs			
never	215 (86.34)	287 (91.69)	502 (89.32)
yes	34 (13.65)*	26 (8.30)	60 (10.67)

****$p < 0.01$; *$p < 0.05$.**

A great majority of girls used illicit drugs for the first time at the age of 14 (27.27%). Some boys used illicit drugs for the first time at the age of 9 (10%) but a great majority of boys did that later, at the age of 16 (30.00%) (Figure 1).

The highest percentage of respondents used illicit drugs only once (48.33%), but 25% used them more than 7 times. There were no statistically significant differences between genders ($\chi^2 = 2.41$, $p > 0.05$).

Considering the number of substance used, one in four adolescents (24.77%) used none of the substances that were a health risk (never smoked cigarettes, never consumed alcohol, and never tried illicit drugs). Among them there was a statistically significant prevalence of females ($\chi^2 = 5.94$, $p < 0.05$). Every second adolescent (48.30%) used at least one substance (either smoked cigarettes, or consumed alcohol, or used illicit drugs), with males having statistically significant higher frequency of one substance used than females ($\chi^2 = 4.41$, $p < 0.05$). There were 19.96% respondents using two substances, and 6.95% using all the three substances (smoking cigarettes, consuming alcohol and using illicit drugs). The differences between genders were not statistically significant (Figure 2).

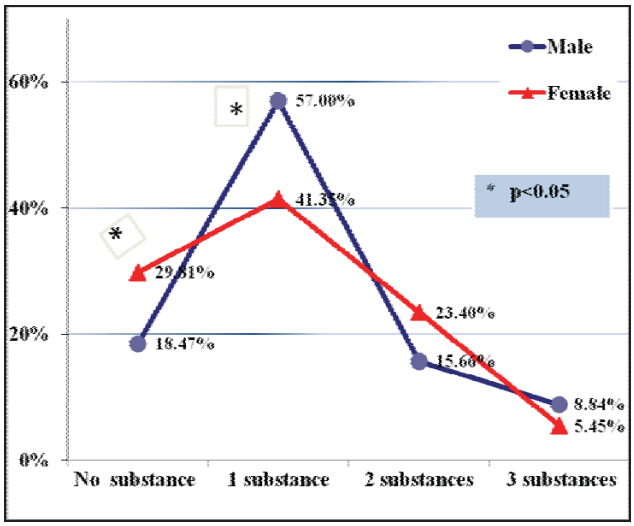

Fig. 2 – Distribution of adolescents according to the number of substances used by gender.

There was a positive correlation between smoking cigarettes, alcohol consumption, and smoking marijuana, but negative between those three and use of other illicit drugs. Those adolescents who smoked cigarettes drunk more alcohol and smoked marijuana frequently. The adolescents who took illicit drugs (except marijuana) smoked and drunk alcohol less frequently (Table 3).

We analysed the correlation between substances use: smoking cigarettes, alcohol consumption, and lifetime use of illicit drugs in adolescents and some social and demographic factors. There was a statistically significant correlation between success at school and the prevalence of substance use. The frequency of substance use was statistically higher in children with poor success at school, so that excellent success at school was a good protective factor for the prevention of substance use. Place of residence (city, village, suburbs) had no effect on the development of substance use. There was a correlation between the family status and smoking of cigarettes, alcohol consumption and lifetime use of illicit drugs. Children who lived in broken homes were more prone to substance use. In relation to the higher economic status there was a statistically significant association with smoking cigarettes and consumption of alcohol, but not with lifetime use of illicit drugs. Significantly more smokers and drinkers were found among those who had much pocket money. Within our respondents the use of illicit drugs was not dependent on the amount of weekly pocket money (Table 4).

Discussion

From all the forms of risky behaviour in adolescence, smoking cigarettes, consumption of alcohol and use of illicit drugs particularly stand out, because of the frequency and the degree of prevalence of use, and because of their impact on youth development in this sensitive stage of growing up [14].

Our results of the prevalence of smoking among adolescents are higher than in the previous studies (2008) in the regional centres in Serbia: Novi Sad (25%), Belgrade (22%), Niš (18.9%) [8] and Kragujevac (21%) [15].

Prevention of smoking among adolescents is a key factor for reducing morbidity and mortality caused by (or associated with) smoking [1, 16, 17], and in many developed countries it has a very important place.

The trend of decreasing prevalence of smoking was been reported in Serbia in the period 2006–2012 from 25.5% [18] to 20% [7]. This trend is a result of implementation of

Table 3

Correlation of lifetime use of illicit drugs with smoking of cigarettes and alcohol consumption

The investigated parameters	Smoked cigarettes	Drinks alcohol
Smoked cigarettes		371**
Drinks alcohol	371**	
Lifetime use of marijuana	0.473**	0.720**
Lifetime use of illicit drugs	-0.415	-0.423**

****Correlation significant at the 0.01 level (Pearson correlation).**

Table 4

Correlation between smoking, alcohol consumption, lifetime use of illicit drugs and social and demographic factors

The investigated parameters	Smoking of cigarettes	Alcohol consumption	Use of illicit drugs
Success in school	-0.236**	-0.211**	0.237**
Place of residence	0.019	0.064	-0.002
Family status	0.094*	0.072	-0.138**
Economic status	0.154**	0.098*	-0.063

*** $p < 0.05$ level**; $p < 0.01$ level (Pearson correlation).**

intensive measures to prevent smoking, including the introduction of statutory ban on smoking in public places [19].

The European average prevalence of smoking among adolescents is 28% (ESPAD study, 2011), which is the same as in our results. A higher prevalence of smokers has been reported in Croatia, Austria, Bulgaria, Czech Republic and Lithuania (38–45%). Bosnia and Herzegovina, Norway, Albania, Montenegro, and Iceland have a lower prevalence of smokers (10–19%) [7].

The ESPAD study shows that the prevalence of cigarette smoking is increasing in many developing countries, while it is declining in developed countries [7].

The trend of decreasing prevalence of smoking was also reported in the USA (MTF study and YRBS study) in the period between 1999 and 2011 from 35% to 18% [9, 10].

The Global Youth Tobacco Survey (GYTS) [20] has shown that the prevalence of smoking among adolescents is higher for boys (28%) than for girls (18%). Our results show that more boys than girls smoke cigarettes daily, while occasional smoking has no significant difference by sex. In many developed European countries the prevalence of smoking among girls exceeds that among boys, but in underdeveloped countries the prevalence is higher among boys than among girls [7, 20].

Alcoholism is one of the most common diseases in modern population. Consumption of alcohol represents one of the most widespread types of risky behaviour among adolescents in our country and in many countries around the world with increasing character.

According to the earlier survey for Serbia, the percentage of adolescents who consume alcohol in the regional centres: Novi Sad (90.7%), Belgrade (90.6%), Niš (87.9%) [8] was significantly higher than in our study.

The results of YUSAD study in Novi Sad (1995–2008) indicated an increasing trend of prevalence of alcohol consumption among adolescents, from 65% to 74% [16] and our results are similar.

Our results show a lower prevalence of occasional alcohol consumption than the one in Europe (average in Europe is 79%). Our prevalence is similar to those in Sweden, Romania (71–74%) [7] and the USA (71%) [9, 10]. A higher prevalence has been noted in two thirds of ESPAD countries (82–93%). A lower percentage of adolescents who consume alcohol have been noted in Sweden, Montenegro Norway, Albania, and Iceland (65–43%) [7].

In comparison to gender structure in most European countries, where the research was made, boys more frequently consume alcohol than girls, which is the same as in our results, but different from three countries in Europe (Iceland, Latvia and Sweden) [7] and also different from studies in the USA where girls consume alcohol more frequently [10].

Many of the adolescents have lifetime use of illicit drugs only once or twice, while others use such drugs more often. In the previous survey in the regional centres in Serbia (2008) [8], 15.1% of adolescents (age 15–19) used illicit drugs at least once in their lifetime (17.2% in Belgrade, 15.8% in Novi Sad and 14% in Niš). Our results show a significantly lower prevalence of lifetime use of illicit drugs among adolescents.

The European average in lifetime use of illicit drugs (18%) [7] is higher than the prevalence in our research. A significantly higher prevalence was found in two-thirds of the ESPAD countries (19–43%) [7] and in the USA (43%) [9, 10]. Serbia (on average, 8%) is among the European countries with the lowest prevalence of lifetime use of illicit drugs (5–9%) [7].

In more than two-thirds of the ESPAD countries significantly more boys than girls (21% boys and 15% girls) try illicit drugs at least once in their lifetime [7].

The adolescents usually experiment with marijuana (boys more often than girls) [7, 8, 21], as well as in our study. In the earlier studies in Serbia there was a reduction in lifetime prevalence of marijuana among adolescents from regional centres (Belgrade, Niš, Novi Sad) from 12.9% to 7.3% in 2008 [21]. Our results show a slightly higher prevalence in lifetime use of marijuana (9%).

The average European prevalence of marijuana consumption is 17% [7] and it is higher than the prevalence of marijuana use in our research and in Serbia [7] (average 7%). The lowest prevalence of marijuana use is in Montenegro, Norway, Bosnia and Herzegovina, Albania (4–5%) and the highest prevalence is in the Czech Republic, France, Monaco (39–40%) [7] and the USA (40%) [9, 10].

Our results show that the highest percentage of adolescents used illicit drugs for the first time between 14 and 16 years, which is similar to the results of Backović et al. [2], Kosić et al. [21].

Our respondents use cocaine, LSD and ecstasy which is similar to the results of the earlier study in Serbia (1.5%) [21], but is lower than the European average which is 3% [7] .The higher prevalence is reported in the USA (4.1%) [9, 10].

Our results indicate that adolescents usually try only one type of illicit drugs (60%), which is more than the European average (18%) [7] but the fact that 22% of adolescents try more than three illegal drugs is not insignificant. In the Czech Republic, France and Monaco, 10% adolescents use illicit drugs 20 times or more [7].

One of the new trends is combining alcohol with different illicit drugs. It is observed that the most common combination is alcohol with pills (sedatives and analgesics without doctor's prescription) or with marijuana among the young people [2]. In an earlier survey in Serbia prevalence of using alcohol with pills was 2.7% [8]. The our respondents use alcohol with pills in 3.36%, which is slightly lower than the European average (5%). The higher prevalence exists in the Czech Republic (16%), and relatively large rates (10%) are also found in Croatia, Hungary, while only 1–2% is found in Belgium, Bosnia and Herzegovina, Iceland, Montenegro, Norway and Ukraine [7].

The results of the ESPAD [7] study in Serbia show a lower prevalence of substance use than indicated by our results, because they covered only adolescents aged 15–16 years.

Cigarette smoking is often associated with the use of other substances, so it is estimated that young people who smoke, consume alcohol three times more and consume

marijuana eight times more than the ones who are non-smokers [2, 11, 22, 23].

Some recent studies confirm that alcohol, marijuana and illicit drugs consumption among young people are connected [24]. Our research demonstrates a connection between smoking and alcohol consumption and using marijuana. Specifically, it was shown that adolescents who consume alcohol and smoke cigarettes more frequently use marijuana. This data is consistent with the results of Slater et al. [25] and Faeha et al. [26] who showed that young people usually use different types of substances.

The results of a meta-analyse [27, 28] indicate that the effect of quitting smoking increases the likelihood of abstinence from alcohol and illicit drugs on average to 25%.

Our results indicate that the adolescents are endangered by the substance use, because 7% of respondents use three substances (smoking cigarettes, drinking alcohol and consuming illicit drugs). One in five adolescents use two substances, regardless of gender and every second adolescent (more boys) uses one substance. Only one quarter of adolescents (more girls) do not use any substance. Due to the lack of data available in the literature, which would include three substances used by adolescents comparisons were not carried out.

The prevalence of substance use among adolescents is associated with several social and demographic factors. The frequency of substance use is higher in adolescents with poor scholarly success. Success at school represents a good protective factor in the prevention of substance use as shown also in the previous research which has been done in this area [4, 12].

Our results show a positive correlation between family status and the use of substances. This implies that stable families are a protective factor in prevention of substance use among young people which is similar to the results of the other authors. Problems in the family, a single parent, divorced parents or living in a foster family represent significant predictors of drug abuse among young people. Adolescents from dysfunctional families tend to start consuming illicit drugs and alcoholic beverages and to drink at earlier age in comparison to adolescents from functional families. Children who grow up in foster families use marijuana more often (38.8%), compared to children from biological families (8.6%) and their first-time-used was earlier (aged between 11 and 14) [29–31]. In contrast, positive, intimate relationships between parents and adolescents are associated with decreased risk of smoking cigarette and alcohol consumption [32].

Our results indicate a statistically significant correlation between the amount of pocket money that adolescents get weekly and smoking cigarettes and alcohol consumption, just as in previous surveys (1995) made in these areas [12]. Low social and economic status is also a significant predictor of illicit drugs abuse among young people [33].

Substance abuse is going towards younger ages and addiction to substance use increases. Therefore it is very important to conduct a comprehensive epidemiological study which would give guidelines for organized and efficient prevention. Measures to prevent and control risk have to be organized and synchronized, and they need to include individuals, families, schools, health services and society. It is particularly important to find a solution for those problems.

Education is proved to be the best way to encourage young people to resist the risky behaviour and to adopt positive lifestyle behaviour. Education is an important prerequisite for the promotion and preservation of health among young people [10, 11, 16].

Prevention activities should be carried out from the early childhood, at all levels of society, simultaneously and continuously. This is the only way to stop spreading of the epidemic of the new century, and of all health and social consequences that it brings.

Conclusion

The prevalence of substances use among adolescents is very high. One third of adolescents smoke cigarettes, two thirds drink alcohol occasionally, and one in ten uses illicit drugs.

Stable family, lower amount of pocket money weekly and good school performance are protective factors in the prevention of substance use among adolescents.

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Correlation between the Finnish Diabetes risk Score and the severity of coronary artery disease

Međusobni odnos Finskog skora rizika od dijabetesa i stepena težine koronarne arterijske bolesti

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Abstract

Background/Aim. The FINish Diabetes RIsk SCore (FINDRISC) which includes age, body mass index (BMI), waist circumference, physical (in) activity, diet, arterial hypertension, history of high glucose levels, and family history of diabetes, is of a great significance in identifying patients with impaired glucose tolerance and a 10-year risk assessment of developing type 2 diabetes in adults. Due to the fact that the FINDRISC score includes parameters which are risk factors for coronary artery disease (CAD), our aim was to determine a correlation between this score, and some of its parameters respectively, with the severity of angiographically verified CAD in patients with stable angina in two ways: according to the Synergy between Percutaneous Coronary Intervention with Taxus and Cardiac Surgery (SYNTAX) score and the number of diseased coronary arteries. **Methods.** The study included 70 patients with stable angina consecutively admitted to the Clinic of Cardiology, Military Medical Academy, Belgrade. The FINDRISC score was calculated in all the patients immediately prior to angiography. Venous blood samples were collected and inflammatory markers [erythrocyte sedimentation rate (ESR), leucocytes, C-reactive protein (CRP), total cholesterol, HDL cholesterol, triglycerides and fasting glucose] determined. Coronary angiography was performed in order to determine the severity of coronary artery disease according to the SYNTAX score and the number of affected coronary vessels: 1-vessel, 2-vessel or 3-vessel disease (hemodynamically significant stenoses: more than 70% of the blood vessel lumen). The patients were divided into three groups regarding the FINDRISC score: group I: 5-11 points; group II: 12–16 points; group III: 17–22 points. **Results.** Out of 70 patients (52 men and 18 women) enrolled in this study, 14 had normal coronary angiogram. There was a statistically significant positive correlation between the FINDRISC score and its parameters respectively (age, body mass index-BMI, waist circumference) and the severity of CAD according to the SYNTAX score ($p < 0.001$) and the number of diseased coronary arteries ($p < 0.001$). The patients with higher FINDRISC score (groups II and III) had more severe and extensive CAD according to the SYNTAX score than the group I. The odds ratio with 95% confidence intervals (CI) between the group III and the group I was 5.143 (95% CI 1.299–20.360, $p = 0.002$) and between the group II and the group I 5.867 (95% CI 1.590–21.525, $p = 0.007$). There were no differences in odds ratio for multivessel disease according to FINDRISC score between the group II and the group III [1.141; (95% CI 0.348–3.734). In the group I mean SYNTAX score was 5.18, and more than 70% of patients had normal coronary angiogram. In the group II mean SYNTAX score was 17.06, and more than 70% of patients had 2-vessel disease and 3-vessel disease, and in the group III mean SYNTAX score was 18.89, and 2-vessel and 3-vessel disease had 36.36% and 31.82% patients, respectively. In multiple regression analysis, where SYNTAX score was dependent variable, and age, BMI, waist circumference, FINDRISC score were independent variables, we found that only FINDRISC score was independent predictor of SYNTAX score. **Conclusion.** The obtained results suggest a statistically significant correlation between the FINDRISC score and its parameters (age, BMI, waist circumference) and the severity of CAD according to the SYNTAX score and the number of diseased coronary arteries. The FINDRISC score may be useful in identifying patients at the high risk for coronary artery disease.

Key words:
coronary artery disease; disease progression; risk assessement; diabetes mellitus, type 2; risk factors.

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Apstrakt

Uvod/Cilj. Finski skor rizika od dijabetesa (FINDRISC) koji obuhvata nekoliko parametara (godine života, istorija arterijske hipertenzije, indeks telesne mase – BMI, fizička (ne)aktivnost, obim struka, konzumiranje voća, ranije registrovana hiperglikemija, porodično opterećenje za dijabetes) ima puno značaja za identifikaciju bolesnika sa poremećajem glikoregulacije i za procenu 10-godišnjeg rizika od nastanka dijabetesa melitusa tipa 2. Pošto skor FINDRISC čine parametri koji su faktori rizika od koronarne arterijske bolesti (KAB), naš cilj bio je da ispitamo međusobni odnos ovog skora i nekih njegovih pojedinačnih parametara i stepena težine KAB kod bolesnika sa simptomima stabilne angine pektoris na 2 načina: prema skoru *Synergy between Percutaneaus Coronary Intervention with Percutaneans Coronary Intervention with Taxus and Cardiac Surgery* (SYNTAX) i prema broju zahvaćenih krvnih sudova srca. **Metode.** Ispitivanjem je obuhvaćeno 70 bolesnika, hospitalizovanih u Klinici za kardiologiju Vojnomedicinske akademije zbog tegoba tipa stabilne angine pektoris, koji su davali odgovore na pitanja iz upitnika FINDRISC, i kojima su određivane vrednosti inflamatornih markera [sedimentacija eritrocita (SE), leukociti, C-reaktivni protein (CRP), lipidni status (ukupni holesterol, HDL holesterol, trigliceridi), kao i glikemija]. Svim ispitanicima je urađena koronarografija radi utvrđivanja stepena težine KAB prema skoru SYNTAX, kao i prema broju zahvaćenih krvnih sudova srca: jednosudovna, dvosudovna ili trosudovna (hemodinamski značajnim stenozama su smatrane stenoze koje zahvataju više od 70% lumena krvnog suda). Svi bolesnici bili su podeljeni u III grupe u zavisnosti od vrednosti skora FINDRISC (grupa I:5–11 poena, grupa II:12–16 poena, grupa III:17–22 poena). **Rezultati.** Ispitivanjem je bilo obuhvaćeno 52 muškarca i 18 žena. Od 70 ispitanika uključenih u studiju, 14 je imalo normalan koronarografski nalaz. Utvrđena je statistički značajna povezanost između skora FINDRISC, kao i njegovih pojedinačnih parametara (godine, indeks telesne mase, obim struka) sa stepenom težine koronarne arterijske bolesti prema skoru SYNTAX ($p < 0.001$), kao i prema broju zahvaćenih krvnih sudova srca ($p = 0,007$). Šansa za postojanje višesudovne bolesti između grupa III i I iznosila je 5,143 (95% CI 1,299–20,360, $p = 0,002$), a između grupe II i grupe I 5,867 (95% CI 1,590–21,525, $p = 0,007$). Nije bilo statistički značajne razlike između grupe II i grupe III 1,141; (95% CI 0,348- 3,734). U grupi I prosečna vrednost SYNTAX skora iznosila je 5,18, a više od 70% bolesnika je imalo normalan koronarografski nalaz. U grupi II prosečna vrednost SYNTAX skora iznosila je 17,06, a više od 70% bolesnika je imalo dvosudovnu ili trosudovnu KAB. U III grupi prosečna vrednost SYNTAX skora je iznosila 18,89, dvosudovnu i trosudovnu KAB imalo je 36,36%, tj. 31,82% ispitanika. U multiploj regresionoj analizi, gde je skor SYNTAX bio zavisna varijabla, a godine života, BMI, obim struka i skor FINDRISC nezavisne varijable nađeno je da je samo skor FINDRISC bio nezavisan prediktor SYNTAX skora. **Zaključak.** Dobijeni rezultati pokazuju da postoji značajna povezanost skora FINDRISC i njegovih pojedinačnih parametara (godine života, indeks telesne mase, obim struka) sa stepenom težine koronarne arterijske bolesti prema SYNTAX skoru, kao i prema broju zahvaćenih krvnih sudova srca. Skor FINDRISC može biti koristan za identifikaciju bolesnika koji imaju povišen rizik od nastanka koronarne arterijske bolesti.

Ključne reči:
koronarna bolest; bolest, progresija; rizik, procena; diabetes melitus, tip 2, faktori rizika.

Introduction

Coronary artery disease (CAD) is the major cause of mortality in the whole world and also in our country. The FINish Diabetes Risk (FINDRISC) score, which includes the following parameters: age, history of hypertension, body mass index (BMI), physical (in)activity, waist circumference, fruit, vegetables or berries consumption, previously registered hyperglycemia, family history of diabetes, is of a great importance in identifying patients with impaired glucose tolerance and a 10-year risk assessment of developing type 2 diabetes in adults.

Independent risk factor surveys [1] were conducted in Finland, comprising 8,268 men and 9,457 women, aged 25–64 years and free of coronary heart disease (CHD) and stroke at baseline. During the 14-year follow-up 699 incident acute CHD events, 324 acute stroke events, and 765 deaths occurred, and the study confirmed that the FINDRISC score is a reasonably good predictor of CHD, stroke and total mortality in apparently healthy population. A recent study [2] has demonstrated that FINDRISC screening tool has high sensitivity and specificity for detecting diabetes mellitus and impaired glucose tolerance (IGT) and it is a feasible noninvasive tool for screening high-risk individuals in an outpatient heart failure cohort, but there are no available data regarding its prognostic validity in primary prevention and cardiovascular risk assessment in patients with stable angina pectoris.

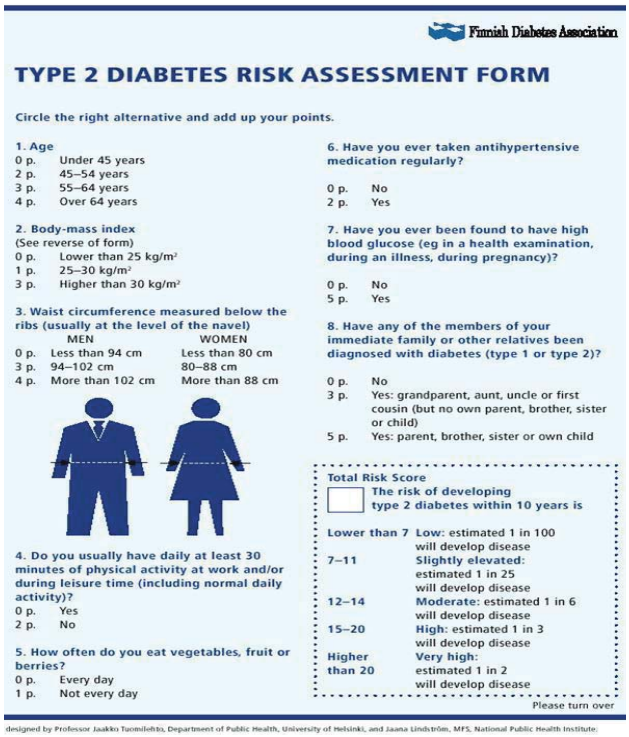
Assessment of CAD severity is a major challenge that cardiologists face every day in their clinical practice. The investigators of the Synergy between Percutaneous Coronary Intervention with Taxus and Cardiac Surgery (SYNTAX) trial developed and validated anatomic scoring system for severity of CAD, which not only quantifies lesion complexity based entirely on angiographic characteristics (the presence of total occlusion, bifurcation or trifurcation, angle and involvement of branch vessels, calcification, lesion length, ostial location, tortuosity and presence of thrombus) [3, 4], but also predicts outcome after PCI in patients with extensive coronary artery disease [5].

Due to the fact that FINDRISC score includes parameters which are risk factors for CAD, we decide do determine correlation between this score, and some of its parameters respectively, with the severity of angiographically verified CAD in patients with stable angina in two ways: according to the SYNTAX score and the number of diseased coronary arteries .

Methods

The study conducted at the Military Medical Academy in Belgrade, included 70 patients with symptoms of stable angina and a number of risk factors for CAD, who were admitted to the Clinic of Cardiology in order to perform coronary angiography. Most patients initially underwent noninvasive cardiac assessment, which include exercise test, or pharmacological dobutamine stress ECHO (assessed as positive for coronary artery disease), while the other had evidence of CAD (previous myocardial infarction, previous percutaneous coronary intervention with stent implantation or revascularization). All the patients with acute or chronic infectious disease, those with chemotherapy or radiotherapy, and those with acute coronary syndrome were excluded. The FINDRISC score was calculated in all the patients immediately prior to angiography. Venous blood samples were collected: inflammatory markers [leucocytes, erytrocyte sedimentation rate (ESR), C-reactive protein (CRP) - we didn`t use high-sensitive CRP, the lowest value was 3.08 mg/dL), total cholesterol, HDL cholesterol, triglycerides and fasting glucose].

