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СРПСКИ АРХИВ

ЗА ЦЕЛОКУПНО ЛЕКАРСТВО Осн.1872

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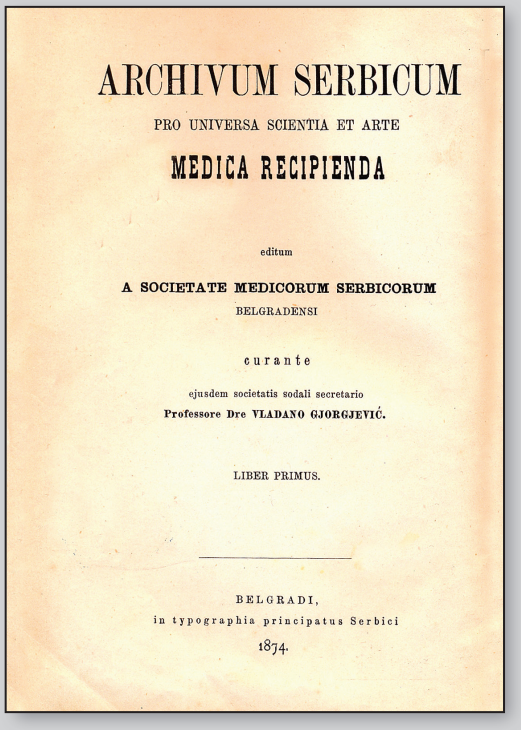

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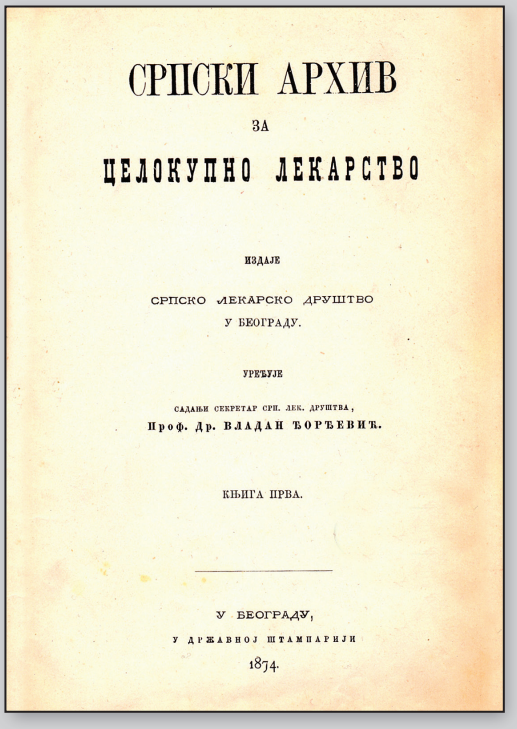
Прва страна првог броја часописа на српском језику



СРПСКИ АРХИВ

ЗА

ЦЕЛОКУПНО ЛЕКАРСТВО

ИЗДАЈЕ

СРПСКО ЛЕКАРСКО ДРУШТВО

У БЕОГРАДУ.

УРЕЂУЈЕ

САДАШЊИ СЕКРЕТАР СРП. ЛЕК. ДРУШТВА,

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КЊИГА ПРВА.

У БЕОГРАДУ,

У ДРЖАВНОЈ ШТАМПАРИЈИ

1874.

The title page of the first journal volume in Latin

Корице/Cover
Оснивач и први уредник
Владан Ђорђевић (1844–1930)
Founder and first editor
Vladan Đorđević (1844–1930)

Српски архив за целокупно лекарство је часопис Српског лекарског друштва основан 1872. године, у којем се објављују радови чланова Српског лекарског друштва, претплатника часописа и чланова других друштава медицинских и сродних струка. Часопис објављује: оригиналне радове, саопштења, приказе болесника, прегледе литературе, актуелне теме, радове из историје медицине, радове за праксу, радове који се односе на језик медицине, радове из медицинске етике (клиничка етика, етика публиковања, регулаторни стандарди у медицини), извештаје с конгреса и стручних састанака, стручне вести, приказе књига и дописе за рубрике Сећање, *In memoriam* и *Promemoria,* као и коментаре и писма Уредништву.

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Поднесени рукопис подразумева да је његово публиковање одобрио одговорни ауторитет установе у којој је истраживање обављено. Издавач се неће сматрати правно одговорним у случају подношења било каквог захтева за компензацију. Треба да се наведу сви извори финансирања рада.

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УВОДНИК • EDITORIAL

ORIGINAL ARTICLES • ОРИГИНАЛНИ РАДОВИ

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UDC: 050:61СРПСКИ АРХИВ ЗА ЦЕЛОКУПНО ЛЕКАРСТВО

УВОДНИК / EDITORIAL

Laboremus! 2. део: Темељи

Миле Игњатовић
Академија медицинских наука Српског лекарског друштва, Београд, Србија;

Срйски архив за целокуйно лекарсйво (*CA*) основан је 19. августа 1872. на првом састанку после оснивања Српског лекарског друштва (СЛД) [1], а 16. септембра 1872. је добио назив који има и данас [2]. Први број часописа је завршен и предат за штампу јуна следеће године, а изашао почетком 1874. То га сврстава међу најстарије часописе у Европи који континуирано излазе.

СЛД је формирано као „српско огњиште науке", али је имало много шири и национални значај. У времену када је било „част и одличје" бити члан СЛД, и краљ и скупштина су схватали национални значај удружења и значајно га помагали. На годишњим састанцима и прославама СЛД по правилу су присутвовали краљ и представници Скупштине Србије. Још пре изласка првог броја часописа „прочита се акт министра просвете којим се Српско лекарско друштво извештава да му је од владе и скупштине одобрено да у државној штампарији свој орган 'Архив за целокупно лекарство' бесплатно штампати може" [3].

Године 1895. издата су два волумена *CA*: као волумен 12. под уредништвом Ђорђа Клинковског [4], као председника СЛД[1], и посебно као волумен 1 реформиран часопис под уредништвом Милана Јовановића Батута (1847–1940). То је исправљено тек 1952. године заснивањем волумена 80. Међутим, брзо је спласнуо Батутов ентузијазам да часопис може издавати у 24 свезака годишње и већ следеће године је предао уређивање часописа Јовану Данићу (1854–1924). Комбинујући стари начин уређивања и публиковања записника са седница СЛД, Данић постепено уводи новине и врло успешно уређује часопис све до смрти, практично 30 година.

Аћим Медовић и Лазар Докић посебно су истицали потребу публиковања оригиналних радова од првих дана часописа [5]. Због тога је часопис и подељен на два дела: „Редовни члан др Докић предлаже да се од сада друштвени 'Архив' штампа у два одељка – у једном да буду сами оригинални чланци, а у другом преводи. Друштво усваја овај предлог" [6]. А Владан Ђорђевић истиче да „тек самостални умни рад, тек самосталном обрађивање извесне научне дисциплине може народу дати достојно место међу носиоцима културе" [7]. Основни проблем, од оснивања часописа, био је недовољан број добрих радова, оригиналних најмање.

На седницама СЛД оцењивана је не само оригиналност радова већ и њихова намена и сврсисходност публиковања. Често су публиковани радови из хигијене јер је то био национални интерес у то време, а хигијеном су се бавили и хирурзи. У СЛД је сматрано националном обавезом подизање општег нивоа медицинског знања и умећа ради лечења. Није било могуће, што је данас често присутно, публиковање радова који никоме не требају (*l'art pour l'art*); данас значајно само ауторима ради неког бенефита. Чак и ако науку посматрате као непрегледну слагалицу или мозаик од безброј елемената или тесара, тј. публикованих сазнања, број очигледно безвредних радова је превелик. Радова који нити доносе нешто ново, нити су интересантни, нити су едукативни. Ти радови спадају у групу тзв. нецитабилних радова, који оптерећују сваки часопис и мора их бити што мање.

Рецензија и критика

CA има посебну вредност, као мало који часопис у Европи, а то је рецензентски поступак од првог броја, пуних 145 година. Наиме, 13. октобра 1872. „друштво одлучује да се мањи чланци који се буду подносили друштву читају и пресуђују у седници, а већа дела да се дају нарочитим референтима на оцену" [8]. Оцењивани су апсолутно сви приложени радови, преводи, књиге. Рецензије су биле јавне; уз посебно неговану критичност чине се вреднијим него данашње „двоструко слепе" рецензије.

Рецензенти радова су најбољи пријатељи аутора и часописа. Они чувају углед свих, јер „штампана реч је нож са две оштрице". Аутори публикују јер имају шта да кажу, из унутрашње потребе да нешто саопште, а не

1 У периоду 1879–1895. часопис је уређиван на састанцима СЛД, чији је председник био Младен Јовановић (1831–1895), а 1895. године Ђорђе Клинковски (1827–1905).

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из личне потребе за избором у звање! Свако сазнање је безвредно док се не изнесе јавно да би га вредновао суд јавности. Притом аутори се не смеју обесхрабривати или како Ј. О. Галперн каже: „Излазећи на чист ваздух социјалног живота, не треба се плашити промаје". Прве радове и критике ће преболети као дечје болести, али ће научити да пишу радове. И то се учи, као и све друго. Уредништво и рецензенти овог часописа ће им помоћи у томе. И тако ћемо доћи опет до приче о науци и јабуци. Ако је сазнање јабука и дате је неком, нећете изгубити јабуку, већ ћете сазнање (јабуку) имати и ви и он. То је својеврсни *perpetuum mobile*, а у тој бесконачности умножавања сазнања наука није трговина нити публиковање пијаца.

Највећи број рецензија (или критика) радова никада не буде објављен. То су најчешће професионалне оцене за поједине издаваче, државне или едукативне институције (рецензије књига, пројеката, дисертација). Рецензије радова за поједине часописе су одраз признања врхунске компетенције, увек су анонимне и никад хонорисане. Рецензенти било којег дела су обавезни да „дају своје компетентно мишљење..., одговорне и озбиљне критике..., а захтева се висока принципијелност, беспрекорна објективност, оправдана строгост" [9].

У свим упутствима за рецензенте данас се запостављају општи утисак о раду и потреба његовог публиковања. Рецензенти се обично усмеравају на анализу појединих делова рада и његову оригиналност, а општи део се оставља уредништву часописа. Тај општи део би обухватао: 1) да ли је рад актуелан, 2) како је компонован, 3) какав је његов садржај и 4) како је формално написан.

Само наизглед противуречно, ово уредништво ће стимулисати и критику радова, јер то даје посебну вредност и тим радовима и часопису. Та критика је последњих година потпуно запостављена свуда у свету. Рајнберг разликује пет типова критичног приказа по садржају и карактеру: 1) анотациони тип приказа (само обавештава, да дело не би остало незапажено, нпр. приказ књиге или писмо уреднику за чланак); 2) критички приказ типа реферата (једноставан као и претходни, преноси суштину дела, са мало критичких примедби; раније често коришћен да би се указало на рад а избегло публиковање превода целог рада); 3) критички прикази полемичког типа (циљ је отварање дискусије-полемике; после 1-2 оваква приказа уредништво прекида полемику одговором аутора рада који је био повод); 4) приказ-критика (*pro et contra*, обично на захтев уредништва часописа); 5) прикази синтетичког типа (највиши ниво; поред критике дела износи и аргументе за „праву истину") [9].

Очигледно је да су темељи часописа изванредни и да се на њима може правити најбоља надградња. Одређи се својих корена водило би нестајању у бескрају сличних. На рачун старе славе у овом послу, у овом времену се не може живети. Мора се стално напредовати или ће вас млађи брутално прегазити и једноставно ћете нестати. Ти млађи жељни успеха су образовани, луцидни, креативни, савремени, неоптерећени неуспехом, бескомпромисни и брутални. Али којим путевима ићи? Најједноставније би било да „идемо утабаним стазама". Међутим, то је по дефиницији анахроно и данас не можете користити старе методе, већ ка старом циљу морамо ићи новим путевима.

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Prognostic value of biomarkers and co-morbidities in patients with acute heart failure – A one-year follow-up study

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SUMMARY

Introduction/Objective Clinical risk stratification of patients hospitalized due to acute heart failure (AHF) applying B-type natriuretic peptide (BNP), troponin I (TnI), and high-sensitivity C-reactive protein (hsCRP) biochemical markers can contribute to early diagnosis of AHF and lower mortality rates.
The aim of this study was to investigate the prognostic significance of biomarkers (BNP, TnI, and hsCRP) and co-morbidities concerning one-year mortality in patients with AHF.

Methods Clinical group comprised 124 consecutive unselected patients, age 60–80 years, treated at the Coronary Care Unit of the Niška Banja Institute, Niš. The patients were monitored for one year after the discharge. During the first 24 hours after admission, BNP, TnI, and hsCRP were measured in fasting serum.

Results Total one-year mortality was 29.8%. The levels of serum BNP were significantly higher in the group of non-survivors compared to the group of survivors (1353.8 ± 507.8 vs. 718.4 ± 387.6 pg/mL, $p < 0.001$). We identified several clinical and biochemical prognostic risk factors by univariate and multivariate analysis. Independent predictors of one-year mortality were the following: BNP, TnI, depression, hypotension, chronic renal failure, ejection fraction, and right-ventricle systolic pressure.

Conclusion The presence of BNP and TnI biomarkers and several co-morbidities such as depression or chronic renal failure have significant influence on one-year mortality in patients with AHF.

Keywords: biochemical marker; cardiac failure; trial

INTRODUCTION

Acute heart failure (AHF) is the leading cause of hospitalization within the age group of over-65-year-olds and it represents a significant economic burden [1]. One of the challenging areas in management of patients with AHF is the involvement of multiple organs and presence of multiple co-morbidities. Care for patients with AHF is complex, involving clinical assessment and prediction as integral parts of daily clinical practice. AHF is associated with a very high mortality rate, and clinical risk stratification after hospitalization due to AHF remains a relevant challenge. In recent years, a growing attention has been paid to new blood-based biomarkers for their ability to risk-stratify patients with AHF. Over the past several years, B-type natriuretic peptide (BNP) and its N-terminal precursor fragment (NT-proBNP) have become the biomarker "gold standards" for predicting risk, with studies demonstrating the value of each test for risk stratification of AHF [2]. Additionally, a decrease in natriuretic peptide levels with proven HF therapy and parallel improvement in prognosis have led to the concept of "biomarker-guided" HF management, with promising results. New biomarkers for HF evaluation include soluble ST2 (sST2), growth/differentiation factor-15 (GDF-15), and highly-sensitive troponinT (hsTnT). Each has a growing set of data supporting its use, and sST2 and troponin measurements have been included in the American College of Cardiology / American Heart Association guidelines for the evaluation of HF [3].

However, the value of any biomarker for risk prediction pertaining to AHF should clearly depend on the degree to which it adds to the prognostic information provided by standard risk factors, co-morbidities, and other available markers.

Several demographic and clinical factors, co-morbidities, and biochemical variables are associated with short- and mid-term mortality in AHF, including measures of renal function, blood pressure, and other relevant predictors [2, 4, 5, 6].

Tha aim of this study was to investigate the prognostic significance of biomarkers (BNP, TnI, and hsCRP) and the influence of co-morbidities on one-year mortality in patients with AHF.

METHODS

This prospective study included 124 consecutive patients within the unselected population, who were admitted to the Coronary Care Unit

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of the Cardiology Department of the Niška Banja Institute during the period from July 1, 2010 to July 1, 2013, with signs and symptoms of AHF. AHF was diagnosed at the admission to the hospital according to the European Society of Cardiology guidelines for ACH, and the same diagnosis (AHF) had to be confirmed at discharge from the hospital [7]. Immediately after admission to the hospital, anthropometric measurements were carried out and existing diseases were registered along with the causes of AHF, precipitating factors, clinical presentation of the patients, as well as 12-lead electrocardiogram. During the first 24 hours after admission, both standard laboratory analysis and analysis of specific biomarkers (BNP, TnI, and hsCRP) were performed. Blood samples were taken in the morning, on an empty stomach, after a night's rest and eight-hour fasting. Creatinine clearance was determined according to the Cockcroft–Gault formula. A BNP fragment (8-29) was determined using Elisys Uno device (Human Diagnostics, Wiesbaden, Germany).

We used ELISA method with the quantitative determination of BNP fragments in biological fluids, with a set of reagents and manufacturers (Biomedica Gruppe, Vienna, Austria) and the reference values were in the 0–2,400 pg/ml range. Measurement of hsCRP was performed on HumaStar 180 analyzer (Human Diagnostics). We used the "sandwich" type ELISA method. Troponin I was determined by ELISA method. During the first 48 hours of hospitalization, all the patients underwent echocardiography. End-diastolic and systolic volumes of the left ventricle (LV) and ejection fraction (EF) were measured by Simpson's biplane method.

During hospitalization, all the patients underwent 24-hour Holter monitoring using a Del Mar device, as well as the analyses of the frequency and complexity of ventricular arrhythmias. After discharged from the hospital, mortality of the patients was being monitored for the following 12 months.

Statistical analysis

To assess the significance of the differences, we used the χ^2 test, Student's t-test, McNamara test, and Mann–Whitney U-test. Univariable and multivariable regression analysis were performed to identify the predictors of one-year mortality. The analysis of survival among the study groups was performed using the Kaplan–Meier method, as well as log-rank test to compare survival rates. The correlations between certain parameters were determined by Spearman's rank correlation coefficient. Testing of the biomarkers (BNP, TnI, and hsCRP) as predictors of mortality was estimated using ROC (receiver operating characteristic) curves by calculating the AUROC (the area under the receiver operating characteristic) curve and by determining the statistical significance of difference of 0.5. The limit value (cut-off point) was determined as the value of the product of optimal sensitivity and specificity.

Statistical analyses were performed using SPSS Statistics for Windows, Version 20.0 (IBM Corp., Armonk, NY, USA) for the level of statistical significance of $p < 0.05$.

RESULTS

The study included 124 patients, 76 males (61.3%) and 48 females (38.7%), with the average age being 60–80 years. The most frequent causes of AHF within the observed population were as follows: coronary heart disease (55%), valvular diseases (19%) and dilatation cardiomyopathy of unknown etiology (19%). The 12-month all-cause mortality was 29.8%.

The levels of serum BNP (Table 1) were significantly higher in the group of non-survivors compared to the

Table 1. Clinical characteristics and biochemical parameters of the patients studied and comparison between one-year survivors and non-survivors

Variables	All n = 124 (%)	Survivors n = 87 (%)	Non-survivors n = 37 (%)	p-value
Age (years)	70.7 ± 9.8	70.9 ± 10.0	70.1 ± 9.3	0.660
Sex (M/W)	76/48	52/35	24/13	0.740
Number of previous hospitalizations	1.8 ± 1.8	1.8 ± 1.7	1.9 ± 2.1	0.807
BMI (kg/m$^{2)}$	27.4 ± 4.9	27.2 ± 4.7	27.9 ± 5.4	0.479
Hemoglobin (g/l)	128.6 ± 19.6	128.5 ± 19.5	128.7 ± 20.2	0.962
Hematocrit (l/l)	0.37 ± 0.07	0.37 ± 0.07	0.36 ± 0.07	0.864
Urea (mmol/l)	8.6 ± 4.7	7.9 ± 4.4	8.9 ± 4.8	0.254
Creatinine (μmol/l)	118.3 ± 53.9	111.6 ± 36.8	121.2 ± 59.7	0.276
GFR (ml/min/1.73 m^2)	60.9 ± 28.3	64.3 ± 27.4	59.4 ± 28.7	0.373
Glycemia (mmol/l)	7.6 ± 3.4	7.7 ± 3.5	7.3 ± 2.9	0.445
Na (mmol/l)	141.5 ± 4.6	141.4 ± 4.6	141.7 ± 4.9	0.712
TC (mmol/l)	4.6 ± 1.4	4.7 ± 1.4	4.6 ± 1.2	0.702
LDL-C (mmol/l)	3.0 ± 1.1	3.0 ± 1.2	2.9 ± 0.9	0.465
HDL-C (mmol/l)	1.1 ± 0.4	1.1 ± 0.3	1.1 ± 0.4	0.519
TG (mmol/l)	1.4 ± 0.9	1.4 ± 0.9	1.4 ± 0.6	0.807
CRF	42 (33.9)	21 (24.1)	21 (56.8)	0.001
CVI	19 (15.3)	13 (14.9)	6 (16.2)	0.857
IM	58 (46.8)	40 (46.0)	18 (48.6)	0.845
AP	58 (46.8)	39 (44.8)	19 (51.4)	0.558
HT	95 (76.6)	66 (75.9)	29 (78.4)	0.821
PAD	46 (37.1)	28 (32.2)	18 (48.6)	0.045
DM	49 (39.5)	36 (41.4)	13 (35.1)	0.553
COPD	37 (29.8)	25 (28.7)	12 (32.4)	0.674
Ventricular arrhythmias	43 (34.7)	32 (36.8)	11 (29.7)	0.538
Hypotension	18 (15)	2 (11)	16 (89)	0.043
Depression	33 (26.6)	22 (25.3)	11 (29.7)	0.012
BNP (pg/ml)	908.0 ± 515.5	718.4 ± 387.6	1353.8 ± 507.8	< 0.001
TnI (ng/ml)	1.7 ± 6.9	1.1 ± 2.7	3.3 ± 11.9	0.402
hsCRP (mg/l)	13.6 ± 15.2	13.0 ± 16.0	15.1 ± 13.2	0.140
SBP (mmHg)	135.9 ± 31.5	144.2 ± 28.5	116.6 ± 30.1	< 0.001
DBP (mmHg)	81.9 ± 17.4	85.6 ± 15.9	73.1 ± 17.9	< 0.001
HR (beats/min.)	95.9 ± 25.8	98.2 ± 25.8	90.5 ± 25.5	0.132

BMI – body mass index; GFR – glomerular filtration rate; CRF – chronic renal failure; Na – sodium; TC – total cholesterol; LDL – low-density lipoprotein cholesterol; HDL-C – high-density lipoproteins cholesterol TG – triglyceride; CVI – cerebrovascular insult; IM – infarctus myocardii; AP – angina pectoris; HT – hypertension; PAD – peripheral arterial disease; DM – diabetes mellitus; COPD – chronic obstructive pulmonary disease; BNP – B-type natriuretic peptide; TnI – troponin I; hsCRP – high-sensitivity C-reactive protein; SBP – systolic blood pressure DBP – diastolic blood pressure; HR – heart rate

Table 2. Predictors of one-year death outcome – univariate Cox regression analysis

Variables	HR	95% CI	p-value
Age	0.999	0.967–1.033	0.961
Sex	0.788	0.394–1.577	0.501
BNP	1.166	1.034–1.311	< 0.001
TnI	1.042	1.011–1.074	0.007
hsCRP	1.008	0.991–1.024	0.375
SBP	0.967	0.953–0.981	< 0.001
DBP	0.948	0.924–0.973	< 0.001
CRF	3.780	1.943–7.356	< 0.001
Ventricular arrhythmias	2.115	1.099–4.071	0.025
Depression	2.602	1.346–5.029	0.004
EF	0.925	0.889–0.962	< 0.001
EDDLV	1.056	1.022–1.091	0.001
ESDLV	1.050	1.021–1.081	0.001
RVSP	1.066	1.043–1.089	< 0.001
LA	1.052	1.025–1.081	< 0.001
LBBB	5.708	2.746–11.863	< 0.001
Diastolic dysfunction	2.946	1.048–8.383	0.041
Hypotension at admission	7.226	3.692–14.143	< 0.001

HR – hazard ratio; CI – confidence interval; BNP – B-type natriuretic peptide; TnI – troponin I; hsCRP – high-sensitivity C-reactive protein; SBP – systolic blood pressure; DBP – diastolic blood pressure; CRF – chronic renal failure; EF – ejection fraction; EDDLV – end-diastolic diameter; ESDLV – end-systolic diameter; RVSP – right ventricular systolic pressure; LA – left atrium; LBBB – left bundle branch block

Table 3. Cox multivariate analysis of mortality predictors – model 1

Variables	HR	95% CI	p-value
BNP	1.166	1.034–1.311	< 0.001
TnI	1.041	1.002–1.082	0.039
hsCRP	1.001	0.970–1.033	0.956
Presence of CRF	1.723	0.821–3.617	0.151
GFR	0.996	0.983–1.009	0.519
EF	0.960	0.923–0.998	0.040

HR – hazard ratio; CI – confidence interval; BNP – B-type natriuretic peptide; TnI – troponin I; hsCRP – high-sensitivity C-reactive protein; CRF – chronic renal failure; GFR – glomerular filtration rate; EF – ejection fraction

Table 4. Cox multivariate analysis of mortality predictors – model 2

Variables	HR	95% CI	p-value
Presence of CRF	3.300	1.662–6.550	0.001
Presence of DM	1.124	0.562–2.248	0.741
Presence of CVI	0.957	0.389–2.353	0.924
Presence of depression	2.050	1.021–4.117	0.043
Presence of COPD	0.972	0.473–1.999	0.939

HR – hazard ratio; CI – confidence interval; CRF – chronic renal failure; DM – diabetes mellitus; CVI – cerebrovascular insult; COPD – chronic obstructive pulmonary disease

group of survivors (1353.8 ± 507.8 vs. 718.4 ± 387.6 pg/ml, p < 0.001). TnI and hsCRP values were higher in the group of deceased patients, with no statistical significance. The mean values of systolic and diastolic blood pressures on admission were the most significant investigated variables in survivors (p < 0.001). The presence of chronic renal failure (CRF), peripheral arterial disease, and depression were significantly more frequent among deceased patients compared to patients who survived (p = 0.001, p = 0.045, and p = 0.012, respectively). Myocardial infarction and angina pectoris were more frequent in the group of deceased patients, with no statistical significance. In other general investigated biochemical parameters and clinical characteristics there were no statistically significant factors related to death (Table 1).

The most important predictors of mortality (p < 0.001) were the following: concentration of BNP, hypotension at admission, left bundle branch block (LBBB), presence of CRF, systolic and diastolic blood pressures at admission, EF, right ventricular systolic pressure (RVSP) and the size of the left atrium (LA). Also, statistically more significant predictors of mortality were TnI, ventricular arrhythmia, depression, left ventricular end-systolic (and end-diastolic) diameter, and the presence of diastolic dysfunction (Table 2).

According to univariate Cox analysis, two multivariate models were formed. In model 1, the used parameters were all biomarkers (BNP, TnI, and hsCRP) as well as the parameter of renal function (the presence of CRF, GFR) and LVEF. In the second model, the presence of the following co-morbidities was tested: CRF, diabetes mellitus, cerebrovascular insult, depression, and chronic obstructive pulmonary disease (Tables 3 and 4).

In the first multivariate model (Table 3), significant predictors of mortality were BNP, TnI, and EF.

In the second multivariate model (Table 4), as the strongest predictor of mortality, adjusted for other tested variables in the model, the presence of CRF and the presence of depression were distinguished.

The risk of death due to the presence of CRF is 3.3 times higher [hazard ratio (HR) 3.300, p = 0.001], and the presence of depression represents a twofold increase in the risk (HR 2.050, p = 0.043).