We used the FINDRISC score (Figure 1) which includes the following parameters: 1) age: 0 point < 45 years; 2 points: 45–54 years; 3 points: 55–64 years, 4 points > 64


Finnish Diabetes Association

TYPE 2 DIABETES RISK ASSESSMENT FORM

Circle the right alternative and add up your points.

1. Age
0 p. Under 45 years
2 p. 45–54 years
3 p. 55–64 years
4 p. Over 64 years

2. Body-mass index
(See reverse of form)
0 p. Lower than 25 kg/m²
1 p. 25–30 kg/m²
3 p. Higher than 30 kg/m²

3. Waist circumference measured below the ribs (usually at the level of the navel)

	MEN	WOMEN
0 p.	Less than 94 cm	Less than 80 cm
3 p.	94–102 cm	80–88 cm
4 p.	More than 102 cm	More than 88 cm

4. Do you usually have daily at least 30 minutes of physical activity at work and/or during leisure time (including normal daily activity)?
0 p. Yes
2 p. No

5. How often do you eat vegetables, fruit or berries?
0 p. Every day
1 p. Not every day

6. Have you ever taken antihypertensive medication regularly?
0 p. No
2 p. Yes

7. Have you ever been found to have high blood glucose (eg in a health examination, during an illness, during pregnancy)?
0 p. No
5 p. Yes

8. Have any of the members of your immediate family or other relatives been diagnosed with diabetes (type 1 or type 2)?
0 p. No
3 p. Yes: grandparent, aunt, uncle or first cousin (but no own parent, brother, sister or child)
5 p. Yes: parent, brother, sister or own child

Total Risk Score
The risk of developing type 2 diabetes within 10 years is

Lower than 7	Low: estimated 1 in 100 will develop disease
7–11	Slightly elevated: estimated 1 in 25 will develop disease
12–14	Moderate: estimated 1 in 6 will develop disease
15–20	High: estimated 1 in 3 will develop disease
Higher than 20	Very high: estimated 1 in 2 will develop disease

Please turn over

designed by Professor Jaakko Tuomilehto, Department of Public Health, University of Helsinki, and Jaana Lindström, MFS, National Public Health Institute.

Fig. 1 – Finish Diabetes Risk SCore (FINDRISC) to assess the 10-year risk of type 2 diabetes mellitus in adults

years; 2) history of arterial hypertension: 0 point: no, 2 points: yes; 3) BMI: 0 points < 25 kg/m^2, 1 point 25–30 kg/m^2, 3 points > 30 kg/m^2; 4) physical activity: 0 point: yes, 2 points: no; 5) waist circumference: in men 0 point < 94 cm, 3 points 94–102 cm, 4 points > 102 cm in women 0 point < 80 cm, 3 points 80-88 cm, 4 points > 88 cm; 6) fruit, vegetables or berries consumption: 0 point: every day; 1 point: not every day; 7) previously registered hyperglycemia: 0 point: no, 5 points: yes; 8) family history of diabetes: 0 point: no, 3 points yes (grandparent, aunt, uncle, first cousin), 5 points: yes (parent, brother, sister, own child).

All the patients were divided into three groups regarding the FINDRISC score (the group I: 5–11 points, the group II: 12–16 points, the group III: 17–22 points). All the subjects underwent coronary angiography to determine the severity of CAD according to the SYNTAX score (version 2.11) and the number of affected coronary vessels: 1-vessel, 2-vessel or 3-vessel disease (hemodynamically significant stenoses: more than 70% of the blood vessel lumen).

Statistical analysis was performed using SPSS statistical software, a significant difference between the groups was determined by factorial analysis of variance (ANOVA), and χ^2 test to assess the significance of the difference frequency. Nonparametric Spearman coefficient r was used for assessment of correlations between the groups. Statistically significant differences are marked with probability $p < 0.05$.

Comparison of the groups was performed with a multivariable logistic regression analysis and the results were described as odds ratios (Mantel-Haenszel common odds ratios - OR) with 95% confidence intervals (95% CIs). We used multiple regression analysis in order to find the best predictor for the SYNTAX score, as dependent variable, and age, BMI, waist circumference and the FINDRISC score as independent variables.

Results

Baseline characteristics of the patient of all the 3 groups are summarized in Table 1. The patients in the group III were significantly older than the patients in the groups I and II. The patients in groups I and III were mainly female. There was a statistically significant positive correlation between some of the FINDRISC score parameters (age, BMI and waist circumference) and the severity of CAD according to the SYNTAX score ($p < 0.05$). Also, there was a statistically significant correlation between fasting glucose, HDL- cholesterol and the severity of CAD according to SYNTAX score ($p < 0.05$). On the other hand, there was no statistically significant correlation between triglycerides, total cholesterol, creatinine, left ventricular ejection fraction (LVEF) and active smoking and the severity of CAD.

We assessed whether the FINDRISC score could be considered a marker of high cardiovascular risk. There was a statistically significant positive correlation between the FINDRISC score and the severity of CAD according to the SYNTAX score ($p < 0.001$) (Figure 2).

Next, we assessed the OR for multivessel disease according to the FINDRISC score in 3 study groups. Comparison of the groups was performed with multivariable logistic regression analysis and the results were described as OR (Mantel-Haenszel common OR) with 95% CIs. The patients with a higher FINDRISC score (groups II and III) had more severe and extensive CAD according to the SYNTAX score than the group I. The hazard ratio with 95% confidence intervals between the groups III and I was 5.143 (95% CI

Table 1

Baseline characteristics of 3 groups of patients

Characteristics	Group I (n = 17) (5–11 points)	Group II (n = 31) (12–16 points)	Group III (n = 22) (17–22 points)	*p*
Age (years), $\bar{x} \pm SD$	57 ± 8.5	58 ± 9.2	64 ± 6.2	0.017
Sex, [n (%)]				
female	7 (41.2)	2 (6.5)	9 (40.9)	0.004
male	10 (58.8)	29 (93.5)	13 (59.1)	
Glucose (fasting) (mmol/L), $\bar{x} \pm SD$	5.08 ± 0.70	4.97 ± 0.64	5.71 ± 0.93	0.002
Triglycerides (mmol/L), $\bar{x} \pm SD$	1.64 ± 0.91	1.80 ± 1.09	1.75 ± 0.68	0.852
Cholesterol (mmol/L), $\bar{x} \pm SD$	5.74 ± 1.25	5.21 ± 1.26	5.40 ± 1.24	0.376
HDL-cholesterol (mmol/L), $\bar{x} \pm SD$	1.19 ± 0.30	1.01 ± 0.16	1.08 ± 0.25	0.032
Creatinine (μmol/L), $\bar{x} \pm SD$	98.18 ± 21.16	96.90 ± 17.73	101.50 ± 15.80	0.655
Leukocytes (x 10^9), $\bar{x} \pm SD$	6.70 ± 0.90	6.89 ± 1.29	7.42 ± 2.43	0.358
ESR (mm/h), mediana (25–75 percentiles)	7 (6–11)	14 (9–17)	14.5 (11–22)	0.136
Active smoking, n (%)	9 (52.9)	24 (77.4)	14 (63.6)	0.213
BMI (kg/m^2), $\bar{x} \pm SD$	26.50 ± 3.09	25.83 ± 2.73	29.72 ± 4.97	0.001
Waist circumference (cm), $\bar{x} \pm SD$	93.18 ± 6.69	96.10 ± 5.36	99.82 ± 6.27	0.004
LVEF (%), $\bar{x} \pm SD$	59.18 ± 4.43	57.52 ± 7.83	57.41 ± 5.70	0.644
Number of diseased coronary arteries, $\bar{x} \pm SD$	0.71 ± 1.16	1.94 ± 0.81	1.95 ± 0.90	< 0.001

HDL – high density lipoprotein; ESR – erytrocyte sedimentation rate; BMI – body mass index; LVEF – left ventricular ejection fraction.

1.299–20.360, $p = 0.002$). The most notable difference in OR for multivessel disease according to FINDRISC score in our study was between the group II and the group I (5.867; 95% CI 1.599- 21.525, $p = 0.007$) as shown in Figure 3. The low-risk patients for CAD were in the group I (FINDRISC score 5–11).

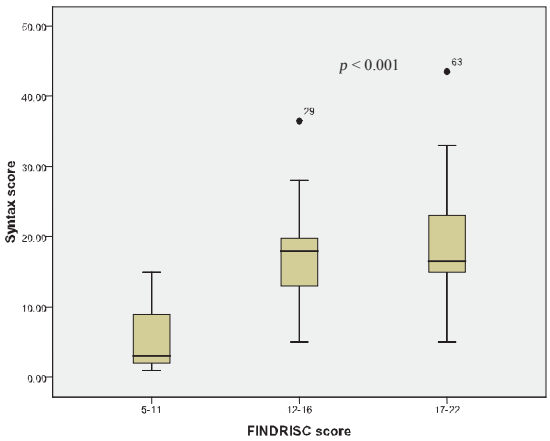


Fig. 2 – Relationship between the Coronary Intervention with Taxus and the Cardiac Surgery (SYNTAX) score and Finish Diabetes Risk SCore (FINDRISC) in the study groups.
The group I: 5–11 points; the group II: 12–16 points; the group III: 17–22 points.

There were no differences in the OR for multivessel disease according to the FINDRISC score between the groups II and III, (1,141; 95% CI 0.348–3.734), which means that all the patients with a score greater than or equal to 12 points were high-risk patients for CAD.

There was a statistically significant positive correlation between the FINDRISC score and the number of affected coronary vessels (total risk $p < 0.001$) (Table 1). In the groups of patients with the score greater than or equal to 12 points more than 70% of the patients had 2-vessel or 3-vessel CAD. In contrast, the patients in the group I had low risk for CAD.

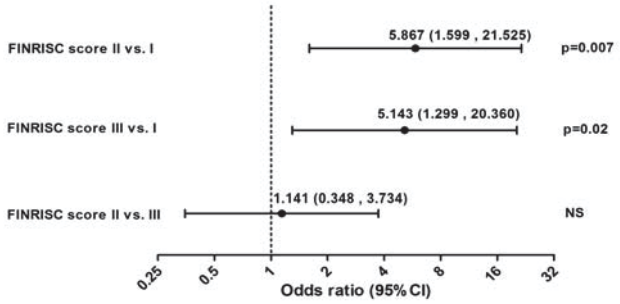


Fig. 3 – Odds ratio with 95% confidence intervals for multivessel disease according to the Finish Diabetes Risk SCore (FINDRISC) in the study groups.
The group I: 5–11 points; the group II: 12–16 points; the group III: 17–22 points.

The patients in the group II (mean value 1.94 ± 08) and the group III (mean value 1.95 ± 0.90) had multivessel CAD in comparison to the patients in the group I (mean 0.71 ± 1.16), i.e. they approximately had 2-vessel CAD. In the group I more than 70% of the patients had normal coronary angiogram; 2-vessel and 3-vessel disease had 17.65% and 11.76% patients, respectively. In the group II more than 70% of the patients had 2-vessel and 3-vessel disease, 25.81% had 1-vessel disease, and only 3% had normal coronary angiogram. In the group III only 4.55% had normal coronary angiogram, 1-vessel disease had 27.27% of the patients, 2-vessel and 3-vessel disease had 36.36% and 31.82% of patients, respectively. CAD was more frequent in the groups II and III than in the group I. The patients who were taking statins, thienopyridines, and nitrates had multivessel disease, which means that they were under optimal medical therapy.

BMI in the group III was higher than in the groups I and II which was statistically significant difference ($p = 0.001$). There was a linear increase in waist circumference through the 3 groups of the patients which was, also, statistically significant ($p = 0.004$).

The values of BMI and waist circumference in the 3 groups of the patients are presented in Figures 4 and 5.

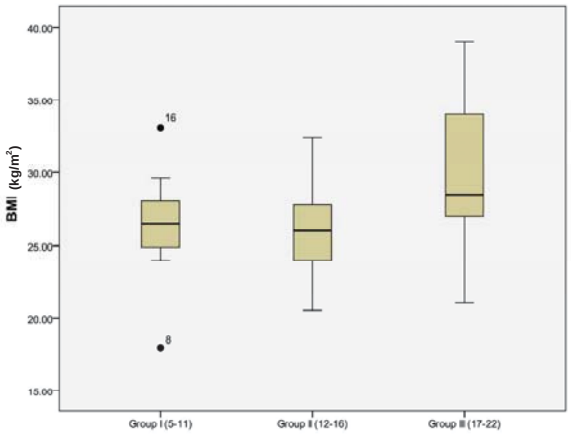


Fig. 4 – Body mass index (BMI) for all the study groups.

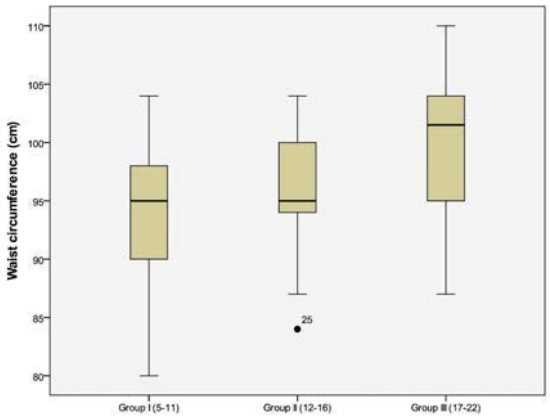


Fig. 5 – Waist circumference for all the study groups.

In multiple regression analysis, where the SYNTAX score was a dependent variable, and age, BMI, waist circumference and the FINDRISC score independent variables we found that only the FINDRISC score was an independent predictor of the SYNTAX score (SYNTAX score = 1.167 + 0.553 × FINDRISC score, $p < 0.001$).

Finally, we studied the correlation between inflammatory markers (ESR, CRP and leukocytes) and severity of angiographically verified CAD according to the SYNTAX score. There was no statistical significance in the values of inflammatory markers between the 3 groups of the patients (Table 1). There was no statistically significant difference in the values of inflammatory markers among the groups I and III: $r = 0.877$ CRP (groups III and I), $p < 0.05$; $r = 0.471$ ESR (groups III and I), $p < 0.05$; $r = 0.790$ Le (groups III and I), $p < 0.05$.

Discussion

To the best of our knowledge, this is the first study about the correlation between the FINDRISC score and the severity of CAD according to the SYNTAX score.

The main result of our study is that there is a statistically significant correlation between the FINDRISC score and its parameters respectively (age, BMI, waist circumference) and the severity of CAD in the patients with stable angina in two ways: according to the SYNTAX score and the number of diseased coronary arteries.

A recent survey [1] has confirmed that FINDRISC screening is a good predictor of coronary heart disease, stroke and total mortality in apparently healthy population, but in our study we tried to determine its prognostic validity in cardiovascular risk assessment in the patients with stable angina. The results obtained in our study are fully consistent with other studies [6], where the correlation between some FINDRISC score parameters, which are classic cardiovascular (CV) risk factors, and CAD were investigated. However, we used the FINDRISC score in order to determine correlation between this score, and the severity of angiographically verified CAD. Our data demonstrated that higher FINDRISC scores indicate a more severe and extensive coronary artery disease according to the SYNTAX score and the number of diseased coronary arteries. The patients in the groups II and III had a higher odds ratio for multivessel disease than in the group I (Figure 3). The most notable difference in our study was between the groups II and I.

There was a statistically significant correlation between some FINDRISC score parameters (BMI, waist circumference) and the severity of CAD. BMI and waist circumference in the group III were higher than in the groups I and II which supports the previously proven association between obesity and the presence of CAD. Obese individuals with increased waist circumference, i.e. those with a higher FINDRISC score, have insulin resistance, which contributes to the development of metabolic syndrome, type 2 diabetes and CAD. A large number of studies [7] have demonstrated that obesity, i.e. BMI greater than 30 kg/m^2, is a significant risk factor for CV disease and death, as well as the development of diabetes. The patients with metabolic syndrome have a two times increased risk of myocardial infarction or stroke compared to healthy population and five times higher risk of diabetes mellitus type 2 (RR 3.77%) [7].

Similar to our study, the large multicenter study (International Day for the Evaluation of Abdominal Obesity – IDEA) [8], which evaluated the correlation between waist circumference and BMI with cardiometabolic risk factors in primary prevention, demonstrated the increased risk of CV disease in patients of both genders, who have a higher waist circumference and BMI. The study, also, showed that the incidence of diabetes can be reduced by 10%, by the reduction of abdominal obesity, which reduces the risk of CAD. Another important study, INTERHEART (a Global Study of Risk Factors for Acute Myocardial Infaction) [9, 10], indicated that abdominal obesity rather than BMI significantly increases the risk of myocardial infarction, which was confirmed in the European Prospective Investigation into Cancer and Nutrition (EPIC-Norfolk) study [11], in 24,508 patients during 9.1 years of follow-up. Abdominal obesity is associated with insulin resistance, which is proinflammatory, proatherogenic [12], with thrombotic potential. Taking into ac-

count the mutual relationship of diabetes and obesity for the development of CV diseases, body weight reduction is very important [13]. Physical activity significantly reduces the risk of type 2 diabetes mellitus, improves insulin resistance, reduces body weight and risk of abdominal obesity. We showed that low risk patients for CAD were in the group of patients with a lower BMI and waist circumference (the group I).

It has been shown that patients with a BMI greater than 25 kg/m^2 have a 3-fold higher risk of developing type 2 diabetes, those with a BMI greater than 30 kg/m^2 have a 10 times higher risk, while those with a BMI greater than 35 kg/m^2 have a 40 times greater risk [14]. A large study (National Health Nutrition Examination Survey) on 15.000 patients [15] demonstrated that increase in waist circumference by 2.5 cm increases TA by 10%, triglycerides by 18%, total cholesterol by 18% and decreases HDL cholesterol by 15%. These results are even more significant considering the fact that the percentage of obese people in the world with a BMI > 25 kg/m^2, from 56% in 1988 was increased to 64% of the total population in 2000.

Our results showed a statistically significant correlation between fasting glucose, HDL- cholesterol and the severity of CAD according to the SYNTAX score ($p < 0.05$). A large metaanalysis, National Cholesterol Education Program (NCEP), conducted in the period 2001–2004, which included 87 studies and 951,083 patients, found that the metabolic syndrome was associated with a 2-fold higher CV risk (RR: 2.35; 95% CI: 2.02–2.73), and 1.5 times higher total mortality (RR: 1.58; 95% CI: 1.39–1.78) compared to healthy population [16, 17].

Elderly subjects are characterized by a high prevalence of CAD but also by a worse prognosis following cardiac events [18]. We found that there was a statistically significant correlation between age and the severity of CAD according to SYNTAX score ($p < 0.05$). In old age advanced atherosclerosis leads to increased incidence of ischemic events, with subsequent hypoperfusion of vital organs.

Most patients with a higher FINDRISC score have a higher odds ratio for developing type 2 diabetes, and greater CV risk, as we found in our study. Macrovascular complications of atherosclerosis are one of the main reasons for patients with type 2 diabetes to have twice or four times greater risk of developing one of the manifestations of ischemic heart disease (angina pectoris or myocardial infarction), and twice or six times greater risk of stroke or peripheral arterial disease [19–21].

Taking into account the mutual relationship of diabetes and obesity for the development of CV diseases, we compared the FINDRISC score, and some of its parameters (age, BMI, waist circumference) as independent variables and the SYNTAX score as a dependent variable. We found that the FINDRISC score was better predictor, providing us more information about the SYNTAX score.

This study investigated the relationship between classic laboratory and metabolic markers that accelerate the process of atherosclerosis (triglycerides, total cholesterol, creatinine, active smoking and inflammatory markers Le, ESR, CRP) and we did not find positive correlation between the 3 groups of patients. But, when we used FINDRISC score, we found that in groups of patients with a score greater than or equal to 12 points more than 70% of patients had 2- vessel or 3- vessel CAD, and severe and extensive CAD according to SYNTAX score.

A number of studies [22] have clearly demonstrated the role of inflammation in all the stages of atherosclerosis, from the formation and rupture of the plaque, to the development of acute coronary syndromes. Given the fact that this study included the patients with stable angina pectoris, we did not find any statistical significance in the values of inflammatory markers (ESR, Le, CRP) among the 3 groups of patients. Another reason may be the fact that we did not use high-sensitive CRP (the lowest value was 3.08 mg/dL) which is the limitation of the study.

Finally, almost all the parameters presented in the FINDRISC score affect a reduced vasodilator response, and paradoxical vasoconstriction in large and in small vessels, even in the absence of structural abnormalities of the blood vessel wall, due to a reduced NO bioavailability [23].

Conclusion

The results of our study showed a statistically significant correlation between the FINDRISC score and its parameters respectively (age, BMI, waist circumference) with the severity of coronary artery disease according to SYNTAX score and the number of diseased coronary arteries.

The FINDRISC score may be useful in identifying patients at the increased risk of coronary artery disease, and with a prognostic value in primary prevention and cardiovascular risk assessment. In the groups of the patients with a score greater than or equal to 12 points more than 70% of patients had 2-vessel or 3-vessel coronary artery disease and more severe coronary disease according to the SYNTAX score.

Our results showED that the incidence and severity of coronary artery disease is associated with a higher FINDRISC score, and that it can be used to estimate the degree of risk for coronary artery disease. The FINDRISC score is also able to identify groups of patients who are at the increased cardiovascular risk, which, under current guidelines do not require further cardiac evaluation.

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Determinants of smoking and smoking cessation among health professionals in Serbia: A cross-sectional study

Faktori pušenja i prestanka pušenja među zdravstvenim radnicima u Srbiji: rezultati studije preseka

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Abstract

Background/Aim. Bearing in mind a high smoking prevalence in Serbia (34% in adult population; men 38%, women 30%) and leading role of health professionals in intervention and prevention, a cross-sectional study was performed smong the representative sample of health professionals in Serbia. The aim of the study was to identify predictors of smoking and smoking cessation prior to the total smoking ban in November 2010. **Methods.** In this nationwide study, 3,084 physicians and nurses from 4 types of institutions and four geographical regions were selected and 2,282 included (response rate 74.0%). Data were collected using a self-administered structured questionnaire. Standard statistical methods were used to calculate prevalence rates, and multivariate logistic regressions to evaluate independent predictors of smoking pattern. Risks were expressed as odds ratios (OR) which represent approximation of relative risks of exposed persons with 95% confidence intervals (95% CI). **Results.** We found a high smoking prevalence of 38.0%, the same for women and men (37.8% and 37.6%, respectively; $p = 0.138$), higher among nurses (41.7%) than physicians (29.1%) ($p = 0.000$), as well as among those employed in general hospitals (42.6%) and institutes of public health (43.8%) ($p = 0.000$). Significantly increased risk of being an ever or current smoker was noticed for nurses (OR = 1.75, 95% CI 1.42–2.14; and OR = 1.91, 95% CI 1.52–2.40, respectively), those employed in general hospitals (OR = 1.37, 95% CI 1.09–1.73 and OR = 1.40, 95% CI 1.09–1.79, respectively), and with worse self-estimated health (OR = 1.15, 95% CI 1.02–1.30; and OR = 1.17, 95% CI 1.02–1.34, respectively). Intentions to quit smoking or to reduce the number of cigarettes were more frequent in women (OR = 1.51, 95% CI 1.01–2.27) and participants who worse evaluated their health (OR = 1.74, 95% CI 1.39–2.18). **Conclusion.** High smoking prevalence in health professionals could be a barrier for the full implementation of smoking ban in health institutions in Serbia. Smoking cessation programs at workplaces, formal education in smoking cessation techniques, and better Law enforcement by health administrations should be implemented.

Key words:
smoking; smoking cessation; prevalence; physicians; nurses; health; legislation

Apstrakt

Uvod/Cilj. Zbog visoke prevalencije pušenja u Srbiji (34% odraslih; 38% muškarci, 30% žene) i vodeće uloge zdravstvenih radnika u prevenciji i odvikavanju od pušenja, sprovedena je studija preseka na reprezentativnom uzorku zdravstvenih radnika u Srbiji Cilj rada bio je utvrđivanje faktora pušenja i prestanka pušenja među zdravstvenim radnicima pre stupanja na snagu zakona koji je zabranio pušenjem na javnim i radnim mestima, 2010. godine. **Metode.** Za studiju je izabran reprezentativni uzorak od 3 084 lekara i medicinskih sestara iz 4 vrste zdravstvenih ustanova u Srbiji. U istraživanju je učestvovalo 2 282 zdravstvenih radnika (stopa odaziva 74,0%). Korišćen je struktuirani upitnik koji su ispitanici sami popunjavali. Korišćene su standardne statističke metode za računanje stopa prevalencije i multivarijantna logistička regresija za procenu nezavisnih prediktora pušenja i prestanka pušenja. Rizik je izračunat kao unakrsni odnos (UO) koji predstavlja približnu vrednost relativnog rizika izloženih osoba sa 95% intervalima poverenja (95% IP). **Rezultati.** Rezultati su pokazali visoku prevalenciju pušenja (38,0%), sličnu

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među muškarcima (37,6%) i ženama (37,8%) (p = 0,138), višu među medicinskim sestrama (41,7%) nego lekarima (29,1%) (p = 0,000), kod zaposlenih u opštim bolnicama (42,6%) i zavodima za javno zdravlje (43,8%) (p = 0,000). Rizik da se bude pušač bilo kad u životu ili trenutno značajno je bio povišen kod medicinskih sestara (UO = 1,75, 95% IP 1,42–2,14 i UO = 1,91, 95% IP = 1,52–2,40), kod zaposlenih u opštim bolnicama (UO = 1,37, 95% IP 1,09–1,73, i UO = 1,40, 95% IP 1,09–1,79) i kod ispitnika koji su lošije procenili svoje zdravlje (UO = 1,15, 95% IP 1,02–1,30; i UO = 1,17, 95% IP 1,02–1,34). Namera da se prekine s pušenjem ili da se smanji broj popušenih cigareta bili su češći kod žena (UO = 1,51, 95% IP 1,01–2,27) i kod ispitanika koji su lošije procenili svoje zdravlje (UO = 1,74, 95% IP 1,39–2,18). **Zaključak.** Visoka zastupljenost pušenja među zdravstvenim radnicima predstavlja prepreku za punu primenu zakona u zdravstvenim ustanovama u Srbiji. Potrebno je sprovoditi programe odvikavanja od pušenja na radnim mestima, metode odvikavanja od pušenja uključiti u program redovnih studija zdravstvene struke, a uprave zdravstvenih ustanova trebalo bi da efikasnije sprovode zakon.