In the investigated population, there was a statistically significant positive correlation between BNP and TnI (p = 0.217, p = 0.015). Both between BNP and hsCRP and between CRP and TnI, there was no statistically significant correlation. EF had significantly negative correlation with BNP (p = -0.396, p < 0.001).

Between BNP and GFR there was a negative correlation, close to statistical significance (p = 0.065).

In the studied population, serum concentrations of brain BNP could represent a mortality marker of hospitalized patients with heart failure [area under the curve (AUC) 0.840, p < 0.001].

In our study the limit value (cut-off) was 905.7 pg/ml, with a sensitivity of 83.8% and specificity of 77%. The other two markers (TnI and hsCRP) had no statistically significant discriminant value (p > 0.05). The value of BNP ≥ 905.7 pg/ml was associated with a higher risk of mortality in patients with AHF (Table 5, Figure 1).

DISCUSSION

The total one-year mortality in the study group was 29.8% – slightly higher than found in previous studies of similar design which included a higher number of respondents [4, 8, 9, 10]. High mortality rate in patients with AHF, shown in all previous studies, is a sign of challenging and limited therapeutic options in the treatment.

Table 5. Parameters in analysis of ROC curve for BNP, TnI, and hsCRP

Predictor	Cut-off	Sensitivity	Specificity	AUC	SE	95% CI	p-value
BNP	905.7	83.80%	77.0%	0.840	0.039	0.765–0.915	< 0.001
TnI	0.04	51.40%	59.8%	0.546	0.058	0.432–0.659	0.422
hsCRP	8.97	59.50%	60.9	0.584	0.058	0.469–0.699	0.140

ROC – receiver operating characteristic; AUC – area under curve; SE – standard error; CI – confidence interval; BNP – B-type natriuretic peptide; TnI – troponin I; hsCRP – high-sensitivity CRP

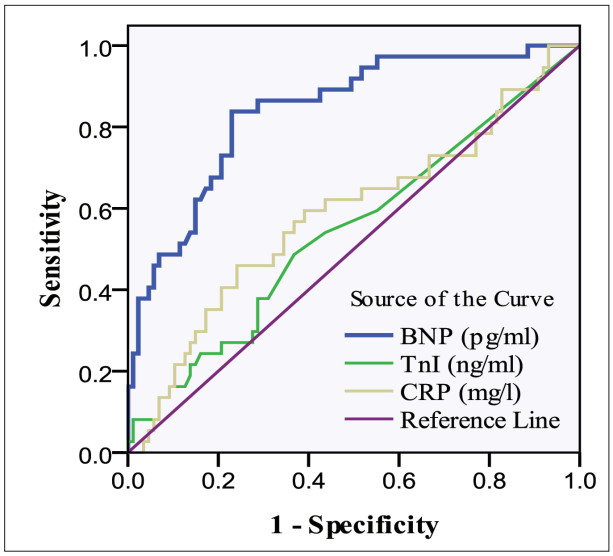

Figure 1. Analysis of receiver operating characteristic curve for BNP (B-type natriuretic peptide), TnI (troponin I), and hsCRP (high-sensitivity CRP)

In our study, the following indicators are shown as strong predictors of mortality in patients hospitalized with AHF in the univariate model: increased concentrations of BNP, hypotension at admission, the presence of CRF, reduced value of EF, systolic and diastolic blood pressure at admission, LBBB, RVSP and the size of LA. Statistically more significant predictors of mortality are troponin I, ventricular arrhythmia, depression, diastolic and systolic left ventricular diameter, and diastolic dysfunction.

Concerning the complexity of the immune/inflammatory/proliferative etiopathogenesis of HF, as well as generalized nature and progressive course of the disease, it is important to monitor the level and change in the level of specific biomarkers (BNP, TnI, hsCRP), because the value of BNP can reflect the progression of the disease, prognosis, and therapeutic approach.

In patients hospitalized due to decompensated HF, high levels of BNP are associated with poorer prognosis. Results similar to our study were presented in a recent pilot study, on a study sample of 187 subjects, and it showed that BNP was an independent predictor of adverse events in patients with acute worsening of chronic HF [11].

In the Organized Program to Initiate Lifesaving Treatment in Hospitalized Patients with Heart Failure (OPTIMIZE-HF) trial [12], the discharge (rather than admission) log-transformed BNP was the most important predictor of one-year mortality (HR of 1.34) and one-year death or re-hospitalization (HR 1.15).

In other studies [10], the values of BNP and NTproBNP at admission have not been shown as predictors of one-year mortality in patients with AHF. Other authors have shown that serial measurements of BNP during hospitalization, as well as measurements of BNP immediately before discharge of patients from hospital, have greater prognostic value than the measurements of BNP immediately after admission to the hospital [13]. In this study, values of BNP were taken once on admission.

In recent years, the cut-off value of BNP > 100 pg/ml has been standardized for diagnosing acute HF with high accuracy at 85% and strong prediction of HF, outperforming the clinical criteria. In our study of cases, the cut-off value for BNP or its fragment (8-29), was 905.7 pg/ml with a sensitivity of 83.8% and specificity of 77%.

In univariate Cox regression analysis, the concentration of BNP was distinguished as the strongest predictor of mortality in our population. Statistically more significant predictor of mortality was troponin I. The weaker statistical significance of troponin can be explained by the fact that ischemia (or myocardial necrosis) is not the cause of all HF; in fact, it appears in only 55% of patients, which has also influenced our results. Our research has shown that there is a direct correlation between BNP and TnI.

The slight increase of troponin is often seen in serious HF or during episodes of decompensation of HF in the absence of acute coronary syndrome or even significant coronary disease [14].

There is clear evidence that even low levels of measurable TnI in patients with HF have important prognostic implications related to mortality and morbidity. In patients who showed clinical improvement after admission, the level of TnI became undetectable after a few days. However, in patients with refractory HF who died in the hospital, detectable levels of TnI persisted during the observation period [15].

In a recent study we investigated the role of TnI in predicting unfavorable outcomes in 238 patients with advanced HF who were suggested cardiac transplantation [15].

Patients with detectable levels of cTnI (from 0.04 ng/ml or more) had higher levels of BNP, hemodynamic compromise and increased mortality rate. The researchers from Acute Decompensated Heart Failure National Registry (ADHERE) studied the correlation between the levels of cardiac TnI and unfavorable events in 84,872 patients with acute decompensated HF [16]. The patients with positive levels of TnI (6.2%) had lower blood pressure (BP) at admission, lower EF, and a higher rate of in-hospital mortality than those with undetectable TnI levels (8% vs. 2.7%, $p < 0.001$). The study confirmed a strong prognostic benefit of increased levels of TnI in predicting mortality in patients hospitalized due to decompensated HF [17].

Prognostic implications of hsCRP in patients with CHF have been tested in a meta-analysis in 6,600 patients [18].

There was a significant association between the increased hsCRP levels and more unfavorable cardiovascular outcomes, including mortality in patients with ischemic and non-ischemic etiology of HF. Unlike some recent studies [4, 19], the results of the Banović et al. study [10] as well as the results of our study, have not shown that hsCRP is a predictor of mortality.

The reasons are not clear enough, because it is not certain yet whether the increased CRP in AHF reflects only the weakening of the heart muscle or it is the result of associated infections. It should be noted that all three specific biomarkers (BNP, TnI, and hsCRP) were higher in the group of deceased patients with AHF. With an increasing number of responders, hsCRP would probably show statistical significance as a predictor of mortality.

The combination of two biomarkers (e.g. BNP and TnI or CRP and BNP) showed better risk stratification than that achieved by using a single biomarker [20]. Better risk stratification allows optimization of therapy or application of more aggressive therapy for patients with higher predictive risk.

Prognostic value of worsening renal function in discharged patients with CHF was based mostly on serum creatinine and estimated glomerular filtration rate [21, 22, 23].

In our study, the presence of renal failure was significantly more frequent in the group of deceased patients with AHF, while the value rates of creatinine, urea, and glomerular filtration did not differ significantly between the deceased and surviving patients with AHF, although they were slightly increased in the group of deceased patients. Multivariate analysis revealed that the presence of CRF increases the risk of death 3.3 times.

The prevalence rates of depression in CHF samples were in the 24–42% range [24].

In our study, depression was present in 19.5% of surviving patients with AHF, compared to 43.2% of deceased patients with AHF, which was statistically significant.

A larger number of studies have shown that depression is a graded, independent risk factor for readmission to the hospital, functional decline, and mortality in patients with congestive heart failure [25, 26]. The patients with HF and depression were found to have the increased concentration of circulating catecholamines, proinflammatory and anti-inflammatory cytokines [27], which can be a cause of poor prognosis.

We need new clinical studies that will involve multifactorial etiology of this syndrome.

CONCLUSION

High rate of one-year mortality in patients with AHF (29.8%), shown not only in our study, is a clear sign of complexity in treating this syndrome. The most common cause of AHF in the observed population is coronary heart disease, followed by valvular heart disease, and dilatation cardiomyopathy of unknown etiology.

The following mortality predictors were distinguished as strong evaluated parameters: concentrations of BNP and TnI, reduced value of EF, hypotension at admission, presence of CRF, and depression. However, renal failure and BNP are common and strong predictors of one-year mortality in hospitalized patients with heart failure, independent of other factors.

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Прогностички значај биомаркера и комобирдитета код пацијената са акутном срчаном инсуфицијенцијом – студија једногодишњег праћења

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Увод/Циљ Стратификација клиничког ризика код пацијената хоспитализованих због акутне срчане инсуфицијенције (АСИ) коришћењем биомаркера натриуретик-пептида Б-типа (БНП), тропонина I (Тн I) и високоосетљивог Ц-реактивног протеина (ЦРП), може допринети раном постављању дијагнозе и нижим стопама смртности.
Циљ ове студије био је да се испита прогностички значај биомаркера БНП, Тн I и ЦРП и комобирдитета на једногодишњи морталитет код пацијената са АСИ.
Методе Клиничка група обухватила је 124 узастопна неселектована болесника старости 60–80 година, лечених у Коронарној јединици. Пацијенти су праћени годину дана након отпуста. Током прва 24 часа наком пријема, БНП, Тн I и ЦРП мерени су у серуму наташте.
Резултати Свеукупни једногодишњи морталитет износио је 29,8%. Нивои серумског БНП-а били су знатно виши у групи болесника који су преминули у односу на групу пацијената који су преживели (1353.8 ± 507.8 тј. 718.4 ± 387.6 *pg/mL*, $p < 0,001$). Независни предиктори једногодишњег морталитета били су: БНП, Тн I, депресија, хипотензија, хронична бубрежна инсуфицијенција (ХБИ), ејекциона фракција, систолни притисак у десној преткомори.
Закључак Присуство оба биомаркера, БНП и Тн I, чак и више коморбидитета, као што су депресија или ХБИ имају значајан утицај на једногодишњу смртност пацијената са АСИ.

Кључне речи: биохемијски маркер; срчана слабост; студија

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Intraoperative and postoperative complications of phacoemulsification in cataract eyes with pseudoexfoliation syndrome

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Introduction/Objective Pseudoexfoliation syndrome (PEX) is an age-related systemic disorder characterized by deposition of fibrillary white flaky material mainly on the lens capsule, corneal endothelium, zonules, ciliary body, iris, and pupillary margin. Zonular weakness progressively increases along with the hardness of the lens, patient's age, and the presence of glaucoma.
The objective of the study is to compare the intraoperative and postoperative complications of phacoemulsification in cataract eyes with PEX with cataract eyes without PEX.
Methods The study enrolled 300 eyes with consequently operated senile cataract and PEX and 300 consequently operated eyes with cataract without PEX who underwent phacoemulsification performed by one experienced surgeon (single-surgeon series). A complete ophthalmological examination of all patients was performed preoperatively, as well as on the first, seventh, and 180th day postoperatively.
Results Significant statistical differences between the observed groups were the following: patients with PXF were older (74.2 ± 8; range 56–82 years vs. 68.1 ± 9.6; range 56–79 years), had smaller pupil diameter, and higher intraocular pressure (IOP) preoperatively (16.1 ± 4.1 vs. 13.8 ± 3.7 mmHg). There were no differences between the groups regarding intraoperative complications. Early postoperative complications were a significant rise of IOP (33 vs. six patients; $p < 0.001$), more frequent postoperative corneal edema (36 vs. 21 patients; $p < 0.036$), and anterior chamber inflammation (17 vs. seven patients; $p < 0.037$) in the PEX group, comparing to the control group. The significant late postoperative complication was elevated IOP (24 vs. five patients; $p < 0.0002$) in patients with PEX.
Conclusion In the hands of an experienced and careful surgeon, phacoemulsification is a safe and beneficial surgery to treat cataract with associated pseudoexfoliation. The greatest problem a surgeon faces is a narrow pupil and zonule instability, and difficulty in recognizing eyes that are particularly at risks, such as those having glaucoma and phacodonesis.
Keywords: phacoemulsification; pseudoexfoliation syndrome; senile cataract

INTRODUCTION

Pseudoexfoliation syndrome (PEX) is an age-related systemic disorder characterized by the presence of fibrillar material that targets all ocular tissue such as lens and iris pigment epithelium, lens capsule, ciliary body, zonules, corneal endothelium, and iris, but it also involves organs other than the eye [1, 2]. Whitish dusty deposits (fibrillary residue) can be observed on the anterior lens capsule, pupillary margin, corneal endothelium, along Schwalbe's line and trabecular meshwork, on the zonules, and vitreous body. Although understanding of this disease has increased considerably, the exact etiology and the structure of the pseudoexfoliative material is still unknown. [3]. It may be a generalized disorder involving abnormal production or turnover of extracellular matrix in the basement membrane [1, 3]. Patients with PEX demonstrated significantly higher zinc and copper levels in aqueous humor; higher copper content in lenses, as well as higher levels of iron and copper in serum were significantly increased in PEX group compared to cataract patients without PEX [4]. There are some reports indicating that infrared radiation contributes to capsular delamination [5].

It has been believed for many years that cataract surgery in patients with PEX carries an increased risk of intraoperative and postoperative complications, thus requiring additional caution and presenting a challenge to the surgeon, especially when extracapsular cataract surgery was performed [6]. Some authors reported a lower rate of intraoperative and postoperative complications when comparing outcome of a modern phacoemulsification with the extracapsular cataract extraction technique [7, 8]

As phacoemulsification surgical technique using ultrasound technology has been the most commonly performed cataract procedure in recent years, the results of this method in patients with PEX have been the subject of many studies. The reports of authors on the incidence of intraoperative and postoperative complications in the eyes with PEX are rather controversial. A few studies that analyzed the results after phacoemulsification cataract surgery indicate that during the surgery certain problems occur due to poorly dilated pupils, weak zonulae, and fragile anterior lens capsule, resulting in

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increased percentage of complications in these eyes [8, 9, 10]. A few recent studies show that phacoemulsification cataract surgery in patients with PXF is a more complicated surgery compared to cataract surgery in a non-PEX eye, but if performed by an experienced surgeon it does not present a significantly higher risk for patients [11, 12].

The aim of this study is to compare the intraoperative and postoperative complications of phacoemulsification in cataract eyes with PEX syndrome with cataract eyes without PEX.

METHODS

In total, 600 eyes with senile cataract that underwent phacoemulsification with implantation of intraocular lenses were included in this study. Patients were divided into two groups: the first group was the group with senile cataract and PEX (300 eyes), and the second, control group (300 eyes), were senile cataract patients without PEX. Phacoemulsification was performed by one surgeon in the period from May 2005 to January 2011 at the Ophthalmology Clinic of the Niš Clinical Center.

Exclusion criteria were traumatic cataract, high values of IOP in patients requiring a previous antiglaucomatous surgery, uncontrolled diabetes and acute cardiovascular events (hypertension resistant to therapy, arrhythmia etc.). The complete ophthalmologic examination was done preoperatively, on the first and the seventh day, as well as six months after the cataract surgery.

All the surgeries were performed using the Millennium apparatus (Bausch & Lomb, Bridgewater, NJ, USA) with "burst" mode and implantation of hydrophilic acrylic flexible lenses. Local peribulbar anesthesia was performed in all the patients, with corneal incision of 3 mm and application of cohesive viscoelastic for performing continuous capsulorhexis.

Student's t-test and χ^2 test were used for statistical analysis of clinical demographic characteristics and frequency of operative complications.

RESULTS

Phacoemulsification was performed in 600 eyes with senile cataract. The group of patients with PEX comprised 180 female and 120 male patients with mean age of 74.2 ± 8 years (range of 56–82 years), and the control group of patients with senile cataract without PEX included 170 female and 130 male patients with mean age of 68.1 ± 9.6 years (range of 56–79 years). The patients in the group with cataract associated with PEX were significantly older in comparison to the control group ($p < 0.01$), but there is no difference in sex distribution ($p < 0.793$).

Pupil diameter and intraocular pressure (IOP) were measured preoperatively. No patients had phacodonesis or lens dislocation, which are parameters that could affect surgery outcome. Patient in the control group preoperatively demonstrated lower IOP and larger pupil diameter, which was a significant difference comparing to the senile cataract group with PEX, who had statistically significantly higher frequency of elevated IOP (≥ 22 mmHg), as shown in Table 1.

Table 1. Preoperative clinical findings in cataract patients with and without PEX

Clinical findings	Senile cataract with PEX (n = 300 eyes)	Senile cataract without PEX (n = 300 eyes)	p-value
IOP (mmHg)	16.1 ± 4.1	13.8 ± 3.7	< 0.001*
Pupil diameter[1]	105 (35%)	38 (12.6%)	< 0.001*
Elevated IOP	30 (10%)	14 (4.7%)	< 0.012*

PEX – pseudoexfoliation syndrome; IOP – intraocular pressure;
[1] Considered to be 5 mm

Table 2. Intraoperative complications in cataract patients with and without PEX who underwent phacoemulsification

Complications	Senile cataract with PEX (n = 300 eyes)	Senile cataract without PEX (n = 300 eyes)	p-value
Incomplete capsulorhexis	9 (3%)	3 (1%)	0.080
Posterior capsule rupture	3 (1%)	1 (0.35%)	0.315
Zonular rupture	3 (1%)	2 (0.7%)	0.563
Vitreous body prolapse	3 (1%)	1 (0.35%)	0.315
Hyphema	1 (0.35%)	0 (0%)	0.316
Residual lens masses	3 (1%)	1 (0.35%)	0.315

PEX – pseudoexfoliation syndrome

Table 3. Early postoperative complications in cataract patients with and without PEX who underwent phacoemulsification (seventh day postoperatively)

Complications	Senile cataract with PEX (n = 300 eyes)	Senile cataract without PEX (n = 300 eyes)	p-value
Corneal edema	36 (12%)	21 (7%)	0.036*
Elevated IOP	33 (11%)	6 (2%)	< 0.001*
Anterior chamber inflammation	17 (5.7%)	7 (3.3%)	0.037*
Fibrinous exudation	5 (1.7%)	3 (1%)	0.476
Hyphema	2 (0.7%)	0 (0%)	0.156
Lens dislocation	3 (1.0%)	0 (0%)	0.082
Cystoid macular edema	5 (1.7%)	2 (0.7%)	0.254

PEX – pseudoexfoliation syndrome; IOP – intraocular pressure

Table 2 lists intraoperative complications in both groups. As it has been shown, there is no statistically significant difference between observed groups regarding incomplete capsulorhexis, zonular rupture, and vitreous body prolapsed. Anterior chamber lens implantation was performed on all patients with posterior capsule rupture.

The patients were postoperatively followed up for six months. Detailed findings are shown in Tables 3 and 4, presenting early and late postoperative complications (on the seventh and 180th postoperative day).

As it has been shown in Table 3, patients with PEX demonstrated significant rise of IOP and more frequent postoperative corneal edema and anterior chamber inflammation compared to the control group in the early postoperative period.

Table 4. Late postoperative complications in cataract patients with and without PEX who underwent phacoemulsification (180th day postoperatively)

Complications	Senile cataract with PEX (n = 300 eyes)	Senile cataract without PEX (n = 300 eyes)	p-value
Postoperative keratopathy	2 (0.7%)	1 (0.35%)	0.562
Elevated IOP	24 (8%)	5 (1.7%)	0.0002*
Lens dislocation	3 (1%)	0 (0%)	0.082
Posterior capsule opacification	7 (2.3%)	5 (1.7%)	0.559
Anterior capsular constriction	2 (0.7%)	0 (0%)	0.156
Macular edema	1 (0.3%)	0 (0%)	0.316

PEX – pseudoexfoliation syndrome; IOP – intraocular pressure

Six months after surgery it was found that 24 patients in the PEX group still had glaucoma, but the number was significantly reduced ($p < 0.01$) in comparison to preoperative findings (n = 30 patients), as shown in Table 4.

DISCUSSION

Patients with pseudoexfoliation syndrome were significantly older, had smaller pupil diameter and higher IOP than controls. These findings are similar to recently published study results [13]. Out of 300 patients with cataract and pseudoexfoliation syndrome, 30 (10%) had capsular glaucoma, which is statistically significantly more frequent than in controls. Mean values of IOP were higher in the group with PEX, which correlates with results from other studies and at the same time justifies the attitudes that strategies should be directed at reducing IOP with medical therapy in patients with elevated IOP in preoperative management treatment.

In 105 patients with cataract and pseudoexfoliation, pupils were less than 5 mm in diameter despite administration of two mydriatics, which imposes a significant problem to the surgeon because it makes the properly sized capsulorhexis more difficult to perform. Apart from the problems concerning capsulorhexis performance, other phases of the surgery are also challenging, considering the tendency of subsequent narrowing of the pupil during the surgery.

In practice, small-sized pupils can be enlarged by high-density viscoelastic agents to perform viscomydriasis, as well as by the use of iris retractors, in order to perform properly sized capsulorhexis. A very important stage in performing ultrasound cataract surgery is continuous curvilinear capsulorhexis, which has been considered to be of great importance, especially in eyes with pseudoexfoliation, zonule laxity, and anterior capsule fragility. In these patients, irregular capsulorhexes and uncontrolled anterior capsule tears that may compromise surgery course have often been described. We performed anterior capsule staining in most patients and achieved significantly better visualization, enabling the surgeon to work more safely and comfortably. Nevertheless, 3% of patients with PEX had incomplete capsulorhexis, which is slightly higher incidence in comparison to the controls. Posterior capsule rupture occurred during lens chopping and anterior chamber intraocular lens was implanted in three cases with PEX and in one case without it. Wong et al. [14] reported similar experience in senile cataract eyes with true exfoliative syndrome.

Zonular weakness or laxity is one of important features in patients with PEX, requiring extreme caution and precision during the procedure. In our group of patients with pseudoexfoliation and cataract, there were no cases of zonular dialysis preoperatively, while three patients manifested zonular weakness during the surgery. All the patients underwent "burst" technique for phacoemulsification, our preferred technique over the "pulse" mode, which means less ultrasound energy use. It is recommended to use adjunctive pupil and zonule support devices [15]. Anterior capsular snap over the capsulorhexis edge has been described as a sign of zonular dehiscence and instability [16].

It is of extreme importance to minimize the risk of zonular dialysis occurrence during emulsification of the lens nucleus, which can be characterized by a greater degree of hardness in some cases. On most of the patients we used the "Phaco quick" technique (85%), "stop and chop" technique in 15%, and "divide and conquer" technique in 5% of cases. The preference was given to the technique that enables faster and more effective nuclear fragmentation, but, ultimately, the recommended technique is the one the surgeon is most comfortable with.

In the phase of viscoelastic aspiration it is extremely important to completely remove viscoelastic substance used in the previous phase of lens implantation since even small residue may result in transitory elevation of IOP.

Early postoperative complications most commonly include postoperative corneal edema and transitory ocular inflammation signs. Zhang and Saheb [17] reported that endothelial cell density is lower in cataract patients with PEX preoperatively, and corneal cell loss is greater postoperatively. They also reported that there is a transient increase in central corneal thickness after cataract surgery in eyes with PEX, as opposed to eyes without PEX.

Lens dislocation and anterior capsular constriction more commonly occur in patients with pseudoexfoliation mostly as the consequence of frequent postoperative inflammation and a smaller capsulorhexis resulting from the narrow pupil.

IOP control in the early postoperative period seems to be important in patients with PEX who underwent cataract surgery [18]. It was shown that a long-term reduction in mean IOP occurred in PEX eyes with and without glaucoma preoperatively, suggesting a protective effect of phacoemulsification on IOP in these eyes [19]. Also, preoperative diagnosis of glaucoma seems to be the only factor to affect the higher postoperative IOP [20]. There were no differences in complications between eyes with PEX and eyes without PEX [21].

CONCLUSION

In our experience, phacoemulsification method can be safely performed in cataract patients with PEX. It is a challenging surgery, but careful preoperative planning and intraoperative care can ensure a successful outcome and safe procedure. Concerning the numerous complications that may occur in these patients, we did not face severe intraoperative complications apart from certain problems in performing capsulorhexis.

By understanding all the specific ocular features in patients with pseudoexfoliation, proper preoperative preparation, application of suitable technique, and surgeon's experience, the optimal outcome can be achieved in patients with senile cataract and PEX. Postoperative one-month follow-up period is of great importance to timely prevent and observe possible complications such as assess endothelial cell function, glaucoma screening, etc. The risks associated with cataract surgery in a PEX patient can be minimized with a proper preoperative, intraoperative, and postoperative care.

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Оперативне и постоперативне компликације факоемулзификације код пацијената са катарактом и псеудоексфолијационим синдромом

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САЖЕТАК

Увод/Циљ Псеудоексфолијациони синдром (ПЕС) представља системски поремећај који се јавља у старијем животном добу а карактерише се наслагама љуспастог материјала на предњој капсули сочива, ендотелу рожњаче, дужици, зонулама, цилијарном телу, ирису и пупиларном рубу. Прогресивна слабост зонула заједно са већом тврдоћом очног сочива, старост и присуство глаукома су чешћи код пацијената са ПЕС-ом.
Циљ рада је упоредити оперативне и постоперативне компликације факоемулзификације код пацијената са катарактом који имају ПЕС у односу на оне који немају ПЕС.

Методе Студија је обухватила 300 узастопно оперисаних очију са сенилном катарактом и ПЕС-ом и 300 узастопно оперисаних очију са сенилном катарактом без ПЕС-а који су оперисали катаракту факоемулзификацијом од стране једног хирурга. Свим пацијентима је рађен комплетан офталмолошки преглед преоперативно, првог, седмог и 180. дана после операције.

Резултати Значајна статистичка разлика између посматраних група преоперативно је постојала у следећем: болесници са ПЕС-ом су били старији (74,2 ± 8,0, распон 56–82 година; тј. 68,1 ± 9,6, распон 56–79 година), имали су мањи дијаметар пупиле и виши интраокуларни притисак (ИОП, 16.1 ± 4.1 тј. 13,8 ± 3,7 *mmHg*). У току операције није било значајних разлика у врсти компликација. Ране постоперативне компликације су биле: значајан раст ИОП-а (33 тј. шест болесника; $p < 0,001$), чешћи налаз корнеалног едема (36 тј. 21 болесник; $p < 0,036$), инфламације у предњој очној комори (17 тј. седам болесника; $p < 0,037$). У касном постоперативном периоду значајно је чешћи био налаз пораста ИОП-а у групи са ПХФ (24 тј. пет болесника; $p < 0,0002$).

Закључак У рукама искусног и пажљивог хирурга факоемулзификација је безбедна и корисна хируршка метода код пацијената са сенилном катарактом и ПЕС-ом. Највећи проблем представља уска зеница, зонуларна нестабилност и правовремено препознавање факодонезе и глаукома код пацијената са ПЕС-ом.

Кључне речи: факоемулзификација; псеудоексфолијациони синдром; сенилна катаракта

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Epilepsy awareness, knowledge, and attitudes among secondary school teachers in Montenegro

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SUMMARY

Introduction/Objective Epilepsy is associated with numerous misconceptions due to its dramatic manifestation and poor knowledge among the public.

The aim of this study was to assess epilepsy awareness, knowledge and attitudes among secondary school teachers.

Methods We conducted a cross-sectional survey by sending a simple self-administered questionnaire to all secondary schools in Podgorica, Montenegro. The questionnaire contained 16 questions regarding epilepsy awareness, knowledge and attitudes, first aid attitudes, as well as demographic and teaching experience data.

Results We analyzed 219 questionnaires. Almost all teachers had heard or read about epilepsy, 57.5% of whom knew someone with epilepsy; 21% had a pupil with epilepsy in their class; more than 50% had witnessed a seizure, with 25% of them linking epilepsy to a central nervous disturbance. Over 60% of teachers chose convulsions/shaking to be a major feature of an epileptic attack. Forty percent of teachers thought epilepsy could be cured. Almost 80% thought people with epilepsy should get married and have children, but only one third would marry a person with epilepsy. Over 13% would object to their child playing with another child with epilepsy, and more than 50% would object if their child married a person with epilepsy. About 35% of teachers suggested putting something in a person's mouth during attack to prevent tongue injury and asphyxiation.

Conclusion Awareness and understanding of epilepsy among teachers were satisfactory, but the results also revealed negative attitudes. Teachers need further education about epilepsy to increase seizure recognition and first aid management, reduce stigma, and intensify acceptance of people with epilepsy.

Keywords: epilepsy; awareness; knowledge; attitudes; teachers; Montenegro

INTRODUCTION

Epilepsy is the most common neurological disorder, affecting over 50 million people worldwide [1]. Unfortunately, it is also poorly understood, even among those who know someone with epilepsy. Attitudes towards people with epilepsy are influenced by the degree of knowledge of the condition [2]; thus, in theory, a higher level of education should correlate with better awareness, knowledge, and attitude regarding epilepsy. However, in reality, the general public frequently hold misconceptions and inappropriate beliefs; this is especially the case among older and less educated members of the community, but also occasionally among those with university education. Danesi [3], Hsieh and Chiou [4], as well as Mekarelli et al. [5], found such misconceptions among school teachers.

School is an important part of every child's life. It is a place where their psychological, social, and educational development begins and it represents a basis for all future accomplishments. Children with epilepsy are particularly vulnerable at the time of puberty, when they might experience stigma, isolation, and the resulting poor self-esteem. It is well established that children with epilepsy have a higher incidence of school underachievement as a result of many factors, including seizures themselves, medications, and psychosocial and behavioral difficulties [6].

Teachers are often seen as role models and their knowledge about and attitudes towards epilepsy can directly impact upon a child's school performance, social skills development and reduction in stigma [4]. Their experience through direct contact with other students can contribute to an improvement of epilepsy management and to their better surveillance and safety. Inadequate teacher's knowledge and negative attitudes can therefore have unforeseeable consequences.

Thus, we designed this study to evaluate the awareness, knowledge, attitudes towards epilepsy, and attitudes concerning first aid among secondary school teachers in Podgorica (the capital of Montenegro), and to correlate these findings with social background and teaching experience.

METHODS

We conducted this cross-sectional study in Podgorica during April 2015. Podgorica is considered an educational center for the entire country, containing 10 of the total of 50 public secondary schools in Montenegro.

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In this study, we included all teachers living in Podgorica, from nine secondary schools. We excluded teachers from one secondary school since this school was specifically designed for children with delay in mental development. Teachers who had children of their own with epilepsy were also excluded. We informed the directors of all secondary schools about the study, and obtained their approval beforehand. Teachers were asked to answer a 16-item questionnaire. Participation was voluntary and anonymous, and all teachers gave written informed consent.

The questionnaire comprised five parts: demographic data of teachers, teaching data, familiarity with epilepsy, understanding of epilepsy, attitudes towards epilepsy, and attitudes towards first aid. Most questions were designed as a choice from "yes," "no," or "not familiar with the disease," while questions regarding etiology of epilepsy, clinical manifestations of an attack, and first aid management permitted multiple answers. The questions were derived from the questionnaires used in previous studies that quantified the knowledge, attitudes, and practice towards epilepsy among selected populations [7–10]. The questionnaire was translated into Montenegrin from the English version and went through translation and back-translation. Finally, the questionnaire was tested with lay volunteers to ensure simplicity of language.

Most of the questions in the returned questionnaires were answered. We discarded from the data analysis those questionnaires in which some or all of the questions in the returned questionnaire were left unanswered.

Statistical analysis

After coding, the data were entered into a computer using MS Excel (Microsoft Corporation, Redmond, WA, USA) and analyzed by SPSS for Windows, Version 16.0 (SPSS Inc., Chicago, IL, USA). Continuous variables were expressed as the range and median, whereas categorical variables were presented as frequencies (%). We used χ^2 test to examine the association between variable's absolute frequencies. When frequencies were less than 5, we applied Fisher's exact test. We used Student's t-test to determine whether differences between means were statistically significant. We considered a p-value of < 0.05 to be a statistically significant association between variables in all tests.

RESULTS

We sent a total of 279 questionnaires to all schools. Of those, 221 teachers responded (aged 24–65 years), with 219 questionnaires being accepted, thus giving a response rate of 78.5%.

Table 1 shows the socio-demographic background and teaching experience of participants. Their mean age was 43.38 ± 10.54 years (range 24–65 years). Most teachers were female (74.9%). Almost two thirds were married (62.1%), predominantly women (75%). Over one third (36.1%) had fewer than 10 years of teaching experience. Male teachers were more likely to have fewer than 10 years of teaching experience ($p < 0.02$), whereas female teachers were more likely to have between 10 and 20 years of teaching experience ($p = 0.005$). There were no statistically significant differences between age and marital status among interviewed teachers.

Almost all the teachers had heard or read about epilepsy (Q1, 97.7%). Female teachers were significantly more likely to have heard of this disease compared to male teachers (99.4% vs. 92.7%, $p = 0.01$). Over half of those who had heard or read about epilepsy (Q2, 57.5%) knew someone with the disorder. Equal numbers of men and women provided positive answers to these questions (60.0% vs. 56.7%, $p = 0.67$). Just over one fifth (Q3, 21%) had a pupil with epilepsy in their class. They were more likely to be female teachers ($p = 0.03$). Fewer than 7% (Q6, 6.4%) of all teachers believed epilepsy was a form of insanity. There were no statistically significant differences between male and female teachers regarding this question ($p = 0.11$). Almost 40% (Q7, 39.7%) of all teachers believed epilepsy could be cured, regardless of gender ($p = 0.12$). Over 50% of teachers (Q8, 57.07%) had witnessed a seizure. Teachers aged over 45 years with over 10 years of experience ($p < 0.05$) answered this question positively. These results are shown in Table 2.

Most teachers (Q9, 73.1%) believed that people with epilepsy could lead as much of a normal life as everybody else. Over three quarters (Q10, 79.5%) thought that people with epilepsy should get married and have children (Q11, 73.5%), but only one third of all teachers said they would marry a person with epilepsy (Q12, 34.7%). Over 10% (Q13, 13.7%) would object if their child played with another child who had epilepsy, but more than a half (Q14, 58.4%) would object if their child married a person with

Table 1. Socio-demographic background and teaching data of teachers

Characteristics		Teachers with students with epilepsy in their class (n = 154)	Teachers without students with epilepsy in their class (n = 65)	p-value
Age (years)		44.88 ± 10.39	39.83 ± 10.13	0.001
Gender	(M/F)	40/114	15/50	0.78
Marital status	Married	97 (63%)	39 (60%)	0.79
	Single	46 (29.90%)	24 (36.9%)	0.39
	Divorced	8 (5.2%)	2 (3.1%)	0.73
	Widow/Widower	3 (1.9%)	0	0.56
Teaching experience	< 10 years	48 (31.2%)	31 (47.7%)	0.03
	10–20 years	53 (34.4%)	20 (30.8%)	0.71
	> 20 years	53 (34.4%)	14 (21.5%)	0.06

epilepsy. Nearly 90% (Q15, 88.1%) thought people with epilepsy should be employed like everybody else. Female teachers were more likely to answer "yes" to this question compared to male colleagues (91.5% vs. 78.2%, p = 0.008), as shown in Table 2.

All teachers with over 10 years of experience had heard of epilepsy, unlike colleagues with fewer years in teaching. It was found that work experience was highly associated with the knowledge regarding this disorder (p = 0.01). Teachers with over 10 years in training were significantly more likely to know someone with epilepsy, compared to colleagues with fewer than 10 years of work experience (p = 0.02). The least experienced teachers had fewer students suffering from epilepsy. Teachers with 10–20 years' experience and in training (p = 0.006) had significantly more students with epilepsy. Almost three quarters (71.6%) of teachers with over 20 years in teaching had seen seizures, compared to 60% of those who had been teaching for 10–20 years. The likelihood of teachers having seen a seizure was significantly related to their teaching experience (p = 0.04). Teachers with a long teaching career were more likely to object to their child marrying a person with epilepsy (p = 0.02). These results are shown in Table 3.

Over 20% of teachers who worked for less than 10 years and over 22% of those who worked for more than 10 years were familiar with causes of epilepsy (p = 0.21). The majority of teachers in both groups considered convulsions/shaking to be the major characteristic of an epileptic attack (79.7% and 87.1%, respectively, p = 0.14). Most respondents believed epilepsy was not a form of insanity (92.4% and 90.0%, respectively, p = 0.55). A third of the teachers who had worked for less than 10 years (34.2%) and less than a half (42.9%) of those who had worked for more than 10 years believed epilepsy could be controlled with medication (p = 0.20). The frequency of correct answers presented in Table 4 is not statistically significant in regard to work experience.

A quarter of all teachers from both groups (with and without students with epilepsy in the class) were familiar with causes of epilepsy (p = 0.90), naming brain disease/disorder/injury in a multiple-choice / single answer format. One fifth believed epilepsy was hereditary, and the rest thought seizures were caused by a combination of emotional stress, birth defects and hereditary factors. Majority of respondents from both groups considered convulsions/shaking to be a major feature of an epileptic attack (84.1%

Table 2. Teachers' answers in relation to gender

Question	YES			p-value
	Total	Male	Female	
Have you ever heard or read about epilepsy?	214 (97.7%)	51 (92.7%)	163 (99.4%)	0.01
Have you ever known anyone who had epilepsy?	126 (57.5%)	33 (60%)	93 (56.7%)	0.66
Have you ever had any students with epilepsy in your classroom?	46 (21%)	9 (16.4%)	37 (22.6%)	0.03
Do you think that epilepsy is a form of insanity?	14 (6.4%)	6 (10.9%)	8 (4.9%)	0.11
Do you think that epilepsy can be controlled with medications?	87 (39.7%)	17 (30.9%)	70 (42.7%)	0.12
Have you ever seen anyone who was having a seizure?	126 (57.5%)	34 (61.8%)	92 (56.1%)	0.45
In your opinion, can people with epilepsy lead a normal life?	160 (73.1%)	40 (72.7%)	120 (73.2%)	0.93
In your opinion, should people with epilepsy get married?	174 (79.5%)	45 (81.8%)	129 (78.7%)	0.70
In your opinion, should people with epilepsy have children of their own?	161 (73.5%)	40 (72.7%)	121 (73.8%)	0.95
Would you marry a person with epilepsy?	76 (34.7%)	17 (30.9%)	59 (36%)	0.58
Would you mind your child playing with a child with epilepsy?	30 (13.7%)	6 (10.9%)	24 (14.6%)	0.37
Would you mind your child getting married to a person with epilepsy?	128 (58.4%)	26 (47.3%)	102 (62.2%)	0.05
Do you think people with epilepsy should be employed in jobs like other people?	193 (88.1%)	43 (78.2%)	150 (91.5%)	0.008

Table 3. Teachers' answers in relation to work experience

Question	YES			p-value
	< 10 years	10–20 years	> 20 years	
Have you ever heard or read about epilepsy?	74 (93.7%)	73 (100%)	67 (100%)	0.01
Did you ever know anyone who had epilepsy?	36 (45.6%)	47 (64.4%)	43 (64.2%)	0.02
Have you ever had any students with epilepsy in your classroom?	8 (10.1%)	16 (21.9%)	22 (32.8%)	0.006
Do you think that epilepsy is a form of insanity?	4 (6.4%)	8 (10.9%)	2 (4.9%)	0.13
Do you think that epilepsy can be controlled with medications?	27 (34.2%)	33 (45.2%)	27 (40.3%)	0.52
Have you ever seen anyone who was having a seizure?	35 (44.3%)	43 (58.9%)	48 (71.6%)	0.004
In your opinion, can people with epilepsy lead a normal life?	58 (73.4%)	51 (69.9%)	51 (76.1%)	0.70
In your opinion, should people with epilepsy get married?	65 (82.3%)	56 (76.7%)	53 (79.1%)	0.69
In your opinion, should people with epilepsy have children of their own?	60 (75.9%)	52 (71.2%)	49 (73.1%)	0.97
Would you marry a person with epilepsy?	32 (40.5%)	22 (30.1%)	22 (32.8%)	0.67
Would you mind your child playing with a child with epilepsy?	9 (11.4%)	11(15.1%)	10 (14.9%)	0.89
Would you mind your child getting married to a person with epilepsy?	40 (50.6%)	40 (54.8%)	48 (71.6%)	0.02
Do you think people with epilepsy should be employed in jobs like other people?	70 (88.6%)	63 (86.3%)	60 (89.6%)	0.26

Table 4. Teachers' answers in relation to work experience

Question	YES		
	< 10 years	≥ 10 years	p-value
What do you think is a cause of epilepsy?	16 (20.3%)	39 (22.2%)	0.21
What do you think an epileptic attack is?	63 (79.7%)	122 (87.1%)	0.14
Do you think that epilepsy is not a form of insanity?	73 (92.4%)	126 (90%)	0.55
Do you think that epilepsy can be controlled with medications?	27 (34.2%)	60 (42.9%)	0.20

Table 5. Teachers' answers in regard to their experience with students with epilepsy

Question	YES		p-value
	Teachers with students with epilepsy in their class	Teachers without students with epilepsy in their class	
What do you think is a cause of epilepsy?	46 (25.3%)	9 (24.3%)	0.90
What do you think an epileptic attack is?	153 (84.1%)	32 (86.5%)	0.71
Do you think that epilepsy is not a form of insanity?	166 (91.2%)	33 (89.2%)	0.69
Do you think that epilepsy can be controlled with medications?	76 (41.8%)	11 (29.7%)	0.17

Table 6. Teachers' answers in regard to their experience with students with epilepsy

Question	YES			p-value
	Total	Teachers with students with epilepsy in their class	Teachers without students epilepsy in their class	
In your opinion, can people with epilepsy lead a normal life?	160 (73.1%)	136 (74.7%)	24 (64.9%)	0.21
In your opinion, should people with epilepsy get married?	174 (79.5%)	150 (82.4%)	24 (64.9%)	0.01
In your opinion, should people with epilepsy have children of their own?	161 (73.5%)	138 (75.8%)	23 (62.2%)	0.08
Would you marry a person with epilepsy?	76 (34.7%)	71 (39%)	5 (13.5%)	0.003
Would you mind your child playing with a child with epilepsy?	30 (13.7%)	23 (12.6%)	7 (18.9%)	0.31
Would you mind your child getting married to a person with epilepsy?	128 (58.4%)	106 (58.2%)	22 (59.5%)	0.89
Do you think people with epilepsy should be employed in jobs like other people?	193 (88.1%)	164 (62.5%)	29 (78.4%)	0.04

Table 7. Proper first aid management in regard to determinants – having a student with epilepsy, being a witness to an epileptic attack, and work experience

Determinants		Proper first aid management (n = 62)	Incorrect first aid management (n = 157)	p-value
Professors who had students with epilepsy in their class		12 (19.4%)	34 (21.7%)	0.7
Professors who witnessed an epileptic attack		31 (50%)	95 (60.5%)	0.15
Teaching experience	< 10 years	24 (38.7%)	55 (35%)	0.69
	10–20 years	18 (29%)	55 (35%)	
	> 20 years	20 (32.3%)	47 (29.9%)	

vs. 86.5%, p = 0.71). Only 8.21% considered loss of consciousness a feature, but 16% chose the combination of convulsions and loss of consciousness. Epilepsy was not considered a form of insanity in a little over 90% of teachers who had students with epilepsy, compared to a similar percentage of those who did not have a pupil with epilepsy in their class (91.2% vs. 89.3%, respectively, p = 0.69). Less than half of all teachers who had students with epilepsy thought epilepsy could be controlled with medication in contrast to one third of those who had no experience with students with epilepsy (p = 0.17). These results are presented in Table 5.

Teachers who had students with epilepsy more often thought people with epilepsy should get married (82.4% vs. 64.9%, respectively, p = 0.01), and they would marry a person with epilepsy, in contrast to their colleagues who were inexperienced (39% vs. 13.5%, respectively, p = 0.003). Teachers who never had a student with epilepsy in their class believed they should be employed in jobs like other people (78.4% vs. 62.5%, respectively, p = 0.04). These results are presented in Table 6.

Almost one third (28.3%) of interviewed teachers knew how to perform proper first aid. One fifth (19.4%) of teachers who had students with epilepsy in their class encircled the correct answer in regard to proper first aid management. One half of teachers who had previously seen an epileptic attack would provide proper first aid in a seizing child. Having a student with epilepsy (p = 0.7), long working career (p = 0.69), or being a witness to an epileptic attack (p = 0.15) are variables which were not statistically significant determinants in regard to first aid management. Correct answers were equally distributed in all three categories (Table 7). Incorrect first aid management included potentially dangerous activities, such as holding a student's

arms and legs and pouring water on a student during an attack. Three teachers did not know what to do with a seizing student.

DISCUSSION

In the eyes of children, teachers are often seen as role models with a lifelong influence. Therefore, evaluating their knowledge and attitudes towards epilepsy is crucial, given that their values may directly impact not only on the children but on their families and communities. This study is the first survey regarding epilepsy awareness, knowledge, and attitudes towards epilepsy among secondary school teachers in Montenegro.

In a present study, teachers had a good awareness of epilepsy. Only three teachers had not heard or read about epilepsy. Female teachers were more aware, but we believe this result is related to a higher percentage of female teachers compared to male colleagues in all secondary schools. More than half had witnessed a seizure. Several studies from both developed and developing countries showed high awareness among public [11–14]. On the contrary, the awareness of epilepsy among schoolteachers in Thailand was only 57.8% [15]. The reason for this disparity is vague; close interpersonal relationships may still be an attribute in our community. Teachers also held appropriate opinions about the nature of epilepsy, naming brain disease (25%), genetics (20%), and birth trauma (5%) as potential causes of epilepsy. These results are not surprising given their level of education, but there are still persisting misconceptions. Although teachers had knowledge, it was stunning to find that over 8% of teachers who had a student with epilepsy believed epilepsy to be a form of insanity. This misconception was present even in those teachers with greater years of teaching experience. Our result is more favorable compared to those from developing countries [15, 16]. However, surveys of public awareness, knowledge, and attitudes towards epilepsy from developed countries reported negative association between epilepsy and insanity [11, 12, 17]. Over 40% of our teachers who had students with epilepsy in their class believed epilepsy can be controlled with medication. In a study conducted among Italian school teachers, it was observed that 46.8% of the teachers believed that epilepsy was incurable [5], while a study conducted in Brazil showed a much greater percentage of teachers believing epilepsy can be treated (90%) [18]. Over 80% of the teachers (those with and those without students with epilepsy in their class) believed that convulsions/shaking is a major feature of an epileptic attack, supporting the well-known misconception that 'people with epilepsy shake,' even though a generalized tonic–clonic seizure is not the most prevalent seizure type.

In contrast to our study, previous similar studies from the USA and Greece found positive attitudes towards students with epilepsy despite significant deficits in general knowledge [19, 20]. Over three quarters of our teachers thought that people with epilepsy should get married and have children, although only one third of teachers who had a student with epilepsy would marry a person with epilepsy. Over 10% of teachers who had a student with epilepsy in their class would object if their child played with another child who had epilepsy, and, strikingly, 50% would object if their child married a person with epilepsy. Negative attitudes in regard to these questions were even higher among teachers who were inexperienced. In the Italian study that included 600 teachers from primary and secondary schools, it was found that 33% of teachers considered epilepsy a moderate-to-strong limitation for marriage and 24.6% of teachers were of the opinion that it would be a limitation for having children [5]. In a study by Mielke et al. [16], 76% of teachers would marry an epileptic person, and 82% of teachers would allow their child to play with an epileptic child. In a survey from Thailand, most teachers would allow their children to associate with a child with epilepsy, but only 41.2% of teachers would allow marriage of their child with an epileptic [15].

Although teachers with over 20 years of experience had more knowledge and more students with epilepsy in their classrooms, it is evident that stigma had remained. These negative attitudes might be a result of the absence of educational programmes in epilepsy for teachers in Montenegro. Nevertheless, negative attitudes of Montenegrin teachers towards students with epilepsy might also be a reflection of what they grew up hearing in community.

Despite this negative attitude, most teachers believed that people with epilepsy could lead as much of a normal life as anybody else, and that they should be employed like other people. We cannot explain this discrepancy.

In regard to first aid measures for a seizing student, less than 20% of teachers who had students with epilepsy in their class and one half of those who had previously seen an attack would turn a pupil on a side and try to prevent further injuries and asphyxiation. Our result is far less favorable compared to a study conducted in Pakistan, according to which almost 60% of teachers would turn a pupil on his side, and over 45% would prevent the tongue rolling back [21]. Over 20% of teachers who had students with epilepsy and over 60% of those who had previously seen an epileptic attack were not familiar with the correct initial procedures and first aid during a seizure. These teachers selected potentially dangerous maneuvers, such as holding a student's arms and legs and pouring water on him during an attack. Other studies found similarly unsatisfactory responses concerning first aid measures in children with epilepsy [5, 7, 15, 19, 20, 22, 23]. In undeveloped countries, these percentages were even higher, like a study from Nigeria, according to which over 80% of teachers were not familiar with first aid management of a seizing child [8]. In Montenegro, paramedics are always called by school staff in any case of emergency, especially seizures. This reflects the amount of panic which a seizure generates. One has to keep in mind that injuries associated with seizures are common among sufferers, and their prevention as well as proper first aid are indispensable. Therefore, teacher education towards a proper first aid seizure management is crucial in eliminating obstacles associated with negative attitudes and fear.

CONCLUSION

The results of the study suggest that though there is a high degree of awareness among secondary school teachers in Montenegro, considerable misconceptions and negative attitudes about epilepsy still persist, despite the level of education of teachers. The major reason for this was insufficient knowledge about epilepsy and lack of education of secondary school teachers during their training. In Montenegro, there are officially no special curricula regarding the education of teachers about the nature of epilepsy, seizure recognition, and first aid management.

We hope that this finding will lead to the inclusion of education about epilepsy in teacher training. Teachers remain an important link between students with epilepsy and the local community, making school less stressful and the community more tolerant for them. Nevertheless, well-informed teachers should be able to recognize all types of seizures, be better able to manage seizing students, and should even be the first to report the disorder in those young people.

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Свест, знање и ставови о епилепсији међу наставницима средњих школа у Црној Гори

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САЖЕТАК

Увод/Циљ Епилепсија је повезана са бројним заблудама које потичу од драматичног испољавања напада и слабог познавања природе болести.

Циљ рада је био да се процене свест, знање и ставови о епилепсији професора средњих школа.

Методе Спровели смо студију пресека у свим средњим школама у Подгорици употребом упитника који су испитаници самостално попуњавали. Упитник је садржао 16 питања.

Резултати Анализирано је 219 упитника. Скоро сви наставници су знали или су читали о епилепсији. Више од половине (57,5%) знало је некога са епилепсијом, а 21% је имало ученика са епилепсијом у свом одељењу. Више од 50% професора је било очевидац напада. Епилепсију као болест централног нервног система је заокружило њих 25%. За преко 60% испитаника грчеви су били главна карактеристика напада. Мање од половине је мислило да епилепсија може да се излечи. Скоро 80% професора је било мишљења да оболели треба да склапају брак и имају децу, али само једна трећина њих би склопила брак. Преко 13% се не би противило да се њихова деца играју са дететом са епилепсијом, а више од 50% би се противило да њихово дете буде у браку са особом која болује од епилепсије. Око 35% наставника је предложило да се у нападу детету стави предмет у уста како би се превенирало западање језика.

Закључак Познавање епилепсије код наставника је било задовољавајуће, али су резултати указали и на негативне ставове. Наставнике треба додатно едуковати о епилепсији, повећати знање о нападима и првој помоћи, а у циљу смањења стигматизације и бољег прихватања особа са епилепсијом.