Ključne reči:
pušenje; pušenje, prestanak; prevalenca; lekari; medicinski tehničari; zdravlje; zakonodavstvo.

Introduction

According to the latest available data adult smoking prevalence in Serbia is still high with a total prevalence rate of smoking 33.6% (38.1% among men, and 29.9% among women) [1]. Data on smoking prevalence and smoking practice among health professionals are limited. A study of employees at institutes of public health in Serbia from 2006 indicated a high smoking prevalence among all employees (43.9%), among physicians (31.1%) and among nurses (48.1%) [2]. A similar high percentage of smokers among medical staff was reported in some Balkans countries and Tunisia [3–6], which is higher than in some high-income countries, where smoking prevalence among physicians and nurses has been substantially reduced in the last decades [7–16]. The 2006 Global Health Professional Survey of students of health sciences in Serbia reported the percentage of current smokers of 34.7% among medical students, 33.8% among students of nursing schools, 29.3% among pharmacy students, and 28.5% among dental students, showing that women smoke more than men in all groups [17].

Smoking prevalence rates and attitudes toward tobacco control policies among health professionals can play an important role in overall public health policy implementation [18]. Medical staff are on the frontline in the primary health care battle and their interventions can be especially effective in helping patients to quit smoking. On the other hand, smoking among health professionals can substantially undermine efforts to reduce smoking and to convince the general population not to smoke.

The aim of this study was to assess the smoking prevalence among health professionals employed in the public health sector of the Republic of Serbia, to identify factors affecting their smoking pattern, and to understand predictors of smoking cessation before the total smoking ban in health institutions that went into effect in November 2010.

Methods

The nationally representative sample was selected among physicians and nurses from four types of national health service institutions (primary health care centers; general hospitals; institutes, clinics or centers within the university teaching hospitals; and public health institutes), and four regions, i.e., Vojvodina (northern part of Serbia), Belgrade, Central Serbia and at the part of Kosovo and Metohia with predominantly Serbian population.

A stratified two-fold random cluster was applied. Sample size was based on the number of physicians (including dentists, and pharmacists) and nurses employed with the National Health Service obtained by the Serbian Institute of Public Health on the July 1, 2009 (20,217 physicians, and 48,613 nurses). In this study we included 20 health institutions. All the physicians and nurses present at work on the day of survey were eligible for the study. The questionnaire was anonymous and no personal data on participants were available.

Fifteen study coordinators were selected among health professionals previously engaged in the tobacco control activities. We provided written instruction and one-day training regarding the purpose and procedures of the study.

To obtain data on sex, age, occupation, type of health institution, years of employment, self-estimated health (very bad = 5, bad = 4, neither bad nor good = 3, good = 2, and very good = 1), sick-leave in previous year (yes/no), and tobacco use we constructed a self-administered questionnaire with 19 questions. Smoking means smoking cigarettes because the use of other tobacco products (e.g., pipes, cigars, shisha, etc) is very rare in Serbia. The participants were classified as current smokers, ex-smokers or never smokers. Current smokers were those who currently smoke regularly (every day) or occasionally (at least one day per week). We obtained the total number of cigarettes smoked daily and the number of cigarettes smoked at work for regular smokers, total number of cigarettes smoked in previous week and separately at work for occasional smokers, and the duration of smoking. For former smokers we obtained information on the year when they quit smoking, and the duration of smoking.

Categorical variables were presented as the numbers and percentages of subjects, and compared by chi square test and relative risks. Continuous variables were described by means and standard deviations and compared using one-way ANOVA with Bonferroni *post-hoc* pairwise comparisons. Prevalence rates are represented with its estimates and 95% confidence intervals (95% CI). We also calculated quit ratios

by dividing the number of former smokers with the number of ever smokers. Multivariate logistic regressions were used to evaluate independent predictors (sex, age, occupation, region, occupational setting, perception of health status, and sick leave during the last year) of smoking, smoking cessation and future intentions with smoking and were reported as odds ratios (OR) and 95% CI. Statistical analysis was performed with SPSS 19.0, a significance level of $p < 0.05$.

Results

The study included 3,084 individuals; out of them 2,282 completed the questionnaire yielding an overall response rate 74%, slightly higher for nurses (76.4%) than for physicians (68.9%). More women participated than men (1,831 and 418, respectively) reflecting the demographics of health employees in Serbia (Table 1); data on sex were missing for 33 participants. The overall smoking prevalence was 38%, similar among women (37.8%) and men (37.6%). Smoking prevalence was significantly higher among nurses (41.7%) than among physicians (29.1%) ($p = 0.000$), in the region of Kosovo and Metohia (46.8%), and at public health institutes (43.8%) and general hospitals (42.6%) ($p = 0.000$). Self-estimated health was the worst in former smokers ($p = 0.029$), while no difference was noticed on sick leave in previous years among different smoking categories ($p = 0.122$).

The quit ratio for all participants was 26.8 ± 1.4, for nurses 24.8 ± 1.4 and somewhat higher for physicians 32.8 ± 2.7.

The smokers on average smoked almost a package *per* day (17.7 cigarettes), men significantly more (23.9) than women (16.2), and physicians more (19.3) than nurses (17.3) (Table 2). The average number of cigarettes smoked daily was higher in Kosovo and Metohia with predominantly Serbian population (19.6) and in Central Serbia (19.0), and among health care workers in general hospitals (19.3) and public health institutes (18.4). About one third of daily number of cigarettes was consumed at work (6.2). The average duration of smoking was 17.6 years, and it was longer for men (20.0 years) than women (17.0 years).

A little more than a half of all the participants thought that they had a problem with smoking at work (55.3%), with no statistically significant difference regarding sex, occupation, type of health institution and region. One third of all participants would like to quit smoking, similarly for men and women. However, more men than women would like to continue smoking unchanged while more women would like to reduce the number of cigarettes smoked ($p = 0.03$). Significantly more physicians would like to quit (40.3%) than nurses (32.3) ($p = 0.02$).

We conducted four separate multivariate analyses to examine factors of being ever, current and former smoker compared to never smoker, as well as current compared to former smoker (Table 3). Multivariate logistic regression models showed that ever or current smoking was significantly associated with the similar factors – occupation, territory, type of health institutions, and self-estimated health. Women were less likely to be ever smokers (OR = 0.74), but nurses were more likely to be ever or current smokers (OR = 1.75 and OR = 1.91, respectively). A significantly elevated risk for ever and current smoking was observed for health professionals from Kosovo and Metohia with predominantly Serbian population (OR = 1.37, and OR = 1.45, respectively), and those employed in general hospitals (OR = 1.37, and 1.40, respectively). Worse self-estimated health was a significant factor for ever and current smoking (OR = 1.15, and OR = 1.17, respectively), while sick-leave was associated only with smoking cessation (OR = 0.70). Factors influencing smoking cessation were also sex (women were less likely to stop smoking, OR = 0.64), and occupation (nurses more often quit smoking, OR = 1.43).

Our results generated from the final multivariate logistic regression analysis showed that women more often had intention to quit smoking compared to men (OR = 1.51) (Table 4). Significant difference was noticed neither for age, type of health institution nor for region and sick leave in the previous year. Those respondents who wanted to quit or reduce the number of cigarettes smoked had worse self-estimate of their health (OR = 1.74).

Discussion

In this representative cross-sectional study among health professionals from the National Health Service in Serbia, we found a high smoking prevalence of 38.0%, nearly the same for women and men. This prevalence is higher than for the general adult population in Serbia based on the data from 2006 (33.6%) and much higher than for adult women population (29.9%) [1]. High smoking prevalence among women has been recorded in many countries in the last decade, mostly due to the changed social role of women, work stress and aggressive tobacco marketing targeting especially women [19, 20].

We also found a considerable difference in smoking prevalence between physicians and nurses, with more nurses smoking than physicians. This has been reported in many other studies [3, 12, 15, 21–23], and is consistent with results from the Serbian national survey indicating higher smoking prevalence among women with college education [24]. The fact that about 30% of the physicians and more than 40% of nurses smoke may have an unfavorable impact on attempts to provide counseling to the patients regarding smoking cessation. It certainly is not a good starting point for the introduction and compliance with the new law totally banning smoking in health institutions in Serbia. Surprisingly, we observed the highest percentage of smokers in public health institutes, which are primarily preventive institutions that deal with healthy life styles of the population, similarly to the findings from 2006 [2].

Smoking prevalence among physicians (29%) was lower than what was recorded for the general population (33.7%), and more than a half physicians have never been smokers. However, this seems to be a pretty high percentage for individuals who know the health risks and have some responsibility for counseling the public regarding smoking.

Tabele 1

Prevalence rates (PR) of smoking status in the population of health professionals and its subgroups

Variable	Total	Current smoker	Ex-smoker	Never smoker	*p*
Participants (n), [PR†]	2,282	866 [38.0; 36.0–39.9]	305 [13.4; 12.0–14.8]	1,111 [48.7; 46.6–50.7]	
Sex‡ n (%)‖, [PR†]					
men	418 (18.6)	157 (18.5) [37.6; 32.9–42.2]	68 (22.6) [16.3; 12.7–19.8]	193 (17.6) [46.2; 41.4–51.0]	
women	1,831 (81.4)	692 (81.5) [37.8; 35.6–40.0]	233 (77.4) 12.7; 11.2–14.3]	906 (82.4) 49.5; 47.2–51.8]	0.138
Age (years), $\bar{x} \pm SD$	41.8 ± 9.8	41.5 ± 9.6	42.6 ± 9.4	41.8 ± 10.0	0.204
Years of employment, $\bar{x} \pm SD$	18.4 ± 9.8	18.5 ± 9.7	19.2 ± 9.5	18.0 ± 9.9	0.167
Occupation, n (%)‖, [PR†]					
physicians	683 (29.9)	199 (23.0) [29.1; 25.7–32.6]	94 (30.8) 13.8; 11.2–16.4]	390 (35.1) 57.1; 53.4–60.8]	
nurses	1,599 (70.1)	667 (77.0) [41.7; 39.3–44.1]	211 (69.2) 13.2; 11.5–14.9]	721 (64.9) 45.1; 42.7–47.5]	0.000
Region, n (%)‖, [PR†]					
Vojvodina	762 (33.4)	266 (30.7) [34.9; 31.5–38.3]	111 (36.4) 14.6; 12.1–17.1]	385 (34.7) 50.5; 47.0–54.1]	
Belgrade	523 (22.9)	216 (24.9) [41.3; 37.1–45.5]	60 (19.7) 11.5; 8.7–14.2]	247 (22.2) 47.2; 42.9–51.5]	
Central Serbia	591 (25.9)	194 (22.4) [32.8; 29.0–36.6]	79 (25.9) 13.4; 10.6–16.1]	318 (28.6) 53.8; 49.8–57.8]	
Kosovo & Metohia	406 (17.8)	190 (21.9) [46.8; 41.9–51.7]	55 (18.0) 13.6; 10.2–16.9]	161 (14.5) 39.7; 34.9–44.4]	0.000
Settings, n (%)‖, [PR†]					
primary health centre	882 (38.7)	302 (34.9) [34.2; 31.1–37.4]	112 (36.7) 12.7; 10.5–14.9]	468 (42.1) 53.1; 49.8–56.4]	
general hospital	721 (31.6)	307 (35.5) [42.6; 39.0–46.2]	101 (33.1) 14.0; 11.5–16.6]	313 (28.2) 43.4; 39.8–47.0]	
university hospitals	293 (12.8)	88 (10.2) [30.0; 24.8–35.3]	47 (15.4) 16.0; 11.8–20.3]	158 (14.2) 53.9; 48.2–59.7]	
public health institutes	386 (16.9)	169 (19.5) [43.8; 38.8–48.8]	45 (14.8) 11.7; 8.4–14.9]	172 (15.5) 44.6; 39.6–49.5]	0.000
Self-estimated health, $\bar{x} \pm SD$	2.18 ± 0.73	2.22 ± 0.77	2.24 ± 0.78	2.14 ± 0.68	0.029
Sick-leave in previous year – Yes, n (%)‖, [PR†]	380 (16.9)	134 (15.6) [35.3; 30.4–40.1]	63 (20.7) 16.6; 12.8–20.3]	183 (16.8) 48.2; 43.1–53.2]	0.122

***Number of participants; †Estimated prevalence rate along with its 95% confidence intervals; ‡Missing data on sex for 33 participants; ‖Vertical percentage (by the total number of participants, total number of smokers, ex-smokers and non-smokers).**

Table 2

Smoking pattern in the group of current smokers (daily and occasionally)

Variable	No of cigarettes consumed daily (0) ($\bar{x} \pm SD$)	No of cigarettes consumed daily at work (0) ($\bar{x} \pm SD$)	Duration of smoking cigarettes† (years) ($\bar{x} \pm SD$)	Smoking as a problem at work† n (%)	Future intentions†		
					Quit n (%)	Reduce n (%)	Continue n (%)
Total	17.7 ± 8.9	6.2 ± 6.1	17.6 ± 8.6	463 (55.3)	284 (34.3)	230 (27.7)	230 (27.7)
men	23.9 ± 11.2	9.9 ± 7.7	20.0 ± 9.4	89 (57.1)	53 (35.1)	45 (29.8)	53 (35.1)
women	16.2 ± 7.7	5.4 ± 5.3	17.0 ±8.4	374 (54.8)	231 (34.1)	270 (39.8)	177 (26.1)
p =	0.000	0.000	0.000	0.341		0.032	
Occupation							
physicians	19.3 ± 10.9	6.7 ± 7.8	18.4 ± 9.2	107 (54.6)	79 (40.3)	58 (29.6)	59 (30.1)
nurses	17.3 ± 8.3	6.1 ± 5.7	17.3 ± 8.4	361 (54.8)	210 (32.3)	261 (40.2)	179 (27.5)
p =	0.007*	0.243	0.114	0.514		0.022	
Region							
Vojvodina (V)	17.1 ± 8.6	4.6 ± 5.1	19.1 ± 9.2	152 (57.8)	100 (38.5)	83 (31.9)	77 (29.6)
Belgrade (B)	15.7 ± 7.7	5.3 ± 5.2	17.7 ± 8.3	107 (50.5)	67 (31.5)	89 (41.8)	57 (26.8)
Central Serbia (CS)	19.0 ± 10.7*	7.5 ± 8.3*	17.6 ± 8.0	110 (57.9)	62 (33.3)	65 (34.9)	59 (31.7)
Kosovo & Metohia (K&M)	19.6 ± 8.7*	8.5 ± 5.4*	15.0 ± 7.9	99 (52.1)	289 (34.2)	82 (43.9)	45 (24.1)
p =	0.000	0.000	0.000	0.279		0.128	
Settings							
Primary health centre (PHC)	15.8 ± 8.2	3.6 ± 4.2	17.8 ± 8.7	169 (56.1)	108 (36.6)	115 (39.0)	108 (36.6)
General hospital (GH)	19.3 ± 9.3*	7.7 ± 5.9*	17.1 ± 8.8	156 (51.5.)	112 (37.2)	104 (34.6)	112 (37.2)
University hospital (UH)	17.8 ± 9.0	6.4 ± 7.0	19.7 ± 8.5	52 (54.7)	30 (34.9)	27 (31.4)	29 (33.7)
Public health institutes (PHI)	18.4 ± 9.5*	8.5 ± 7.7*.	16.7 ± 7.7	91 (55.8)	39 (23.8)	73 (44.5)	52 (31.7)
p =	0.000	0.000	0.04	0.515		0.035	

***Statistically significant difference; 0 – daily smokers only; †daily and occasional smokers; *K/V (*p* = 0.015); *H/PHC (*p* = 0.000); *PHI/PHC (*p* = 0.000), and *PHI/UH (*p* = 0.045); *K&M/V (*p* = 0.000), and *K&M/B, (*p* = 0.000); *CS/V (*p* = 0.000), and *CS/B (*p* = 0.002); *GH/PHC (*p* = 0.000); *PHI/PHC (*p* = 0.000), and *PHI/UH (*p* = 0.045).**

Table 3

Multivariate analysis of the factors explaining different smoking pattern among employees in health institutions in Serbia

Variables	Ever *vs* never			Current *vs* never			Ex smokers *vs* never			Current *vs* ex-smokers		
	p	OR†	(95% CI)	*p*	OR	(95% CI)	*p*	OR	(95% CI)	*p*	OR	(95% CI)
Sex												
men‖												
women	0.013*	0.74	(0.59–0.94)	0.058	0.78	(0.60–1.01)	0.011	0.64	(0.46–0.90)	0.303	1.20	(0.85–1.71)
Age	0.041	1.01	(1.00–1.02)	0.123	1.01	(1.00–1.02)	0.059	1.01	(1.00–1.03)	0.339	0.99	(0.98–1.01)
Occupation												
physicians‖												
nurses	0.000*	1.75	(1.42–2.14)	0.000*	1.91	(1.52–2.40)	0.025	1.43	(1.05–1.95)	0.057	1.37	(0.99–1.89)
Region												
Vojvodina‖												
Belgrade	0.210	1.18	(0.91–1.54)	0.098	1.27	(0.96–1.79)	0.817	0.95	(0.63–1.44)	0.104	1.42	(0.93–2.16)
Central Serbia	0.255	0.88	(0.70–1.20)	0.295	0.87	(0.68–1.12)	0.447	0.88	(0.63–1.23)	0.817	1.04	(0.72–1.48)
Kosovo & Metohia	0.020	1.37	(1.05–1.78)	0.010*	1.45	(1.10–1.93)	0.695	1.08	(0.73–1.62)	0.165	1.33	(0.89–1.98)
Settings												
primary health center‖												
general hospital	0.006	1.37	(1.09–1.73)	0.009*	1.40	(1.09–1.79)	0.098	1.34	(0.95–1.90)	0.769	1.05	(0.74–1.50)
university hospital	0.893	1.02	(0.77–1.34)	0.613	0.92	(0.68–1.26)	0.250	1.27	(0.85–1.89)	0.220	0.76	(0.49–1.18)
institute of Public Health	0.060	1.30	(1.00–1.70)	0.051	1.34	(1.00–1.78)	0.550	1.14	(0.74–1.76)	0.622	1.12	(0.72–1.74)
Self-estimated health	0.028*	1.15	(1.02–1.30)	0.025*	1.17	(1.02–1.34)	0.188	1.14	(0.94–1.38)	0.839	1.02	(0.85–1.22)
Sick-leave in previous year												
no‖												
yes	0.663	1.05	(0.83–1.33)	0.698	0.95	(0.73–1.23)	0.080	1.35	(0.96–1.90)	0.050*	0.70	0.49-1.00

*** – Statistically significant difference; OR – odds ratio; 95% CI – 95% confidence Interval; ‖reference group.**

Table 4

Multivariate analysis of the predictors of future intentions to quit or reduce smoking compared to intention to continue smoking unchanged among employees of health institutions in Serbia

Variables	*p*	OR	(95% CI)
Sex			
men‖			
women	0.050*	1.51	(1.01–2.27)
Age	0.846	1.00	(0.98–1.02)
occupation			
physicians‖			
Nurses	0.519	0.88	(0.59–1.31)
Region			
Vojvodina‖			
Belgrade	0.377	1.24	(0.76–2.03)
Central Serbia	0.922	1.02	(0.66–1.58)
Kosovo & Metohia	0.128	1.43	(0.90–2.28)
Settings			
primary health centre‖			
general hospital	0.286	0.79	(0.52–1.21)
university hospital	0.068	0.60	(0.35–1.04)
institute of public health	0.159	0.71	(0.44–1.15)
Self-estimated health	0.000*	1.74	(1.39–2.18)
Sick-leave in previous year			
no‖			
yes	0.094	0.69	(0.44–1.07)

*** – Statistically significant difference; OR – odds ratio; 95% CI – 95% confidence interval; ‖reference group.**

High smoking rates among physicians have been reported in some other countries, such as in Turkey, Tunisia, Pakistan, Italy, Bosnia and Herzegovina, Greece and China [3–6, 21, 25–28]. In these countries, except in Italy, there was a huge difference between men and women, with men smoking more than women. This differs substantially from high smoking prevalence among women – health professionals in our study indicating that smoking is socially acceptable and/or reflects the changing social role of women in the transitional society of Serbia. Such a high smoking prevalence among Serbian health professionals is in contrast with many countries with a longer history of tobacco control and effective enforcement of smokefree legislation (e.g., the US, Australia, Brazil or France), in which reduction in smoking rates among health professionals was followed by the reduction in general population [11, 12, 14, 29, 30]. In some of these countries, smoking prevalence in medical doctors is far below 15%, and in nurses below 20%.

The majority of smokers in our study group were regular daily smokers, only 14 (1.6%) participants were occasional smokers who smoked on average 9.7 cigarettes weekly and 4.5 cigarettes at work weekly (data not presented). This is contrary to the trend in some other countries that reported increasing number of occasional smokers in health care settings and decreasing number of regular smokers [7, 22, 23]. Among regular daily smokers, men smoke more cigarettes *per* day (23.9), smoke more at work (9.9) and have had a longer duration of smoking (20 years) than women. An interesting finding was that despite reporting lower smoking prevalence, physicians smoke more cigarettes *per* day (19.3) than nurses (17.3), which is higher than reported in some other studies [4–7, 19, 22, 23, 30], but similar to data from Bosnia and Herzegovina [3].

We found that the mean duration of smoking was 17.6 years and the mean years of employment 18.5, suggesting that becoming regular smoker for many employees coincided with the start of employment, indicating that changing their behavior and overcoming nicotine addiction won't be an easy task. However, a quarter of smokers (24.2% among physicians and 23.3% among nurses) reported that they did not smoke at work, which shows their attempt to set a good example. A little more than a half of all respondents mentioned that smoking was a problem at work, with little variation by gender, occupation, settings or region, probably because the study was performed a year before the new Law on Protection of Citizens from Exposure to Tobacco Smoke fully entered into force in November 2010. The old law (1995) that banned smoking in enclosed premises only partially restricted smoking in health institutions with a very low compliance; smoking was actually allowed almost everywhere.

There were no differences in future intentions regarding smoking cessation in men and women, with a third of all smokers wanting to quit. Compared to men women more often wanted to only reduce the number of cigarettes than to quit. Regarding occupation, physicians more frequently expressed the intention to quit, while nurses preferred to reduce the daily number of cigarettes, reported also in some studies [3, 4, 21], while in others more than a half of all smokers wanted to quit [23, 28, 30, 31].

Among physicians and nurses, a similar percentage of former smokers (13.8% and 13.2%, respectively) was reported, 85% of whom had quit more than a year ago. The percentage of ex-smokers among health professionals (13.4%) was much lower than in the general Serbian adult population – 25% [24]. This is supported by the low quit ratios

in our study, both for physicians and nurses, lower than reported in the US or in Germany [12, 32]. Such a low percentage of former smokers was also reported in some studies that had high smoking prevalence among health professionals, such as in Tunisia and Jordan [6, 28], while higher percentage of ex-smokers has been reported more often [4, 7, 9, 12, 21, 30, 33, 34]. The multivariate analysis also showed that women were less likely to be ex-smokers compared to men, and physicians compared to nurses. Since ex-smokers rated their own health worse, we can speculate that the main reason for quitting could be already damaged health. Many other studies reported that the main reason for quitting smoking was currently bad health [29–31, 35]. Similarly, our findings indicated that a worse self-estimate of health was a predictor of future intention to quit or reduce the number of cigarettes. On the other hand, we also noticed that two third of all former smokers quit after 2003, which coincided with more intensive tobacco control activities by the Ministry of Health and its National Committee for Tobacco Prevention suggesting that the media campaigns and other related activities strengthen their motivation to quit.

Although health hazards from smoking and exposure to second hand smoke were well-known to the majority of health professionals in 2009, i.e. at the time the study was performed, an unacceptable percentage continued to smoke. We regarded this as a great obstacle to the compliance with the new law that totally banned smoking in all health institutions, including backyards and front doors. Smoking doctors and nurses are less likely to ask their patients about smoking, counsel them about smoking hazards, or actively participate in smoking cessation programs. Such a high prevalence of smoking especially among nurses, that constitute the majority of workforce in health institutions, may substantially reduce the chances of health institutions to become 100% smoke-free. Moreover, it is not easy to convince the general population to give up smoking and accept healthy life styles, when health professionals continue to smoke.