Кључне речи: епилепсија; свест; знање; ставови; наставници; Црна Гора

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Patients' symptoms as a reason for consulting a doctor and obtaining a diagnosis of obstructive sleep apnea

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SUMMARY
Introduction/Objective Obstructive sleep apnea (OSA) is characterized by a number of symptoms of which the patient is sometimes not aware.
The aim of this study was to determine the symptoms due to which patients came to our sleep department, and to examine to which extent patients' self-awareness plays a role in diagnosing OSA.
Methods The study included 388 patients who came to the Sleep Department of the Clinic of Pulmonology, Skopje, Macedonia, from 2012 to 2016, with suspicion of OSA. Medical history was taken from all patients and polysomnography was performed in order to diagnose OSA. All patients with symptoms of OSA and Apnea–Hypopnea Index score of over 5 were diagnosed with OSA.
Results We identified a list of 23 symptoms that lead patients to visit a doctor. The most common symptom was snoring, which occurs in 86% of patients. It is followed by a feeling of under-sleeping with 68% and witnessed apnea with 63%. A total of 258 patients were diagnosed with OSA. The most important primary symptoms that led OSA-positive patients to our clinic were snoring, witnessed apnea, and daytime sleepiness. The percentage of snoring was decreasing with disease severity. Percentage of witnessed apnea and daytime sleepiness were increasing with disease severity. Self-awareness of symptoms led a majority of the patients to come to the Sleep Department.
Conclusion Patients who have symptoms such as snoring, witnessed apnea, and daytime sleepiness are likely to suffer from OSA. Most of the patients are aware of their symptoms and seek help from a doctor.
Keywords: sleep apnea; snoring; primary symptom

INTRODUCTION

Obstructive sleep apnea (OSA) is defined as an intermittent cessation of breathing during sleep [1]. Sleep apnea is characterized by the cessation of airflow of over 10 seconds up to two minutes or longer [2]. The prevalence of OSA in the world is 2–5% of the general population [3]. A growing number of authors point out that its prevalence is 5% and greater among men and 2% and greater among women [4]. According to this statistic, we can assume that there are between 40,000 and 100,000 persons suffering from OSA in Macedonia. Obstructive sleep apnea is characterized by a number of symptoms of which the patient is sometimes not aware [5]. The most common are snoring, witnessed apnea, daytime sleepiness, feeling under-slept upon waking, the weakening of intellectual abilities, dry mouth or throat in the morning [6, 7, 8]. Most of the patients had never heard of obstructive sleep apnea and had not been aware of having symptoms that may indicate this disease. Unfortunately, doctors rarely think of OSA and symptoms that patients complain of are often considered to be symptoms of other diseases. Population-based epidemiology studies and observations of OSA patients have consistently shown the prevalence of hypertension, type II diabetes, cardiovascular disease, and stroke to be higher in people with OSA [9]. The prevalence of hypertension is significantly higher in patients with OSA than the general population. OSA prevalence is especially high in patients with hypertension resistant to drugs. OSA was diagnosed in 83% of patients with uncontrolled hypertension (three or more antihypertensive drugs) [10]. The risk of myocardial infarction in patients with OSA is five times higher than the general population. Chances to survive myocardial infarction in patients with OSA are much smaller than in patients with no OSA. Seventy-five percent of patients with angiographically proven coronary artery disease have OSA [11]. Sleep disordered breathing is significantly more common in patients who have had a stroke or a transient ischemic attack than in the general population, occurring in 32–63% of stroke patients, and is associated with increased mortality and worse functional outcomes in these patients. [10]

The aim of this study was to determine the most common symptoms due to which patients with suspicions of OSA came to our Department, to identify the primary symptom for which they underwent polysomnography (PSG), and to see if there is a difference in symptoms in PSG-positive patients in comparison to negative patients. Furthermore, we wanted to examine to which extent patients self-awareness plays a role in reaching the diagnosis.

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METHODS

The study was prospective and performed at the Clinic of Pulmonology from 2012 to 2016. The study included 388 patients who came to the Sleep Department with suspicion of OSA. Medical history was taken from all the patients, after which PSG was performed on all patients. When taking patient anamnesis, data was grouped into four sets. The first set of information included standard data for age and sex. The second set of collected data included information about all OSA symptoms such as snoring, witnessed apnea, daytime sleepiness, morning dry mouth or throat, feeling under-slept in the morning, and others. The third set of collected data included information about the primary symptom and was classified as a distinct category of data information. Primary symptom was defined as a single primary issue that led patients to the hospital. Fourth set of information included data of patient's self-awareness of symptoms. OSA was diagnosed by PSG, which represents the golden standard for the diagnosis [12, 13]. In this study we used Respironics Alice 5 Diagnostic Sleep System polysomnograph (Koninklijke Philips N.V., Amsterdam, the Netherlands). All results from PSG were scored manually according to standard criteria. Apnea, hypopnea and arousals were also identified according to the standard criteria [14]. All results were summarized according to the Apnea–Hypopnea Index (AHI). All patients with symptoms of OSA and AHI score of over 5 were diagnosed with OSA [13].

RESULTS

The study included 388 patients, who came to the Sleep Department with suspicion of OSA. The average age was 44.8 ± 13.7 years, and 301 were males and 78 females. Medical history was taken from all patients with suspicion of OSA, and PSG was performed on all of them in order to diagnose OSA. All the patients with symptoms of OSA and AHI score of over 5 were diagnosed with OSA. A total of 258 patients were diagnosed with OSA with AHI > 5 per hour, 107 patients had negative AHI < 5 per hour, 18 patients were diagnosed with respiratory effort-related arousals (RERA), obesity-hypoventilation syndrome was found in four patients, and one patient had central sleep apnea (CSA). Patients with RERA, CSA, and obesity-hypoventilation syndrome were not taken into account in statistical analysis.

We identified 23 symptoms that led the patients to the Sleep Department. Twelve most common symptoms are listed in Table 1. The most common symptom was snoring, which occurred in 86% of the patients, followed by a feeling of under-sleeping, with 68%, and witnessed apnea, with 63%. The remaining 10 symptoms (headache, dizziness, bruxism, stuffy or blocked nose, nose bleeds, restless legs, numbness in hands and feet, excessive dreaming, regurgitation of food, and nightmares) occurred in less than 1% of cases and are not listed in Table 1.

After PSG, the patients were divided into two groups. The first group included patients with negative PSG with AHI < 5, and the second group included patients with positive PSG with AHI > 5, whom we diagnosed with OSA. Table 2 shows six most common symptoms that we found in booth groups.

Table 1. Percentage of 13 most common symptoms for all patients with clinical suspicion of OSA who presented at the Sleep Laboratory before they underwent overnight PSG

1.	Snoring	86%
2.	Feeling of lack of sleep in the morning	68%
3.	Witnessed apnea	63%
4.	Daytime sleepiness	55%
5.	Abrupt awakening from sleep because of choking	38%
6.	Abrupt awakening not associated with choking	29%
7.	Inability to fall asleep	21%
8.	Dry mouth and/or throat in the morning	20%
9.	Fatigue	19%
10.	Short sleep	18%
11.	Anxiety	15%
12.	Chest pain	9%
13.	Fear of falling asleep	5%

OSA – obstructive sleep apnea, PSG – polysomnography

Table 2. List of most common symptoms in patients with and without OSA

	OSA negative AHI < 5	OSA positive AHI ≥ 5
1.	Snoring	Snoring
2.	Abrupt awakening not associated with choking	Daytime sleepiness
3.	Feeling of lack of sleep in the morning	Witnessed apnea
4.	Inability to fall asleep	Feeling of lack of sleep in the morning
5.	Anxiety	Abrupt awakening from sleep because of choking
6.	Daytime sleepiness	Dry mouth and/or throat in the morning
7.	Other	Other

OSA – obstructive sleep apnea, AHI – Apnea–Hypopnea Index

We identified primary symptoms for all the patients and we attempted to classify the primary symptoms as a distinct category. Primary symptom was defined as a single primary issue that led patients to the hospital. In fact, most patients could clearly identify their primary symptom. A smaller number of patients had two or more symptoms. In these patients we had to identify only one major symptom that we considered to be primary. For example, if a patient had snoring, witnessed apnea, daytime sleepiness, and a feeling of under-sleeping in the morning but his wife told him that he had been suffocating while sleeping and that is the reason he is visiting a doctor, then witnessed apnea was registered as the primary symptom. Or if we had a patient who complains of abruptly awakening from sleep, cannot fall asleep, and experiences anxiety, and the main reason for visiting a doctor was abrupt awakening from sleep, then we took this symptom as the primary one. The most common primary symptom for all the patients was snoring, with 31%, followed by witnessed apnea, with 25%, abrupt awakening from sleep because of choking, with 19%, etc. (Table 3)

Table 3. Percentage of primary symptoms for all patients with clinic suspicion of OSA who presented at the Sleep Laboratory before they underwent overnight PSG

1.	Snoring	31%
2.	Witnessed apnea	25%
3.	Daytime sleepiness	19%
4.	Abrupt awakening from sleep because of choking	18%
5.	Abrupt awakening not associated with choking	9%
6.	Inability to fall asleep	6%

OSA – obstructive sleep apnea, PSG – polysomnography

Table 4. Primary symptom that occurs in different groups according to the severity of OSA

Severity	Snoring	Witnessed apnea	Daytime sleepiness	Other
AHI (5–14)	65.8%	12.2%	7.3%	14.7%
AHI (15–30)	60.1%	17.7%	13.5%	8.7%
AHI (> 30)	29.2%	34.8%	27.8%	8.2%

OSA – obstructive sleep apnea, AHI – Apnea–Hypopnea Index

Table 5. The primary symptom that occurs in patients with AHI <5

OSA severity	Snoring	Abrupt awakening not associated with choking	Feeling of lack of sleep in the morning	Inability to fall asleep	Other
AHI < 5	43.9%	23.4%	13.1%	10.3%	9.3%

AHI – Apnea–Hypopnea Index

Table 6. Motivation for patients to seek help from a doctor

1.	Patients aware of their symptoms seeking help	72%
2.	Patients unaware of their symptoms, but alerted by their partner	22%
3.	Patients unaware of their symptoms, but alerted by the media	4%
4.	Patients unaware of their symptoms, but alerted by their doctor	2%

After PSG, we divided the patients into two major groups. The first group comprised patients with AHI < 5 (negative PSG in 107 patients), and the second group comprised patients with AHI > 5 (positive PSG in 258 patients). Patients who were diagnosed with OSA according to the severity of AHI were divided into three groups. In the first group with mild OSA (AHI ≥ 5, but < 15 per hour) there were 41patients (15.9%). In the group with moderate OSA (AHI ≥ 15, but < 30 per hour) there were 23 patients (8.9%), and in the group with severe OSA (AHI ≥ 30 per hour) there were 194 patients (75.2%).

Table 4 presents the percentage of primary symptoms in different groups of patients divided according to severity of OSA. We found that snoring as the primary symptom is highest in mild OSA, and is decreasing as severity of OSA increases, while the results are opposite with witnessed apnea and daytime sleepiness as a primary symptoms.

Results from 107 patients with AHI < 5 are shown in Table 5. The most common primary symptom in this group of patients was snoring, with 43.9%, followed by abrupt awakening not associated with choking, with 23.4%, and a feeling of under-sleeping in the morning, with 13.1%.

The results that we collected from all 388 patients which refer to the question if they were aware of their symptoms are shown in Table 6. The majority of patients (72%) had been aware of their symptoms, while the percentage of patients who were "forced" to go to the doctor by their partners and had not been aware of their symptoms amounted to 22%. Four percent of patients had not been aware of their symptoms but were alerted by the media and come to our department. Two percent of patients had not been aware of their symptoms, but the symptoms were discovered by their doctors, who referred the patients to our department.

DISCUSSION

Most of patients had never heard of OSA and do not know that they have symptoms that may indicate this disease. Unfortunately, doctors rarely think of OSA and symptoms that patients are complaining of are often considered to be symptoms of other diseases [15]. Franklin et al [3] pointed out that OSA is primarily regarded as a male disorder, which is comparable with results of our study. Almost 80% of patients that we included in this study were male. OSA is characterized with a number of symptoms. From the study we can see that we identified 23 different symptoms. The most common symptoms are snoring, a feeling of under-sleeping in the morning, witnessed apnea, and daytime sleepiness. Once we separated OSA-positive and negative patients, we found that snoring is the most common symptom in booth groups. Myers et al. [12] pointed that snoring is the most common symptom in sleep apnea patients but is not useful for establishing the diagnosis.

But after snoring we have difference in order of appearance of symptoms in booth groups. In the negative OSA group, symptoms that followed snoring were abrupt awakening from sleep not associated with choking and feeling under sleeping in the morning. In the positive OSA group next following symptoms were witnessed apnea, daytime sleepiness. We have similar results with primary symptoms. Most common primary symptoms in all patients were snoring, witnessed apnea, daytime sleepiness, abrupt awakening from sleep because of choking, abrupt awakening not associated with choking and cannot fall asleep. Most common primary symptoms in patients with OSA were snoring, witness apnea and daytime sleepiness. The percentage of snoring as the primary symptom reduced, as severity of the OSA increased. While the percentage witnessed apnea and daytime sleepiness was increasing, as severity of the OSA was increasing. In the study of Li et al. [16], the most common primary symptom is witnessed apnea with 32.9% and then snoring followed by 28.9%. In the same study, patients with AHI > 30 have daytime sleepiness as most common primary symptom, followed by witnessed apnea and snoring. Myers et al. [12] pointed out that the most useful observation for identifying patients with obstructive sleep apnea was witnessed apnea. The most common primary symptoms in patients with AHI < 5 were snoring, abrupt awakening from sleep not associated with choking, a feeling of under-sleeping in the morning, and inability to fall asleep. In these patients, the

symptoms are likely due to other diseases and we should conduct research in other directions as well. The differences in primary symptoms are obvious, and they might lead the doctor to suspect which patient will be positive or negative to OSA. From the results we obtained from medical history questionnaires, in which patients were asked about the motivation to seek help from a doctor, we can conclude that 72% of the patients were aware of their symptoms. Arnardottir et al. [17] reported that in a middle-aged general population, approximately 20% of subjects had moderate-to-severe OSA, but the majority of them were neither symptomatic nor sleepy and did not have impaired vigilance. We can also conclude that doctors only in a minority of patients, amounting to only 2%, had a suspicion that the symptoms are due to OSA. Although this percentage is very low, Rosen et al. [18] published that the prevalence and recognition of sleep disorders in a community-based outpatient health setting was 0.1%. Patients lose a lot of time before a doctor recognizes the disease and refers them to a sleep disorder department [19]. We had 4% of patients who were not aware of their symptoms but were alerted by media and came to our department. Public awareness of this widespread disease will rise with its presence on television and in printed media, and many patients will come to the right place on time. The majority of papers like this one stress the need for more education of general and clinical physicians. In this way they will recognize the disease early enough to diagnose OSA and to provide proper treatment to OSA patients [20].

CONCLUSION

In the group with confirmed OSA, the most common symptoms were snoring, apnea confirmed by a witness, and daytime sleepiness. In the OSA-negative group, in addition to snoring, the most common symptoms were suddenly waking up from sleep not associated with suffocation, a feeling of lack of sleep in the morning, and inability to fall asleep. Many patients are aware of their symptoms and seek help from a doctor. Greater education of hospital doctors and doctors in the primary health care in recognizing the symptoms of OSA are needed if patients are to be promptly referred for diagnosis and treatment.

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Симптоми због којих се пацијенти обраћају лекару за дијагностиковање опструктивне ноћне апнее

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САЖЕТАК

Увод/Циљ Опструктивна апнеја се јавља у току сна (ОАС) карактеришу је симптоми којих понекад ни сам болесник није свестан.

Циљ ове студије је да одреди симптоме због којих се пацијент јавља и да се испита до ког степена самосвесност пацијента има улогу у дијагностиковању ОАС.

Методе У студију је било укључено 388 пацијаната који су се јавили на Одељење за медицину спавања на Клиници за пулмологију у периоду од 2012. до 2016. године, а код којих се посумњало на ОАС. Пацијентима је узета анамнеза за ОАС и урађена је полисомнографија. Свим пацијентима са симптомима ОАС и код којих је апнеја-хипопнеја индекс (АХИ) био изнад 5 дијагностикована је ОАС.

Резултати Идентификована су 23 симптома због којих су се пацијентици јавили лекару. Најчешћи симптом је хркање (86% случајева). Затим следе осећај ненаспаваности (68% случајева) и апнеја регистрована од сведока (63%). Код 258 пацијената је дијагностикована ОАС. Најчешћи примарни симптоми били су хркање, апнеја регистрована од сведока и дневна поспаност. Проценат хркања се смањивао са тежином болести. Проценат апнеје регистроване од сведока и дневна поспаност су се повећавали са тежином болести. Највећи број пацијената се јављао јер су свесни својих симптома.

Закључак Код пацијената који имају симптоме као што су хркање, апнеја регистрована од сведока и дневна поспаност постоји велика вероватноћа да болују од ОАС. Велики део пацијената је свестан својих симптома и тражи помоћ лекара.

Кључне речи: апнеја у спавању; хркање; примарни симптом

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Impact of sodium profiling on ambulatory blood pressure in patients on maintenance hemodialysis

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SUMMARY

Introduction/Objective Most patients with end-stage renal disease (ESRD) have hypertension. However, dialysis-related strategies to optimize blood pressure in these patients remain controversial. The current study aims to investigate the influence of dialysate sodium profiling on ambulatory blood pressure (ABP) in patients on maintenance hemodialysis, when there are no adequate dialytic and economic resources or high patient compliance.

Methods This prospective, single-center study enrolled 60 hypertensive ESRD patients. Subjects received maintenance dialysis with regular dialysate sodium concentration (140 mmol/L) during the initial three months after the enrollment, and were randomly assigned to continue regular sodium dialysate (group A) or switch to sodium profiling (group B) for duration of three months. ABP, heart rate (HR), pre-/postdialysis serum sodium levels, antihypertensive treatment dosages, and interdialytic weight gain (IDWG) etc. were recorded after treatment assignment.

Results Thirty patients each were enrolled in groups A and B. The characteristics at baseline were not significantly different between the two groups. Compared to patients in group A three months later, patients in group B had lower systolic ABP ($p = 0.00$), HR ($p = 0.04$), IDWG ($p = 0.04$), and antihypertensive medication dosages ($p = 0.04$). Throughout the treatment duration, no significant inter-group differences were observed for pre-/post-dialysis serum sodium and intradialytic complications. Additionally, no significant correlations were found between systolic or diastolic ABP and other variables studied in this study.

Conclusion In this study, we found that dialysate sodium profiling successfully ameliorated hypertension and reduced BP medications without altering natremic levels or increasing complications among patients on maintenance hemodialysis during the three months. Dialysate sodium profiling was relatively safe in this duration.

Keywords: ambulatory blood pressure; end-stage renal disease; sodium profiling; hemodialysis

INTRODUCTION

Hypertension is frequently observed in patients with advanced chronic kidney disease (CKD) and end-stage renal disease (ESRD). More than 90% of patients with advanced CKD have hypertension, which persists even after progression to ESRD [1]. In addition, blood pressure (BP) variability is a risk factor for adverse outcomes among hypertensive patients; patients on maintenance dialysis are more susceptible to adverse impact of BP fluctuations due to increased vascular stiffness and dialysis-related body fluid shift. In 2009, a meta-analysis mostly incorporating randomized controlled trials found that reduction of BP is closely associated with lower risk of cardiovascular events, better all-cause mortality, and cardiovascular mortality among patients on maintenance dialysis [2].

Rahman et al. [3] found that hypertensive ESRD patients had significantly higher blood volume, plasma volume, and extracellular fluid levels than normotensive patients with ESRD. In ESRD patients undergoing maintenance hemodialysis, it is now recognized that refractory hypertension and sodium and fluid retention might result from impaired homeostasis associated with loss of renal function. Sodium and fluid retention plays an important role in the pathogenesis of hypertension in ESRD patients, and the sodium-potassium balance is crucial to endothelium-dependent vascular dilatation. Determinants of salt and water retention in ESRD patients primarily include dietary sodium intake, sodium gain from high-sodium dialysate, and the dialytic modality and schedule (usually thrice weekly) for sodium clearance [4, 5].

Greater ultrafiltration during dialysis and dry weight lowering is beneficial for BP reduction, decreasing predialysis systolic BP, left ventricular volume, and antihypertensive medication dosage. A recent study lends support to this theory by showing that enhanced dialysis sodium removal can ameliorate arterial stiffness, left ventricular hypertrophy, and interdialytic weight gain (IDWG) [6]. Thus, maintaining the balance of sodium and extracellular fluid plays an important role in IDWG modulation. Del Giudice et al. [7] found that

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low dialysate sodium concentrations improved BP control among patients on maintenance dialysis. However, shorter duration of dialysis and increased ultrafiltration rate following technological advancements in the field of hemodialysis have brought about hypotension, disequilibrium syndrome, and muscle cramps. Adjustment of dialysate sodium concentration can be a potential approach to achieve this balance [8]. Sodium profiling is usually used in normovolemic patients with hypotension or normal blood pressure who are instable during dialysis session, and some researchers suggest that sodium profiles reduce dialysis complications and provide patient comfort. They thus believe that such procedures are beneficial to patients facing difficulty in reaching dry weight due to the adverse effects of dialysis.

In addition, recent studies suggest the superior prognostic importance of ambulatory BP (ABP) monitoring compared to single BP measurement and its facilitation of adequate BP control in patients on dialysis [9]. Guidelines from the American Heart Association and European Hypertension Society recommend ABP monitoring in all hypertensive patients [10].

People in Chinese Shandong province usually have a dietary habit of high salt, and the IDWG of our patients was in the range of 0.5–3.5 kg or more. Large IDWG requires high ultrafiltration rate, which is always followed by dialysis instability, some patients even could not eliminate the superfluous fluid in one dialysis course. But according to Kidney Disease Outcomes Quality Initiative (KDOQI) guidelines, patient's IDWG should not exceed 1 kg during the week and 1.5–2 kg during the weekend. IDWG of more than 4.8% (i.e. 3.4 kg in a 70 kg person) is a reflection of excessive sodium and water intake, and is associated with increased mortality.

A large percentage of patients in our dialysis center comprised refractive hypertension, probably because of superfluous fluid and sodium. Although we prescribed them with restricted sodium and water diet, tried to use 138 mmol/l or lower dialyzate sodium, tried to normalize Kt/V and to increase the frequency of dialysis etc, there was no ideal result, which might be due to low compliance with clinicians' advices. Additionally, as a result of our country's limitations in dialytic and economic resources, we neither have enough dialysis machines to meet longer dialysis session time nor can we afford high flux dialyzers every time. In this dialysis center, the routine dialysis time was four hours, and the center has three courses every day, with no nocturnal hemodialysis, although it is well known that longer dialysis session time may cure dialysis hypertension, and dialysis time should be at least four hours (KDOQI). Therefore, we performed this study and tried to find a suitable way to relieve their hypertension in our center.

There is a paucity of studies that evaluate the effectiveness of dialysate sodium profiling in controlling interdialytic ABP, and we sought to investigate this issue in patients on maintenance dialysis, to instruct clinical intervention. The primary endpoints of this effective study were changes in clinical parameters, including ABP, heart rate (HR), and IDWG. Secondary endpoints included changes in antihypertensive treatment dosage, serum sodium, and dialysis adequacy in this study. Safety endpoints included intradialytic hypovolemia-related complications, such as hypotension and cramping events per month.

METHODS

Study population

The current study was approved by the institutional review board of Qingdao University, Shan-Dong Province, People's Republic of China, and all participants provided written informed consent. The study was conducted with adherence to the principles of the Declaration of Helsinki.

This prospective, single-center study enrolled patients undergoing maintenance hemodialysis at the blood purification center of the affiliate hospital for the Qingdao University. Inclusion criteria were the age of 18 years or older, dialysis for more than 12 months, mean hemoglobin of 110–120 g/L with stable erythropoietin dosage, and daily residual urine output less than 100 mL. Subjects were included when mean systolic ABP levels were above 150 mmHg. Patients with a history of arrhythmia (atrial fibrillation or frequent premature complexes), major cardiovascular or cerebrovascular events within three months preceding the enrollment, active infection or concurrent malignancy, and prominent subcutaneous edema or pericardial effusion on cardiac ultrasonography were excluded.

Study procedure

All participants underwent a washout period of three months with a regular dialysate sodium concentration (140 mmol/L) before commencing study treatment. All patients were prescribed low salt, limited fluid intake before this trial, and did not perform any alterations in dietary habits or new dietary instructions. All subjects underwent maintenance hemodialysis using polysulfone-based membrane thrice weekly for four hours, low flux dialyzers routinely and high dialyzers twice a month. Subjects were randomly assigned to either regular sodium dialysate (group A) or sodium profiling (group B) groups. Dialysate for group A patients comprised a bicarbonate-based formula, with a fixed sodium, potassium, and calcium levels of 140 mmol/L, 2.5 mmol/L, and 1.5 mmol/L, respectively. Dialysate for group B patients consisted of an adjustable formula, which comprised an initial sodium level of 148 mmol/L and a linear reduction to 132 mmol/L over the treatment period, and potassium and calcium levels of 2.5 mmol/L and 1.5 mmol/L, respectively. The blood flow rate for each patient ranged between 200 mL/min. and 280 mL/min.; dialysate flow rate and temperature were 500 mL/min. and 37°C, respectively, with anticoagulation using unfractionated or low-molecular-weight heparin.

In study subjects, pre- and post-dialytic serum sodium and urea were measured once a month at the central laboratory of the hospital. The formula Kt/V was utilized

to evaluate the adequacy of each dialysis session. Kt/V was calculated according to a natural logarithm formula Kt/V = Ln (R - 0.008 × t) + (4 - 3.5 × R) × UF/W, where R is postdialysis blood urea nitrogen and predialysis blood urea nitrogen, t is session length in hours, UF is ultrafiltration volume in liters, and W is postdialysis weight in kg [11].

Study endpoints

Parameters included in primary and secondary endpoints were monitored. On the interdialytic day of the first week of each month, sitting BP and HR were measured after patients rested for 10 minutes at three time points (morning, noon, and evening) and the mean of the three measurements was recorded as the value for that day. Mean values of ABP and HR were documented from data obtained on consecutive interdialytic days in that week. The patients were censored if their ABP decreased to less than 130/80 mmHg during the intervention period or after three months of treatment.

Antihypertensive medication dosage was quantified with the defined daily dose (DDD) approach for each type of medication [6]. For patients on more than one antihypertensive medication, we calculated the sum of DDD from all medications to represent the antihypertensive dosage. IDWG was calculated from the difference between body weight measured after one dialysis and that measured before the next session, which was divided by their dry weight and the mean of measurements from consecutive interdialytic days of the first week of each month was calculated.

Safety endpoints included intradialytic hypovolemia-related complications. We recorded the monthly frequency of dialysis-related hypotension and muscle cramping. Dialysis-related hypotension was defined as a decrease in systolic BP of more than 20 mmHg or a decrease in mean BP of more than 10 mmHg, accompanied by symptoms of hypotension requiring clinical interventions. Muscle cramping referred to intradialytic painful muscle contractions unaccompanied by hypotension, amenable to improvement by a local massage and saline infusion.

Statistical analysis

We used SPSS Statistics for Windows, Version 17.0 (SPSS Inc., Chicago, IL, USA) for statistical analysis. Continuous variables are expressed as mean ± standard deviation; normal distributions of the variables were examined. Comparison of data normal distributions was done by Student's independent t-test. Blood urea nitrogen, DDD, IDWG were not normal distributions and were tested by Mann–Whitney U-test. Categorical data were analyzed by χ^2 test. Pearson analysis and multi-factor regression analysis were performed to find the correlation between ABP and other variables. A p-value of less than 0.05 was considered statistically significant.

RESULTS

Sixty patients [male, 27; mean age 45 ± 12.3 (range 25.5–65) years] on maintenance hemodialysis were enrolled. The mean duration of dialysis was 5.7 ± 2.6 years (range 1.5–10.5 years). ESRD etiologies in these patients were: 35 (58.3%) chronic glomerulonephritis, 10 (16.7%) diabetic nephropathy, and 15 (25%) hypertensive glomerulosclerosis.