According to the opinion poll performed a year after the law went into force in 2011 by the Ministry of Health of the Republic of Serbia, public support for this law was high and stable over the time (77% in 2010, 81% in 2011 and 82% in 2012). Since 2010, out of 10,873 inspections in health institutions only a very small number of infringements of law were noted (20 cases of cigarettes or ashtrays visible, 6 cases of smoking inside health institutions, 4 cases of missing name of the responsible person and 2 cases of missing no smoking signs). Having in mind that compliance with laws in the country is generally very low, compliance with the new law in health institutions could be accepted as quite good. Smoking is not obviously apparent in health institutions, however, our survey would indicate that it must be occurring, probably in some remote offices or spaces, especially in the afternoon or evening shifts and therefore more intensive efforts to encourage compliance are required.

There are some limitations in our study. The study relied on self-reported responses from a questionnaire and smoking status was not biochemically verified. These could lead to under-reporting of smoking among health professionals. Although such a bias may occur, we do not believe that it is serious because in the time of study smoking was still regarded as "normal" behavior in Serbia. A majority of studies that evaluated the validity of self-reported smoking status found it as an acceptable method of gathering information [36–39], although not all such reports agreed [40, 41]. In addition, the questionnaire was anonymous and the confidentiality was assured. It may be, however, that the smoking rates among health care professionals are higher than indicated by our survey.

The strength of our study includes a large nationwide sample of health professionals and health institutions and geographical distribution of participants. The response rate was pretty high – 74%, higher than reported in the majority of studies on smoking among health professionals. We were able to assess the smoking prevalence in physicians and nurses across the country and evaluate their capacity to give up smoking, and thus enable better enforcement of smoking ban in health institutions.

Conclusion

Our results indicate that a high smoking prevalence among health professionals, particularly in nurses, is still considerable and could be a barrier for the full implementation of smoking ban in health institutions in Serbia. There is a need for developing and performing special smoking cessation programs for health professionals on their workplaces, as a part of workplace health promotion activities. Formal education in tobacco control and particularly in different smoking cessation methods should be a part of regular high school and university curricula, as well as later continuous education. This is also an opportunity for adoption of the evidence based clinical guidelines that will specifically target nurses and physicians. After adoption of the law that totally banned smoking in health institutions, health administration should strengthen their efforts to enforce the law.

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Experimental pleural empyema model in rabbits: Why, how and what are the next steps

Eksperimentalni model empijema pleure kod kunića: zašto, kako i šta dalje

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Abstract

Bacgraund/Aim. The use of new therapeutic methods to prevent development of fibrothorax as the final complication of the human pleural infections requires research with experimental animals. The aim of this study was to standardize the procedures for the establishment of our own experimental model of empyema in rabbits, since it should be able to offer similar conditions found in human pleural infections. **Methods.** This experiment included 15 chinchilla rabbits, weighing from 2.3 to 2.8 kg. There were 12 rabbits in the experimental group, while 3 rabbits formed the control group. On the first day, we administered 0.4–0.5 mL of turpentine in the right pleural space of the rabbits from the experimental group in order to provoke sterile exudative pleurisy. After 24 h we injected 1 mL of *Staphylococcus aureus* and 1 mL of *Escherichia coli* bacteria in the same concentration of 4.5×10^8 bacteria/mL. Thoracocentesis for the pleural fluid analysis was performed 24, 48, 72, and 96 h after bacteria instillation. In these pleural samples we estimated the number of leucocytes and the values of lactate dehydrogenase (LDH), glucose and pH in pleural fluid, as well as the presence of bacteria. We did not protect the animals with antibiotics, and on the day 7 of the experiment they were sacrificed with the lethal dose of barbiturate (*iv*). The lung from the empyemic side of all experimental animals and the lung of one control animal were histopathologically examined. **Results.** A total of 4 animals had a small amount of clear pleural fluids or there was no fluid obtained with thoracocentesis 24 and 48 h after the bacteria instillation. after the bacteria instillation. In the remaining 8 rabbits 24 h after bacteria administration the mean values (± SD) of the parameters monitored were as follows: Le $34.75 \pm 6.13 \times 10^9$/L, LDH 17,000 ± 4,69 U/L, glucose 1.23 ± 0.45 mmol/L, and pH 6.975 ± 0.15. The obtained values met the criteria for the evaluation of effusion as pleural empyema or complex and complicated pleural effusion (LDH > 1000 U/L, glucose < 2.31 mmol/L and pH < 7.20). Bacterial cultures were positive in 5 out of 8 first pleural samples and in only 2 samples after 48 h of bacteria administration. There was a positive correlation between the number of leukocytes and the LDH value ($r = 0.071$, $p < 0.001$), and a negative correlation between the number of leukocytes and the glucose level ($r = 0.864$, $p < 0.001$), and the leukocytes number and pH of the pleural fluid ($r = 0.894$, $p < 0.001$). The mean glucose value increased after 48 h (3.23 ± 0.44 mmol/L), and the pH value rose after 72 h (7.22 ± 0.03) which was beyond the empyema level. **Conclusion.** The creation of the experimental empyema model is a very delicate work with uncertain success. Its value and importance are crucial for pleural pathology research. With the intention to obtain a more empyemic pleural reaction we created a model with two different human pathogen bacteria. We generated the satisfactory results, but not as good as those contained in some of the reference literature data.

Key words:
empyema, pleural; animal experimentation; rabbits; pleural effusion; bacteria.

Apstrakt

Uvod/Cilj. Primena novih metoda lečenja u cilju sprečavanja razvoja fibrotoraksa, kao krajnje komplikacije empijema zahteva prethodno ispitivanje na eksperimentalnim životinjama. Cilj rada bio je standardizovanje postupaka za ustanovljavanje pouzdanog eksperimentalnog modela empijema kod kunića. **Metode.** U eksperimentu je korišćeno 15 činčila kunića mase od 2 300 do 2 800 g. Eksperimentalnu grupu činilo je 12 kunića, a kontrolnu tri. Prvog dana eksperimenta, u desni pleuralni prostor kunića iz eksperimentalne grupe stavljeno je 0,4–0,5 mL terpentina u cilju izazivanja sterilnog eksudativnog pleuritisa. Nakon 24 sata u pleuralni prostor stavljen je 1 mL *Staphylococcus aureus-a* i 1 mL *Escherichia coli* bakterija iste koncentracije ($4,5 \times 10^8$ bakterija/mL). Torakocenteza je rađena 24, 48, 72 i 96 časova nakon primene bakterija radi dobijanja

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uzoraka pleuralne tečnosti. U ovim uzorcima određivane su vrednosti leukocita (Le), laktat dehidrogenaze (LDH), glukoze, pH u pluralnoj tečnosti i prisustvo bakterija. U toku eksperimenta životinje nisu dobijale antibiotik, a žrtvovane su sedam dana od primene bakterija letalnom dozom barbiturata *iv*. Patohistološki su pregledana sva pluća sa empijemom svih eksperimentalnih životinja, kao i pluća jedne kontrolne životinje. **Rezultati.** Kod četiri životinje dobijen je oskudan bistar sadržaj, ili sadržaja nije bilo prilikom punkcija 24 i 48 časova od primene bakterija. Kod preostalih 8 eksperimentalnih životinja srednja vrednost Le u izlivu 24 časa nakon primene bakterija bila je: $34,75 \pm 6,13 \times 10^9$/L, LDH $17,000 \pm 4,69$ U/L, glukoze $1,23 \pm 0,45$ mmol/L, a pH $6,975 \pm 0,15$. Navedene vrednosti ispunjavaju kriterijume za proglašavanje izliva empijemskim ili kompleksnim, komplikovanim izlivom (LDH > 1 000 U/L, glukoza < 2,31 mmol/L i pH < 7,20). Bakteriološke kulture bile su pozitivne kod pet od osam prvih uzoraka, a nakon 48 časova samo kod dva uzorka pleuralnog izliva. Utvrđeno je postojanje pozitivne korelacije između broja leukocita i vrednosti LDH ($r = 0,071$, $p < 0.001$), a negativne korelacije između broja leukocita i vrednosti glukoze ($r = 0,864$, $p < 0.001$) i broja leukocita i vrednosti pH izliva ($r = 0,894$, $p < 0.001$). Vrednost glukoze nakon 48 sati ($3,23 \pm 0,44$ mmol/L) i pH nakon 72 sata ($7,22 \pm 0,03$), izašle su iz okvira empijemskih vrednsoti. **Zaključak.** Kreiranje eksperimentalnog modela empijema veoma je delikatan posao, sa neizvesnim uspehom. Dobar eksperimentalni model od suštinskog je značaja za proučavanje pleuralne patologije. U cilju što boljeg pleuralnog odgovora napravili smo model sa dve humane patogene bakterije. Dobili smo zadovoljavajuće rezultate, mada slabije od onih koji su objavljeni u literaturi.

Ključne reči:
empijem; životinje, zaštita; zečevi; pleura, izliv; bakterija.

Introduction

Pleural empyema is a serious inflammatory disease which is characterized by the presence of purulent effusion in the pleural space and, almost always arises as a complication of some other disease or condition. The clinic phases of empyema are acute, intermediate and chronic and these are counterparts of exudative, fibropurulent and fibrous pathologic stage of the disease [1, 2].

Despite effective antibiotic therapy and different drainage options which are available today for drainage of pleural infectious space, pleural empyema remains a serious medical problem with morbidity and mortality up to 20%. Half of all empyemas are consequences of inappropriate pneumonia treatment. Other causes may be a thoracic trauma, surgical procedures and infection spreading from the surrounding organs [3–5].

Pleural effusion is found in 9–30% of cases of bacterial pneumonia. The frequency is much higher among hospitalized patients (33–66%). Patients with unilateral and bilateral parapneumonic effusion have 3.7 and 6.5 times higher mortality rate, respectively [6, 7].

Thoracic drainage along with antibiotics is the most frequent initial empyema treatment. The choice of treatment modality depends on the severity of the disease and the stage of illness at the moment of diagnosis, as well as on patient's overall condition.

In addition to the thoracic drainage there are other treatment modalities, such as: drainage with intrapleural application of fibrinolytics, video-assisted thoracoscopic surgery (VATS) debridement, thoracotomy with decortication, open drainage and thoracoplasty [8–10]. The main goal of empyema treatment is cleaning of purulent pleural content, followed by lung reexpansion intending to avoid complications and the need for second operation [1, 2, 11].

Despite the fact that up to 20% of pleural parapneumonic effusions progress to empyema and that up to 40% of empyemas need a more aggressive surgical treatment, most of thoracic surgery centers usually have a few empyema patients. That is why there are very few randomized prospective trials with the purpose to compare efficacy of empyema treatment modalities [12].

The first experimental empyema model was made by Graham and Bell and they performed it on dogs almost a century ago. The rabbit turpentine model, the pig model with umbilical cord and the rabbit model with nutritive agar were made in the last 30 years of the last century. These models were used for the basic empyema research at the beginning of the last century [13], for studying therapeutic and drainage procedures efficacy [3, 14] and antibiotics used in the empyema treatment [9, 15].

Richard W. Light established well-known criteria for parapneumonic effusions and empyemas classification. A parapneumic effusion will be classified as an empyema if its glucose level is lower than 40 mg/dL or 3.0 mmol/L, pH value under 7.0 and lactate delydrogenase (LDH) value above 1000 I/U. Beside these biochemical parameters, positive bacteriological cultures or frank pus in pleural space have the same importance [16].

In the last few years the experimental empyema model has been used for basic research again, but this time on the cellular level, studying the role of some pro-inflammatory and pro-fibrotic cytokines and possibilities of the therapeutic use of their antagonist or inhibitors.

Most promising is the use of transforming growth factor-β (TGF-β) antagonists to prevent proliferation of fibroblasts and deposition of extracellular matrix which are responsible for fibrothorax formation [16].

It is obvious that therapeutic potentials of the abovementioned surgical procedures leave room for the research of new substances, which could be applied in empyema treatment. Our goal was to make a reliable experimental empyema model that would enable us to participate and continue these studies.

Methods

Experimental animals

The research was approved by the Military Medical Academy Ethics Committee. We used 15 four-month-old male chinchilla rabbits, weighing 2.3-2.8 kg. The rabbits

were from the Institute for Medical Research farm of the Military Medical Academy. They were placed in separate boxes. They were given food and water *ad libitum*. We randomised two groups: the experimental one with 12 rabbits with intrapleural application of bacteria, and the control one with 3 rabbits without application of bacteria.

Bacteria preparation

In this experiment we used *Staphyloccocus aureus* ATCC 25923 strain, and *Esherichia coli* ATCC 25922 strain. Concentration of the bacteria was determined under McFarland scale (Biomerieux, Densi chek plus McF). We administered 1 mL saline solution with 4.5×10^8/mL (1.5 McF) *Staphyloccocus aureus* and 1 mL saline solution with *Esherichia coli* bacteria in the same concentration. The day before the application bacteria were seeded on fresh blood agar.

Empyema induction

The rabbits from the experimental group were anesthetized with ketamine, 35 mg/kg, (Laboratorio Sanderson, Santiago Chile), and acepromazine 0.1 mg/kg (Ceva Tiergesundheit GmbH Dusseldorf, Germany). The right chest wall from the sternal to scapular line of each rabbit was shaved and scrubbed with povidon iodine (Betadine®).

The animals were placed in the supine position on the operating table. With the surgical knife, we made a small, 5 mm long incision, between the middle and lower third of the right hemithorax, 2.5–3 cm from the sternal edge. We performed thoracocentesis with a Mediflon *iv* catheter 18 G × 1.3 × 45 mm, flow 90 mL/min (Eastern Medikit Ltd. Gurgaon India). After we had excluded the presence of pneumothorax with probe aspiration, we injected 0.5–0.6 mL of turpentine through the catheter in the right pleural space of each experimental rabbit. After turpentine application the catheter was rinsed with 0.5 mL of saline solution. The aim of turpentine application was to cause a chemical pleural lesion followed by sterile effusion. Skin suture was not necessary. We did not inject turpentine and bacteria into the rabbits from the control group and did not perform any thoracocentesis.

After 24 h we conducted the same anesthesia procedures and thoracocentesis and then 8 experimental rabbits were administered 2 mL of saline solution with the abovementioned bacteria concentration. The catheter was rinsed with 0.5 mL of saline solution. The animals were gently rotated manually in order to spread the bacterial solution uniformly in the pleural space.

Experimental animals were given 50 mL of saline solution and 50 mL of 5% glucose solution deep in the subcutaneous tissue on a daily basis in order to prevent dehydration.

Empyema verification and pleural specimens

After 24 h applying bacteria 8 rabbits from the experimental group were anesthetized again, and subjected to thoracocentesis. We evacuated 2 mL of pleural effusion from each rabbit and divided this specimen into 4 parts: for microbiological, biochemical, immunological and hematological analysis. We analyzed values of with blood cells (WBC), LDH, glucose and pH of the effusion. Pleural fluid samples were put into blood agar in order to observe bacterial colonies growth. Empyema was considered where the effusion specimen was apparently purulent, or if the glucose was < 2.3 mmol/L (normal range: 4.1–5.9 mmol/L), pH < 7.10 and LDH > 1 000 U/L. Both lungs of all experimental animals were histopathologically examined.

Glucose and LDH values were measured under the Advia 1800 Chemistry System, (Siemens Germany), and WBC under the Advia Hematology System (Siemens Germany) device. Pleural fluid pH values were measured on a digital pH meter (Model 4500, Beckman, USA). Thoracocentesis in 8 experimental rabbits was performed 24, 48, 72 and 96 h after the bacteria administration.

Animals' well-being and the end of experiment

The experiment lasted 7 days and in this period we respected all the principles of animals' well-being. All experimental animals were sacrificed with a lethal intravenous dose of pentobarbital injected through the ear vein, then we performed autopsy and took lungs for histopathological examination.

Histopathological examination

The lungs from empyemic side of all experimental animals and the lung of one control animal were taken for histopathological examination.

We used 4 tissue samples of each rabbit lung specimen, parafinized them and hematoxylin eosin (HE) stained. We randomized 5 HE slides of each tissue sample and did microscopic measurement on 4 microscopic fields. In that way we had done 20 measurements of pleural thickness of each tissue sample.

Statistical analysis

The results were presented as mean values with standard deviation. The significant differences between more than 2 groups were analyzed using one-way ANOVA test (*post-hoc* Tukey test). The relationships among variables were evaluated by Pearson's correlation analysis. A p value of less than 0.05 was taken to be significant. The obtained data were processed through the Star for Windows, R.4.5. software package.

Results

In 4 rabbits the pleural specimens were scarce and clear or there was no pleural effusion following the thoracocentesis 24 and 48 h after the bacteria administration. After the autopsy we did not find any intrapleural changes other than pleural congestion with a few mL of clear effusion and intense smell of turpentine. These animals were excluded, and there were 8 rabbits left in the experimental group.

The mean values ± SD of leukocyte count, LDH, glucose and pH in the pleural fluid specimens obtained by thoracocentesis in 8 rabbits 24, 48, 72 and 96 h after the bacteria administration are shown in Table 1. The dynamics of leukocyte, glucose and pH values alterations in the function of time are shown in Figures 1, 2 and 3.

Table 1

Values of pleural fluid parameters for 8 rabbits over time induced by *Staphylococcus aureus and Escherichia* coli application

Parameters	Hours			
	24	48	72	96
WBC ($\times 10^9$/L), $\bar{x} \pm$ SD	34.75 ± 6.13	27.62 ± 4.62	15.62 ± 4.20	5.62 ± 2.87
LDH (U/L), $\bar{x} \pm$ SD	17.00 ± 4.69	10.12 ± 4.51	6.50 ± 2.72	4.00 ± 1.92
Glucose (mmol/L), $\bar{x} \pm$ SD	1.23 ± 0.45	3.23 ± 0.44	4.15 ± 0.42	4.83 ± 0.46
pH, $\bar{x} \pm$ SD	6.97 ± 0.15	7.10 ± 0.09	7.22 ± 0.03	7.28 ± 0.02

WBC – white blood cells; LDH – lactate dehydrogenase.

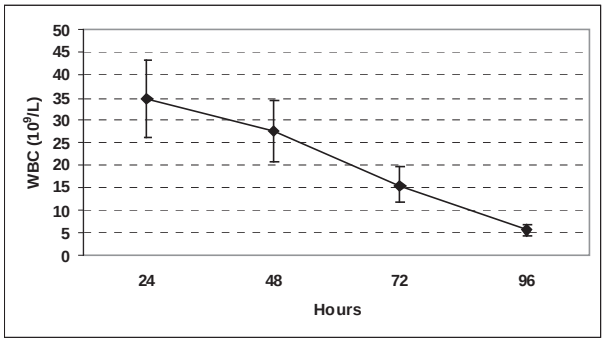

Fig. 1 – The values ($\bar{x} \pm$ SD) of pleural fluid leukocytes count *vs* time after intrapleural bacterial injection in 8 rabbits. WBC – white blood cells.

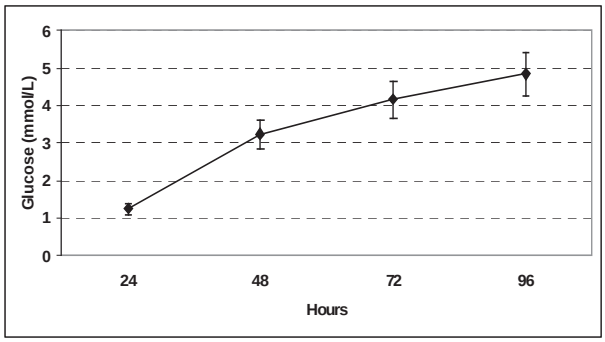

Fig. 2 – The values ($\bar{x} \pm$ SD) of pleural glucose *vs* time after intrapleural bacterial injection in 8 rabbits.

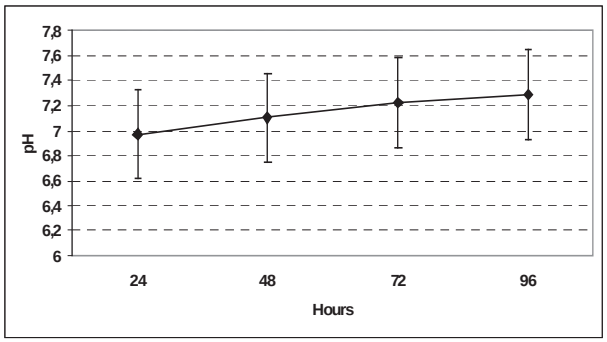

Fig. 3 – The values ($\bar{x} \pm$ SD) of pleural effusion pH *vs* time after intrapleural bacterial injection in 8 rabbits.

The mean value of leukocytes found after 24 h was: 34.75 ± 6.13 $\times 10^9$/L, LDH 17,000 ± 4.69 U/L, glucose 1.23 ± 0.45 mmol/L and pH 6.97 ± 0,15. These values entirely met the Light's criteria for complex and complicated parapneumonic effusions and empyema, and 5 of 8 samples showed bacterial growth on blood agar.

The mean value of leukocytes found in pleural fluid after 48 h was: 27.62 ± 4.62 $\times 10^9$/L, LDH 10.12 ± 4.51 U/L, glucose 3.23 ± 0.44 mmol/L and pH 7.10 ± 0.09. High levels of leukocytes and LDH were retained, and low values of glucose and pH were the consequence of intense glucose metabolism and a higher production of acid metabolic products (Figures 2 and 3). Two of eight specimens showed bacterial growth on the blood agar plate.

After 72 and 96 h the mean glucose and pH values were 4.15 ± 0.42 mmol/L, 4.83 ± 0.46 mmol/L and 7.22 ± 0.03, 7.28 ± 0.02, respectively, coming out from the empyema limits. Leukocytes dropped to 15.62 ± 4.20 $\times 10^9$/L after 72 h, and came into the normal range of 5.62 ± 2.87 $\times 10^9$/L 96 h after the bacteria injection. There was no bacteria growth after 72 and 96 h.

We found a significant statistical difference for all the values of leukocytes as well as glucose at any thoracocentesis time ($p < 0.05$). No significant statistical difference was found between LDH values measured after 48 and 72 h as well as 72 and 96 h after the bacteria injection. There was no significant statistical difference for pH values in the pleural samples after 24–48, 48–72 and 72–96 h from bacteria administration.

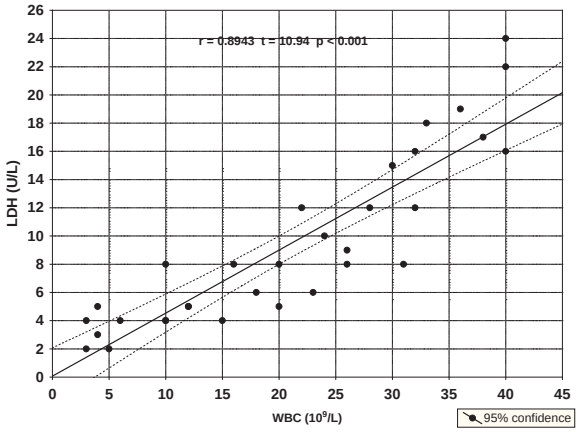

Fig. 4 – A positive correlation between leukocytes count and lactate dehydrogenese (LDH) value in experimentally induced pleural effusion in 8 rabbits.

We found a positive correlation between leukocytes count and the LDH values ($r = 0.071$, $p < 0.001$) (Figure 4), but a negative correlation between leukocytes count and the glucose level ($r = 0.864$, $p < 0.001$) (Figure 5) and between leukocytes count and the pH level in the pleural effusion ($r = 0.894$, $p < 0.001$) (Figure 6).

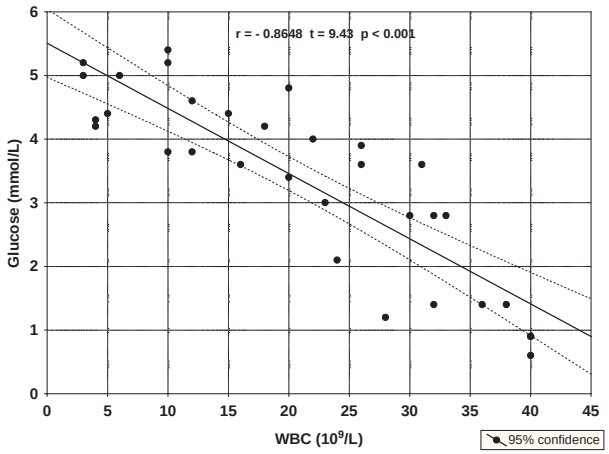

Fig. 5 – A negative corelation between glucose level and leukocytes count in experimentally induced pleural effusion in 8 rabbits.

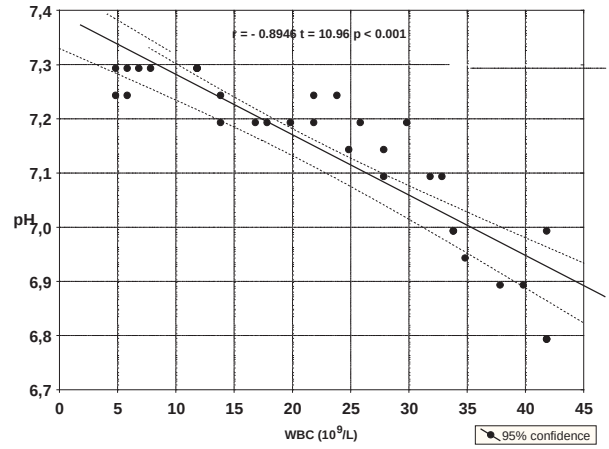

Fig. 6 – A negative correlation between leucocytes count and pH value in experimentally induced pleural effusion in 8 rabbits.