Baseline parameters: inter-group comparison

Of the 60 participants, 30 were assigned to group A (46.7% male) and 30 to group B (43.3% male). Mean age and duration of dialysis were 45.4 ± 13.1 and 5.8 ± 2.7 years for group A, 44.7 ± 11.6 and 5.7 ± 2.6 years for group B. No significant inter-group differences existed for baseline characteristics, including hemoglobin, total protein, albumin, calcium, phosphates, potassium, urea, creatinine, total protein, antihypertensive medication dosage, IDWG status, intradialytic hypotension, and muscle cramping (Table 1). Pretreatment ABP, HR, pre- and postdialysis

Table 1. Patients' baseline characteristics ($\bar{x}$ ± SD)

Variable	group A	group B	p-value
Hemoglobin (g/L)*	97.3 ± 8.6	99.1 ± 9.4	0.442
Total Protein (g/L)*	67.4 ± 5.2	65.3 ± 4.2	0.090
Albumin (g/l)*	35.5 ± 3.5	36.6 ± 2.9	0.190
Calcium (mmol/L)*	2.22 ± 0.47	2.35 ± 0.36	0.234
Phosphates (mmol/L)*	1.65 ± 0.23	1.54 ± 0.31	0.124
Potassium (mmol/L)*	5.0 ± 0.8	4.9 ± 1.2	0.705
Blood urea nitrogen (mmol/L) #	25.5 ± 10.4	22.7 ± 12.5	0.349
Serum creatinine (umol/L)*	890.2 ± 150.9	907.5 ± 143.7	0.651
Estimated dry weight (kg)*	60.5 ± 10.7	59.2 ± 8.4	0.602
Kt/V*	1.36 ± 0.10	1.41 ± 0.11	0.070
Pre-dialysis serum sodium (mmol/L)*	138.7 ± 3.8	139.3 ± 3.3	0.516
Post-dialysis serum sodium (mmol/L)*	135.8 ± 3.8	137.6 ± 3.4	0.052
Antihypertensive medication dosage (DDD)#	4.5 ± 1.9	4.5 ± 2.1	1.000
Intradialytic hypotension (event/month)¤	16	20	0.503
Intradialytic cramping (events/month)¤	12	16	0.448
Interdialytic weight gain (%)#	4.32 ± 1.25	4.39 ± 1.49	0.8444
Systolic blood pressure (mmHg)*	152.67 ± 17.53	154.48 ± 16.40	0.6811
Diastolic blood pressure (mmHg)*	104.34 ± 15.49	109.46 ± 13.20	0.1735
Heart rate (beats/min.)*	76 ± 7	78 ± 9	0.168

DDD – daily defined dose;
*Data were normal distributions and comparisons were made using Student's independent t-test;
#Data were not normal distributions and comparison were made using Mann–Whitney U-test;
¤Data were categorical and comparisons were made using χ^2 test; p-value of less than 0.05 was considered statistically significant

serum sodium, and Kt/V did not differ between group A and B patients.

Endpoints

After dialysate sodium profiling for three months, group B patients did not have significant alterations in their pre-/postdialysis serum sodium, dialysis adequacy measures, IDWGs, and intradialytic hypotension / muscle cramping compared to group A (Table 2).

However, group B patients had significantly lower systolic ($p < 0.01$), diastolic ABP ($p < 0.01$) and HR ($p = 0.04$) compared to patients in group A. Antihypertensive medication dosage also decreased significantly in group B patients compared to group A ($p = 0.04$) (Table 1).

IDWG for group B patients decreased from 4.4 ± 1.5 kg at baseline to 3.8 ± 1.2 kg at three months, with a significantly lower level at three months than in patients in group A ($p = 0.04$) (Table 2).

Correlation analysis

We performed Pearson analysis and multi-factor regression analysis, but did not find any significant relation between systolic or diastolic ABP and other variables (data not show).

Table 2. Inter-group comparison of parameters at post-assignment

Variable	Group A	Group B	p-value
Kt/V*	1.38 ± 0.10	1.40 ± 0.10	0.442
Pre-dialysis serum sodium (mmol/L)*	139.5 ± 2.8	138.7 ± 2.9	0.281
Post-dialysis serum sodium (mmol/L)*	136.5 ± 2.8	136.3 ± 3.0	0.790
Antihypertensive medication dosage (DDD)#	4.4 ± 2.3	3.3 ± 1.7	0.039
Intradialytic hypotension (events/month)¤	20	12	0.156
Intradialytic cramping (events/month) ¤	8	16	0.102
Interdialytic weight gain (%)#	4.44 ± 1.17	3.80 ± 1.18	0.039
Systolic blood pressure (mmHg)*	154.67 ± 18.32	138.00 ± 18.19	0.000
Diastolic blood pressure (mmHg)*	109.47 ± 14.88	85.34 ± 12.90	0.000
Heart rate (beats/min.)*	78 ± 6	75 ± 8	0.039

DDD – daily defined dose;
*Data were normal distributions and comparisons were made using Student's independent t-test;
#Data were not normal distributions and comparison were made using Mann–Whitney U-test;
¤Data were categorical and comparisons were made using χ^2 test; p-value of less than 0.05 was considered statistically significant

DISCUSSION

This is an effective study designed to compare the effect of sodium profiling with sodium dialyzate concentration of 140 mmol/L, which is the routine sodium dialyzate concentration in our dialysis center, as well as many centers in our country, maybe some other countries as well. This study tried to use adjustable sodium dialysis, adjusted the ultrafiltration according to the IDWG and the tolerance of each patient, to exclude more fluid in one dialysis course, get near to the dry weight, lowering the ABP, without increasing dialysis adverse events. We previously found that dialysate sodium profiling can reduce postdialysis BP in hypertensive patients on maintenance hemodialysis [12]; in the current study, we further identified that dialysate sodium profiling, compared to regular dialysate sodium status, effectively decreases interdialytic ABP and use of antihypertensive medication. Another study similarly showed that low-sodium dialysate can reduce morning and night ABP over a period of six months [13]. Inrig et al. [14] conducted a randomized study, comparing hemodialysis patients receiving low- and high-sodium dialysate for three weeks, and found that low-sodium dialysate leads to a mean 9-mmHg decrease in systolic BP, consistent with our findings. Our study extends their findings by demonstrating the similarly favorable effect of dialysate with adjustable sodium on ABP parameters, antihypertensive medication dosages, and IDWG in patients on maintenance hemodialysis.

In sodium profiling, hypernatremic dialysates are used at the beginning of the dialysis process. Sodium content of the dialysate is then gradually reduced to allow excess sodium to be removed from the blood. High sodium concentration in this method facilitates the movement of water from the interstitial space into the intravascular space and results in better maintenance of intravascular volume and fewer adverse effects during dialysis. In this study, we used high-sodium dialysate (148 mmol/L) initially during each session, and linearly reduced dialysate sodium concentrations over time (finally to 132 mmol/L) to facilitate removal of excessive sodium. This maneuver did not affect serum sodium and intradialytic BP after three months; the systolic and diastolic ABP and HR all significantly improved after dialysate sodium profiling, whereas these parameters did not alter in the control group.

Intradialytic complications such as hypotension and muscle cramping are frequently associated with dialysis intolerance, the reduction of blood flow and dialysis duration, leading to lower dialysis adequacy (Kt/V) [15]. From this study, we found that, compared to regular dialysate sodium group, dialysate sodium profiling did not increase these complications significantly, which is consistent with results from previous studies [11]. However, despite the fact that there are already multiple studies addressing the effect of dialysate sodium profiling on improving hypertension in patients on maintenance hemodialysis, some believe that high concentrations of sodium in sodium profiles will lead to not only increased thirst and interdialytic weight gain (which means the need for removing greater volumes of fluids to reach dry weight and higher frequency of hypotension) but also hypertension [11]. Thus, sodium in sodium profiles nowadays should only be used in short time, with adequate assessment of patients' response. Once there is improvement in fluid load and ABP, dialysis mode should be back to the routine mode.

Therefore, more studies, especially those with large sample size, conducted at multiple centers, and of longer duration, are needed to verify the benefit of this approach. Other issues such as ultrafiltration profiling and individualized sodium profiling regimens also await further investigation. Our study is limited in several aspects. The case number is modest, and cardiac as well as vascular stiffness parameters were not collected during the study. However, our findings have merit because adjustable sodium dialysate for BP control is scarcely addressed in the literature.

CONCLUSION

This study found that, in patients on maintenance hemodialysis, adjustable sodium dialysis can reduce ABP, HR, antihypertensive medication dosage, and IDWG. These findings contribute to the current literature by showing that adjustable dialysate sodium has a beneficial effect on hemodynamic parameters similar to that with low-sodium dialysate in patients on hemodialysis, and we did not increase dialysis adverse events in the three months. Further study is needed to confirm our results.

NOTE

Zhang Yue-yue and Zhai Li-hui have equally contributed to this paper and should be considered to be first co-authors.

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Утицај натријума на целодневни крвни притисак болесника на дуготрајној хемодијализи

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САЖЕТАК

Увод/циљ Већина болесника са терминалном болешћу бубрега (ТББ) има хипертензију. Међутим, стратегија за дијализу везана за оптимизацију крвног притиска код ових болесника остаје контроверзна.

Циљ овог рада је да испита утицај подешавања натријума у дијализату на крвни притисак (КП) мерен целог дана код болесника на дугорочној хемодијализи у условима неодговарајућих дијализних и економских ресурса.

Методе Проспективна студија у једном дијализном центру обухватила је 60 хипертензивних болесника са ТББ. Прва три месеца сви су били су на дијализи са натријумом у дијализату од 140 *mmol/L*, а потом насумично подељени у групу А (иста дијализа) и групу Б (подешен натријум у дијализату). Групе су дијализиране три месеца, када су им праћени следећи параметри: целодневни КП, пулс, натремија пре и после дијализе, дозе антихипертензивних лекова (АЛ) и међудијализни прираст телесне масе (МДТМ).

Резултати Почетне вредности контролисаних параметара нису се значајно разликовале између група. После три месеца болесници групе Б су у односу на групу А имали значајно нижи систолни КП ($p = 0,02$) и пулс ($p = 0,03$) у односу на њихове почетне нивое, мањи прираст МДТМ ($p = 0,04$) и мање дозе АЛ ($p = 0,04$). Између група нису уочене значајне разлике у натремији пре и после дијализе, као ни у учесталости интрадијализних компликација. Такође, нису пронађене значајне везе између систолног и дијастолног КП и других праћених варијабли у овој студији.

Закључак Подешавање натријума у дијализату успешно смањује хипертензију и дозе АЛ, без мењања натремије и повећања учесталости компликација хемодијализе.

Кључне речи: холтер крвног притиска; крајњи стадијум бубрежних болести; натремија; хемодијализа

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Jeune syndrome with renal failure

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SUMMARY

Introduction/Objective Jeune syndrome (JS) is a rare hereditary ciliopathy characterized by asphyxiating thoracic dystrophy, shortened limbs and brachydactyly. Extraskeletal anomalies such as chronic renal failure (CRF), hepatic fibrosis, and retinitis pigmentosa may be a part of the JATD phenotype.
The aim of this study is to present long-term follow-up of JS patients with early progressive kidney disease.

Methods This is a retrospective study of pediatric patients with JS and CRF who were treated at the University Children's Hospital between January 1980 and December 2014. The patients' data were retrospectively reviewed from the medical records.

Results There were thirteen patients from 11 families, five girls and eight boys mean aged 4.3 years at the time of diagnosis. All of the patients had characteristic skeletal findings, retinal degeneration and an early onset of CRF at age range from 1.5 to 7 years. Five patients had neonatal respiratory distress and congenital liver fibrosis was diagnosed in five patients. One patient died due to complications of CRF, while others survived during follow-up of mean 11 years. *IFT140* mutations were found in four genetically tested patients.

Conclusion The average incidence rate of JS with renal phenotype in Serbia was about 0.2 per one million of child population. Long-term survival of JS patients depends on renal replacement therapy, while skeletal dysplasia, growth failure, respiratory and eyes problems have impact on the patients' quality of life.

Keywords: asphyxiating thoracic dystrophy; osteochondrodysplasia; ciliopathies; neonatal respiratory insufficiency; terminal renal failure

INTRODUCTION

Jeune syndrome or asphyxiating thoracic dystrophy (JATD) belongs to a group of osteochondrodysplasia. It was first described in 1955 by Jeune et al. [1]. In 1992, the International Working Group on Constitutional Diseases of Bone classified Jeune syndrome as one of six short-rib dysplasia syndromes with or without polydactyly into Type I (Saldino–Noonan), Type II (Majewski), Type III (Verma–Naumoff), Type IV (Beemer–Langer), Jeune, and Ellis – van Creveld [2]. They all show a recessive mode of inheritance, frequently caused by mutations in primary cilia intraflagellar transport (IFT) genes [3, 4]. Primary cilia are non-motile hair-like sensory organelles on the surface of most cells of mammals, birds, amphibians, and fish. They have a structure of nine doublet microtubules emerging from a basal body that contains a pair of centrioles [5]. A microtubule- and ATP-dependent IFT governs bidirectional (anterograde and retrograde) cargo transport and delivery processes that are essential for primary cilia growth and maintenance and governs a variety of important cell signaling events that are key to normal human development [5, 6].

The main recognizable clinical feature of JATD visible immediately after birth is a small, narrow chest and variable limb shortness, while extra skeletal organ involvement may occur later in life [7]. Fatal, early neonatal respiratory insufficiency may occur. Respiratory problems may be difficult to manage, but bilateral thoracic expansion offers an effective reduction in ventilator requirements in children with severe condition [8].

Approximately, one third of JATD patients display a nephronophthisis-like nephropathy with progression to terminal renal failure. There isn't enough data in literature concerning their long-term follow-up.

The aim of this study is to describe thirteen JATD patients with renal phenotype who were followed up mean 11.2 years (range of 1–26 years).

METHODS

This is a retrospective study of pediatric patients with JATD who were treated at Departments of Nephrology and at Center for dialysis and transplantation of University Children's Hospital between January 1980 and December 2014. The patients' demographic data, clinical characteristics at presentation, laboratory data and radiographic findings, treatments, and

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outcomes were recorded through retrospective review of their medical records. Early course of the disease for four patients was previously reported [9].

RESULTS

We evaluated 13 patients from 11 families, five girls and eight boys. Their clinical characteristics are presented in Table 1 and Table 2. Five patients were familial cases; two sisters and two brothers, and one patient was a cousin of two affected brothers. The others were single cases in the family. Ages at the time of diagnosis of JATD ranged from 1.5 to 8 years, mean age was 4.3 years. In all patients, the diagnosis was made by pediatric nephrologists when the patients presented with chronic preterminal renal failure.

All of the patients had characteristic skeletal findings including a narrow bell-shaped chest with short and horizontal ribs that variably reduced the diameter of the thoracic cage, short limbs and brachydactyly, while only one patient had polydactyly. The patients shared similar radiological features, including irregular costochondral junctions, elevated clavicles, short iliac bones with a trident-shaped acetabular roof of the pelvis, short and wide long bones of the extremities, and cone-shaped epiphyses of phalanges of hands and feet (Figures 1–4).

Five patients had neonatal respiratory distress but only two of them remained prone to respiratory problems later in the disease course. All except one patient were growth retarded (height below the third percentile). In addition, all patients had retinal degeneration and chronic renal failure, and five patients had congenital liver fibrosis.

Genetic analysis was performed in four patients. *IFT140* mutations were found in all of them [10].

Only one patient died due to heart failure in pre terminal renal failure, while others survived during mean 11 years of follow-up. Renal replacement therapy was predominant for patients' survival, while skeletal dysplasia, growth failure, respiratory and eye problems had impact on the patients' quality of life. All the patients had normal psychosocial development. The three oldest patients had university education, normal jobs and normal, independent social life.

DISCUSSION

JATD is a rare autosomal recessive disorder with a variable prevalence estimated at one per 70,000–150,000 live births [4, 8]. It has been described that about 30% of JATD patients will have renal involvement [8]. During the study period, only 13 patients with JATD and renal involvement were identified at the Dialysis and Transplantation Center of the University Children's Hospital in Belgrade, which serve all pediatric patients with terminal renal failure in Serbia. Thus, the average incidence rate of JATD with renal insufficiency in Serbia was 0.4 per year or approximately 0.2 per one million of child population.

The diagnosis of JATD is based on clinical and radiological findings. A key factor in the early diagnosis of JATD is skeletal dysplasia manifested as abnormal small thorax causing a reduced thoracic capacity, short limbs and brachydactyly, with occasional postaxial or axial polydactyly of the hands [8]. All of our patients had thoracic and limb abnormalities, but only one patient was found to have polydactyly.

Early postnatal survival of patients with JATD is often dictated by respiratory insufficiency due to the restrictive chest cage. From the literature data the survival rates in infancy are 40–80% [7, 8]. Treatment is usually palliative respiratory support, while in severe cases bilateral thoracic reconstructive surgery offers satisfying functional and esthetic results [7, 8]. Respiratory problems tend to become less pronounced with age due to improved mechanical properties of the chest wall with growth. However, majority of the patients maintained a restrictive lung function

Table 1. Clinicalof characteristics of patients with Jeune syndrome (JATD)

JATD patients	1	2	3	4	5	6	7	8	9	10	11	12	13
Sex	M	M	F	M	M	M	M	M	M	F	F	F	F
Age at presentation (years)	8.5	3	3	4.5	6	6	2.5	5	1.5	3.5	7	3	6
Follow-up (years)	8	2	26	13	13.5	14	4.7	3	1	5	21	14	21
Genetic analysis*	–	–	–	*IFT140*	*IFT140*	*IFT140*	–	–	–	–	–	–	*IFT140*
NRI	+	+	–	+	–	–	–	+	–	+	–	–	–
Small thorax	+	+	+	+	+	+	+	+	+	+	+	+	+
Brachydactilia	+	+	+	+	+	+	+	+	+	+	+	+	+
Polydactylia	–	–	+	–	–	–	–	–	–	–	–	–	–
Cone-shaped epiphyses	+	+	+	+	+	+	+	+	+	+	+	+	+
Shortened legs	+	+	+	+	+	+	+	+	+	+	+	+	+
Short stature	+	+	+	+	+	–	+	+	+	+	+	+	+
Facial dysmorphism	+	+	+	+	+	+	+	+	+	+	+	+	+
Impairment of vision	+	+	+	+	+	+	+	+	+	+	+	+	+
Congenital hepatic fibrosis	+	+	+	–	+	–	–	–	–	+	–	–	–

JATD – Jeune asphyxiating thoracic dystrophy; M – male; F – female; NRI – neonatal respiratory insufficiency; * Genetic analysis in England [11]

Table 2. Symptoms and signs of renal disease

JATD patients	1	2	3	4	5	6	7	8	9	10	11	12	13
Age (years) when first symtoms were recognised	7	2.5	2	2	2.5	4	2	3	1.5	2	2	2	3
Polyuria	+	+	+	+	+	+	+	+	+	+	+	+	+
Polydipsia	+	+	+	+	+	+	+	+	+	+	+	+	+
Enuresis	+	–	–	+	–	+	+	–	–	–	–	+	–
Renal ultrasound showing reduced size of kidneys, unclear CM differentiation	Yes	Yes	Yes + Cysts at the CM junction	Yes + Cysts at the CM junction	Yes + Cysts at the CM junction	Yes + Cysts at the CM junction	Yes + Cysts at the CM junction	Yes + Cysts at the CM junction	Yes + Cysts at the CM junction	Yes	Yes + Cysts at the CM junction	Yes	Yes
CRF, age (years)	8.5	3	3	4.5	6	6	2.5	5	1	3	6	3	6
TRF, age (years)	9	4	5	4.5	6	6	2.8	5	–	5.9	10	6	6
Peritoneal dialysis	-	-	-	–	–	–	–	+	–	–	–	+	–
Hemodialysis	+	+	+	+	+	–	+	–	–	+	+	–	+
Kidney transplantation age (years)	–	–	10	5	9.5	7	3.5	7	–	6	10	11.5	9
Follow-up after transplantation (years)	–	–	15	11.5	12.5	6	3	0.1	_	1.5	12	4.5	17
Family renal history	–	–	–	Mother:renal cyst	–	Mother:scarred right kidney	Cousen with JATD 8 and 9; Mother: renal cyst	JATD 8 and 9 are brothers and their cousen is JATD 7	JATD 8 and 9 are brothers and their cousen is JATD 7	–	JATD 11 and 12 are sisters	JATD 11 and 12 are sisters	–
Outcome	Lost to follow-up	Lost to follow-up	Short stature, severe vision impairment, finished faculty	Short stature, eyeglasses for vision correction	Short stature, severe obesity	Eyeglasses for vision correction	Eyeglasses for vision correction	Short stature, eyeglasses for vision correction	Died at the age of 1.5 years	Short stature, eyeglasses for vision correction	Short stature, eyeglasses for vision correction, finished faculty	Short stature, eyeglasses for vision correction	Short stature, eyeglasses for vision correction, finished faculty
GFR (ml/min/1.73 m^2)	/	/	90	88	82	122	102	72	–	71	85	94	90

JATD – Jeune asphyxiating thoracic dystrophy; M – male; CRF – chronic renal failure; TRF – terminal renal failure; CM – corticomedullary; GFR – glomerular filtration rate

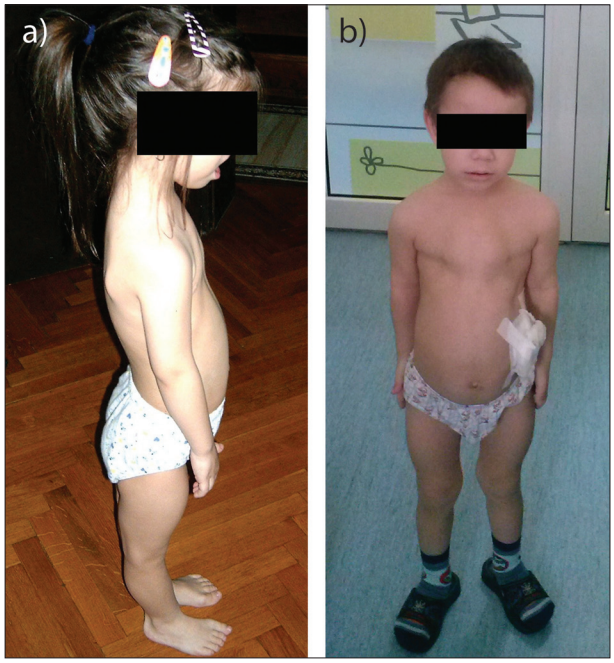

Figure 1. Clinical features of Jeune syndrome patients; a–b) Phenotypes of patients JATD 8 and JATD12 at five years of age; note disproportionate short stature with short extremities, relatively narrow thoraces with a protruding abdomen

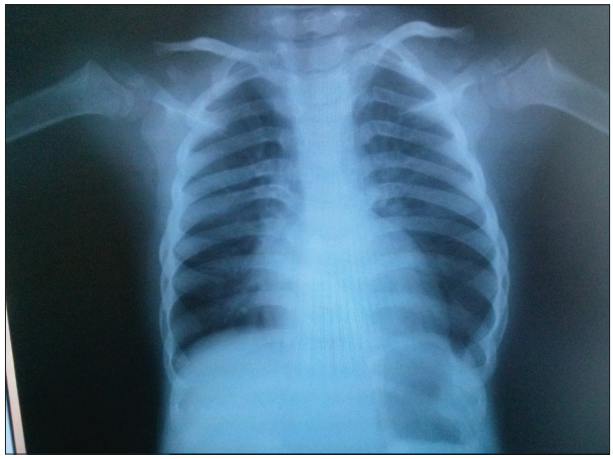

Figure 2. Thoracic features of Jeune syndrome: chest X-ray taken at the age of five years in patient JATD 8; note the narrow thorax, short ribs and elevated clavicles

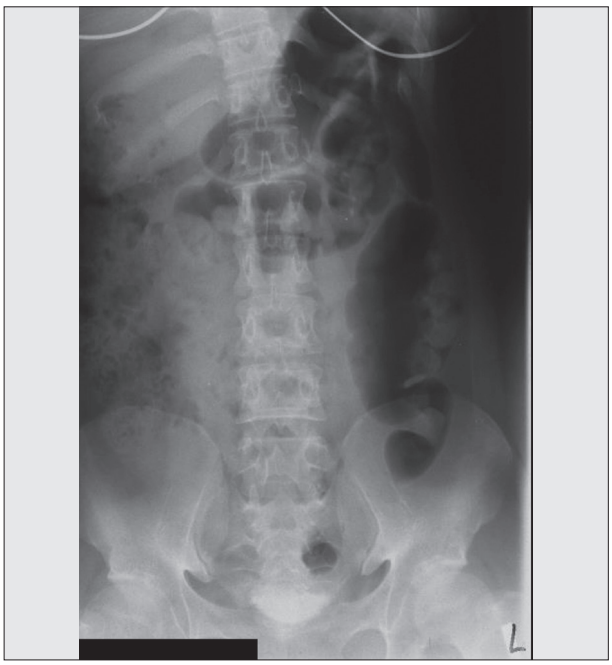

Figure 3. Characteristics of the pelvis of Jeune syndrome: X-ray of the pelvis of patient JATD 12 at the age of 20 years; note the typical trident appearance of the acetabula

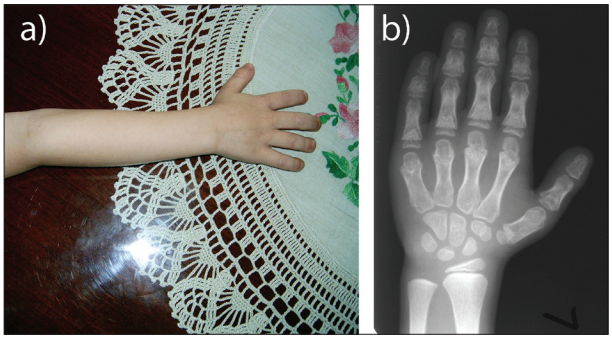

Figure 4. Brachydactyly in Jeune syndrome: a) typical broad hand with short fingers of the patient JATD 8; b) X-ray of the left hand of the same patient; note short and dysplastic phalanges

impairment resulting from lung hypoplasia. In our series, only five patients (38.5%) experienced respiratory problems in the infancy, and none died because of these. Two patients were prone to respiratory tract infection during further follow-up.

In contrast to milder respiratory problems in our patients, compared with those described in most articles [7, 8, 10–13], extraskeletal features affecting the eyes and kidneys were highly expressed later in life.

Retinal abnormalities are reported in 15–50%, but it may represent under-ascertainment [14, 15, 16]. All of our patients had documented retinal pigmented dystrophy by fundoscopy and/or electroretinography, but only half of them suffered of night blindness, usually manifested after the age of two years. Defective rhodopsin transport via the connecting cilium in retinal photoreceptor cells is proposed as the basis for the development of retinal dystrophy [16]. The ophthalmologic problems may cause gait instability which may prone them to trauma. That happened to one of our patients (patient No. 10), who had head trauma with subarachnoid hemorrhage and consecutive operative treatment at the age of 3.5 years. Five patients needed ophthalmologic correction by glasses due to hypermetropia.

Renal involvement has been reported in 17–20% of JATD patients [10–14, 17–20]. The kidneys are usually of normal or reduced size. The histological findings on renal biopsy include atrophic and cystic dilatation of the tubules, diffuse interstitial fibrosis, periglomerular fibrosis, and glomerular sclerosis. Cysts may develop typically at the corticomedullary junction [17]. Usually, clinical renal problems do not manifest until the second year of life and renal failure manifests at a median age of 13 years. Our patients had early onset of renal failure occurring at age range from 1.5 to seven years. Initial symptoms were relatively mild, started at approximately age of two years, and consisted of polyuria, polydipsia, and enuresis. That may be the explana-

tion of delayed diagnosis until the advanced stage of renal failure. No effective prophylaxis or treatment is available for renal involvement in JATD other than supportive care once chronic renal failure develops and dialysis and transplantation for terminal renal failure prove ineffective. One of our patients died at the age of 1.5 years due to cardiovascular complications of chronic renal failure. Other patients were on renal replacement therapy. Ten patients were successfully transplanted. As found by other authors [19], no recurrence of JATD was noted after transplantation.

JATD can be associated with periductal liver fibrosis, due to which patients develop hepatomegaly and moderate portal fibrosis with mild bile duct proliferation [8, 21]. Bile duct involvement in these cystic kidney diseases may be explained by the ciliary theory because the epithelial cells lining bile ducts (cholangiocytes) possess primary cilia. Five of our patients had mild liver affection.

The association of pancreatic fibrosis with Jeune syndrome has been described, but it has not been appreciated as a stable and important manifestation of this disorder [22]. None of our patients had pancreatic involvement.

Most of our patients exhibited short stature that can be explained by skeletal dysplasia and renal failure. Two patients had obesity that was difficult to treat with dietary measures. All patients had normal psychosocial development, doing well in school.

A lot of research has been done concerning a genetic diagnosis of JATD. A locus for JATD was mapped to chromosome *15q13* [23]. Work in animal models such as knockout mice suggests that defective IFT leads to impaired hedgehog signaling, which disturbs chondrogenic and osteogenic cellular proliferation and differentiation, leading to chondrodysplasia phenotypes [24, 25, 26].

Recently, five genes causing JATD have been reported, all encoding proteins involved in IFT (*DYNC2H1*, *IFT80/WDR56*, *IFT139/TTC21B*, *IFT144/WDR19*, *IFT140*) [4, 10, 15, 27–33]. *DYNC2H1* is a major gene responsible for JATD [27, 28, 29]. Phenotype–genotype correlations were recognized [27]. *IFT140* mutations in JATD seem to be causal only for a specific subset of cases with severe renal and prominent retinal involvement, in the context of heterozygous mutations potentially modifying the skeletal phenotype [10]. These mutations were documented in four of our patients, who were the only ones in whom genetic testing was done [10]. Having in mind that clinical features of the remaining patients, such as a non-lethal thorax-related clinical course, no polydactyly, retinal dystrophy, and an early onset of severe renal failure, were similar to those with documented *IFT140*, we can speculate that they also share the same genotype.

It is important to establish a correct diagnosis since JATD might recur within the family [8, 13, 33]. In our series of JATD patients, there were two sisters and two brothers. A prenatal sonographic diagnosis of Jeune syndrome at as early as 14 weeks of gestation in a fetus at risk for this condition has been reported [34]. Key factors in the prenatal diagnosis are the features of the skeletal dysplasia, polyhydramnios, and unidentifiable fetal respiratory movements [34, 35, 36].

Our survey demonstrates very encouraging results for long-term survival of children with JATD. However, their overall health-related quality of life (HRQOL) was significantly lower compared to that in healthy children. Short stature, skeletal deformities, visual impairment, as well as renal and extrarenal comorbidities, had negative impact on physical, emotional, and social functioning. The positive observation is that nearly all children attend schools with a standard education program, which is an important factor in preparing them for participation in adult life. In general, patients who underwent kidney transplantation had better quality of life than dialysis patients did. Therefore, early transplantation in those with terminal renal failure and adequate psychosocial support of the patients and their family can help to decrease the negative effects of the disease on the quality of life. Multicenter studies and use of a specific pediatric HRQOL assessment instrument are needed to develop JATD-specific interventions to optimize HRQOL.

The strengths of this study include, firstly, quite a high number of patients with similar renal phenotype, and, secondly, their long term follow-up. However, our study has limitations that should be considered. Its retrospective design may be related to potential under- and incomplete reporting, and unknown genotype of all patients.

CONCLUSION

We presented a group of JATD patients with early onset of severe renal failure, while respiratory problems were absent or mild. Long-term survival of these patients depends on renal replacement therapy. Further genetic investigations are necessary to examine whether these patients with renal phenotype share the same genotype.

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Женов синдром са бубрежном инсуфицијенцијом

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САЖЕТАК

Увод/Циљ Женов синдром (ЖС) ретка је хередитарна цилиопатија коју карактеришу асфиктична торакална дистрофија, скраћени екстремитети и прсти. Екстраскелетни поремећаји као што су хронична бубрежна инсуфицијенција (ХБИ), фиброза јетре и пигментни ретинитис пигментоза могу бити део ЖС фенотипа.

Циљ ове студије је да прикаже дуготрајно праћење ЖС болесника са раним прогресивним обољењем бубрега.

Методе Ово је ретроспективна студија педијатријских болесника са ЖС и ХБИ који су лечени на Универзитетској дечјој клиници у периоду од јануара 1980. до децембра 2014. године.

Резултати Укупно је било 13 болесника из 11 фамилија, пет девојчица и осам дечака просечног узраста 4,3 године у време дијагнозе болести. Сви болесници су имали карактеристичне промене скелета, ретиналну дегенерацију и рану ХБИ у узрасту 1,5–7 година. Пет болесника је имало неонатални респираторни дистрес и конгенитална фиброза јетре је дијагностикована код пет болесника. Један болесник је умро, док су остали преживели у току просечног праћења од 11 година. ИФТ 140 мутације су откривене у четири генетски тестирана болесника.

Закључак Просечна инциденца ЖС са реналним фенотипом у Србији је око 0,2 на милион дечје популације. Дуготрајно преживљавање ових болесника зависи од успеха терапије функције бубрега, док скелетна дисплазија, застој у расту, респираторни и очни проблеми утичу на квалитет живота.

Кључне речи: асфиктична торакална дистрофија; остеохондродисплазија; цилиопатије; неонатална респираторна инсуфицијенција; терминална бубрежна инсуфицијенција

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Scintigraphic, histopathologic, and biochemical evaluation of lycopene effects on renal ischemia reperfusion injury in rats

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SUMMARY

Introduction/Objective Medical protection of kidneys against ischemia reperfusion injury is very important. Many agents have been used for the protection of ischemia reperfusion renal tissue injury. We aimed to evaluate the radioprotective effect of lycopene on kidneys in ischemia reperfusion injury with histopathological, biochemical, and scintigraphic parameters.

Methods Twenty-one Wistar male albino rats were divided into the following three groups: lycopene, control, and sham group. In the lycopene group, lycopene was started three days before right renal ischemia reperfusion injury and continued for 15 days. In the control group, right renal ischemia reperfusion injury was applied with no medication. In the sham group, neither right renal ischemia reperfusion injury nor medication were applied. On the 15th day, all rats were sacrificed after ^{99m}Tc-dimercaptosuccinic acid (DMSA) scintigraphies were taken. Histopathological, biochemical, and scintigraphic evaluations were made.

Results The histopathological score was lower in the lycopene group. In biochemical analysis, myeloperoxidase levels were lower in the lycopene group than in the control group, but not statistically significant. Malondialdehyde and nitrite levels were lower in the lycopene group than in the control group. The postoperative mean ^{99m}Tc-DMSA uptake values were 44.82 ± 1.84 in the lycopene group, 38.92 ± 1.17 in the control group, and 50.21 ± 1.35 in the sham group. DMSA uptake values were higher in the lycopene group than in the control group.

Conclusion Lycopene seems to be an effective agent for protection of kidneys in ischemia reperfusion injury as demonstrated by the histopathological, biochemical, and scintigraphic parameters.

Keywords: renal ischemia/reperfusion; kidney; lycopene

INTRODUCTION

Acute renal failure from ischemic damage to the kidney cause morbidity and mortality. Tubular epithelial cell death is the most common cause of this renal failure [1]. Even after reperfusion of extended ischemia of kidney, injury occurs [2]. Although reperfusion is essential for the survival of ischemic renal tissue, it may cause additional damage to kidney [3]. Renal damage caused by ischemia reperfusion injury (IRI) occurs in surgical procedures in which the kidney remains without blood supply for some time. IRI is the most common cause of acute renal failure after renal transplantation, major abdominal and vascular surgery, coronary bypass graft surgery, trauma, and sepsis [4]. IRI seen in kidney transplantation effects the short- and long-term graft survival [5]. IRI results in persistent intrarenal vasoconstriction, injury of microvascular endothelial and tubular epithelial cells [6]. Reperfusion of ischemia causes a rapid burst of free radicals, which is responsible for endothelial injury and edema. Reperfusion damages endoplasmic reticulum, leading to autophagosome formation and cellular disintegration [7].

Medical protection of the kidney against IRI is very important for nephron sparing surgery and renal transplantation, so many studies continue in this area. Many agents have been used for the protection of ischemia reperfusion renal tissue injury, such as recombinant human manganese superoxide dismutase, n-acetylcysteine and desferrioxamine, telmisartan, exendin-4, and sitagliptin [8, 9, 10].

In this study we aimed to evaluate the radioprotective effect of lycopene on the kidney after unilateral ischemia due to renal artery temporary clamping through the use of histopathological evaluation, malondialdehyde (MDA), myeloperoxidase (MPO) and total nitrite level analysis and ^{99m}Tc-dimercaptosuccinic acid (DMSA) scintigraphy uptake.

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METHODS

This experimental study was approved by the Ankara Training and Research Hospital Local Ethics Committee of Animal Experiments and conducted in Hüsnü Sakal Experimental and Clinical Practice Center at the same hospital. The study group consists of total of 21 Wistar male albino rats (260 ± 45 grams, three to five months old). The rats were acclimated for at least one week prior to the study and housed under standard laboratory conditions [constant temperature (21°C ± 2°C) with relative humidity of 50–60%, with 12-hour light and dark cycles). During the study, the animals had ad libitum access to water and standard food.

Three groups of rats were formed and evaluated. Group 1 (n = 9) was administered lycopene [5 mg per kg of body weight per day (LYC-O-MATO®, GNC Holdings Inc., Pittsburgh, PA, USA)] starting three days before right renal ischemia reperfusion injury and this was continued for 15 days. Group 2 was the control group (n = 9), to which right renal ischemia reperfusion injury was applied without any medication. Group 3 (n = 3) was the sham group, to which neither right renal ischemia reperfusion injury nor medication were applied.

For static renal scintigraphic examination, ^{99m}Tc-DMSA images were obtained preoperatively and on postoperative day 15 for each group. Surgery and ^{99m}Tc-DMSA scintigraphy were applied under anesthesia of 40 mg/kg ketamine hydrochloride (Ketalar, Parke-Davis/Eczacıbaşı, Istanbul, Turkey) and 5 mg/kg xylazine (Rompun, Bayer, Istanbul, Turkey) applied intramuscularly. The subjects were sacrificed by decapitation under 50 mg/kg, intraperitoneally, with propofol anesthesia (Abbott Laboratory Corporation, Istanbul, Turkey). After the sacrification for each group, left and right kidneys were removed surgically for histopathological examination.

Surgical method

DMSA renal scintigraphy was performed in all rats before the surgical procedure. General anesthesia was administered intramuscularly as a combination of 40 mg/kg ketamine hydrochloride (HCl) (Ketalar, Parke-Davis/Eczacıbaşı) and 10 mg/kg xylazine HCl (Rompun, Bayer). The incision area was shaved and the surgical site was cleaned preoperatively with soap and povidone-iodine. A sterile environment was prepared. By laparotomy via midline incision, the right kidney was reached and the right renal pedicle was isolated. The right pedicle was occluded for 45 minutes to induce ischemia and then subjected to reperfusion for six hours (I/R groups). After the reperfusion, DMSA renal scintigraphy was performed (Figure 1). Before the sacrification, blood samples had been taken from all the rats, and the values of urea and creatinine (mg/dL) parameters were determined in plasma to assess the renal activity. We performed the right nephrectomy 24 hours after all the rats were sacrificed. Surgically excised right kidney tissues obtained at 24 hours were placed into aseptic containers for biochemical examination and

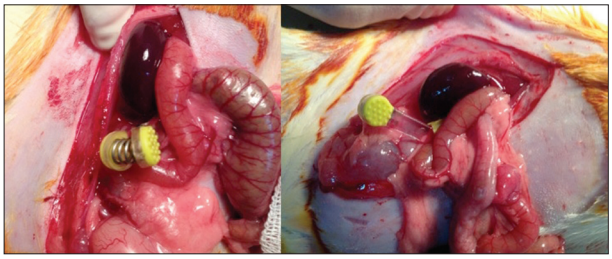

Figure 1. Placing atraumatic microvascular clamps to the renal pedicle for renal ischemia

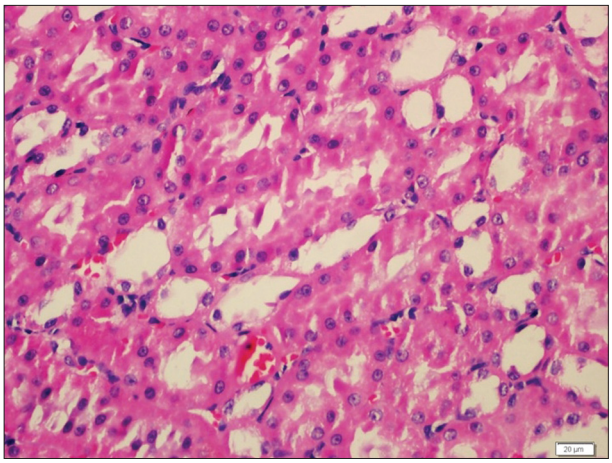

Figure 2. Areas of unaffected tubules displaying normal nuclei with open chromatin, retained brush borders, and cytoplasmic integrity (H&E, × 400)

histopathological analysis (placed in liquid nitrogen for immediate freezing and protein denaturation) and were stored at -80°C in special containers and racks in freezer. The right kidney was evaluated histopathologically and biochemically for oxidative stress markers in the tissue, including the level of MDA, which are indicators of lipid peroxidation, and MPO and total nitrite.

Histopathological evaluation

Kidney tissues obtained after sacrificing the rats were fixed immediately in 10% formaldehyde and were processed in paraffin tissue blocks and macroscopic sections were taken to include the renal cortex and pelvis. Sections of 5 µm thickness cut from formalin fixed paraffin-embedded blocks were stained with hematoxylin and eosin. Histopathological examination was performed in 40–100–200–400 × original magnification with light microscopy (Figure 2). For the histopathological score the following criteria were used:

- Normal histology: 0 points;
- Swelling of tubule cells, loss of brush border and nuclear condensation of ≤ 1/3: 1 point;
- In addition to the changes in 1 point, 1/3–2/3 tubule changes: 2 points;
- Tubular changes more than 2/3: 3 points.

All kidneys were examined in 100 areas with a maximum score of 300.

Biochemical Evaluation

Tissue samples were stored at -80°C until analysis. Kidney samples were homogenized with 0.15M KCl at a rate of 1/10 (weight per volume). MDA, MPO, and total nitrite levels were measured in the tissue samples.

Determination of MPO level

MPO activity was obtained spectrophotometrically by determining the decomposition of hydrogen peroxide using o-dianisidine as the hydrogen donor. Tissue samples of approximately 50 mg were taken, weighed and homogenized three times for 30 seconds at 4°C in 1 ml of ice-cold 0.5% hexadecyltrimethylammonium bromide in 50 mmol/L phosphate buffer (pH 6). The homogenate was subjected to three freeze/thaw cycles and centrifuged for 15 minutes at 40,000 × g. MPO activity was determined by the addition of 0.1 ml of the supernatant to 2.9 ml of 50 mmol/L phosphate buffer containing 0.167 mg/mL o-dianisidine dihydrochloride and 0.0005% hydrogen peroxide. The change in absorbance at 460 nm over a five-minute period was measured at 25°C. The data are expressed as the change in absorbance per minute per gram of tissue (Δabs/min/g of tissue) [11].

Determination of MDA level

MDA levels were calculated by the fluorometric method, developed by Wasowicz et al. [12]. After the reaction of thiobarbituric acid with MDA, the reaction product was extracted in butanol and was measured spectrofluorometrically at wavelengths of 525 nm for excitation and 547 nm for emission. As standard, 0–5 μmol/L 1,1',3,3'-tetraethoxypropane solutions were used. For the measurement of tissue MDA, 50 μL of homogenate was added and introduced into 10 mL glass tubes containing 1 ml of distilled water. After the addition of 1 ml of the solution containing 29 mmol/L thiobarbituric acid in acetic acid and mixing, the samples were placed in a water bath and heated for one hour at 95–100°C. After the samples cooled, 25 μL of 5 mol/L HCL was added and the reaction mixture was extracted by agitation for five minutes with 3.5 mL n-butanol. After separation of the butanol phase by centrifugation at 1,500 × g for 10 minutes, the fluorescence of the butanol extract was measured with F-2500 fluorometer (Hitachi Ltd., Chiyoda, Tokyo, Japan) at wavelengths of 525 nm for excitation and 547 nm for emission. As standard, 0–5 μmol/L 1,1',3,3'-tetraethoxypropane solutions were used. MDA levels are given as μmol per gram of wet tissue.

Determination of total nitrite level

A total of 300 μL of homogenate was added to 300 μLl of KH2PO4/K2HPO4 buffer (pH 7.5), 50 μl of 2 mmol/L NADPH, 50 μl of 50 μmol/L FAD, and 50 μl of 1 unit/mL aspergillus nitrate reductase. This was incubated at room temperature for one hour followed by the addition of 500 μl of 0.2 mol/L ZnSO4 and 70 μL 2 mol/L NaOH to deproteinate the sample. After centrifugation, 0.75 mL of the supernatant was added to 1 mL of 1% sulphanilic acid (in 4 mol/L HCl). After 10 minutes at room temperature, 0.75 mL of freshly prepared 1% N-(1-Naphthyl)ethylenediamine was also added. The resultant color change was measured at 548 nm using a spectrophotometer. Nitrite concentration was calculated from 5, 12.5, 25, 50 μmol/L sodium nitrite standards [13]. Total nitrite levels are given as nmol per liter per gram of wet tissue.

^{99m}Tc-DMSA scintigraphy evaluation

Preoperatively and on postoperative 15th day of the renal injury, adequate hydration with sterile saline and induction of anesthesia were carried out before the scintigraphic study. A commercially available kit of DMSA (MON. DMSA KIT, Monrol Eczacıbaşı, Istanbul, Turkey) was labeled with ^{99m}Tc in accordance with the manufacturer's leaflet for use. A 37 MBq (1 mCi) dose of ^{99m}Tc-DMSA in a 0.1 ml volume was administered to the tail vein. Two hours after injection, static renal images were taken under anesthesia. Renal static scintigraphies were performed using a single-head e.Cam gamma camera (Siemens Healthcare, Erlangen, Germany) equipped with pinhole collimator, energy peak adjusted to 140 keV ± 20% with 256 × 256 matrix and 2.67 zoom factor for five minutes in posterior position. ROIs (regions of interest) were drawn on the anterior and posterior images and the geometric mean of these values was accepted as the relative uptake percentage of the kidneys.

Statistical analysis

Statistical analysis was performed with SPSS Statistics for Windows, Version 20.0 (IBM Corp., Armonk, NY, USA). Continuous and categorical variables are expressed as the mean value ± standard deviation and frequency (%) of numbers. Group comparisons for each parameter were done using the nonparametric Mann–Whitney U-test and the Kruskal–Wallis tests. A p–value less than 0.05 was considered to be a statistically significant difference.

RESULTS

The histopathological score was 45.00 ± 16.58 in the lycopene group, 95.56 ± 40.34 in the control group, and 30.00 ± 5.00 in the sham group (Table 1). The histopathological score was lower in the lycopene group than in the control group ($p < 0.001$) and higher in the control group than in the sham group ($p = 0.012$). The histopathological score of the

Table 1. Histopathological scores of the groups; values are given as mean ± standard deviation (SD)

	Minimum score	Maximum score	Mean score ± SD
Lycopene group	10	60	45 ± 16.58
Control group	75	200	95.56 ± 40.35
Sham group	25	35	30 ± 5

Table 2. Statistical analysis (p-values) of biochemical parameters and histopathological scores between the groups

Groups	MPO level level	MDA level	Nitrite level	Urea (mg/dL) level	Creatine level level	Microscopic score
Lycopene group vs. control group	0.07	0.009*	0.003*	0.306	0.424	0.000*
Lycopene group vs. sham group	0.782	0.013*	0.926	0.013*	0.012	0.093
Control group vs. sham group	0.079	0.021*	0.013*	0.012*	0.011*	0.012*

MPO – myeloperoxidase; MDA – malondialdehyde

Table 3. Biochemical parameters of the groups; values are given as mean ± standard deviation (SD)

Groups	MPO level (Δabs/min/g tissue)	MDA level (μmol/g wet tissue)	Nitrit level (nmol/l/g wet tissue)	Urea level (mg/dL)	Creatine level (mg/dL)
Lycopene group	0.08 ± 0.09	4.61 ± 2.10	0.05 ± 0.02	175.56 ± 52.94	1.46 ± 0.47
Control group	0.11 ± 0.03	6.85 ± 1.68	0.09 ± 0.02	144.44 ± 27.89	1.24 ± 0.19
Sham group	0.06 ± 0.03	13.36 ± 3.56	0.04 ± 0.01	50.00 ± 10.00	0.8 ± 0.00

MPO – myeloperoxidase; MDA – malondialdehyde

lycopene group was higher than that of the sham group, but it was not statistically significant (p = 0.093) (Table 2).

In biochemical analysis, the mean value of MPO level (Δabs/min/g of tissue) was 0.08 ± 0.09 in the lycopene group, 0.11 ± 0.03 in the control group, and 0.06 ± 0.03 in the sham group. The MPO level was lower in the lycopene group than in the control group, and higher in the control group than in the sham group, but not statistically significant. The mean value of the MDA level (μmol/g of wet tissue) was 4.61 ± 2.10 in the lycopene group, 6.85 ± 1.68 in the control group and 13.36 ± 3.56 in the sham group. The MDA level was lower in the lycopene group than in the control group (p = 0.009) and the sham group (p = 0.013), and lower in the control group than in the sham group (p = 0.021). The mean value of the nitrite level (nmol/l/gm wet tissue) was 0.05 ± 0.02 in the lycopene group, 0.09 ± 0.02 in the control group, and 0.04 ± 0.01 in the sham group. The nitrite level was lower in the lycopene group than in the control group (p = 0.003) and higher in the control group than in the sham group (p = 0.013). The mean value of urea level (mg/dL) was 175.56 ± 52.94 in the lycopene group, 144.44 ± 27.89 in the control group and 50 ± 10 in the sham group. The urea level was higher in the lycopene group than in the control group but not statistically significant, and higher in the control group than in the sham group (p = 0.012). The mean value of creatine level (mg/dL) was 1.46 ± 0.47 in the lycopene group, 1.24 ± 0.19 in the control group and 0.8 ± 0 in the sham group. The creatine level was higher in the lycopene group than in the control group but not statistically significant, and higher in the control group than in the sham group (p = 0.011) (Tables 2 and 3).

In the scintigraphic analysis, the preoperative mean uptake values were 50.16 ± 1.49 in the lycopene group, 50.56 ± 1.38 in the control group and 50.18 ± 0.34 in the sham group. Preoperative ^{99m}Tc-DMSA uptake values were not statistically significant between the groups (p = 0.767). The postoperative mean uptake values were 44.82 ± 1.84 in the lycopene group, 38.92 ± 1.17 in the control group and 50.21 ± 1.35 in the sham group. There was statistically significant difference between the lycopene and control groups, higher in the lycopene group (p < 0.001). The uptake values of the lycopene and control groups were lower than those of the sham group, as expected (p = 0.013, both) (Figure 3).

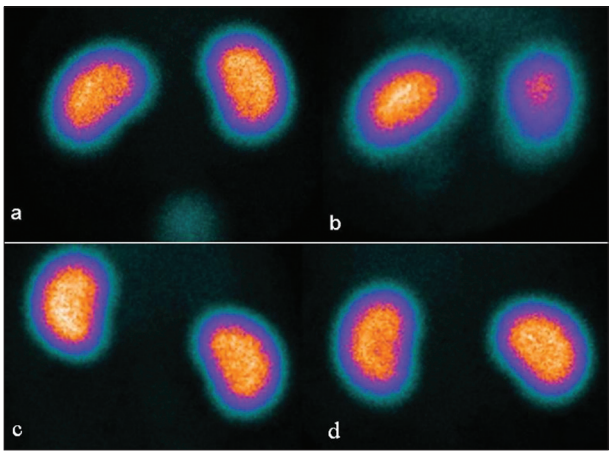

Figure 3. Preoperative (a) and postoperative (b) DMSA scintigraphy in posterior image of a rat in the control group (uptake value of the right kidney; preoperative = 37.45%, postoperative = 48.89%), preoperative (c) and postoperative (d) DMSA scintigraphy in posterior image of a rat in the lycopene group (uptake value of the right kidney; preoperative = 47.56%, postoperative = 50.63%)

DISCUSSION

In our study, lycopene showed histopathological protective effect in IRI of the kidney. In the histopathological evaluation, the histopathological score was lower in the lycopene group than in the control group, and histopathological score of the control group was higher than that in the sham group. MPO level in the lycopene group was lower than in the control group, but not statistically significant. MDA and nitrite levels in the lycopene group were lower than in the control group. These biochemical parameters indicate that lycopene has protective effect against IRI. ^{99m}Tc-DMSA uptake of the effected kidneys were higher in the lycopene group than in the control group, which indicates lycopene has protective effect on renal parenchyma.

In animal models, reperfusion of the ischemic kidney is followed by tissue destruction and many morbidities ensue. Neutrophils accumulate in the area of ischemic tissues after reperfusion and tissue damage is mediated through neutrophil-mediated oxidative stress. Reactive oxygen species are produced at the sites of inflammation by neutrophils and cause formation of lipid peroxides, damage

of cell membrane and destruction of antioxidative defense mechanism. Reperfusion may increase the damage after ischemia in tissues. After reperfusion of ischemic tissue, inflammatory and metabolic damage occurs as a result of disruption of cellular integrity. When the cascade begins, various distant organs, such as lungs, liver and heart, are affected by many activated system and toxic mediators [14].