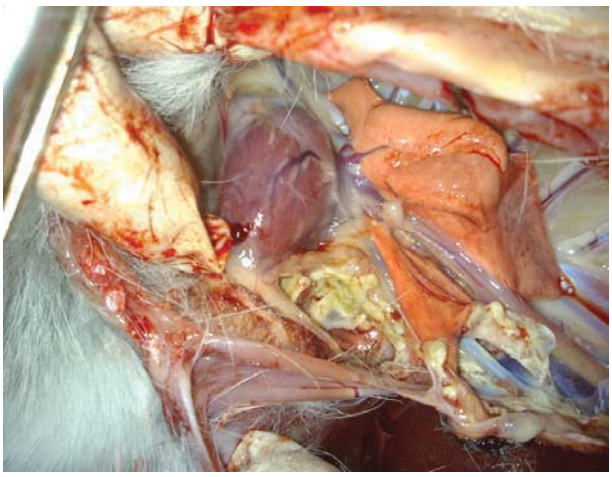

Fig. 8 – Empyemic space with fibrin and purulent detritus. The control lung did not sustain any macroscopic change.

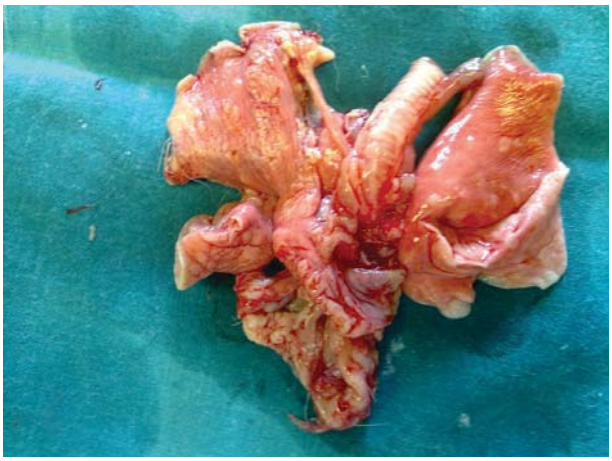

Fig. 9 – Experimentally induced purulent pleural infection causes inflammatory infiltration in subvisceral part of the lung parenchima at the point of the thickest fibropurulent layer.

The animals from the experimental group and one rabbit from the control group were sacrificed on the day 7 of the autopsy. Five of 8 rabbits had purulent effusion, detritus and fibrin layers in the pleural space (Figures 7 and 8). In 3 animals pleural adhesions were dominant with a few millilitres of turbid effusion (Figure 9).

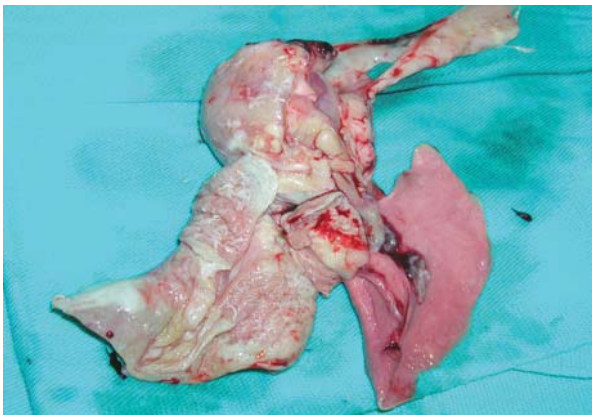

Fig. 7 – Rabbit empyema lung covered with fibrin layers and purulent detritus.

Microscopic examination of HE slides revealed thickness of visceral pleura with prevalent granulocytes infiltrate, fibroblasts and endotel cells proliferation along with purulent detritus on the pleural surface (Figure 10).

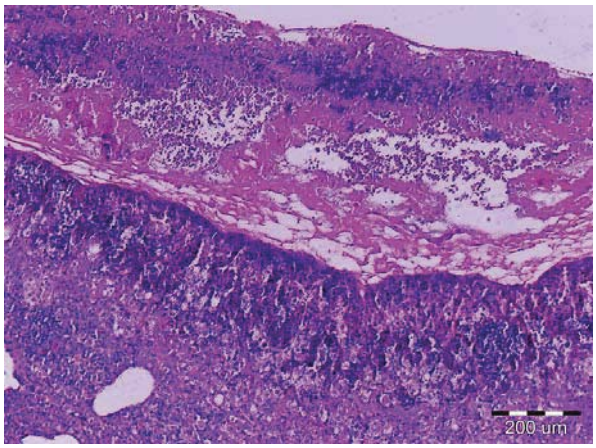

Fig. 10 – Thickened visceral pleura because of submesothelial inflammatory infiltration (HE stain, ×5).

The mean value ± SD for visceral pleura thickness in the experimental group (n = 8 rabbits) was 144.18 ± 24.64 μm. The mean value ± SD of fibroblast number in thickened visceral pleura of the experimental group (n = 8 rabbits) was 196.27 ± 41.20. Macroscopic intrapleural and microscopic lung findings of the control animal were quite normal and the mean value of 20 measurements for visceral pleura thickness was 38.54 μm and the mean value for fibroblast number was 11.30.

Discussion

Despite the extensive experience in the use of Büllau underwater thoracic drainage and decortication, as well as the introduction of new treatment methods such as fibrinolytic therapy video-assisted thoracoscopic surgery (VATS), empyema patients still remain a serious medical problem and therapeutic challenge [17–19].

The American College of Chest Physicians published a clinical practice guideline on the medical and surgical treatment of parapneumonic effusions (PPE), and after comprehensive analysis they found only 3 relevant randomized controlled studies [20]. Ten years after this meta analysis, the situation in this field has not changed substantially [11, 3].

It is well-known that 5–20% of hospitalized patients with parapneumonic effusions progress to empyema, and no thoracic surgery center has enough patients *per* year to perform a prospective study. In addition, it is very difficult to make stratification of these patients because of significant differences among them.

The most convincing examples of a different approach to empyema treatment include the use of antibiotics only [21], or repeated thoracocentesis in the treatment of complicated parapneumonic effusions and empyema [3, 22], as well as appliance of video-assisted thoracoscopic surgery in chronic empyema treatment [1, 11].

In the absence of quality research studies, recommendations for pleural infection treatment depends on pathophysiologic research, small studies and experts' opinions [23].

These circumstances were the reason for experimental empyema model development, which would be able to offer similar conditions found in human pleural infection. These models have been used for almost a century to solve the problems and controversies in empyema treatment.

Graham and Bell [13] made the first experimental empyema model in 1918 to investigate the causes of a very high mortality rate (up to 60%) among American soldieries in the World War I. They used dogs in their experiment and concluded that the open pleural drainage, prematurely performed, was the main cause of such a high mortality rate. Then they formed the Empyema Commission which introduced the closed tube underwater pleural drainage in empyema treatment, and the mortality rate dropped down to 10%.

During their work investigators came to know that any direct intrapleural application of bacteria without causing a sterile exudative pleurisy before such application would end with animal's death due to sepsis or with spontaneous healing without signs of pleural infection [24, 3].

In late 1970's, Sahn and Potts [25] made the turpentine rabbit experimental empyema model. Empyema was induced with 10^9 *K. pneumoniae* injected in the pleural space. Sterile pleurisy was provoked with 0.3 mL of turpentine 96 h before the bacterial injection.

In this paper the authors estimated the influence of administered turpentine on leucocytes number, pH, pO2 and pCO2 values in pleural exudates in the first 96 h. They revealed that in turpentine-provoked pleural effusions there was no increase of leucocytes number and bacteria growth which are responsible for metabolic activity. That was why the values of estimated the parameters did not exceed the normal range. It was very important to find that chemical injury of mesothelial cells did not affect parameters which we used for empyema classification. This model was used for research of pharmacological characteristics and therapeutic efficacy of gentamicin [15]. Turpentine rabbit experimental empyema model with 10^{10}/mL *Escherichia coli* was applied for the research of clarithromycin efficacy, newer quinolones and azithromycin penetration in the empyema fluid [9, 26, 27]. This model was also used to study linezolid and ertapenem pharmacokinetics in parapneumonic effusions [28]. This model has been proved as a better one compared with our model with two bacteria. What is next? We will try to create experimental empyema model with 10^{10}/mL *Escherichia coli*, because there is 10 times more bacteria than we accepted as optimal concentration.

The pig experimental empyema model had used an umbilical cord instead of the turpentine in order to provoke sterile effusion. Mavreudis et al. [29] applied this model to prove that the possibility for empyema induction depends on the number and strain of administered bacteria. In the case of application of *B. fragilis* bacteria, no animal developed empyema, but in combination with *S. aureus* or *E. coli,* one third of animals developed empyema. One million bacteria caused empyema in half of all animals, but with a hundred million bacteria, all animals suffered from empyema. considering these results, we decided to take 9 times more bacteria in a single (2 mL) intrapleural inoculums (4.5×10^8/mL) *S. aureus* [9].

A similar research was conducted with the rabbit turpentine empyema model [30]. The authors have studied how particular bacteria influence the severity and evolution of empyema. They found that the same number (10^8) of administered bacteria *H. influenze*, *S. aureus and B. fragilis* caused empyema in one third, one half and two thirds of animals, respectively. A combination with two bacteria was more successful in empyema induction and 11 of 12 animals evolved empyema.

Taking experience from our previous experiment when we used only *S. aureus* in intrapleural injection and gained more than one third of negative results, we decided to make a new experimental empyema model using two bacteria: *S. aureus and E. coli*. With the intention to make a greater chance for empyema induction we applied again 9 times more bacteria (1.5 Mc Farland/ml bacterial concentration) than it was recommended for this bacterial combination. Despite this fact we succeeded to induce empyema only in 8 of 12 experimental animals.

Sasse et al. [24] published their negative experiences in empyema induction by application of a monoculture bacterial specimen upon previously applied different cofactors such as mineral oil, *E. coli* supernatant, nutrition agar and talc. These authors published that pH value in their turpentine model effusion was under 7.1 only within the first 6 h, and after 24 h it rose to 7.25. The glucose level was 45 mg/dL only in the first 24 h and after that exceeded 100 mg/dL.

We got better results using the turpentine model with two bacteria compared with the above-mentioned data. In our experimental model the pH value 48 h after bacteria administration was 7.10, and 7.22 after 72 h. The glucose level was 1.23 mmol/L 24 h after we administered bacteria in pleural space, and 3.23 mmol/L after 48 hours. Glucose value returned in the normal range (4.1–5.9 mmol/L) 72 h after bacteria administration and was 4.15 mmol/L.

A positive correlation found between a growing number of leukocytes and LDH values could be explained with intense cellular destruction in an acute inflammation.

Also, with the growth of leukocytes count a glucose level became lower, which was in concordance with the results of other authors [4]. This might be explained with phagocytosis and intense bacterial metabolism which was followed by a growing intensity of glycolysis. In those conditions a bigger production of acid metabolic products was present so the measured values of pleural fluid pH were the lowest on the first day, when the intensity of inflammation was obviously the highest, and after that they became gradually bigger.

Other authors [4] using the turpentine model with *Streptococcus pneumoniae* determined the level of glucose, the value of LDH and the number of leucocytes in pleural effusion samples. The values of glucose they found were under 1.4 mmol/L at 72 h after bacteria administration, whereas the highest number of leucocytes was 5.95×10^9/L. The numbers of leucocytes that we found in effusion samples were 35×10^9/L at 24 h and 5.62×10^9/L at 72 h after administered bacterial inoculums.

In their latest rabbit experimental empyema model Sasse et al. [24] used nutritive brain heart infusion (BHI) agar aimed at keeping bacteria inside the pleural space without prior causing of chemical pleurisy. In this experiment they administered 10^8 *Pasteurella multocida*, a very virulent rabbit pathogen. Using this model the authors studied thoracocentesis effectiveness and its possibility to replace thoracic drainage in the empyema treatment, and the importance of timing for thoracic drainage to improve drainage efficacy [3, 14]. This model was also used for studying antibiotics' effectiveness by measuring their concentration in the pleural space after the parenteral administration was conducted [9, 15, 31]. On this model Sasse et al. [24] studied the role of TGF- β in the process of pleural fibrosis. These authors documented that the level of TGF-β1 correlates with microscopic thickness and the number of fibroblast in visceral pleura. This research suggests that TGF-β1 inhibition can reduce the intensity of residual pleural fibrosis.

In this empyema model the authors pH values lower than 7.1 in the samples collected 24, 48, 72 and 96 h after bacteria administration. Glucose level obtained in the effusion sample 48 h after bacteria injection was 43 mg/dL, a little above the empyema level (40 mg/dL). After 72 h, glucose values came into normal range. The glucose values obtained in this experimental empyema model were very similar to those we got in our empyema model.

After they sacrificed experimental animals, they found a dense purulent content, as well as fibrin adhesions within all pleural spaces of experimental rabbits. The rabbits from the control group did not have any intrapleural pathological changes.

We found purulent exudates and fibrin adhesions in 5 of 8 rabbits. Three rabbits had as dominant fibrin adhesions with a very little turbid pleural effusion. Two control rabbits also had no intrapleural pathological findings.

In this experimental model modification the authors placed a chest tube in the pleural space and then fixed it in the subcutaneous tissue of the scapular region, using the drain for the substances application and taking pleural fluid samples. Using this model of experimental empyema Zhu et al. [19] compared efficacy of the separate and common use of recombined human deoxyribonuclease and tissue plazminogen activator in the treatment of fibropurulent stage of empyema. We did not fix the drain in the subcutaneous tissue due to possible formation of fibrin septa that could block the discharge of pleural fluid samples.

Moxifloxacin efficiency research can serve as evidence of the turpentine model reliability. Twenty six rabbits were used for turpentine empyema model with *Streptococcus pneumoniae* and another 26 rabbits for empyema model with *Pasteurella multocida* in nutritive agar. In the results analysis after moxifloxacin administration they did not make differences between these 2 models of experimental empyema [31].

Despite many attempts to provoke experimental empyema by using *P. multocida* we did not succeed because the rabbits were dying even after inoculation of 10^5 bacteria. Bacteria application in solution of BHI agar did not result in the desired effect of keeping bacteria in the pleural space as all animals died with the signs of sepsis.

What is next? Rabbit experimental empyema model with *P. multocida* in BHI agar is one of the most efficient models and we will try to establish it again, now with new standardised bacterial cultures.

In the last 10 years empyema research has become basic again. Studying the role of cytokines, the powerful mediators of inflammation and tissue reparation, lowered these researches to the cellular and molecular levels.

Researches have not continued with the expected intensity and the present situation leaves enough room to continue this work the accomplishments of which would be implemented in practice in the area of the empyema treatment.

The fact that without these reliable experimental empyema models these researches would not be feasible accentuates their necessity and importance. In particular, we have been interested for the influence of some cytokines on the development of pleural fibrosis and possibilities of their inhibition. For these researches we created not so efficient rabbit experimental empyema model with *S. aureus*.

We plan to investigate the influence of TGF-β on inflammatory-proliferative processes and angiogenesis and complex cytokines network in the purulent pleural infection. Because of that we made our own model with two bacteria. Unfortunately we are not entirely satisfied with the results.

What are the next steps? The third answer to this question would be creation of the rat experimental empyema model.

Conclusion

With the intention to obtain a reliable empyemic model with more intensive pleural reaction we created the model with two different human pathogen bacteria and used 9 times higher number of bacteria than recommended in published papers. The combination of two human pathogens (*E. coli and S. aurous)* has already been proven as very effective in experimental emphysema causing. We generated acceptable, but not satisfactory results. They were a little better than in our experiment with monobacterial empyema, but not as good as we expected and came across in published respectable articles. We did not succeed in provoking an empyema in 40% of experimental animals. Obviously, the number of bacteria and bacterial combination of human pathogens are not sufficiently reliable. We plan to continue our research concerning cytokines network and angiogenesis in pleural inflammation and we need a better and more reliable experimental empyema model. Our previous experiences justify new decision making.

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P R E L I M I N A R Y
R E P O R T

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A preliminary study on local administration of dexamethasone after tooth extraction – Better preservation of residual alveolar ridge?

Preliminarno ispitivanje lokalne primene deksametazona posle ekstrakcije zuba – bolja očuvanost rezidualnog alveolarnog grebena?

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Abstract

Background/Aim. It is important that the height of the edentulous alveolar ridge after tooth extraction remains at a reasonable acceptable level for as long as possible. The aim of this study was to report preliminary results of the clinical effect of local oral submucous administration of dexamethasone after tooth extractions in order to prepare alveolar supporting tissues for acceptance of removable dentures. **Methods.** In a total of 15 patients (11 partially and 4 completely edentulous) the quantity of 0.25 mL to 0.5 mL of dexamethasone was injected bucally and orally in the region of the tooth socket after complicated extractions. **Results.** Healing of extraction wounds was uneventful in all the patients, without pain or local inflammation. **Conclusion.** Dexamethasone can be locally applied to oral tissues to prevent post-extraction inflammation and extensive resorption of the residual alveolar ridge. The obtained results are promising for patients undergoing classic prosthodontic rehabilitation soon after tooth extraction, demonstrating that there are no adverse effects after local oral corticosteroids administration.

Key words:
oral surgical procedures, preprosthetic; tooth extraction; alveolar process; rehabilitation; dexamethasone; treatment outcome.

Apstrakt

Uvod/Cilj. Od ključnog značaja je da visina bezubog alveolarnog grebena posle vađenja zuba ostaje što duže na prihvatljivom nivou. Cilj rada bio je da se prikažu preliminarni rezultati efekata lokalne submukozne primene deksametazona na tkiva iz kojih su ekstrahovani zubi radi pripreme alveolarnih tkiva i nosećih tkiva za prihvatanje zubnih proteza. **Metode.** Kod ukupno 15 pacijenata (11 krezubih i 4 bezuba) dato je od 0.25 mL do 0.5 mL deksametazona *per injectionem* bukalno i oralno u alveolarne čašice posle komplikovanih ekstrakcija zuba. **Rezultati.** Zarastanja rana kod svih pacijenata bila su neometana, bez bolova ili lokalnih upala. **Zaključak.** Deksametazon može biti lokalno dat u oralna tkiva sa ciljem prevencije postekstrakcione upale i izražene resorpcije rezidualnog alveolarnog grebena. Rezultati studije su obećavajući za lečenje pacijenata koji će biti stomatoprotetski rehabilitovani neposredno posle ekstrakcija zuba i ukazuju na to da nema neželjenih efekata prilikom lokalne primene kortikosteroida na tkiva iz kojih su ekstrahovani zubi.

Ključne reči:
hirurgija, oralna, preprotetske procedure; zub, ekstrakcija; alveolni nastavak; rehabilitacija; deksametazon; lečenje, ishod.

Introduction

Corticosteroids otherwise sovereign anti-inflammatory and anti-oedematous drugs, are frequently used in oral surgery to prevent, or at least minimize, postoperative pain and oedema due to surgical trauma [1–3]. They can be used locally or systemically, administered by injection or orally [4]. Although corticosteroids have several general effects [5], their single use (preoperative or postoperative) is supposed not to have any undesired effect on the adrenal-pituitary regulation of steroid secretion [1].

The problem of edentulous ridge reduction has been noted in the dental literature for many years. A number of authors have discussed this problem due to difficulties for orofacial rehabilitation by means of proper denture design [6, 8]. It is well known that marked atrophy and reduction of alveolar bone following tooth loss complicate prosthodontic rehabilitation [7, 9–11]. Difficulties have been encountered even in taking impressions of edentulous jaws with reduced and resorbed edentulous ridges [11], as well as in achieving and maintaining the stability of fabricated acrylic dentures, particularly in the mandible [11]. Consequently,

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edentulous patients with the resorbed residual ridges have serious problems in chewing with dentures [9, 11, 12]. For these reasons it is crucially important that the height of the edentulous alveolar ridge, after tooth extraction, remains at a reasonable acceptable level for as long as possible. Additionally, it is necessary that fabricated acrylic dentures, through constant pressure on the edentulous ridge, do not cause delayed resorption of residual ridge.

It is possible that steroids, due to their marked local anti-inflammatory effect could lessen the ridge resorption after tooth extraction as one of the possible reasons for this event is local tissue inflammation as a consequence of tissue injury. The aim of this study was to report preliminary results of the clinical effect of local oral submucous administration of dexamethasone after tooth extractions in order to prepare alveolar supporting tissues for the acceptance of removable dentures.

Methods

A total of 15 otherwise healthy patients (without visible symptoms of local osteoporosis, or any other intraoral disorder except tooth caries or periodontal disease), 11 partially and 4 completely edentulous (9 women aged 45–52 years, and 6 men aged 54–63 years), undergoing tooth extraction prior to classic prosthodontic rehabilitation, were included into the study. Altogether, 23 teeth were removed, 3 teeth at the most in a single patient. In each patient, at least one extraction was difficult, meaning the need for a bone portion removal with burs, or removal of a part of buccal or lingual cortical plate.

To prevent an extensive post-extraction bone resorption and possible complications of extraction wound healing, immediately after the completion of difficult tooth extraction, a submucous 0.5 mL dexamethasone injection (Dexason®, Galenika, Belgrade) was administered, bucally and orally (altogether 4 mg of dexamethasone), beside the socket of the tooth that was removed with difficulties (Figure 1).

All the patients were followed-up regularly, and prosthodontic rehabilitation with removable partial or full dentures started two weeks afterwards.

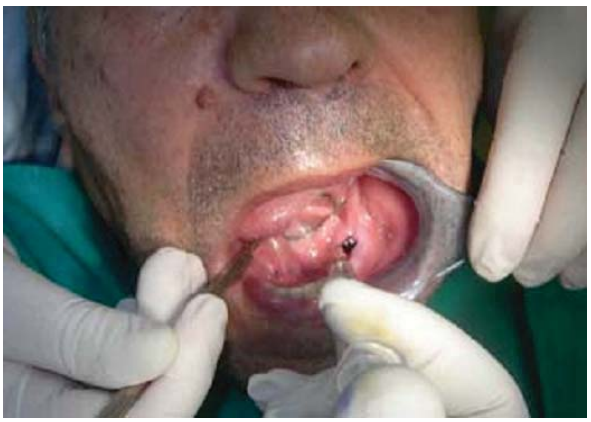


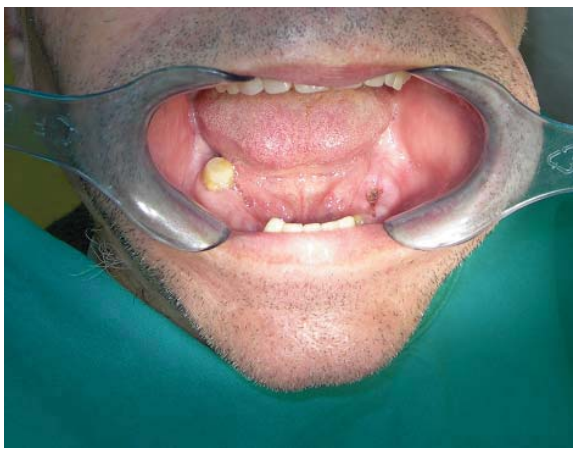
Fig. 1 – Dexamethasone injection beside the post-extraction wound.

Results

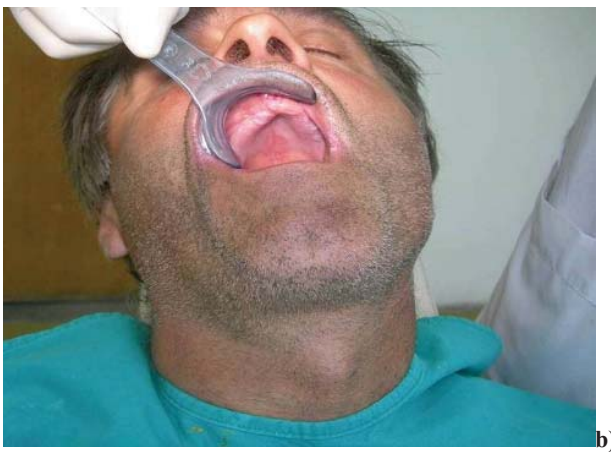
After administration of dexamethasone, no symptoms of local pain or inflammation, or any other complication, were noted (Figure 2).

The bones of the alveolar ridge were solid enough to enable making of dentures without the need of any additional plastic procedure, and control radiographs (Figure 3) revealed the absence of signs of undesired bone remodelling or extensive resorption.

Fig. 3 – Control radiogram after the wound had been healed.



a)



b)

Fig. 2 – The lower (a) and upper (b) jaw 2 weeks after local administration of dexamethasone.

Figure 4 shows the favorable finding of the upper jaw in one patient, 2 weeks after local administration of dexamethasone.

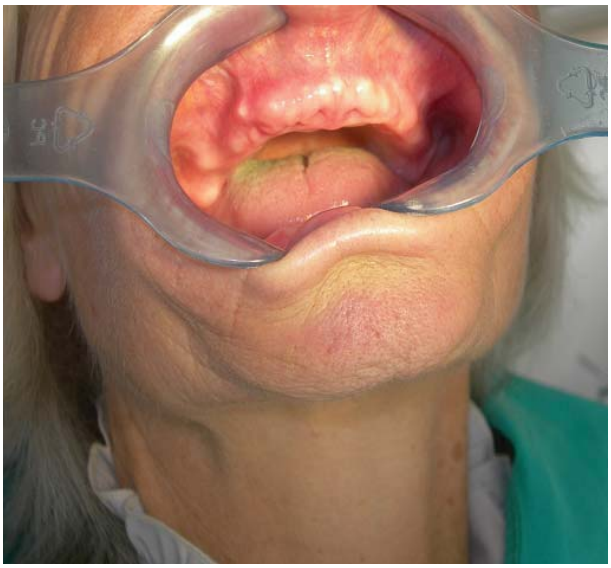


Fig. 4 – The upper jaw of a female patient 2 weeks after local administration of dexamethasone.

Consequently, all the patients were successfully rehabilitated with removable dentures (Figure 5).

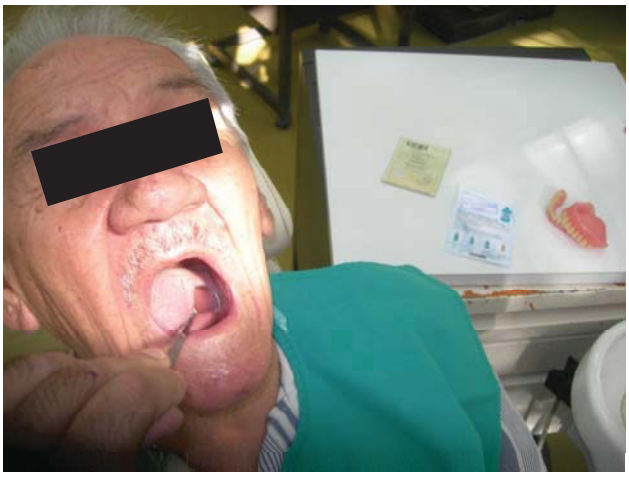


Fig. 5 – Local finding after the complete dentures had been made.

Discussion

The anti-inflammatory effect of corticosteroids has been proved for many local or systemic disorders [13, 14]. Therefore, indications for the use of corticosteroids due to their anti-inflammatory effect are wide, ranging from any kind of local inflammation to many postoperative complications in oral and maxillofacial surgery. Therefore, the use of corticosteroids has also been recommended for pain reduction, oedema, and trismus following oral surgical procedures [1, 2, 4].

Dexamethasone was injected beside the post-extraction socket so that it could demonstrate maximal anti-inflammatory effect and remain locally and not rinsed by salivary flow. It was injected in a total dose that is usually used for single intraoral application when anti-inflammatory dexamethasone effect is desired [1–4]. It was considered not to use dexamethasone if patients had any possible contraindication for steroid use [5, 15], and such patients were excluded from the study.

Although the anti-inflammatory effect of dexamethasone is well-known, there are no studies investigating its possible influence on the reduction of post-extraction alveolar ridge resorption. In spite the fact that all the 15 treated patients in this preliminary study had difficult tooth extractions, the wound healing in the post-extraction period was uneventful, and the residual alveolar ridge did not demonstrate signs of excessive resorption or malformation. We believe that this is mainly due to anti-inflammatory effect of the used dexamethasone. However, the number of treated patients is relatively small, and a double-blind controlled trial is under way to confirm possible favourable effect of dexamethasone in wound healing after tooth extraction.

Conclusion

Dexamethasone can be locally applied to oral tissues to prevent post-extraction inflammation and extensive resorption of the residual alveolar ridge. The obtained results are promising for patients undergoing classic prosthodontic rehabilitation soon after tooth extraction, demonstrating that there are no adverse effects of such corticosteroids use.

Acknowledgment

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CASE REPORT

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Congenital cholesteatoma of the middle ear – uncommon clinical presentation

Neobična klinička prezentacija kongenitalnog holesteatoma srednjeg uva

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Abstract

Introduction. Congenital cholesteatoma of the middle ear is un uncommon and yet not well-defined disease. Only few cases of cholesteatoma in the *fossa ovalis* with unusual clinical presentation have been reported in medical literature. **Case report.** We reported a 16-year-old girl with congenital cholesteatoma in the *fossa ovalis* with minimal clinical presentation. A small mass was found occluding the *fossa ovalis* and mimicking otosclerotic process within tympanic cavity. The operation started as stapedotomy, and when the process was confirmed it converted to mastoidectomy *via* the retroauricular approach. **Conclusion.** The diagnosis of congenital cholesteatoma in children should always be considered, even if the clinical symptoms imitate other ear disorders, in our case otosclerosis.

Key words:
cholesteatoma; congenital abnormalities; ear, middle; diagnosis; otorhinolaryngologic surgical procedures.

Apstrakt

Uvod. Kongenitalni holesteatom lokalizovan u kavumu timpani retko je oboljenje, još uvek nerazjašnjene etiologije. Do sada je objavljeno samo nekoliko radova u medicinskoj literaturi o kongenitalnom holestatomu u ovalnom prozoru sa minimalnom kliničkom prezentacijom. **Prikaz bolesnika.** Prikazana je 16-godišnja devojčica sa kongenitalnim holesteatomom lokalizovanim u ovalnom prozoru sa minimalnim kliničkim simptomima. Pronađen je mali holesteatom koji je u popunosti ispunjavao fosu ovalis i imitirao otosklerotični proces u kavumu timpani. Operacija je započeta kao stapedotomija, a kada je proces konstatovan, nastavljena je kao mastoidektomija kroz retroaurikularni pristup. **Zaključak.** Trebalo bi uvek razmotriti dijagnozu kongenitalnog holesteatoma kod dece, čak i kada simptomi imitiraju neko drugo oboljenje srednjeg uva, u našem slučaju otosklerozu.

Ključne reči:
holesteatom; anomalije; uvo, srednje; dijagnoza; hirurgija, otorinolaringološka, procedure.

Introduction

Congenital cholesteatoma is relatively uncommon condition. It is defined as the whitish mass behind an intact eardrum, in a patient with no history of ear trauma or previous surgery and with no retraction pocket, perforation or granulation tissue that can be detected on the surface of an eardrum [1]. The term "congenital" has been used rather conventionally because the pathogenesis of congenital cholesteatoma remains unclear. Various hypotheses suggest anything from development during the fetal period to a condition acquired during infancy [2]. Almost any recently published paper emphasizes the importance of early diagnosis and intervention, as late diagnosis may be associated with extensiveness of the disease.

In spite of reports suggesting the age at the time of surgery being an important factor that affects treatment outcome, and widely accepted fact that congenital cholesteatoma increases over the time, there is no firm evidence in the literature confirming such relationship.

Usual age at the time of diagnosis is 4–5 years, and 70% of patients are asymptomatic at that point [3].

We reported a patient with congenital cholesteatoma in the *fossa ovalis* with minimal clinical symptoms, presenting as an otosclerotic process within tympanic cavity.

Case report

A 16-year-old female patient was referred to the Clinic for Otolaryngology due to the right-sided hearing loss that

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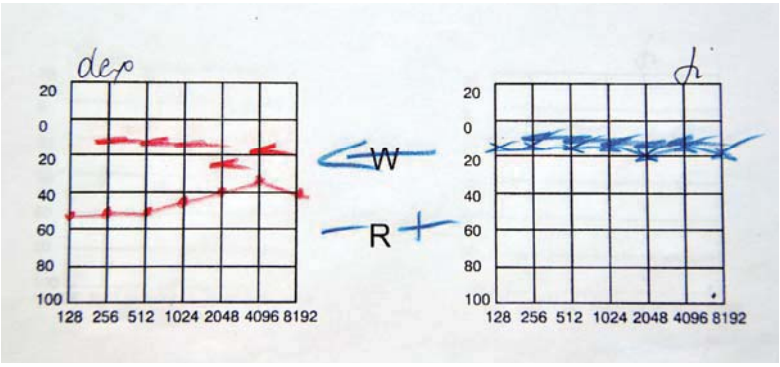
was diagnosed two years before. The patient had no previous history of *otitis media*, middle ear trauma, or any previous ear surgery.

Otoscopic examination showed intact tympanic membranes on both sides, although the right membrane had tympanosclerotic plaque in its anterior-inferior quadrant. There were no symptoms related to the vestibular system, nose or throat. Audiometric evaluation revealed mild conductive hearing loss of about 40 dB on the right side with a dip of bone conduction at 4 kHz (Figure 1). Stapedius reflex was absent on the same side. The results of general physical examination and routine laboratory and blood chemistry tests were within normal range.



Frequency (Hz) Frequency (Hz)

Fig. 1 – Preoperative liminar tonal audiogram.

Computed tomography (CT) of the temporal bone was performed and the findings in the middle ear appeared normal, with normally aerated and pneumatized mastoid cells.

Based on these findings, explorative surgery was performed on the right ear with the presumptive diagnosis of otosclerosis. Open type cholesteatoma was found obliterating oval window (Figure 2). The long process of incus and head

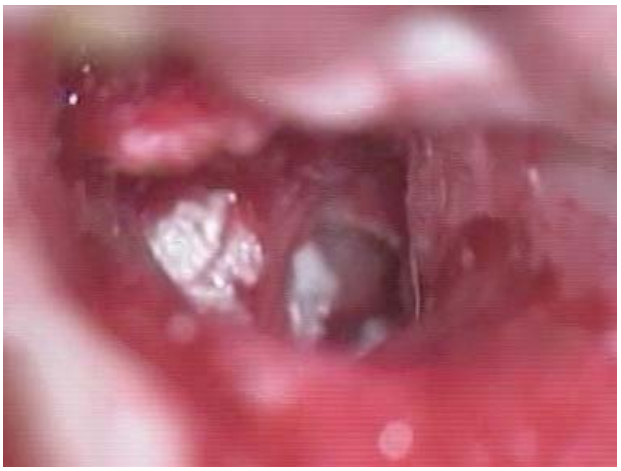


Fig. 2 – Intraoperative findings showing a cholesteatoma in the *fossa ovalis.*

of stapes was missing. Due to the findings, operation was continued as mastoidectomy and posterior timpanotomy in order to completely remove the process. After removal of cholesteatoma, mobile stapes footplate was covered with temporalis fascia and TTP™ (Tuebingen Titanium Prosthesis, AERIAL) was implanted between the malleus and stapes footplate.

Four years after the surgery, there were no signs of relapse and liminar tonal audiometry of the right ear showed closure of air bone gap within the 10 dB.

Discussion

Generally, the initial otologic symptom in a patient with open type of cholesteatoma is hearing loss, and in some cases, a white mass can be seen through tympanic membrane [3]. Many of these patients are unaware of their hearing loss, especially because it is manifested in childhood. Most of congenital cholesteatoma in the middle ear are detected early in life, but our patient was 16-years-old when first referred to our Clinic, and had no previous history of any ear, nose or throat condition.

The primary localization of open type cholesteatoma, as suggested by many authors, is around the oval window, the same as in the presented patient. From there, it may spread to epitympanic recess, mastoid antrum, or to mesotympanum [4, 5].

Congenital cholesteatoma commonly develops in the isthmus of the tympanic cavity and the most frequent ossicular malformations are involvements of a long process of the incus and suprastructures of stapes in more than 60% of patients [3, 6, 7] corresponding to the junction of the first and second branchial arches. As congenital cholesteatoma tends to spread into the posterior-superior part of tympanic cavity, the fact that the suprastructure of stapes was affected in the presented patient suggested congenital origin of cholesteatoma.

Mastoid pneumatization in our patient was normal and these findings are in accordance with Iino et al. [8] and other authors [9, 10] who reported better pneumatization in patients with congenital, compared to acquired cholesteatoma.

The initial symptom, intact tympanic membrane, audiological findings, and normal CT scans suggested an otosclerotic process in the middle ear, the diagnosis being supported by the age of the patient at the time of detection of hearing loss. However, these findings were proved to be misleading in the presented patient. There are few other cases in the lit-

erature describing congenital cholesteatomas that have been silent for many years or have been accompanied with minimal clinical presentation [4, 11].

Conclusion

The diagnosis of congenital cholesteatoma should always be considered, even if the clinical symptoms mimic other ear disorders, in our case otosclerosis. In some cases, such as this one, even CT findings are not able to detect cholesteatoma. Therefore, clinicians should always keep high level of cautiousness when dealing with young patients who present with hearing loss as the only symptom in order to deliver early treatment and improve final outcome.

Acknowledgements

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CASE REPORT

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Sarcoidosis of the pleura – A case report

Sarkoidoza pleure

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Abstract

Introduction. Pleural involvement is an uncommon manifestation of sarcoidosis. It may manifest as pleural effusion, pneumothorax, pleural thickening and nodules, hydropneumothorax, trapped lung, hemothorax, or chylothorax. The incidence of pleural effusion with sarcoidosis ranges from 0% to 5% but has been reported to be as high as 7.5%. Pleural effusions complicate sarcoidosis in < 3% of patients. **Case report.** We reported a 64-year-old male patient with chronic multiorgan sarcoidosis. This patient developed pleural sarcoidosis with massive pleural effusion several years after the diagnosis of sarcoidosis. A definitive diagnosis of a sarcoid pleural effusion was based on a biopsy demonstrating noncaseating granuloma. The patient responded well to the treatment (methotrexate and methylprednisolone) with a complete withdrawal of pleural effusion following five weeks of the treatment beginning. **Conclusion.** The presented patient is a rare case of pleural involvement of sarcoidosis with massive effusion, who responded well to the treatment.

Key words:
sarcoidosis; pleural effusion; biopsy; therapeutics.

Apstrakt

Uvod. Zahvaćenost pleure je retka manifestacija sarkoidoze. Može se manifestovati kao pleuralni izliv, pneumotoraks, pleuralno zadebljanje, noduli, kao hidropneumotoraks, zarobljena pluća, hemotoraks ili hilotoraks. Učestalost pleuralnog izliva kod sarkoidoze kreće se od 0% do 5%, ali može biti i do 7,5%. Zahvaćenost pleure kod sarkoidoze javlja se kod < 3% bolesnika. **Prikaz bolesnika.** Prikazan je bolesnik, star 64 godine, sa hroničnom multiorganskom sarkoidozom. Bolesnik je razvio pleuralnu sarkoidozu sa masivnim pleuralnim izlivom nekoliko godina nakon postavljanja dijagnoze sarkoidoze. Definitivna dijagnoza sarkoidoznog pleuralnog izliva postavljena je na osnovu biopsije koja je pokazala nekazeifikovani granulom. Bolesnik je dobro reagovao na lečenje metotreksatom i metilprednisolonom, sa kompletnim povlačenjem pleuralnog izliva posle pet nedelja lečenja. **Zaključak.** Ovo je redak slučaj pleuralne zahvaćenosti sarkoidozom sa masivnim pleuralnim izlivom koji je dobro reagovao na lečenje.

Ključne reči:
sarkoidoza; pleura, izliv; biopsija; lečenje lekovima.

Introduction

Pleural involvement is an uncommon manifestation of sarcoidosis. It may be manifested as a pleural effusion, pneumothorax, pleural thickening and nodules, hydropneumothorax, trapped lung, hemothorax, or chylothorax [1–4]. Clinically significant pleural manifestations occur in 2% to 4% of patients with sarcoidosis [1, 2, 4, 5–9]. With the introduction of computed tomography (CT) scans, especially high resolution CT, awareness of pleural manifestations of sarcoidosis has increased, thus allowing detection of more subtle cases of pleural involvement. Pleural manifestations of sarcoidosis may arise at the initial presentation, or at a later stage in the development of known sarcoidosis. The development of pleural sarcoidosis does not appear to have any clear prognostic value. Often the diagnosis is based on clinical findings without histologic proof because when the disease is present elsewhere, most pleural sarcoidosis does not require a biopsy to treat the patient properly. A total of 145 biopsy-proven cases of pleural involvement with sarcoidosis have been reported up to 2000 [4].

Case report

A 64-year-old male patient, had been treated since 1982 for type II diabetes. In 1992 a bronchoscopy with a transbronchial biopsy was performed because of both-sided lung lesions and hilar adenopathy, and sarcoidosis of the lung was

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verified. He was treated with corticosteroids for several months successfully. A few years later, because of complaints of cardiac rhythm disorder, holter monitoring and radionuclid ventriculography were done and confirmed the myocardial sarcoidosis. From that moment, on several occasions he was treated due to clinical, biochemical and radiographic signs of deterioration of chronic multiorgan sarcoidosis. Chest radiography from 2000 presented the stable phase of sarcoidosis when the patient was receiving a maintenance dose of corticosteroids. At that time, remission of sarcoidosis was verified. Whenever trying to discontinue steroid treatment, the sarcoidosis relapsed, and deterioration became evident. Thus, by 2002 the patient was treated with corticosteroids, but because of a glycoregulation impairement as a consequence of this long-term treatment, the corticosteroids were discontinued and methotrexate introduced. In October 2002 a relapse of sarcoidosis was verified with radiographic findings of marked hilar adenopathy and both-sided interstitial changes in the lung parenchyma (Figure 1).

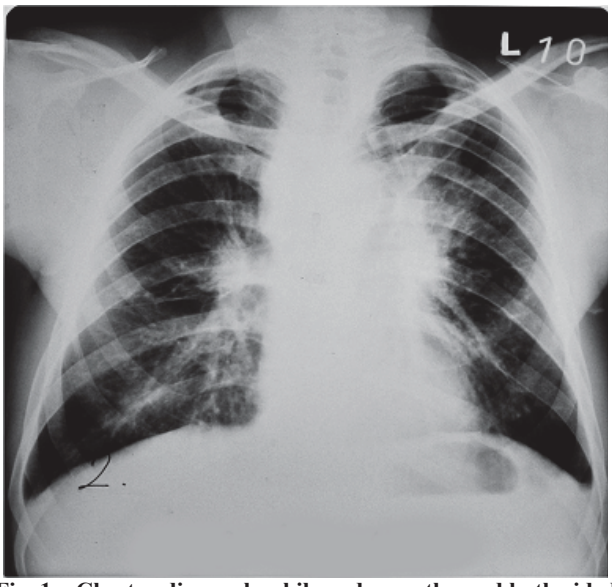

Fig. 1 – Chest radiography: hilar adenopathy and both-sided interstitial changes in the lung parenchyma.

The serum angiotensin converting enzyme (ACE) level was 39 U/L, and the calcium level in 24 h urine was 7.41 mmol/L. All the other clinical and biochemical findings were normal. A bronchoscopy was performed, the endoscopic finding showed extramural compression to the inner wall of the left main bronhcus. A histologic analysis of transbronchial biopsy confirmed sarcoidosis. The methotrexate treatment dose was increased with the favorable effect of sarcoidosis remission achieved soon. Then the drug dose was set to the maintenance one.

Almost a year later at the check-up, the patient complained of progressive dyspnea and chest radiography revealed the shadow of a left-sided massive pleural effusion (Figure 2).

The serum level of ACE was 124 U/L (normal range less than 52 U/L) and the calcium 24-urine level was elevated – 7.2 mmol/L. The patient was hospitalized again, underwent thoracocenthesis when 2 L of serous content were evacuated, characterized as a paucicellular, lymphocyte-predominant exudate. Then a pleuroscopy with pleural biopsy was performed. The histologic finding was noncaseating granulomas, indicative for pleural sarcoidosis (Figure 3).

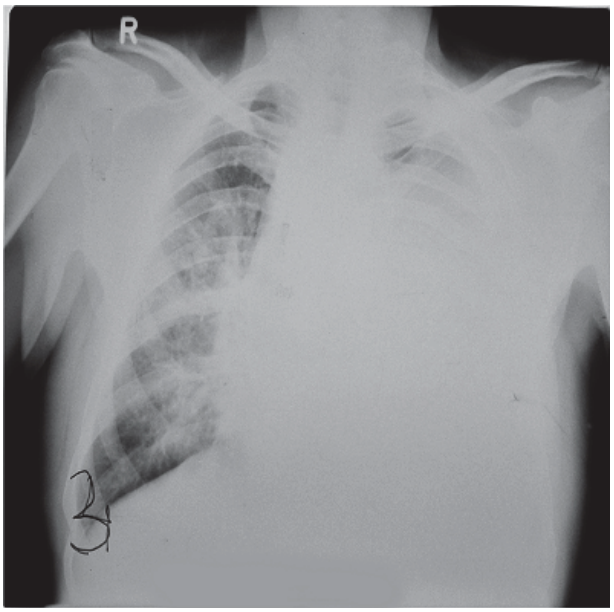

Fig. 2 – Chest radiography: left-sided massive pleural effusion.

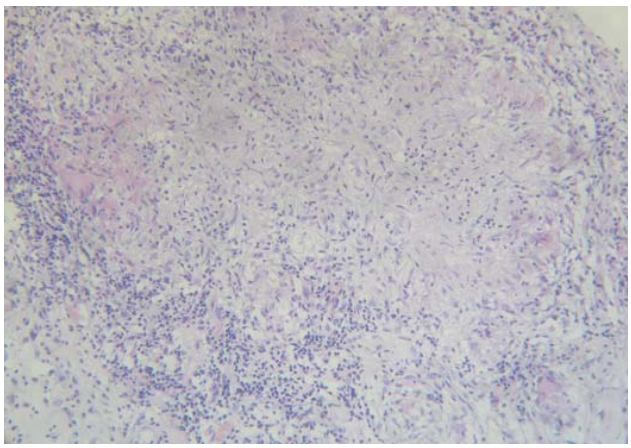

Fig. 3 – Histologic finding of noncaseating granulomas (HE, × 100)

Other causes of pleural effusion were excluded. Following the diagnosis, the methotrexate dose was increased to 10 mg *per* week and 20 mg daily of methylprednisolone was introduced. After 5 weeks of the treatment, a complete withdrawal of the pleural effusion was evident (Figure 4). After that the methotrexate dose was set to the maintenance one of 5 mg *per* week, and methylprednisolone to 15 mg every second day. After three months methylprednisolone was withdrawn.

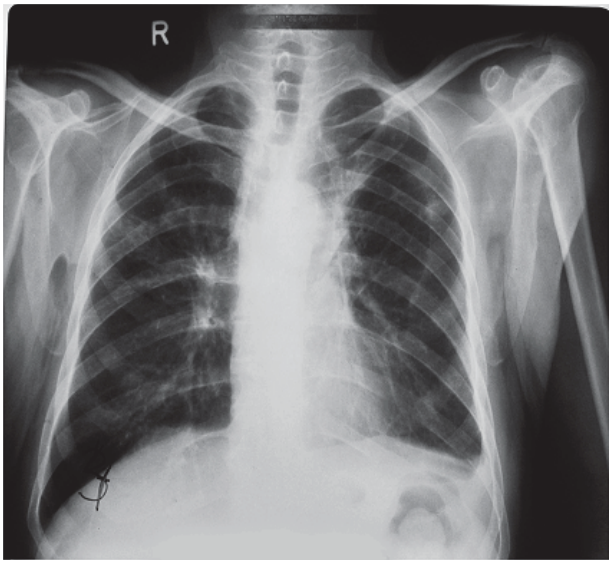

Fig.4 – Chest radiography: complete withdrawal of the pleural effusion.

Discussion

The incidence of pleural effusion with sarcoidosis ranges from 0% to 5% [2, 4, 9] but has been reported to be as high as 7.5% [8]. Pleural effusions complicate sarcoidosis in < 3% of patients and, when present, are usually asymptomatic [1, 4]. We presented the case of sarcoid-related massive pleural effusion as the clinical manifestation of a relapse of sarcoidosis with progressive dyspnea as its only symptom. An analysis of the published references up to 1985 that included reports of pleural involvement with sarcoidosis [4–6, 8–11] shows that out of a total of 3,146 sarcoidosis cases, 76 (2.4%) of them had pleural effusions [10]. However, in another report only three pleural effusions were detected among 2,775 patients with pulmonary sarcoidosis [12]. Because sarcoid-related pleural effusions are rare, it should not be assumed that a pleural effusion occurring in a sarcoid patient is sarcoid-related; other causes of pleural effusion should be considered [2]. We undertook all the available investigations to rule out tuberculosis and malignancy which are the most frequent causes of massive pleural effusion in our population, as well as other granulomatous diseases. The definitive diagnosis of sarcoid pleural effusion relies on a biopsy demonstrating noncaseating granuloma, with the exclusion of alternate granulomatous diseases. In a recent prospective study, thoracic ultrasonograms were performed in 181 consecutive outpatients with sarcoidosis [2]. Pleural effusions were detected in five (2.8%) but only three (1.1%) patients were attributed to sarcoidosis; two were a manifestation of congestive heart failure. The mechanism of pleural effusion formation in patients with sarcoidosis is presumably similar to that of other infiltrative diseases. Involvement of the pleura may lead to increased capillary permeability. Superior vena cava obstruction [13], endobronchial sarcoidosis leading to bronchial stenosis and lobar atelectasis [14], trapped lung [15, 16] and lymphatic disruption with the development of chylothorax have been reported as a cause of sarcoid-related pleural effusions.

Our patient had left-side massive pleural effusion unlike the majority of published cases. Sarcoidosis-related pleural effusions occur slightly more commonly in the right lung (45%) than in the left lung (33%) [4]. The reason for the right-sided predominance is unclear and is not related to organ involvement. Bilateral effusions have been reported in 22% of cases [4].