Effects of IRI on kidneys have been studied by various methods such as biochemical assays, histopathological examination, and scintigraphic imaging, but there is no consensus for the best method for the evaluation of impaired renal functions [15]. After reperfusion of ischemic kidney, MDA and MPO levels increase and injure the renal tissue to a greater extent. Reactive oxygen species released by the neutrophils increase the tissue damage further during reperfusion [14, 16]. MDA,which is a marker of lipid peroxidation, increases during reperfusion after an ischemic renal episode [17]. MPO activity increases if neutrophil infiltration into the tissues occurs as in IRI, and total nitrite level is the marker of total lipid membrane damage [18].

Lycopene is the most common studied compound on the preventive effects of dietary intake of tomato products [19, 20]. Lycopene is the most potent antioxidant among various carotenoids. Lycopene has the best relative radical scavenging abilities among the carotenes. Carotenoids prevent damage to DNA, proteins, cell membranes, lipids, and other structures, which occurs after oxidative injury. Lycopene has higher singlet oxygen quenching ability than β-carotene and α-tocopherol by its high number of conjugated double bonds [21]. Lycopene has antiproliferative properties to protect the development of prostate cancer and inhibits cholesterol synthesis and enhances low density lipoprotein degradation [22]. Lycopene has also many other functions in the immune system, metabolic pathways, and cell–cell communication. Lycopene normalizes the change of intrathymic T-cell differentiation seen in tumorigenesis [23].

Due to antioxidative properties, lycopene mediates free radical scavenging activity and results in the reduction of infarct volume in ischemia reperfusion brain injury [22]. Lycopene ameliorated the ischemia reperfusion induced tissue damage and was found to protect the germ cells after testicular torsion [24]. However, in another study, lycopene was not effective for testicular torsion in the long term, there was no improvement in the groups treated with lycopene for therapeutic purposes [25]. Liu et al. [26] identified the pro-regenerative, anti-apoptotic and anti-oxidant properties of mesenchymal stromal cells in IRI. Lycopene ameliorated lysosomal membrane damage as well as alterations in cardiac enzymes, lipid profile, and oxidative stress markers. Yue et al. [27] thought that lycopene protects myocardium against hypoxia reoxygenaration induced apoptosis by maintaining the mitochondrial function. Lycopene was also found effective in pancreatitis by inhibition of neutrophil infiltration and lipid peroxidation [20]. Bayramoglu et al. [21] used lycopene in IRI of liver in different doses of 2.5 and 5 mg/kg body weight. Improvements of alanine aminotransferase, aspartate aminotransferase, lactate dehydrogenase, and MDA levels were partial and dose dependent. Lycopene showed the protective effect as a decrease in MDA level, nitrite level, and histopathological score, and an increase in DMSA uptake of the kidney.

Pektaş et al. [17] evaluated the effectiveness of lycopene in IRI with biochemical and histopathological parameters and found that lycopene may have a protective effect on IRI. Kaya et al. [28] also used high dose of lycopene (100 mg/kg) in a single dose in IRI of the kidney. They also used biochemical and histopathological parameters and mentioned that lycopene administered prior to renal IRI prevented renal damage. A few studies used ^{99m}Tc-DMSA scintigraphy for the evaluation of IRI of the kidney. DMSA scintigraphy was found to be an effective non-invasive method in the evaluation of kidney restoration after IRI injury [29]. In our study, we also used DMSA scintigraphy in addition to biochemical and histopathological parameters.

CONCLUSION

In conclusion, lycopene seems to be an effective agent for protection of the kidney in reperfusion injury after renal ischemia, as demonstrated by the histopathological, biochemical, and scintigraphic parameters. However, further larger studies are necessary for clinical use.

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Сцинтиграфска, патохистолошка и биохемијска процена ефекта ликопена код реперфузионих оштећења бубрега после исхемије код пацова

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САЖЕТАК

Увод/Циљ Заштита бубрега од реперфузионих оштећења после исхемије је врло значајна. За ову заштиту су коришћена многа средства.

Циљ рада је процена радиопротективног ефекта ликопена на реперфузиона оштећења бубрега после исхемије.

Методе Група од 21 мужјака вистар албино пацова подељена је у следеће три групе: ликопенску, контролну и псеудогрупу. Пацовима у ликопенској групи ликопен је даван три дана пре и 15 дана после реперфузионог оштећења бубрега, контролној групи није даван ликопен после оштећења, а пацови псеудогрупе нису имали исхемију бубрега и није им даван ликопен. После 15 дана урађена је сцинтиграфија са ^{99m}Tc-ДМСК (димеркаптосукцинска киселина), а потом су пацови жртвовани и урађена су патохистолошка и биохемијска истраживања.

Резултати Патохистолошки скор је био нижи у ликопенској групи. Биохемијска анализа је показала да је ниво мијелопероксидазе био нижи у ликопенској групи него у контролној, али не статистички значајно. Нивои малондиалдехида и нитрата су били нижи у ликопенској него у контролној групи. Прихват ^{99m}Tc-ДМСК у ликопенској групи био је 44,82 ± 1,84, у контролној групи 38,92 ± 1,17 и 50,21 ± 1,35 у псеудогрупи.

Закључак Ликопен би могао бити ефикасно средство за заштиту бубрега од реперфузионог оштећења после исхемије, што је показано патохистолошким, биохемијским и сцинтиграфским параметрима.

Кључне речи: бубрежна исхемија / реперфузија, бубрег, ликопен

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Effect of alcohol on insulin secretion and viability of human pancreatic islets

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SUMMARY

Introduction/Objective There are controversial data in the literature on the topic of effects of alcohol on insulin secretion, apoptosis, and necrosis of the endocrine and exocrine pancreas.
The goal of this research was to determine how alcohol affects the insulin secretion and viability of human adult pancreatic islets in vitro during a seven-day incubation.

Methods Human pancreatic tissue was digested with Collagenase XI, using a non-automated method. Cultures were incubated in Roswell Park Memorial Institute (RPMI) medium containing alcohol (10 µl of alcohol in 100 ml of medium). Insulin stimulation index (SI) and viability of the islets were determined on the first, third, and seventh day of cultivation.

Results Analysis of the viability of the islets showed that there wasn't significant difference between the control and the test group. In the test group, viability of the cultures declined with the time of incubation. SI of the test group was higher compared to the control group, by 50% and 25% on the first and third day of cultivation, respectively. On the seventh day, insulin secretion was reduced by 25%. The difference was not statistically significant ($p > 0.05$). In the test group, significant decline in insulin secretion was found on the third and seventh day of incubation ($p \leq 0.05$).

Conclusion Alcohol can increase or decrease insulin secretion of islets cultures, which may result in an inadequate response of pancreatic β-cells to blood glucose, leading to insulin resistance, and increased risk of developing type 2 diabetes.

Keywords: alcohol; insulin secretion; viability; insulin resistance; type 2 diabetes

INTRODUCTION

Alcohol consumption is a part of the tradition and customs of many communities worldwide. It is customary to take a glass of drink as an aperitif, and wine or beer during or after meals. Alcohol is taken primarily for good mood or for better digestion. The question is how and to what extent the amount of consumed alcohol affects insulin secretion of human adult pancreatic islets. Perfusion model of the rat pancreas and measure of basal insulin secretion showed that ethanol reduced glucose-induced insulin secretion by means of dose-related effect [1].

Dembele et al. [2] examined the effects of ethanol and fatty acids on β-cell (cell line from rats) metabolism and survival. It was observed that both substances generate cellular oxidative stress, and affect mitochondrial function. Ethanol causes β-cell death by apoptosis, while fatty acids cause cell death predominantly by necrosis. Pancreatic β-cell dysfunction is a prerequisite for the development of type 2 diabetes Alcoholism is a risk factor and ethanol increases oxidative stress in β-cells. Cells in such a state increase expression of PHB (poly3-hydroxybutyric acid) synthase genes, to protect them from the harmful effects of ethanol [3].

Studies in rats in vivo showed that long-term alcohol feeding suppresses apoptosis in the pancreas; however, it increases the sensitivity of acinar cells to endotoxin-induced injury that may cause pancreatitis in alcoholics [4]. Alcohol causes reactive hypoglycemia by attenuating the release of counter regulatory hormones, redistribution of pancreatic blood flow and direct stimulation of insulin secretion. Signaling of ethanol-induced insulin secretion from rat insulin-secreting cell lines (INS-1 and INS-1E) bypasses calcium and protein kinase C (PKC) involving steps. An extra pool of secretory vesicles not available for glucose is exploited for exocytose after ethanol stimulation [5]. Study of an in-vitro isolated rat pancreas perfusion system showed that ethanol decreases glucose-stimulated insulin secretion. Second phase secretion (30–60 minutes) was inhibited at both low (100 mg/dl) and high (1,000 mg/dl) alcohol concentration [6]. Several studies have shown that ethanol causes insulin resistance in the liver and skeletal muscle by interfering with insulin signaling [7, 8]. One study, examining the influence of alcohol, ethanol concentration of 20 mM or 80 mM for 24 or 48 hours in β-cell lines and isolated murine islets showed that chronic exposure to ethanol causes β-cell dysfunction by re-

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ducing not only insulin secretion but also β-cell insulin content by interfering with muscarinic signaling and PKC activation but not the K-ATP channels [9]. Light-to-moderate alcohol consumption (for women < 28, 28–64, > 64 g/week) for healthy women was associated with enhanced insulin sensitivity, reduced basal insulin secretion rate, and lower fasting plasma glucagon concentration, providing consistent mechanisms for the reduced risk of type 2 diabetes [10]. Data for the effect of alcohol consumption on insulin secretion are conflicting; some studies show the absence of an association in men [11, 12]. The final product of ethanol oxidation is acetate, which is converted into acetyl-CoA in peripheral tissue. Acetate may modulate fat oxidation and decrease lipolysis. Therefore, an increase in skeletal muscle oxidative capacity after alcohol intake has been suggested even though evidence is limited in humans [13, 14].

The aim of the present study is to examine effect of ethanol on viability and insulin secretion of human adult pancreatic islets in the culture during short-term incubation period (seven days) to solve the controversy present in the literature.

METHODS

Human adult pancreatic tissue was procured from the Institute for Gastrointestinal Diseases, Clinical Center of Serbia, Belgrade. Tissue samples were collected from live donors, after total or subtotal pancreatectomy for cysts or tumors [15]. In tumors, healthy tissue was obtained near the line of the resection. Histopathological analysis of the tissue samples showed no tumor cells. All procedures were performed in accordance with the rules of Ethical Committee of the School of Medicine in Belgrade. Written consent was obtained from all patients.

Pancreatic tissue was transported in physiological solution from the Institute for Gastrointestinal Diseases to the Laboratory for Cell Culture at the Clinic for Endocrinology, Diabetes and Metabolic Diseases. The material was kept in a refrigerator at 4°C (cold ischemia). Biometric data of the material (tissue weight, cold and warm ischemia) are given in Table 1.

Table 1. Biometric data of samples

Parameters	Tissue weight (g) mean ± SE	Warm ischemia (min.) mean ± SE	Cold ischemia (min.) mean ± SE
Control group	5.03 ± 0.57	117.2 ± 17.14	70.2 ± 28.33
Group with alcohol	2.26 ± 0.48	128.63 ± 20.06	112.13 ± 35.70
Statistical significance ($p \leq 0.05$)	0.195	0.061	0.1

Digestion of pancreatic tissue

For pancreatic tissue digestion we used Collagenase XI – product number C7657 (Sigma-Aldrich, St. Louis, MO, USA), activity > 1,200 collagen digestion units per mg solid; 2–5 FALGPA hydrolysis units per mg solid). This enzyme preparation also contained clostripain, nonspecific neutral protease and tryptic activities. Isolation of pancreatic islets was performed in aseptic conditions in a laminar flow hood by the non-automatic method [16]. The tissue was transferred to Hanks solution (Sigma-Aldrich) and mechanically chopped. This material was collected with a pipette and put in test tubes containing the Collagenase solution (5 mg/ml concentration). The duration of incubation was 30 minutes at 37°C with occasional mechanical stirring. After the incubation, the content of the test tube was centrifuged at 400 × g for 10 minutes at 15°C. Supernatant was decanted and the remaining islets were rinsed several times with Hanks solution to eliminate excess of lipids and collagenase. After rinsing, islets were resuspended in the final RPMI 1640 medium (Sigma-Aldrich) supplemented with 0.1% L-glutamine, 5.5 mM glucose, 25 mM hepes, 100 U/ml penicillin, 100 μg/ml streptomycin, and 10% fetal calf serum (FCS, Sigma-Aldrich). The islets were incubated in Falcon 3013 plastic flasks, volume 50 cc (Thermo Fisher Scientific, Waltham, MA, USA) at 37˚C in a 5% CO_2 and 95% humidity atmosphere for seven days. RPMI medium was changed every 24 hours of incubation. Warm ischemia time is the time measured from the beginning of the mechanical mincing of the tissue, including the isolation procedure, to the moment when the islets were placed in the culture medium. A total of 20 cultures were incubated in the standard medium (control group), and 27 cultures were incubated in the medium with ethanol (test group) in standard conditions for seven days. In the test group, a certain concentration of ethanol was added to the culture medium using Widmark formula. It was taken undre the assumption that a man of an average weight of 75 kg drinks two glasses (2 × 0.5 dl) of 40% alcoholic drink per day, which would make his blood alcohol concentration 0.62‰.The authors have chosen this formula so that the influence of alcohol in in vitro researches would be the closest to in vivo conditions. In order to achieve specified concentrations, 10 μl of pure ethanol was added to 100 ml RPMI medium to give 0.00165 M (0.16 mM) solution of alcohol. Ethanol was added to the medium during the incubation every 24 hours when replacing RPMI medium, and before the start of glucose stimulation.

Determination of the viability of isolated human adult islets

Viability of the islets was determined by dithizone (DTZ) staining on the first, third, and seventh day after their isolation.

Preparation of the dithizone solution

Fifty milligrams of DTZ was dissolved in 10 ml of DMSO and 10 ml of Hanks solution. The solution was sterilized by passage through nylon filters with pore size of 0.20 μm. Samples (1 ml of each culture) were stained with 0.2 ml of DTZ solution and placed in an incubator at 37°C in a 5% CO_2 and 95% humidity atmosphere for 30 min. Stained

islets were rinsed in Hanks solution and resuspended in 1 ml of RPMI medium. Islets viability was determined using a stereo-light microscope and special counting microchambers [17].

Determination of the functional capacity and insulin secretion

To determine preservation of the functional capacity of the isolated islets, glucose-stimulated insulin secretion was measured on the first, third and seventh day of cultivation [18, 19]. A static glucose stimulation assay was performed. Samples (approximately 1,000–2,000 islets per culture) were incubated for one hour in low glucose (2.8 mM/L RPMI) medium with ethanol (10 µl ethanol in 100 ml RPMI), then one hour in high glucose (20 mM/L RPMI) medium and one hour in low glucose medium again. After each step of stimulation, the cultures were centrifuged at 400 × g, for 10 minutes at 15°C. Supernatant was decanted and stored at -18°C for insulin quantification. Insulin content was determined by radioimmunoassay (RIA INSULIN PEG). Sensitivity of the assay is 0.60 mIU/L and detection range 0.6–300 mIU/L. Relative insulin release was expressed as insulin stimulation index (SI) and calculated as the ratio of insulin release during high glucose stimulation to insulin release during low glucose stimulation.

Statistical analysis

All results are expressed as mean ± standard error. P-value of less than 0.05 was considered to be statistically significant. For statistical analysis of the data, analysis of variance (ANOVA, Fisher) was used.

RESULTS

Islet viability

The percentage of viable islets in the cultures was determined on the first, third and seventh day after isolation. Viability of the islets was determined by DTZ staining and results are presented in Table 2. Immediately after isolation (day 1) percentage of viable islets incubated in normal medium, without alcohol (control group) and in medium containing alcohol (test group) was 51.58 ± 2.16 and 64.68 ± 5.99%, respectively, and the difference was not statistically significant (p = 0.090). On the third day of cultivation, numbers of distinctly stained islets in control and test groups were similar (57.99 ± 6.49 and 54.23 ± 4.58%, respectively, p = 0.654). On the seventh day of incubation, viability was higher in the control group (39.99 ± 6.27%) compared to the test group (37.79 ± 5.64%), and the difference was statistically not significant (p = 0.800).

Comparison of viability by days of incubation (first and third, first and seventh, third and seventh) in the control group showed no statistically significant difference (p > 0.05). In the test group, there is statistically significant difference between the first and the seventh day (p = 0.011).

Insulin secretion

To determine the functional capacity of the isolated islets, a static glucose test was performed on the first, third and seventh day of cultivation. SI values calculated for both groups and days of cultivation are presented in Table 3. On the first day of stimulation, SI for the control group was 0.60 ± 0.13 and for the test group SI was 1.22 ± 0.27. The difference was not statistically significant (p = 0.257). On the third day of stimulation, SI value for the control group was 0.80 ± 0.16, and for the test group it was 1.01 ± 0.13 (p = 0.215). On the seventh day of stimulation, SI for the control group was 0.80 ± 0.11, and for the test group SI = 0.60 ± 0.16 (p = 0.547).

Comparison of functional capacity by days of incubation (first and third, first and seventh, third and seventh) in the control group showed no statistically significant difference (p > 0.05). In the test group, there is a marginal statistical significance between the first and the seventh day (p = 0.058). Between the third and the seventh day of cultivation there is a statistically significant difference (p = 0.028), while between the first and the third day there is no statistically significant difference (p = 0.626).

Table 2. Percentage of colored islets by culture, viability of the human adult pancreatic islets in the culture, for the control group and the group incubated with alcohol during seven days of cultivation

Parameters	Time of cultivation % of viability (mean ± SE)			Comparison by days of incubation (p-value)		
	1st day	3rd day	7th day	1st and 3rd day	1st and 7th day	3rd and 7th day
Control group	51.60 ± 2.16	58.00 ± 6.49	40.0 ± 6.27	0.376	0.119	0.081
Test group with ethanol	64.70 ± 5.99	54.30 ± 4.58	37.80 ± 5.64	0.216	0.011*	0.053
p-value	0.090	0.654	0.800			

Table 3. Insulin stimulation index in the control group and cultures with ethanol (the test group) on the first, third and seventh day of incubation

Parameters	Time of cultivation Stimulation index (mean ± SE)			Comparison by days of incubation (p-value)		
	1st day	3rd day	7th day	1st and 3rd day	1st and 7th day	3rd and 7th day
Control group	0.60 ± 0.13	0.80 ± 0.16	0.80 ± 0.11	0.616	0.506	0.966
Test group with ethanol	1.20 ± 0.27	1.00 ± 0.13	0.60 ± 0.16	0.626	0.058	0.028*
p-value	0.257	0.215	0.547			

DISCUSSION

The percentage of distinctly stained cells in the cultures containing ethanol (test group) on the first day of incubation was higher by 25% compared to the control group (medium without ethanol), but on the third and seventh day of incubation, the percentage of stained cells was lower in the test group by 6.38% and 5.5%, respectively. Since the difference is not statistically significant, we can conclude that there is no difference in cell viability between the control and the test group for all days of incubation (first, third, and seventh). Viability in the control group showed no statistical significance by days of incubation. However, in the test group, viability was the highest on the first day of cultivation and declined on the third and the seventh day by 16% and 42%, respectively. The difference was statistically significant ($p = 0.011$). These results are acceptable because islet viability is determined by DTZ, which binds to zinc in insulin. On the first day of incubation, β-cells contain the highest concentration of insulin. In test group, alcohol increases the permeability of the membrane and facilitates degranulation and the release of insulin, so depot of insulin is gradually reduced by the length of incubation (from the first to the seventh day).

SI was higher in the test group compared to the control group by 50% and 25% on the first and third day of incubation, respectively, while on the seventh day SI was lower by 25% in the test group. There is no statistically significant difference in SI between control and test groups. However, in the test group there was a decline in insulin secretion during the cultivation by 17% and 50% on the third and seventh day, respectively, compared to the first day ($p = 0.058$). There is also statistically significant decline in insulin secretion by 40% between the third and seventh day ($p = 0.028$).

Similar results are presented in another paper, where insulin secretion in cell culture increases after 24 hours of incubation with lower alcohol concentration (20 mM) [9]. However, higher concentration of alcohol (80 mM) has the opposite effect. After 48 hours of incubation, concentration of alcohol reduces the level of insulin secretion compared to the control group. Results of other authors confirm that ethanol reduces insulin secretion [1, 6]. It should be emphasized that reduction in the number of stimulated β-cells during the incubation due to the effects of natural processes of apoptosis, and necrosis does not affect the obtained values of insulin secretion, expressed in the form of SI, which represents the ratio between the values of insulin secretion after stimulation with high glucose concentration and values after low glucose stimulation. Hence, secretory capacity does not depend of the total number of stimulated cells. Although in laboratories worldwide there is a practice to associate SI values with a strict number of stimulated cells [19].

Some authors try to explain how alcohol causes increased or decreased insulin secretion in response to glucose stimulation. Stimulation of insulin secretion with diacylglycerol is explained by the release of Ca from endoplasmic reticulum [20]. Response to high glucose stimulation decreases due to reduced levels of cyclic adenosine monophosphate (cAMP) caused by reduced levels of adenosine triphosphate (ATP) [2, 3, 21].

In standard conditions, glucose-stimulated insulin secretion increases oxygen consumption and ATP. This is associated with potassium (K^+)-induced membrane depolarization, leading to rapid entry of Ca^{2+} ions into the cell through voltage-dependent channels. Fusion of secretory granules containing insulin with the cell membrane depends of calcium ions. cAMP emphasizes action of glucose and amino acids, and stimulates the release of Ca^{2+} ions from intracellular organelles, or may activate a kinase that phosphorylates one of the components of microfilaments/tubules system, so that this structure becomes contractile and sensitive to Ca^{2+} ions. If the Na^+ outside the cell is replaced with other monovalent cation, then the effects of glucose and other secretagogues are reduced. Na^+ concentration may regulate the intracellular Ca^{2+} using a co-transportation system [21]. It is possible that the effect of certain concentrations of ethanol somehow bypasses the dependence of Ca^- release from the synthesis of cAMP.

Higher SI values in the test group (SI higher by 50% on the first day of incubation) mean increased insulin secretion of pancreatic islets in response to high glucose stimulation (see chapter Methods). It is probable that alcohol consumed immediately before or during ingestion can have a protective effect in type 2 diabetes because increased insulin release reduces the harmful effects of postprandial hyperglycemia. It is known that hyperglycemia has a toxic effect on β-cells because it increases the percentage of their apoptosis [22]. The body is struggling to maintain a normal blood sugar level, so a chronic state of hypoglycemia occurred after chronic alcohol consumption, especially if alcohol is taken without food, can lead to insulin resistance, which exists in the pathogenesis of type 2 diabetes [23]. Acute alcohol consumption increases insulin secretory capacity of islets, which could lead to hypoglycemia in patients with normal glycemic control. In vivo researches have shown that ethanol increased insulin secretion during glucose tolerance test [24, 25].

Symptoms of hypoglycemia are very similar to the symptoms of alcohol condition. However, alcohol consumption after meals may improve insulin secretion thereby reducing potential harmful effects of increased concentrations of blood glucose. Consumption of alcohol without food increases insulin secretion and leads to discharge depot of insulin, which can lead to insulin resistance. The effect of alcohol mainly depends on the administered dose in the body. Adverse effects are manifested in the increased consumption of alcohol causing accumulation of nitric oxide (NO) in the organism that has a detrimental effect on the whole body. Increased NO synthesis under the influence of inducible nitric oxide synthase is associated with various cytotoxic damages [26]. Larger amounts of NO produced in macrophages that infiltrate the endocrine pancreas tissue can lead to damage of β-cells and increased apoptosis of these cells [27]. In the initiation of apoptosis, accumulation of calcium ions in the cytosol has particular

importance [26]. Also, long-term alcohol consumption could lead to β-cell sensitivity to the presence of endotoxins in the blood, thereby increasing the percentage of necrosis of pancreatic islets [2, 4]. It is known that the presence of bacteria in the pancreas can also affect insulin secretion and the development of diabetes, and chronic alcohol consumption would only intensify this effect [28].

Our experiment showed that insulin secretion declines during cultivation, especially on the seventh day (Table 3). Inadequate response to glucose stimulation may lead to hyperglycemia and insulin resistance that are prerequisite for development of diabetes type 2. Similar results were obtained by other authors [29–32]. In addition to the impact on carbohydrate metabolism, experiments on rats showed considerable impact of alcohol on lipid metabolism. These results demonstrated that visceral fat is more susceptible to alcohol toxicity compared to subcutaneous fat, and disruption of adipose lipogenesis contributes to the pathogenesis of alcoholic lipodystrophy [33].

Results suggesting a protective role of alcohol consumption in the development of type 2 diabetes [34, 35, 36] should be interpreted with caution. To determine the true impact of alcohol on insulin secretion, results obtained from animal models and animal cell lines should be taken with reserve, because alcohol consumption is characteristic of humans and there are differences in metabolism. Considering that authors express alcohol concentration in different units, perhaps there is a need for standardization.

CONCLUSION

Based on the presented results we can conclude that alcohol concentration 0.165 mM slightly increases insulin secretion in the culture on the first and third day of incubation. The longer the cultivation, the greater the decline in insulin secretion in the test group compared to the control group. The effect of alcohol on insulin secretion is most noticeable in the test group, clearly showing the long-term effects of alcohol, which causes a decline in insulin secretion.

Alcohol can increase or decrease insulin secretion of human pancreatic islets, which may result in an inadequate response of pancreatic β-cells to blood glucose, leading to insulin resistance, and increased risk of developing type 2 diabetes.

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Утицај алкохола на инсулинску секрецију и вијабилност хуманих острваца панкреаса

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САЖЕТАК

Увод/Циљ У литератури постоје прилично контрадикторни подаци на тему ефеката алкохола на лучење инсулина, апоптозе и некрозе ендокриног и егзокриног панкреаса. Циљ овог истраживања је да се утврди како алкохол утиче на лучење инсулина и одрживост острваца адултног хуманог панкреаса *in vitro* током седам дана инкубације.

Методе Ткиво хуманог панкреаса је разложено колагенезом XI, користећи неаутоматизовану методу. Културе су биле инкубиране у *RPMI* раствору који садржи алкохол (10 *μl* етанола у 100 *ml* раствора). Инсулински стимулациони индекс и одрживост острваца су одређивани првог, трећег и седмог дана култивације.

Резултати Анализа одрживости острваца показала је да не постоји значајна разлика између контролне и тестиране групе. У тестираној групи одрживост култура опала је са временом инкубације. Капацитет инсулинске секреције тестиране групе био је већи у односу на контролну групу за 50% првог и 25% трећег дана култивације. Седмог дана инсулинска секреција се смањила за 25%. Разлика није била статистички значајна ($p > 0,05$). У тестираној групи откривен/пронађен је значајан пад инсулинске секреције трећег и седмог дана инкубације ($p \leq 0,05$).