The onset of pleural effusion ranges from being coincidental with the first diagnosis of sarcoidosis [17] to occurring several years after the diagnosis was made. The latter was the case we have described, our patient had progressive and profound dyspnea lasting for several weeks. In most cases published, the effusions were an incidental finding [2, 4, 5, 9, 17–20]. Patients with a sarcoid pleural effusion usually have extensive parenchymal disease (radiographic stage 2 or stage 3) as it was the case in our report, but also frequently have extrathoracic sarcoidosis [8, 9, 11, 21]. In a series of pleural sarcoidosis with biopsy of visceral and parietal pleural surfaces performed, most cases were radiographic stage II and III sarcoidosis [22]. It appears that with progression of the parenchymal disease, the prevalence of pleural effusions decreases, while that of pleural thickening and pneumothorax increases [4]. Nevertheless, sarcoid-related pleural effusions can occur in all Scadding radiographic stages. There are no specific radiologic features of the pleural effusions that occur in sarcoidosis to suggest the cause, except for the presence of associated parenchymal disease or intrathoracic lymphadenopathy. Although being generally small to moderate, occasionally effusions can be massive [5, 9, 11, 18–21], the same situation being with our patient who had left-sided massive sarcoid-related pleural effusion; sometimes they can be bilateral [5, 8, 11, 14, 16, 20–23] and rarely loculated [24]. Sarcoid-related pleural effusions have been described as exudates or transudates; our patient had serous, paucicellular, lymphocyte-predominant exudate. However, most series [4, 5, 8, 9, 11, 13, 14–16, 18–22, 25–28] have not reported the criteria used to classify these pleural effusions. Higgins et al. [2] have thus summarized the pleural fluid characteristics of all sarcoid-related pleural effusions reported in the literature. The majority of sarcoid-related pleural effusions are exudative. The appearance of the pleural fluid is most commonly serous, sporadically it is serosanguinous [11, 15, 19, 22] and an extremely rare finding is a bloody pleural effusion. The nucleated cell count is typically low at ≤ 1,100 cells/μL. Lymphocytosis occurs in two thirds of cases [1, 2, 4, 5, 9], with predominance of CD_4 lymphocytes [1, 4]. Few cases of pleural fluid eosinophilia have been reported [29–31]. The typical finding in sarcoid pleural effusions is a paucicellular, lymphocyte-predominant exudate, with a pleural fluid/serum protein ratio more consistently in the exudative range than the pleural fluid lactate dehydrogenase (LDH) criterion, as was the case with outpatient's effusion. The dyssynchrony between the pleural fluid protein and LDH ratios suggests that the pathogenesis of sarcoid-related pleural effusions is most consistent with increased capillary permeability with minimal pleural space inflammation. A definitive diagnosis of sarcoid pleural effusion in the presented case relied on the pleural biopsy sample demonstrating noncaseating granulomas, with the exclusion of granulomatous diseases of

known etiology, which is in accordance with the standard diagnostic approach. When it comes to treatment, our patient was treated for chronic sarcoidosis with methotrexate for a longer period of time up to the development of pleural effusion. As sarcoid pleural effusion was diagnosed, the drug dose was increased to 10 mg *per* week and for the following 3 months methylprednisolone was also prescribed in daily doses of 15 mg every second day. With the withdrawal of effusion, methotrexate was set to the maintenance dose of 5 mg *per* week. According to the literature, sarcoid pleural effusions may resolve spontaneously or require corticosteroids for resolution. The majority of these effusions, unlike the presented case, resolve spontaneously. The time of spontaneous resolution is variable, but most resolve in 1 to 3 months [2, 5, 8, 9, 18, 27]. However, there are reports of resolution at 2 weeks with corticosteroid therapy [20] and for as long as 6 months with or without corticosteroids [9, 14]. If the effusion is symptomatic and recurrent, steroid therapy is recommended for symptomatic relief and to hasten the resolution of the effusion. Incomplete resolution of these effusions has been reported with eventual progression to chronic pleural thickening [8] or a trapped lung [15, 16]. Decortication has been successful in relieving dyspnea in a patient who had lung entrapment from sarcoidosis [1, 4, 15, 27].

Conclusion

The presented patient is a rare case of pleural involvement of sarcoidosis with massive effusion, who responded well to the treatment.

Acknowledgements

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Statement of interest

The authors declare no conflict of interest.

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CASE REPORT

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Orbital lymphoma associated with Graves' disease: A case report

Orbitalni limfom udružen sa Gravesovom bolesti

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Abstract

Introduction. The presence of bilateral exophthalmos and palpebral, periorbital edema associated with hyperthyroidism is most often considered as an initial sign of Graves' ophthalmopathy. However, in up to 20% of cases, Graves' ophthalmopathy might precede the occurence of hyperthyroidism, which is very important to be considered in the differential diagnosis, especially if it is stated as unilateral. Among other less common causes of non-thyroid-related orbitopathy, orbital lymphoma represents rare conditions. We presented of a patient with Graves' disease, initially manifested as bilateral orbitopathy and progressive unilateral exophthalmos caused by the marginal zone B-cell non-Hodgkin lymphoma of the orbit. **Case report.** A 64-year-old man with the 3-year history of bilateral Graves' orbitopathy and hyperthyroidism underwent the left orbital decompression surgery due to the predominantly left, unilateral worsening of exophthalmos resistant to the previously applied glucocorticoid therapy. A year after the surgical treatment, a substantial exophthalmos of the left eye was again observed, signifying that other non-thyroid pathology could be involved. Orbital ultrasound was suggestive of primary orbital lymphoma, what was confirmed by orbital CT scan and the biopsy of the tumor tissue. Detailed examinations indicated that the marginal zone B-cell non-Hodgkin lymphoma extended to IV – B-b CS, IPI 3 (bone marrow infiltration: m+ orbit+). Upon the completion of the polychemiotherapy and the radiation treatment, a complete remission of the disease was achieved. **Conclusion.** Even when elements clearly indicate the presence of thyroid-related ophthalmopathy, disease deteriorating should raise a suspicion and always lead to imaging procedures to exclude malignancy.

Key words:
exophthalmos; diagnosis, differential; graves disease; histological techniques.

Apstrakt

Uvod. Zbog ispoljavanja bilateralnog egzoftalmusa i periorbitalnih edema udruženih sa hipertireoidizmom najčešće se inicijalno postavlja sumnja na Gravesovu oftalmopatiju. Ipak, Gravesova orbitopatija kod 20% bolesnika može prethoditi ispoljavanju hipertireoidizma i predstavljati diferncijalnodijagnostički problem, naročito ako je unilateralna. Takođe, i pored primene intenzivne farmakološke terapije, kod 3% bolesnika sa Gavesovom orbitopatijom može doći do progresije bolesti koja u ozbiljnim slučajevaima zahteva zračnu ili operativnu terapiju. Prikazan je bolesnik sa Gravesovom bolešću, inicijalno ispoljenom bilateralnom orbitopatijom sa progresivnim pogoršanjem unilateralnog egzoftalmusa, uzrokovanog B-ćelijskim nehočkinskim, limfomom orbite marginalne zone. **Prikaz bolesnika.** Kod bolesnika, starog 64 godine, sa 3-godišnjom evolucijom bilateralne Gravesove orbitopatije i hipertireoze, predominantno levostrano unilateralno ispoljilo se pogoršanje egzoftalmusa, rezistentno na primenjenu glukokortikoidnu terapiju, zbog čega je učinjena levostrana dekompresija orbite. Godinu dana nakon operativnog lečenja ponovo se razvio značajan egzoftalumos levog oka, što je pobudilo sumnju na drugu etiologiju. Ultrazvučni pregled orbite ukazao je na primarni limfom orbite, što je CT skenom i biopsijom tumorskog tkiva potvrđeno. Detaljna ispitivanja ukazala su na B-ćelijski nehočkinski limfom marginalne zone, proširenog IV – B-b CS, IPI 3 (infiltracija koštane srži: m+orbita+). Nakon primenjene polihemioterapije i radijacione terapije orbite ostvarena je kompletna remisija bolesti. **Zaključak.** Mada Gravesova bolest jeste najčešći razlog bilateralne orbitopatije progresivno pogoršanje orbitopatije zahteva detaljnu analizu u cilju isključivanja drugih ređih etiologija.

Ključne reči:
egzoftalmus; dijagnoza, diferencijalna; gušavost, egzoftalmička; histološke tehnike.

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Introduction

Thyroid-related orbitopathy (TRO) is the most common cause of extraocular muscle abnormality [1]. It tipically presents as proptosis, eyelid inflammation and chemosis, motility disturbances and in severe cases, decreased visual acuity [2]. Orbital imaging clasically shows well-defined extraocular muscle swelling, usually *musculus rectus medialis* and inferor, and periocular fat tissue edema [3]. Its strong association with autoimmune thyroid disease and chronic lymphocytic infiltration suggests shared antigens for both conditions with frequent serum antibodies against thyroid-stimulating hormone (TSH) receptors, thyroglobulin and thyroid microsomal antibodies [4]. Reach lymphocytic infiltration might be a predisposing risk factor for the later development of a malignant lymphocyte clone and orbital lymphoma [5].

We reported a patient with unilateral, low-grade marginal zone B-cell lymphoma simulating unilateraly worsening TRO.

Case report

A 64-year-old man, presented with an excessive lacrimation and discrete palpebral edema with bilateral conjunctival suffusion in November 1999. He was treated for the bilateral conjunctivitis. Steroid/antibiotic eyedrops administered for the presumptive diagnosis of allergic conjunctivitis did not relieve his symptoms. By the end of January 2000, the patient developed the manifestations of hypermetabolism, observed in the form of anxiety, insomnia, sporadic palpitations, tachycardia and weight loss. The patient was seen by an endocrinologist who diagnosed Graves' disease with associated ophthalmopathy.

An objective examination revealed marked periocular swelling, conjunctival hyperemia and chemosis, bilateral exophthalmos with considerable proptosis of the left eye but without any motility disturbances.

The first grade diffuse goiter was determined by palpation; it was more consistent and avascular, whilst the heart rate was 88 beats per minute. Other clinical findings were found to be within normal ranges. Evaluation of thyroid function evidenced hyperthyroidism with suppressed serume TSH level: 0.02 μIU/mL, and T4: 191 nmol/L (60.0–120.0 nmol/L); T3: 3.9 nmol/L (0.6–2.1 nmol/L). The thyroid-specific antibody test was not carried out at the time of diagnosis due to technical reasons.

An initial bilateral enlargement of the thyroid gland of hypoechogenic texture without nodules, but with highly increased vascularisation was verified by thyroid ultrasound. Ophthalmological examination confirmed the bilateral proptosis predominantly to the left eye, oculus sinister (OS) 24 mm, oculus dexter (OD) 20 mm (base 107 mm).

The diagnosis of Graves' disease with associated ophthalmopathy was made, and the patient was placed on propylthiouracil treatment without applying any therapy specific for orbitopathy.

Over the next year, antithyroid therapy application ensured a stable thyroidmetabolic status with the TSH level of 1.23 μIU/L, normal values of free thyroxine iodine fractions. Inspite of achiving the euthyreoid status, the gradual progression of proptosis of the left eye was evident, (OS 26 mm, OD 20 mm, base 107 mm) and the ultrasound and computed tomography (CT) scan of the orbit revealed a marked enlargement of the inferior, medial and lateral rectus muscles bilaterally and more pronounced on the left eye, along with the enlargement of the retrobulbar fat tissue compressing the left bulbous and displacing it downwards. The same findings were confirmed by magnetic resonance imaging (MRI). Upon the completion of the corticosteroid therapy, the regression of the exophthalmos was achieved, but, in May 2002, the exophthalmos was seen to progress on the left side again. Measurements by Hertel exophthalmometry at the base were 107 mm – OS 28 mm, OD 20 mm. Due to the possible damage to the left optical nerve, the orbital decompression surgery was performed. The definite histopathological findings of a part of the ocular muscle showed the lymphocytic infiltration specific to Graves' disease. The patient's postoperative recovery went well, with the expected regression of the left-sided exophthalmos.

In December 2002, left eyeball protrusion was observed to progress again.

The orbital ultrasound findings indicated the presence of the retrobulbar tumor mass of a low reflectability, with a lobular appearance and internal septations, which by their characteristics were suspectable to orbital lymphoma (Figure 1). The

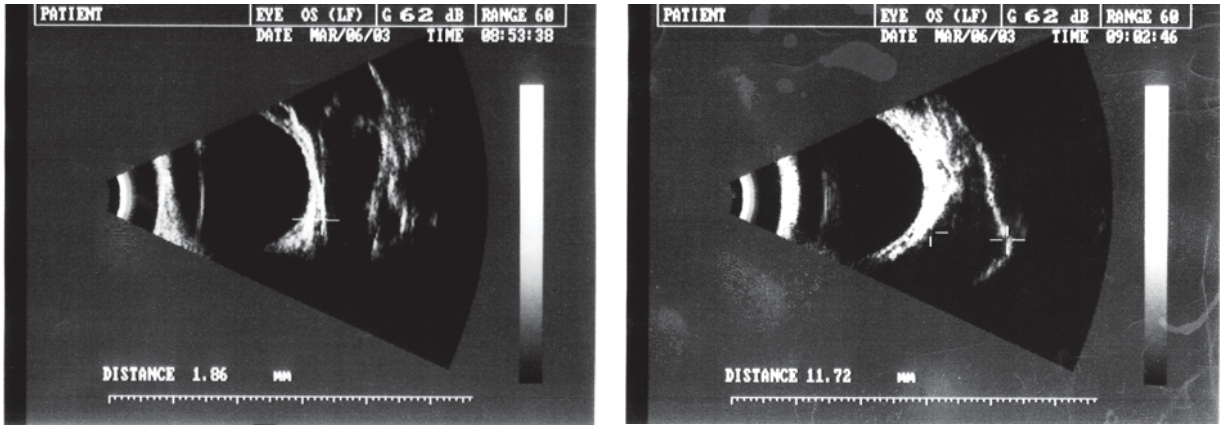


Fig. 1– Ultrasound of the left eye showing hypoechoic lesion of the lateral wall of the left orbit with low reflectability and internals septations.

orbital CT scan demonstrated the protrusion of both globes of the eyes, more pronounced on the left one, and the extraocular muscles' enlargement with the two nodules, one 38 × 16 mm nodule localized to the exterior wall of the left orbit, and another one of 15 × 10 mm in diameter found in the medial angle (Figure 2). The tumor grew and extended around the surrounding anatomical structures (*nervus opticus sinister*) which resulted in a concentric narrowing of the left view field (Figure 3).

The controlled laboratory test results showed that the erythrocyte sedimentation rate was 50 mm/hr, fibrinogen level was 5.2 g/L, haptoglobin level was 4.34 g/L, low immunoglobulin levels were – IgG 4,56 (8–17) g/L, IgA (1–4,90) 0.731 g/L, IgM (0.5–3.2) 0.441 g/L. Other laboratory findings were within the reference ranges. The chest x-ray and the ECG were normal. The serum levels of T3: 1.50 nmol/L; T4 : 95.3 nmol/L; TSH: 0.95 μIU/mL were also within the normal limits. The Goldman visual field testing

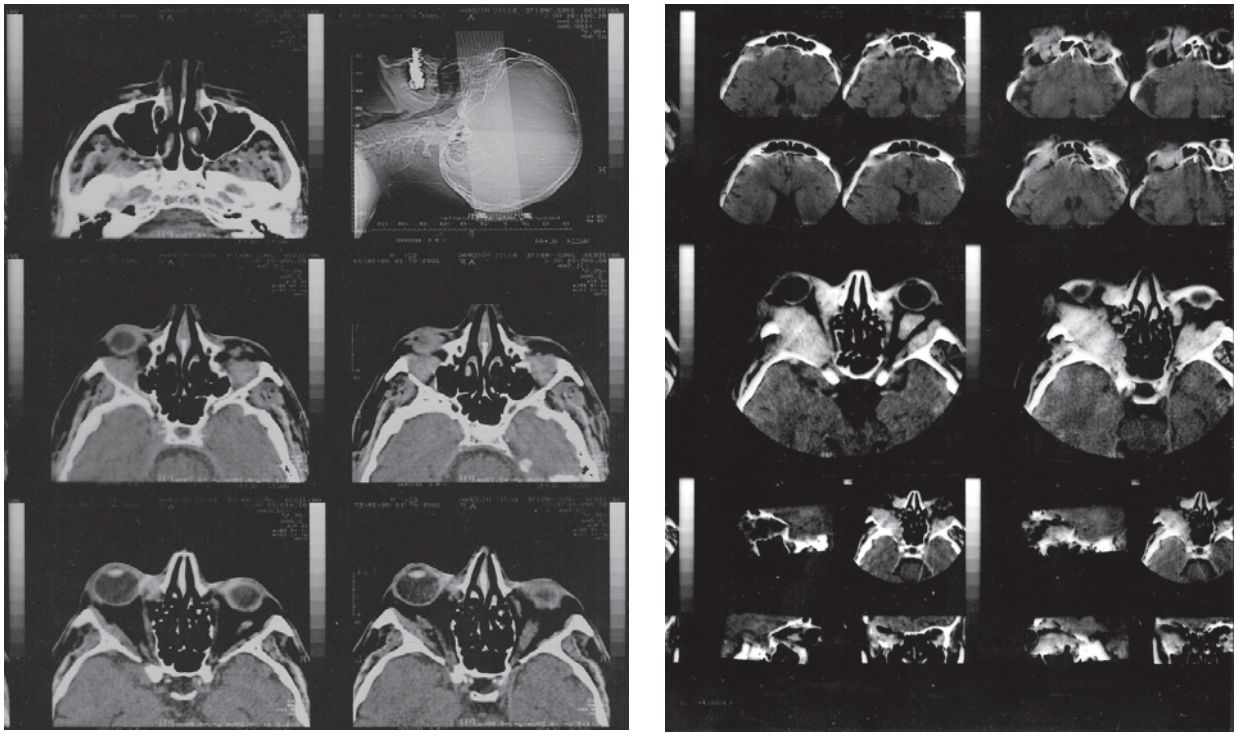


Fig. 2 – Computed tomography depicting protrusion of the left bulb with tumor mass lesion diameter 3.8 × 1.6 cm of the lateral wall of the left orbit. The lesion is strongly enhanced after contrast injection.

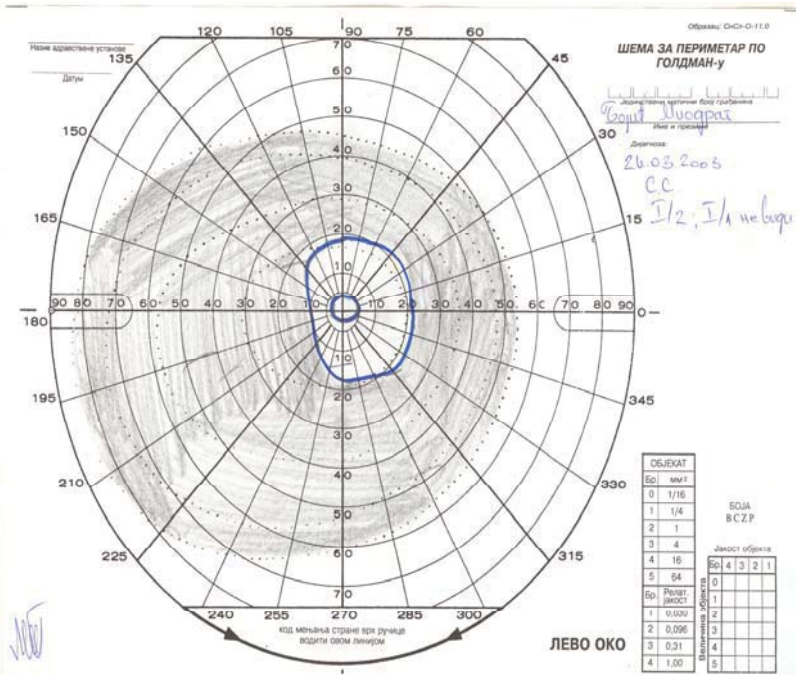


Fig. 3 – Goldmann visual field testing indicated the concentric visual field narrowing up to 30 degrees from the point of fixation to the right, and 15 degrees from the fixation point on the left.

indicated the concentric visual field narrowing up to 30 degrees from the point of fixation to the right, and 15 degrees from the fixation point on the left. The visual evoked potentials (VEP) o. dex – latent conductivity ratio of 115 m/sec / 6.64 μV (normal values); VEP o. sin – latent conductivity rate of 129 m/sec / 7.16 μV, what was the sign of prolonged conductivity due to the compression of the left optical nerve. The Hess-Lancaster test was within normal ranges, as well.

The biopsy of tumor changes protruding from the left bulbus confirmed a non-Hodgkin B-cell, marginal zone lymphoma, with a low degree of malignancy (low grade type). Histopathology disclosed several nodular lymphoid infiltrate, also within orbital fat tissue, small to medium sized atypical lymphocytic cell population, nearly monomorphic centrocellular images, with focally distributed sheets of small lymphocyte cells, scattered clear-cell monocytoid like lymphocyte, and a few histiocytic cells in periphery (Figures 4a and b). Mitotic index, Ki-67 was low, bellow 10%. The immunophenotyping analysis of the tumor cells demonstrated the CD-79 alfa expression, CD20-positive in the percentage of over 80%, CD-43 cells of 43% and the dispersed small CD3-positive lymphocyte cells. Staging of the disease including haed, neck, chest, abdomen and small pelvis CT scans were whitin the normal ranges, but the biopsy of the bone marrow confirmed bone marrow infiltration by the small lymphocyte cells of some 95% with the immunophenotypisation: CD20+, CD3-, CD5-, CD43-, CD 23-, and Cyclin dl, what indicated the spreading of the disease: IV B –b CS (m+ orbit+) IPI 3-.

Esophagogastroscopy revealed grade 1 and grade 2 esophageal varices extending 25 cm from the top of the esophagus; a spontaneous Mallory Weis tear at 1 h and 11 h; a friable, polypoid mass about 5 mm in diameter confined to the posterior wall of the stomach subcardially, at the distance of 43 cm. Some 16 years before, the patient underwent Bilroth surgery, when a biopsy sample was taken from the stoma.

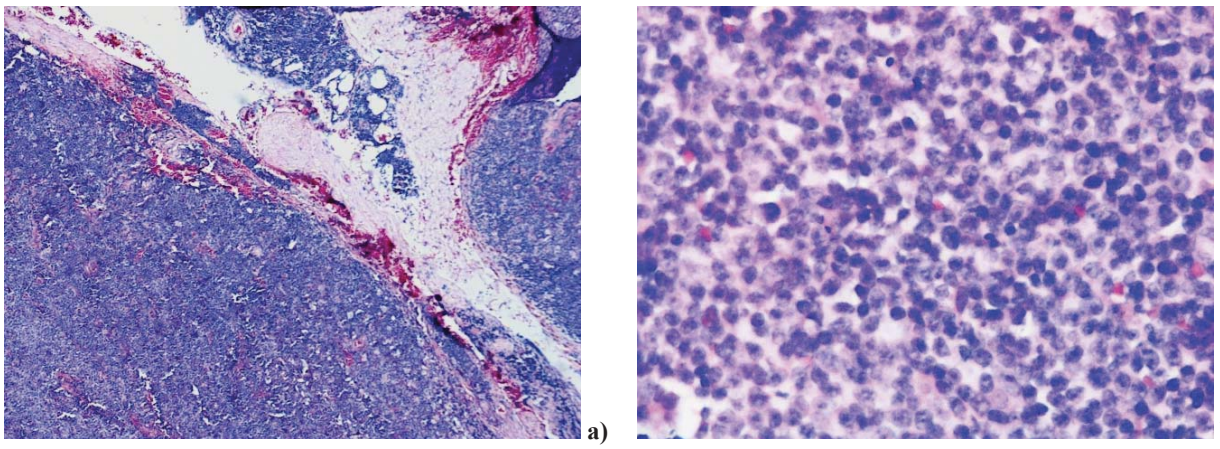
The council of doctors decided on the CHOP chemotherapy regimen followed by the radiation therapy for the orbit. Due to the expressed myelosuppression, the orbital radiation therapy was terminated after the completion of the second chemotherapy cycle, but it was reinitiated as soon as the bone marrow was recovered, and applied along with the chemotherapy. The remission in the patient was maintained after the two years of the initial treatment.

Discussion

Orbital lymphoma and lymphoma of the orbital adnexa are relatively rare conditions, and account for approximately 0.1% of all lymphomas [5]. The prevelance is more frequent in patients with previous several autoimmune diseases such as Hashimoto thyroiditis and Graves disease, Sjögren's syndrome and coeliac disease.

The study that included 369 patients with periocular lymphoma (1979–1999 year) found a considerably higher prevelance of the previous thyroid disease (in 5.0% of patients), whilst the autoimmune thyroid disease was confirmed in 2.5% of the patients, TRO was seen in 1.6 percent of cases. The average latency period of TRO before the diagnosis of periocular lymphoma assessed to be 17.5 years (11–27 years), and in the majority of patients it was identified as marginal zone lymphoma [5].

In the presented case, the period of the development of Graves' disease and lymphoma was markedly short. Even though it was the case of an extended disease (IV B-b), it was initially manifested as a localized, unilateral condition involving the left periocular area. So, a question arises whether the disease was present at the time when the orbital decompression surgery was performed, since the biopsy included only the right *musculus rectus medialis* and not the periocular fat tissue. However, despite the well-preserved thyroid function and metabolism, the first systemic manifestations of lymphoma in the form of the weight loss, weakness and adinamia were observed by the end of 2002, i.e. seven months after the orbital decompression surgery. It, therefore, may be assumed that the primary localization of



a) b)

Fig. 4 – a) Histopathology of the lid biopsy tissue showing diffuse inflammation and invasion by atypical monomorphyc lymphocyte cells [May-Grünwald-Giemsa (MCG), ×20]; b) Hight power magnification of the biopsy revealing tightly packed, homogenous small to medium sized lymphocytes. Many of cells showed neoplastic appearance with clear nuclei containing multiple nucleoli (monocytoid like lymphocytes) (MCG, ×20).

lymphoma was initially the periorbital tissue with further progression of the disease.

Bartalena et al. [6] also described a case of bilateral exophthalmos, in a patient wrongly assumed that it was Graves' disease, even though it was the case of a non-autoimmune thyroid disorder, i.e. an autonomously hyperfunctioning adenoma and subclinical hyperthyroidism, that could not cause bilateral ophthalmopathy.

Since the previous treatment of Graves' ophthalmopathy proved to be unsuccessful, some other etiologies as possible causes were considered, but only after exophthalmos deterioration [7, 8]. Similarly, in our case, the patient had a previously confirmed Graves' disease with histopathologically proven lymphocytic infiltration of the muscles, but the clinical presentation and progression of exophthalmos was sugestive of some extrathyroidal causes. Morphological imaging, first ultrasound and than orbital CT scan and MRI, also biopsy of the tumor mass, confirmed orbital lymphoma.

Within typical manifestations and biochemical evidence of hyperthyreoidism, bilateral ocular inflammation is likely to be interpreted as Graves' ophthalmopathy. A review of 1,849 cases of orbital muscle enlargement revealed thyrod orbitopathy in 95% and other muscle disease in 5%. The three leading causes of non TRO were nonspecific myositis (43%), dural and carotid cavernous fistula (22%) and neoplasms (18%). Intramuscular lymphoma was seen in 0.2% [9].

About 85% of primary orbital lymphomas are low-grade, such as marginal zone lymphomas, difusse lymphoplasmocytic or follicle cell lymphomas [9]. The majority of patients had localized, IE stage diseases, with good prognosis after the completion of the local radiation therapy for the orbit [10].

In this case, the patient has a systemic spread of low-grade marginal zone B-cell lymphoma and received a combination of polychemiotherapy and radiotherapy. Complications occured after second cycle of chemiotherapy, but after recovery and continuing the combined therapy, the complete remission and favourable outcome was achived.

Conclusion

Even when the elements clearly indicate the presence of the thyroid-related ophthalmopathy, disease deterioration, especially unilaterally, should raise a suspicion and always lead to imaging procedures to exclude malignancy. Biopsy and adequate pathological sampling will be needed to make the diagnosis of lymphoma. In case of encertainty, regular and timely referral to the endocrinologist and ophthalmologist is mandatory.

Even though orbital lymphoma is localized in the majority of described studies, in-depth examination is required to be conducted to ascertain the degree of the disease in all cases.

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CASE REPORT

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Improvement of post-hypoxic action myoclonus with levetiracetam add-on therapy: A case report

Poboljšanje akcionog posthipoksičnog mioklonusa primenom dodatne terapije levetiracetamom

Ksenija Božić, Ksenija Gebauer-Bukurov, Lorand Sakalaš, Ivana Divjak, Aleksandar Ješić

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Abstract

Introduction. Chronic post-anoxic myoclonus, also known as Lance-Adams syndrome, may develop following hypoxic brain injury, and is resistant to pharmacological therapy. **Case report.** The patient we presented developed post-anoxic action myoclonus with severe, completely incapacitating myoclonic jerks. Myoclonus did not respond to the treatment with commonly used agents, i.e. valproate and clonazepam alone or in combination. Improvement of the action myoclonus was observed only after adding levetiracetam. **Conclusion.** Although Lance-Adams syndrome may not be fully curable at this point, levetiracetam appears to be a promising agent that can significantly improve functional level and overall quality of life of patients with this disorder.

Key words:
myoclonus; anoxia; syndrome; diagnosis; drug therapy; drug resistance; treatment outcome.

Apstrakt

Uvod. Hronični postanoksični mioklonus, poznat i kao Lance-Adamsov sindrom, može se javiti nakon hipoksične povrede mozga i rezistentan je na farmakološku terapiju. **Prikaz bolesnika.** Prikazan je bolesnik sa postanoksičnim mioklonusom i izraženim miokloničnim trzajevima koji su doveli do njegove potpune onesposobljenosti. Mioklonije nisu reagovale na uobičajene lekove kao što je valproat i klonazepam pojedinačno ili u kombinaciji. Poboljšanje akcionog mioklonusa postignuto je tek nakon uvođenja levetiracetama u dodatnoj terapiji. **Zaključak.** Iako još nije moguće potpuno izlečenje sindroma Lans-Adams, levetiracetat znatno popravlja funkcionalni i nivo ukupnog kvaliteta života bolesnika sa ovim poremećajem.

Ključne reči:
mioklonus; anoksija; sindrom; dijagnoza; lečenje lekovima; lekovi rezistencija; lečenje, ishod.

Introduction

Post-hypoxic action myoclonus (PAM), first described by Lance and Adams in 1963, is characterized mainly by action myoclonus and associated cerebellar ataxia, mild intellectual deficit and resistance to conventional pharmacological agents [1]. This syndrome is caused by anoxia of the central nervous system, generally in the course of primary respiratory failure. It is a rare condition, with fewer than 150 cases reported in the literature. We presented a male patient who survived hypoxic brain injury, after which he developed severe action-triggered jerks unresponsive to standard anticonvulsants, and had partial improvement only after levetiracetam was added. We discussed clinical and electroencepahalographic features of our patient in the light of the relevant published data.

Case report

According to available medical history data, a 58-year-old male injured in a car-accident (March 2009) experienced anoxia during surgery on his fractured zygomatic bone in a local hospital. For the following five days the patient remained in coma, receiving respiratory support. While in coma, eight hours after the surgery, the patient developed seizure-like generalized tonic-clonic movements. He was treated with phenobarbital and diazepam injections. Electroencephalography (EEG) was not performed at this time, and brain computed tomography (CT) was reported as normal.

After five days, when the patient regained consciousness, he had no hemiparesis or aphasia, but generalized myoclonus was present accompanied by dysmetria, dysarthria and impaired swallowing. Because of myoclonus he was un-

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able to sit from a supine position, stand up after sitting in a wheelchair, walk without aid, or perform simple, coordinated manual tasks. Myoclonic jerks would disappear only during sleep. Repeated brain CT showed abnormal, frontal and temporal lesions corresponding to contusion.

Brain magnetic resonance imaging (MRI) and magnetic resonance angiography (MRA) performed three weeks after the anoxic event demonstrated a few lacunar infarcts in the cerebral white matter without signs of postcontusion lesions, and with insignificant bilateral stenosis of the common and internal carotid arteries (Figure 1).

physiological evaluation at our tertiary clinic. Simple EEG demonstrated paroxismal abnormalities in the form of rapid series of spikes and polyspikes on slow-wave background activity. The EEG spikes were highly frequent, generalized, with an amplitude maximum at the vertex and often followed by slow waves (Figure 2). Myoclonic jerks and paroxismal EEG abnormalities were not strictly related to each other. Somatosensory evoked responses (SSEP) were normal.

The diagnosis of post-hypoxic action myoclonus was established, and discontinuation of phenobarbital and carbamazepine was advised. Drugs known to control myoclo-

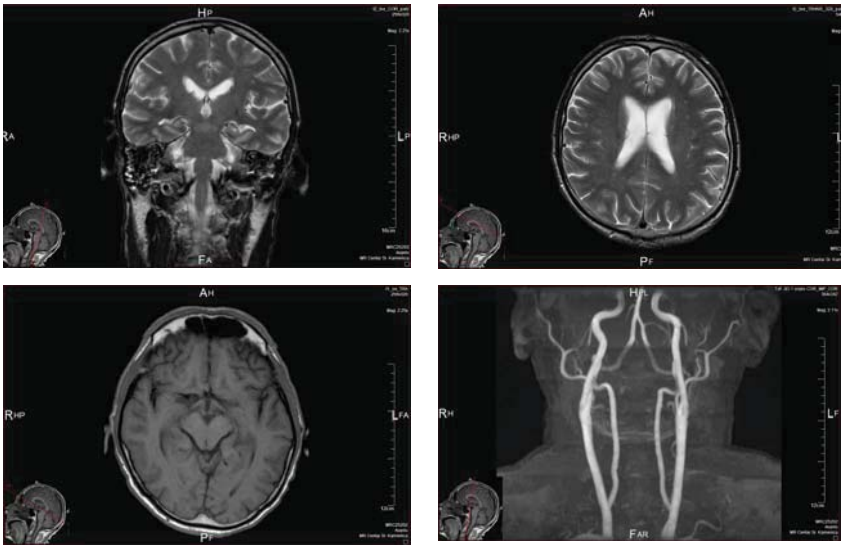

Fig. 1 – Lacunar infarcts in the cerebral white matter demonstrated by brain magnetic resonance imaging (MRI); magnetic resonance angiography (MRA) without significant pathological changes.

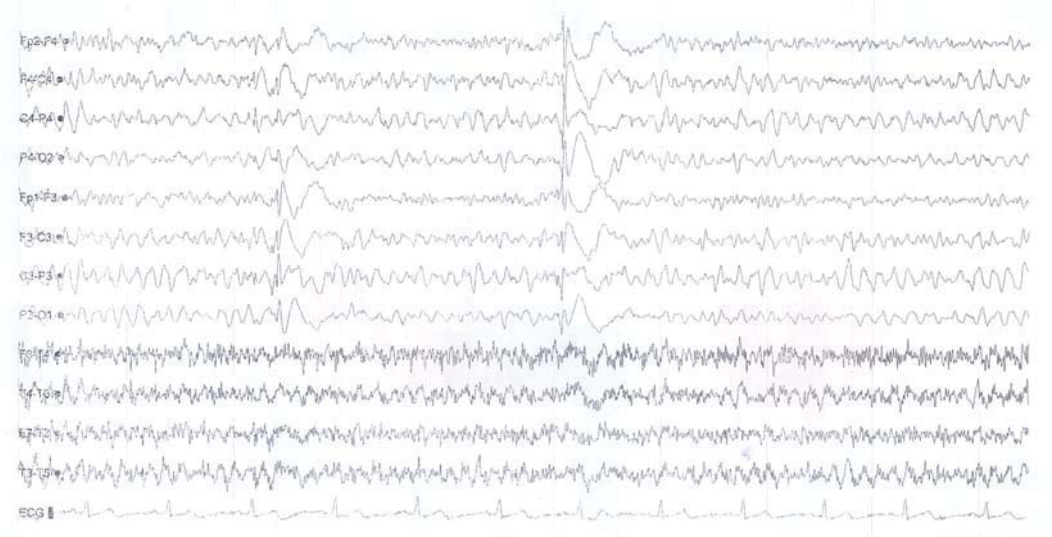

Fig. 2 –Electroencephalography (EEG) before levetiracetam (LEV) was added (longitudinal montage; 30 mm/sec, 7 µV/mm, 70.0 Hz, 1,600 Hz, Notch Off).

Despite the treatment with phenobarbital (100 mg/day p.o.), diazepam (20 mg/day i.m.) and carbamazepine (300 mg/day p.o.) the patient continued to have multifocal myoclonus that increased with voluntary movements and nurse's manipulation. The patient was therefore referred to electronus were administered progressively, first sodium valproate (VPA) (1000 mg/day p.o.) and then clonazepam (CZP) (2 mg/day p.o.), thereafter the frequency and severity of myoclonus slightly decreased. The patient's speech and swallowing improved, he was able to turn himself in bed, sit up

and stand up for a while, but he was unable to walk without aid.

At this point he was discharged from the local hospital and for the following six months he was receiving VPA (1750 mg/day), CZP (6 mg/day), lamotrigine (LTG) (200 mg/day) and sertraline (50 mg/day). However, there was no further improvement, and due to action myoclonus the patient was bed-bound and completely dependent. Levetiracetam (LEV) (250 mg b.d.) was added to the therapy, to which an immediate response was seen. The LEV dose was gradually increased up to 3,000 mg/day and a marked clinical improvement of action myoclonus was achieved. On EEG, central spiking was dramatically reduced, small-amplitude intermediate centro-temporal slow activity over both hemispheres on normal background activity was noted (Figure 3). The patient's overall quality of life was considerably improved. He could sit and stand up without support and had no significant cognitive impairment. Dysarthria and daily activities, such as eating and dressing, improved as well. However, he was still mildly ataxic, able to walk only about 150 meters independently, prone to sudden falling because of "sudden weakness in his legs" (negative myoclonus), still requiring supervision.

Clinical presentation of PAM is quite distinct and in most cases, like in our case, the diagnosis can be established on the clinical ground alone. In chronic PAM, myoclonus is noted within a few days to some months after the acute episode. The myoclonus may be accompanied by dysmetria, dysarthria and ataxia, with relatively preserved higher cognitive functions [2, 3]. Several types of myoclonus can be observed in this syndrome: cortical, subcortical, brainstem, reticular myoclonus, and exaggerated startle. Myoclonus may originate from either cortical or subcortical foci, although both forms may coexist [4]. Myoclonus may be focal, multifocal, segmental or generalized, it may be spontaneous or stimulus-sensitive, but typically is precipitated by movement intention and voluntary action [2]. Both positive and negative myoclonus may exist, separately or in association. Negative myoclonus may involve the hamstrings and quadriceps muscles, producing a characteristic "bouncing" gait and/or sudden falls [5, 6]. Lapses of postural control due to negative myoclonus may play an important role in producing the clinical picture of more obvious muscle jerking [7]. Clinical course of chronic PAM is variable. Gradually, myoclonus and neurological deficits improve, although the brainstem reticular reflex myoclonus may be associated with a poorer prognosis [2, 5]. In our patient, myoclonus was multifocal, action- and stimulus-sensitive, bilateral and generalized. Action jerks were more prominent distally and occurred particularly at the onset of a limb movement. His facial muscles were involved as well, affecting speech and swallowing. He was mildly ataxic without cognitive impairment. As it is typical with negative myoclonus, in our patient lapses in contraction of anti-gravity muscles became more apparent when the patient attempted to walk. The mechanism underlying this movement disorder remains largely unknown. Alterations in multiple neurochemical systems have been reported in animal and human studies, pointing out that abnormalities

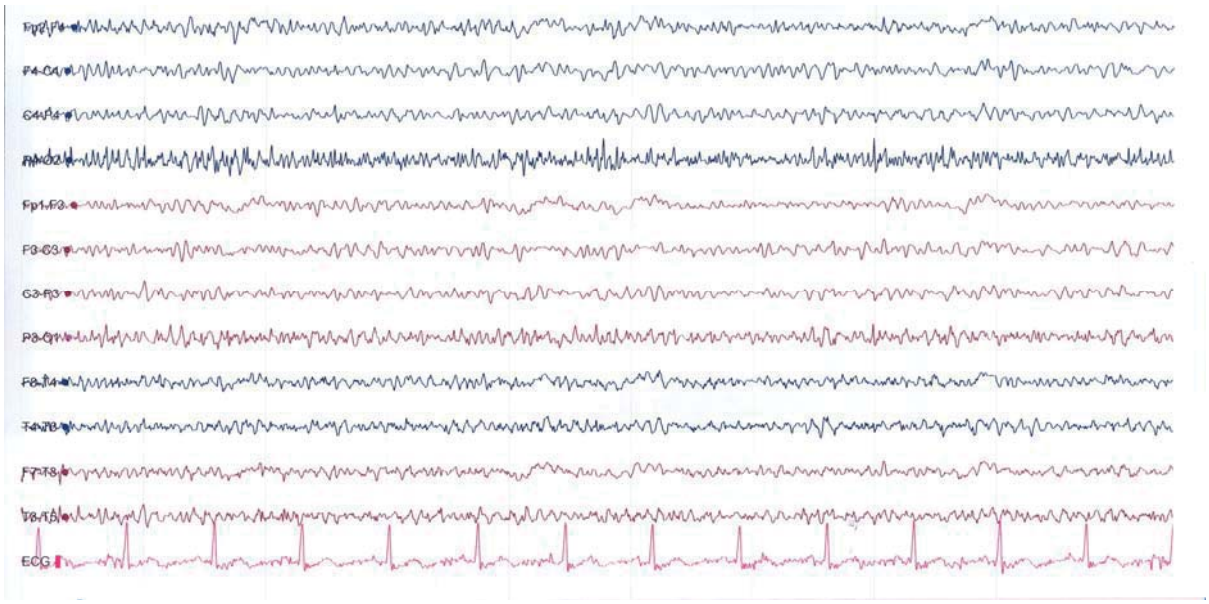

Fig. 3 – Electroencephalography (EEG) after levetiracetam (LEV) was added (longitudinal montage; 30 mm/sec, 7 µV/mm, 70.0 Hz, 1,600 Hz, 50Hz).

The patient's condition remained constant on the therapy (VPA 1,500 mg/day + CZP 6 mg/day + LEV 3,000 mg/day) for the past two years.

Discussion

Post-anoxic action myoclonus is a distinct entity that can develop in survivors of anoxic brain injury. It is a rare but devastating complication of near-fatal cardiopulmonary arrest (e.g., cardiac arrest, anesthesia accident during surgical procedures, asthmatic attacks, airway obstruction and drug intoxication).

within the serotonin system and/or a loss of GABA-ergic inhibition may influence the pathophysiological mechanism of PAM [8, 9]. According to recent neuroimaging data, it is likely that subcortical neuronal networks including the ventrolateral thalamus are involved [10, 11].

Imaging findings of brain CT and MRI in patients with PAM are usually unremarkable. MRI may occasionally show loss of grey-white matter distinction and selective neuronal injuries in the grey deep nuclei, but usually it is not specific of PAM [2, 5]. In agreement with published data, in our patient brain MRI revealed only a few small lesions in the cerebral white matter consistent with infarction.

In chronic PAM EEG background activity is usually normal, occasionally associated with spikes or spike-waves that are enhanced by movement or other stimuli [2, 12]. In some cases, EEG-EMG polygraphy with back-averaging and giant somatosensory evoked potentials are required to confirm the diagnosis (i. e., cortical origin of action myoclonus). In our patient, the standard EEG was initially abnormal. Abnormalities consisted of bilateral spikes, sharp waves or spike/poly-spike slow-wave complexes mainly over the vertex on slow (theta/alpha) background activity. These were only occasionally accompanied by muscle jerks. Cortical somatosensory evoked potentials (SSEPs) were normal. The EEG-EMG polygraphy with back-averaging of the EEG activity preceding jerks were not done.

Therapy of chronic PAM is difficult and empiric. Because of the relative rarity of the syndrome, case-controlled data are lacking. Several treatment options have been previously proposed, however, the results are inconsistent. Drugs that augment GABA-ergic transmission are useful in all types of myoclonus, and CZP and VPA are the first-line treatments [13, 14]. Approximately 50% of patients respond to treatment, although often partially [5]. Standard anticonvulsants, such as phenobarbital, carbamazepine and lamotrigine, were not efficient in our patient, which is in accordance with previous findings [15]. Efficacy of piracetam, as a very potent antimyoclonic agent, was reported many years ago, first in patients with Lance-Adams syndrome [16]. However, very high doses of piracetam (20–45 g/day) needed to reach efficacy is not very practical and can impede compliance [17–19].

Novel anti-epileptic medications such as levetiracetam and zonisamide (ZNS) have recently been described to be useful in the control of myoclonus disorders, including Lance-Adams syndrome [20, 21]. A body of evidence suggests that LEV may be effective in both positive and negative myoclonus [22, 23] as well as in patients with post-hypoxic and postencephalitic myoclonus [24]. The exact mechanism of action of LEV is unknown. It was found to be effective for the treatment of PAM in higher doses (3000–4000 mg/day) than those used for seizures [25, 26], although Krauss et al. [24] achieved a good response with low doses (1000 mg/day). In our patient improvement of action myoclonus ensued in the first day of LEV therapy. We used relatively high doses of LEV add-on therapy (3000 mg/day) and successfully controlled the action myoclonus without any unwanted side effects. Interestingly, LEV was not so effective for negative myoclonus in our case. However, although our patient did not recover completely, the treatment with LEV add-on therapy seems to have significantly improved his quality of life.

Conclusion

Although Lance-Adams syndrome may not be fully curable at this point, levetiracetam appears to be a promising agent that can significantly improve functional level and overall quality of life of patients with this disorder.

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ERRATUM

In the contents of the Vojnosanit Pregl 2014; 71(4), on the page CCCXXXII, the Serbian title of the article by Sladjana Petrović, Aleksandar Tasić, Dragan Mihailović, Nikola Živković, Marija Vitanović, Dragan Stojanov D. Bilateral giant angiomyolipomas revealed after massive retroperitonel hemorhhage – A case report [Veliki bilateralni angiolipomi posle masivne retroperitonelane hemoragije],

Should read as:

Veliki bilateralni angiolipomi otkriveni posle masivne retroperitonelane hemoragije

I N M E M O R I A M

prim. dr RISTO IVANOVSKI pukovnik u penziji (1914–2013)

Dana 17.06.2013. godine u Skoplju je preminuo doajen anesteziologije na tlu prethodne Jugoslavije, primarijus dr Risto Ivanovski. Rođen je 1914. godine u Prilepu. Srednju školu završio je u Bitolju, a Medicinski fakultet u Beogradu 1942. godine. Započeo je specijalizaciju iz interne medicine u Vojnoj bolnici u Nišu, a 1946. godine upućen je na specijalizaciju iz anesteziologije u Glavnu vojnu bolnicu Jugoslovenske armije (JA) u Beogradu.

Dr Risto Ivanovski, dr Sever Kovačev i dr Milan Jovanović bili su prva trojica specijalista iz anesteziologije u ondašnjoj Jugoslaviji i učenici dr Patricka Shacklton-a i dr Davis Russel-a, istaknutih britanskih anesteziologa koje su u našu zemlju uputile britanske vlasti na predlog generala Gojka Nikoliša, načelnika Saniteta JA. Oni su u Beogradu udarili temelj savremene anesteziologije na tadašnjem prostoru bivše SFRJ. Dr Sever Kovačev i dr Milan Jovanović su se demobilisali, a dr Ivanovski je kao pukovnik ostao pripadnik vojnog saniteta do penzionisanja 1978. godine.

Po završetku specijalizacije 1948. godine upućen je u Zagreb sa ciljem da organizuje Odeljenje za anesteziologiju u Vojnoj bolnici u Zagrebu. Budući da je za vreme šestogodišnjeg boravka u Hrvatskoj bio jedini anesteziolog, sarađivao je sa mnogim hrvatskim bolnicama, čak i za vreme godišnjih odmora. Posebno dobru saradnju ostvario je sa Hirurškim odeljenjem bolnice „Braća dr Sobol“ u Rijeci, gde je održao niz predavanja o endotrahealnoj anesteziji koju je prvi i izveo na tlu tadašnje Hrvatske.

O endotrahealnoj anesteziji od njega je učio poznati hrvatski anesteziolog, dr Ljuba Ribarić, koji ga je smatrao svojim „anesteziološkim ocem“.

U Skoplje dolazi 1954. godine, gde ostvaruje dobru saradnju sa hirurzima dr Ervinom Ginzbergom i dr Brankom Oberhofenom u oblasti grudne i kardijalne hirurgije.

Bio je učitelj prve generacije makedonskih anesteziologa, a vodio je i tečajeve za medicinske sestre i tehničare. Sudelovao je u osnivanju Vojne bolnice u Skoplju. Kada je 1962. godine osnovano Makedonsko anesteziološko društvo, on je bio njegov prvi predsednik. Ponosno je isticao da je u Društvu počeo raditi sam, a da sada, u 21. veku ovo Društvo ima više od 200 članova.

Dr Risto Ivanovski autor je dvadesetak radova u kojima je pisao o tadašnjim anesteziološkim problemima. Bio je na usavršavanju u Velikoj Britaniji (London, Edinburg) i Danskoj. Poslednju godinu radnog staža bio je angažovan u Alžiru kao predstavnik Vojnosanitetske službe naše zemlje.

Iako je u zemljotresu u Skoplju 1963. godine izgubio suprugu i ćerku, a on i sin bili teže povređeni, uspeo je da kao snažna ličnost prebrodi sve tegobe i uđe u stotu godinu života.

Lik i delo ovog velikana – humaniste ostaće trajno u sećanju svih koji su ga poznavali. Do kraja njegovog života i duboko u njegovom srcu bili su Srbija, Beograd i njegove kolege i saradnici iz vojnog saniteta.

pukovnik u penziji
prof. dr sc. med
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Poziv za reklamiranje u 2014. godini

U prilici smo da vam ponudimo mogućnost oglašavanja i reklamiranja proizvoda i usluga u časopisu „**Vojnosanitetski pregled**“ (VSP). To je sigurno najbolji vid i najzastupljeniji način upoznavanja eventualnih korisnika sa vašim uslugama i proizvodima.

Časopis „**Vojnosanitetski pregled**“, zvanični organ lekara i farmaceuta Vojske Srbije, naučno-stručnog je karaktera i objavljuje radove iz svih oblasti medicine, stomatologije i farmacije. Radove ravnopravno objavljuju stručnjaci iz vojnih i civilnih ustanova i iz inostranstva. Štampa se na srpskom i engleskom jeziku. Časopis izlazi neprekidno od 1944. godine do sada. Jedini je časopis u zemlji koji izlazi mesečno (12 brojeva), na oko 100 strana A4 formata, a povremeno se objavljuju i tematski dodaci (suplementi). Putem razmene ili pretplate VSP se šalje u 23 zemlje sveta. Radove objavljene u VSP-u indeksiraju: *Science Citation Index Expanded (SCIE)*, *Journal Citation Reports/Science Edition*, *Index Medicus (Medline*), *Excerpta Medica (EMBASE), EBSCO* (preko ove baze VSP je dostupan *on line* od 2002. godine u *pdf* formatu) i *Biomedicina Serbica*.

Cene reklama i oglasa u časopisu „Vojnosanitetski pregled“ u 2014. godini su:

1.	Oglas u crno-beloj tehnici A4 formata za jedan broj	20 000,00 dinara
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Parts of the manuscript are: **Title page; Abstract with key words; Text; References**.

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Examples of references:

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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.

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

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

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

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

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Tabele

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