Закључак Алкохол може повећати или смањити инсулинску секрецију културе острваца, што може довести до неадекватног одзива *β*-ћелија панкреаса на глукозу у крви, што даље доводи до инсулинске резистенције и повећане могућности за развијање дијабетеса типа 2.

Кључне речи: алкохол; инсулинска резистенција; инсулинска секреција; одрживост; дијабетес тип 2

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Pertussis incidence rates in Novi Sad (Serbia) before and during improved surveillance

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SUMMARY

Introduction/Objective The Global Pertussis Initiative (GPI) proposed clinical case definitions for pertussis diagnosis in three different age cohorts in order to improve surveillance of pertussis especially in older children, adolescents, and adults.

The main goal of this research was to compare the burden of pertussis in the city of Novi Sad before and after the introduction of improved surveillance using the GPI clinical case definitions of pertussis.

Methods Baseline data on pertussis were obtained from routine (non-sentinel) reporting before improved surveillance was introduced. From September 16, 2012, clinical case definitions proposed by GPI were applied within improved (sentinel and hospital) surveillance, while surveillance clinical case definitions were not introduced within non-sentinel. To confirm the suspected diagnosis, sampling of nasopharyngeal swab and/or blood was obtained from all cases. The choice of laboratory method (PCR or ELISA) depended on the duration of coughing and the age of the patients. Data were statistically processed by SPSS Statistics, version 22.

Results During the 12-year period before the introduction of improved surveillance, only two clinical pertussis cases were registered. In contrast, during the two-year period of improved surveillance, a total of 14 (season 2012/13) and 146 (season 2013/2014) confirmed pertussis cases were reported. Significant differences were determined in distribution of pertussis according to the type of surveillance and the level of health care.

Conclusion Introduction of clinical case definitions proposed by GPI improved the quality of surveillance and enabled an insight in the distribution of pertussis in all age groups and at all levels of health care.

Keywords: pertussis; surveillance; epidemiology

INTRODUCTION

Before vaccines became widely available, pertussis was one of the most common childhood diseases worldwide. Following large-scale vaccination during the 1950s and 1960s, a dramatic reduction (> 90%) in incidence and mortality of pertussis was observed in the industrialized world. Estimates from WHO suggest that in 2008 about 16 million cases of pertussis occurred worldwide, 95% of which were in developing countries, and that about 195,000 children died from this disease [1]. In developed countries, pertussis is increasingly reported in older children, adolescents and adults [2]. The true burden of pertussis is unknown and is still significantly underestimated.

Today, many different case definitions are used throughout the world. Most case definitions are supplemented with laboratory and epidemiological data so that reports may be categorized as confirmed, probable, or suspect. The Global Pertussis Initiative (GPI) described the difficulties in defining pertussis from a clinical perspective. In recognition to the fact that the signs and symptoms of pertussis differ by age, GPI has tailored criteria for pertussis diagnosis in three age cohorts (0–3 months, four months to nine years, and ≥ 10 years) [3].

Unlike in many other European countries, quality of surveillance in Serbia has not influenced the immunization strategy against pertussis. Immunization against pertussis in Serbia is mandatory according to the Law on Protection of Population Against Communicable Diseases. The primary series comprises three doses of DTP (combined diphtheria-tetanus-whole cell pertussis) or DTaP (combined diphtheria-tetanus-acellular pertussis) vaccine given at two months of life with an interval of four weeks between subsequent doses. Currently, only booster against pertussis is given during the second year of life, one year after the third dose in the primary series. Immunization coverage with primary series and revaccination in the Autonomous Province of Vojvodina (APV) is over 95% [4].

In 2012, in addition to mandatory routine, non-sentinel surveillance, improved surveillance of pertussis was implemented and funded in the APV, as a part of Special Public Health Program. It included hospital surveillance (all

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hospitals in the province) and sentinel surveillance (city of Novi Sad). This paper reports on the results of improved surveillance and routine surveillance (non-sentinel) conducted in the city of Novi Sad.

The main goal of this study is to compare the burden of pertussis before and after the introduction of improved surveillance of pertussis and to determine differences in registered overall and age-specific incidence rates between the improved and routine, non-sentinel surveillance of pertussis.

METHODS

The research was carried out by the Institute of Public Health of Vojvodina in Novi Sad, the main administrative center of the APV in northern Serbia. According to the 2011 census there are 341,624 people living in Novi Sad**.** Data relevant to the study were collected in collaboration with the primary, secondary, and tertiary healthcare facilities.

Determination of the burden of pertussis before the introduction of improved surveillance

In order to investigate the burden of pertussis in the period from January 1, 2001 to September 15, 2012, reports from the routine, non-sentinel reporting system were obtained. The source of data for routine, non-sentinel surveillance was mandatory notification of pertussis, as reported to the Institute of Public Health of Vojvodina in accordance with the Law on Protection of Population from Infectious Diseases [5]. The Law mandates reporting of communicable diseases and does not determine diagnostic criteria or clinical case definitions.

Determination of the burden of pertussis after the introduction of improved surveillance

The research was carried out in two periods: the first one in the 2012/13 season – from September 16, 2012 until September 15, 2013, and the second one in the season of 2013/14 – from September 16, 2013 until September 15, 2014. During these two seasons, in addition to the routine non-sentinel surveillance, sentinel surveillance was carried out at the primary healthcare level in the city of Novi Sad. At secondary and tertiary healthcare level, hospital surveillance was improved in the entire province. Within the sentinel and hospital surveillance, clinical case definitions proposed by GPI were used, while within the routine surveillance the only criteria for reporting was clinical diagnosis based on the opinion of the physician examining the patient. A subset of hospitalized patients, only those from Novi Sad, was analyzed in this paper.

Description of components in improved surveillance in the city of Novi Sad

Sentinel surveillance

Sentinel surveillance was carried out through an existing network of sentinel physicians in Novi Sad Health Centre (10 general practitioners and five pediatricians) who treat patients with developed cough and other symptoms and signs corresponding to the GPI clinical case definition of the disease. Population under sentinel surveillance included patients cared for by sentinel physicians, 22,830 patients during the 2012/13 season (6.7% of the total population of the city of Novi Sad) and 22,385 patients during 2013/14 season (6.6% of the population). Sentinel surveillance population was stratified for data analysis by age groups: 0–12 months of age, 1–6 years, 7–19 years, and 20 years or older (Table 1).

Hospital surveillance

Hospital surveillance was carried out in cooperation with stationary healthcare facilities where patients from Novi Sad were hospitalized with cough and other symptoms and signs that correspond to the GPI clinical case definition of pertussis.

Epidemiological investigation of close contacts

Epidemiological investigation of close contacts was conducted in accordance with the Law on Protection of Population from Infectious Diseases in the Republic of Serbia. The collection of epidemiological and clinical data was conducted by the epidemiologist of the Institute of Public Health in collaboration with the patient's primary care physician.

Close contacts of confirmed pertussis cases (family, other groups) were checked for prolonged cough (more than two weeks), without increased temperature or with minimally increased temperature. Biological samples were

Table 1. Population under sentinel and routine, non-sentinel surveillance according to age groups

Age group	Population of Novi Sad according to 2011 Census	Population under routine non-sentinel surveillance in 2012/13 season	Population (%) under sentinel surveillance in 2012/13 season	Population under routine non-sentinel surveillance in 2013/14 season	Population (%) under sentinel surveillance in 2013/14 season
	n	n	n (%)	n	n (%)
0–12 months	3,673	3,233 (88.0)	440 (12.0)	3,235 (88.1)	438 (11.9)
1–6 years	21,580	18,519 (85.8)	3,061 (14.2)	18,444 (85.5)	3,136 (14.5)
7–19 years	44,519	42,194 (94.8)	2,325 (5.2)	41,384 (93.0)	3,135 (7.0)
20+ years	271,852	254,848 (93.7)	17,004 (6.3)	256,176 (94.2)	15,676 (5.8)
Total	341,624	318,794 (93.3)	22,830 (6.7)	319,239 (93.4)	22,385 (6.6)

obtained from close contacts in order to confirm the diagnosis of the disease. Laboratory analyses were conducted regardless of whether the symptoms fitted the clinical case definition. Contacts, including the ones that were established through hospital surveillance, were further classified on sentinel and non-sentinel contacts dependent whether primary patient was cared for by sentinel or non-sentinel physician. Classification of contacts was done so that the more accurate rates could have been calculated in sentinel and non-sentinel population.

Target group

Target group of the research were all patients from sentinel and hospital surveillance with symptoms and signs of clinical disease that fulfilled the GPI clinical case definition of pertussis, as well as patients detected in routine surveillance based on clinical diagnosis set by the physician.

Pertussis case definition

In accordance with the recommendations of the GPI, the clinical case definitions of pertussis presented in Figure 1 were implemented [3].

Sampling and transport of patient material

To confirm the suspected diagnosis, sampling of nasopharyngeal swab (NPS) for polymerase chain reaction (PCR) and/or blood (pertussis serology) was obtained from all cases.

Sampling was conducted by sentinel physician at the Health Centre or by trained laboratory staff of the Institute of Public Health. In case the patient was hospitalized, blood and NPS specimens were obtained by the health facilities competent staff.

Before transport to the Institute of Public Health of Vojvodina, all samples were stored in a refrigerator at the point of sampling, and transported in a hand refrigerator within 48 hours after sampling.

Laboratory testing of samples and interpretation of results

Sample testing was carried out at the Institute of Public Health of Vojvodina. The choice of laboratory method depended on the duration of coughing and the age of the patients included in the survey:

1. For patients of all ages in whom the onset of cough was less than three weeks prior to the testing, and for those up to three months of age, regardless of the duration of cough, the testing of NPS was performed by PCR. The following commercial kits were used: Bordetella R-gene™ (ARGENE, BioMerieux, Marcy-l'Étoile, France) and *Bordetella pertussis* / *B. parapertussis* / *B. bronchiseptica* Real-TM (Sacace Biotecnologies Srl., Como, Italy).

2. Among patients aged four months and older, with a duration of cough of more than three weeks, and where the application of the last dose of DTP/DTaP vaccine had been more than 12 months ago, testing of the serum samples was done by using the following commercial kits: Anti-Bordetella pertussis toxin ELISA (IgG) with four calibrators 5 IU/ml, 25 IU/ml, 100 IU/ml, and 200 IU/ml; Anti-Bordetella pertussis toxin ELISA (IgA) with four calibrators 2 IU/ml, 10 IU/ml, 25 IU/ml, and 50 IU/ml (Euroimmun, Lübeck, Germany). If anti-PT IgG was 40, according to manufacturer's instructions the result did not indicate acute infection. If anti-PT IgG was ≥ 100, the result indicated acute infection. If anti-PT IgG was between 40 and 100 IU/ml, detection of anti-PT IgA was performed. If anti-PT IgA exceeded the age-dependent reference range, the result indicated positive result and acute infection. If anti-PT IgA was below the-age dependent reference range, the result did not indicate acute infection.

3. Among persons aged four months and older in whom the occurrence with a duration of cough of more than three weeks, and where the last dose of DTP/DTaP vaccine was administered less than one year ago, the testing of the samples to pertussis was done by using the same commercial kits as mentioned above. Additionally, in case the findings in these patients were inconclusive, secondary

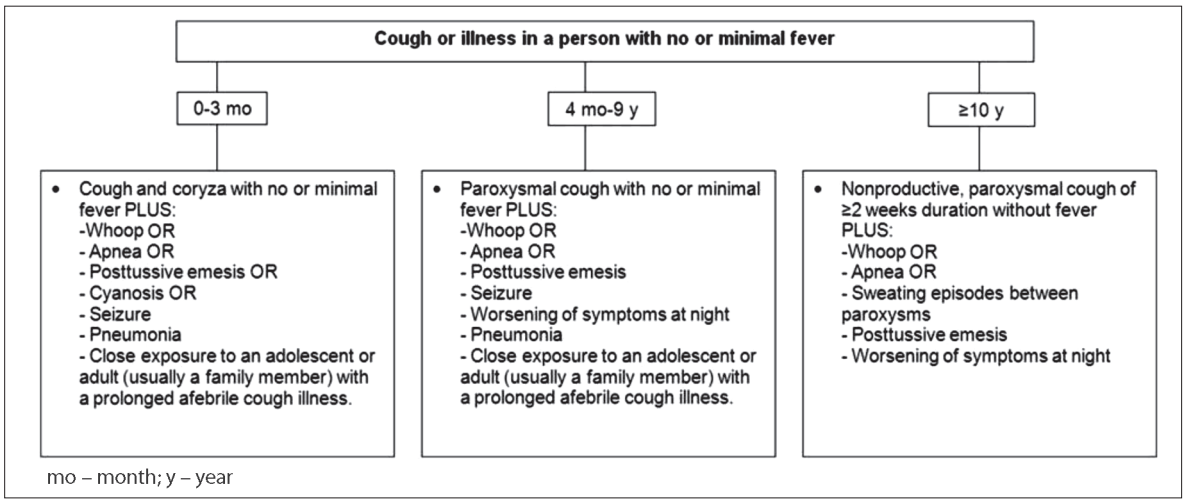

Figure 1. Implemented clinical case definitions of pertussis in accordance with the recommendations of the GPI [3]

Table 2. Suspect and confirmed pertussis cases according to the type of surveillance in both seasons

Type of surveillance	Season 2012/13 Sept. 16, 2012 – Sept. 15, 2013		Season 2013/14 Sept. 16, 2013 – Sept. 15, 2014	
	Number of suspected cases	Number (%) of confirmed cases	Number of suspected cases	Number (%) of confirmed cases
Sentinel	34	3 (8.8)	208	47 (22.6)
Close contacts	9	4 (44.4)	15	8 (53.3)
Subtotal	43	7 (16.3)	223	55 (24.7)
Hospital	25	4 (16.0)	100	53 (53.0)
Close contacts	6	3 (50.0)	8	3 (37.5)
Subtotal	31	7 (22.6)	108	56 (51.6)
Non-sentinel	0	0 (-)	61	26 (42.6)
Close contacts	0	0 (-)	22	9 (40.9)
Subtotal	0	0 (-)	83	35 (42.2)
Total	74	14 (18.9)	414	146 (35.3)

cases were investigated and laboratory-confirmed in order to establish epidemiological link with the inconclusive case so that the true number of pertussis cases (including vaccine failures) could have been ascertained and the true epidemiology of the disease established.

A confirmed case of pertussis was every suspected case with laboratory confirmation of pertussis. Suspected cases of pertussis with negative laboratory results were not classified as pertussis and further clinical and laboratory follow-up was conducted towards determination of true etiology of the disease. Laboratory findings were reported back to the caring physicians.

Data processing and analysis of the survey results

The obtained data were statistically processed using SPSS Statistics for Windows, Version 22.0 (IBM Corp., Armonk, NY, USA). The results are presented as a frequency, pertussis incidence (per 100,000 persons) with 95% confidence interval (Wilson score). The difference among registered number of confirmed pertussis cases was tested by the χ^2 test. Two-tailed p-values < 0.05 were considered to be significant.

Nominators were the numbers of confirmed pertussis cases in total sentinel population and in the age groups, and respective numbers in non-sentinel population under routine surveillance. Denominators for calculation of rates were as follows: the total population monitored by the sentinel surveillance in a given season and population of Novi Sad according to 2011 census subtracted for the number of people monitored through the sentinel surveillance (Table 1).

RESULTS

Burden of pertussis before the introduction of improved surveillance

In the period from 2001 to September 15, 2012 in the city of Novi Sad, only two pertussis cases were reported. The diagnosis was based exclusively on clinical grounds. Both cases were unvaccinated children four months of age who required hospitalization.

Burden of pertussis after the introduction of improved surveillance

A total of 14 and 146 pertussis cases were confirmed during the 2012/13 and 2013/14 seasons, respectively. During the first season (2012/13) of improved surveillance based on GPI clinical case definition, a total of 34 and 25 suspected cases within sentinel and hospital surveillance were reported, respectively. Laboratory confirmation of pertussis was obtained for three and four cases in sentinel and hospital surveillance, respectively. Highest percentage of confirmed cases after epidemiological investigation was determined among close contacts of confirmed pertussis cases with four and three cases determined among close contacts of confirmed cases in sentinel and hospital surveillance (Table 2). During the same period of observation there were no suspected cases registered in the routine, non-sentinel surveillance.

During the 2013/14 season, out of 61 suspected cases indicated in the non-sentinel surveillance, pertussis was confirmed in 26 (42.6%). During the same period, there were 47 (22.6%) and 53 (53%) confirmed cases registered out of 208 and 100 suspected cases in sentinel and hospital surveillance, respectively. Among the close contacts, out of 45 suspected cases, there were 20 confirmed cases registered, with eight (53.3%), three (37.5%), and nine (40.9%) originating from confirmed cases detected through the sentinel, hospital, and non-sentinel surveillance, respectively.

In the 2012/13 season, one half of confirmed cases was determined through epidemiological investigation of close contacts with one being a member of sentinel and six members of non-sentinel population. Among laboratory confirmed cases, three were detected through sentinel surveillance in primary health care, while the remaining four were registered in hospitals and were members of non-sentinel population. All four pertussis cases from non-sentinel population were missed in routine surveillance because all of them were sent to hospitals under other diagnosis (Table 3).

In the 2013/14 season, 73 (50%) of all confirmed cases were detected by primary health care level physicians through sentinel or non-sentinel, routine surveillance. Sig-

Table 3. Distribution of confirmed pertussis cases from sentinel and non-sentinel population according to the type of surveillance and level of health care during the period of improved surveillance

Season	Type of surveillance and level of health care	Total n (%)	Incidence per 100,000 population	Sentinel n (%)	Incidence per 100,000 population	χ^2 p-value	Non-sentinel n (%)	Incidence per 100,000 population	χ^2 p-value
2012/13	Primary level	3 (21.4)	0.9	3 (75)	13.1		0 (0)	0	
	Hospital	4 (28.6)	1.2	0 (0)	0		4 (40)	1.3	
	Epidemiological investigation of close contacts – field investigation	7 (50)	2	1 (25)	4.4	*	6 (60)	1.9	*
	Overall	14 (100)	**4.1**	4 (100)	**17.5**		10 (100)	**3.1**	
	No. of people under surveillance	**341,625**		**22,830**			**318,795**		
2013/14	Primary level	73 (50)	21.4	47 (87)	210	$\chi^2 = 29.438$ $p < 0.001$	26 (28.3)	8.1	$\chi^2 = 22.196$ $p < 0.001$
	Hospital	53 (36.3)	15.5	2 (3.7)	8.9		51 (55.4)	16	
	Epidemiological investigation of close contacts – field investigation	20 (13.7)	5.9	5 (9.3)	22.3		15 (16.3)	4.7	
	Overall	146 (100)	**42.7**	54 (100)	**241.2**		92 (100)	**28.8**	
	No. of people under surveillance	**341,625**		**22,385**			**319,240**		

* It was not possible to calculate the probability due to small size of the sample

nificant differences in distribution of confirmed pertussis cases from sentinel and non-sentinel population according to the type of surveillance and level of health care were determined. Sentinel physicians detected confirmed cases more frequently than non-sentinel physicians.

In 36.3% of all cases, suspect cases were detected according to the GPI clinical case definition in hospitals. Primary health care level physician did not suspect pertussis in these cases. Patients were sent to hospitals under diagnoses other than pertussis. Among hospitalized patients, only two (3.7%) out of 54 cases from the sentinel population were unrecognized by sentinel physicians. The majority of 51 (55.4%) out of 92 cases from of non-sentinel population were confirmed during hospitalization.

Patients with confirmed pertussis after epidemiological investigation of close contacts make up 13.7% of the overall number of confirmed cases in 2013/14 season. They did not seek medical attention from their primary health care level physician.

Dependent on the type of surveillance and clinical case definition used, significant differences in age-specific incidence and burden of the disease in the population of Novi Sad were registered when compared between sentinel and routine surveillance during the season of 2013/14 (Table 4).

Pertussis was reported in all age groups through sentinel surveillance. The highest age-specific incidence rates were noted in school-age children and adolescents (988.8/100,000). A high age-specific incidence rate was also found in unimmunized or incompletely immunized children of the youngest age group (newborns and infants), 456.6/100,000. The lowest age-specific incidence rate was registered in adults (38.3/100,000).

Through non-sentinel, routine surveillance, pertussis was not registered in the youngest age group and in adults. Registered age-specific incidence rates in children 1–6 years of age (16.3/100,000) and in children 7–19 years of age (55.6/100,000) were several times lower than those registered through sentinel and hospital surveillance in respective age groups.

Age distribution of patients registered in hospitals confirms the presence of pertussis in all age groups. The highest age-specific incidence in hospitalized patients was registered in the youngest age group (136.1/100,000). Distribution of confirmed cases from sentinel and non-sentinel population detected in hospitals was statistically significant in the youngest age group.

Among 20 cases registered in close contacts of confirmed cases, there were no confirmed cases in the youngest age group, while 12 were adults. Distribution of confirmed cases among close contacts from sentinel and non-sentinel population was highly statistically significant in age groups of 1–6 and 7–19 years.

DISCUSSION

Comparative analyses of results shows that registered burden of pertussis and distribution of cases in population depend on the type of surveillance and applied clinical case definition. Sentinel surveillance showed that all age groups were affected and that pertussis is widely distributed in our population. Detection of pertussis in the first season of improved surveillance in hospitals and through sentinel network of physicians most probably contributed to the registration of the disease within non-sentinel, routine surveillance at primary healthcare level during the second season of improved surveillance. Epidemiological investigation of close contacts of confirmed cases showed that adults represent reservoir of the infection for younger age groups and for the sustainable transmission of disease in the whole population. Clinical case definitions proposed by the GPI seem more adequate in recognition of severe forms of the disease.

Table 4. Incidence and age-specific incidence of pertussis in sentinel and non-sentinel population in the 2013/14 season

Age group	Type of surveillance and origin of population	Population	Confirmed cases	Incidence per 100,000			χ^2 p-value
			n	Value	95% CI lower limit	95% CI upper limit	
0–12 months	Sentinel	438	2	456.6	125.3	1,649.4	$\chi^2 = 14.78$ $p < 0.001$
	Non-sentinel	3,235	0	0			
	Total	**3,673**	2	54.5	14.9	198.3	
	Hospital – sentinel	438	0	0			$\chi^2 = 14.78$ $p < 0.001$
	Hospital – non-sentinel	3,235	5	154.6	66	361.3	
	Total	**3,673**	5	136.1	58.2	318.3	
	Contacts – sentinel	438	**0**	0	-	-	-
	Contacts – non-sentinel	3,235	**0**	0	-	-	
	Total	**3,673**	**0**	0	-	-	
	Overall	3,673	7	190.6	92.3	392.9	
1–6 years	Sentinel	3136	8	255.1	129.3	502.6	$\chi^2 = 30.01$ $p < 0.001$
	Non-sentinel	18,444	3	16.3	5.5	47.8	
	Total	**21,580**	11	51	28.5	91.3	
	Hospital – sentinel	3,136	0	0			$p = 0.41$
	Hospital – non-sentinel	18,444	4	21.7	8.4	55.8	
	Total	**21,580**	4	18.5	7.2	47.7	
	Contacts – sentinel	3,136	2	63.8	17.5	232.2	$\chi^2 = 6.566$ $p = 0.01$
	Contacts – non-sentinel	18,444	1	5.4	1	30.7	
	Total	**21,580**	3	13.9	4.7	40.9	
	Overall	**21,580**	**18**	**83.4**	**52.8**	**131.8**	
7–19 years	Sentinel	3,135	31	988.8	697.5	1,400.1	$\chi^2 = 209.511$ $p < 0.001$
	Non-sentinel	41,384	23	55.6	37	83.4	
	Total	**44,519**	54	121.3	93	158.2	
	Hospital – sentinel	3,135	0	0			$p = 0.108$
	Hospital – non-sentinel	41,384	34	82.2	58.8	114.8	
	Total	**44,519**	34	76.4	54.7	106.7	
	Contacts – sentinel	3,135	2	63.8	17.5	232.3	$\chi^2 = 8.298$ $p = 0.004$
	Contacts – non-sentinel	41,384	3	7.2	2.5	21.3	
	Total	**44,519**	5	11.2	4.8	26.3	
	Overall	**44,519**	**93**	**208.9**	**170.6**	**255.8**	
20+ years	Sentinel	15,676	6	38.3	17.5	83.5	$\chi^2 = 98.054$ $p < 0.001$
	Non-sentinel	25,6177	0	0			
	Total	**271,853**	6	2.2	1	4.8	
	Hospital – sentinel	15,676	2	12.8	3.5	46.5	$\chi^2 = 3.729$ $p = 0.053$
	Hospital – non-sentinel	256,177	8	3.1	1.6	6.2	
	Total	**271,853**	10	3.7	2	6.8	
	Contacts – sentinel	15,676	1	6.4	1.1	36.1	$p = 0.702$
	Contacts – non-sentinel	256,177	11	4.3	2.4	7.7	
	Total	**271,853**	12	4.4	2.5	7.7	
	Overall	**271,853**	**28**	**10.3**	**7.1**	**14.9**	

Epidemiological situation of pertussis in Novi Sad was not known well until 2012, though the change in epidemiological characteristics of pertussis occurred in countries with high vaccination coverage [2]. Due to the lack of laboratory diagnostics and consequently insufficient reporting, in the period before the improved surveillance, the disease was registered discontinuously as sporadic cases without laboratory confirmation. Therefore, the change of epidemiological characteristics of pertussis was not registered in our country, unlike the shift towards an increase in pertussis incidence to older age groups in some other countries [6]. Within sentinel surveillance, where indication for laboratory analyses was determined in line with GPI clinical case definition, confirmed cases were detected in both seasons (2012/13 and 2013/14). During the first season of improved surveillance (2012/13), non-sentinel physicians did not detect any suspect cases of pertussis, just like in the period before the improved surveillance. Therefore, none of the cases were confirmed through routine surveillance. Although the incidence registered through sentinel surveillance in the sample of population cannot be compared to the true incidence but only esti-

mated, disease distribution and rates are similar to those registered in other European countries [7]. All hospitalized cases initially referred to the hospital under clinical diagnosis of prolonged cough without initial suspicion of pertussis were considered unrecognized or missed at the primary healthcare level. A total of 55 out of 57 pertussis cases registered in a hospital setting were missed by non-sentinel physicians and only two cases were considered missed in the sentinel surveillance in 2013/14 season because the final diagnosis was set in the hospital.

Highest age-specific incidence rates were detected in school-age children and adolescents in 2013/14 season. These findings, in the setting of high vaccination coverage, may be considered as a consequence of waning vaccine-induced immunity similar to findings of some researchers, where vaccine-induced immunity lasted for four to 12 years, i.e. six to 10 years [8, 9].

Infants are at the highest risk of pertussis and severe forms of the disease and relatively high age-specific incidence of hospitalized cases in non-sentinel population as well as high incidence in sentinel population shows that pertussis still represents a problem among infants too young to be completely immunized according to schedule of immunization [8].

The lowest age-specific incidence rates in adults was most probably the consequence of unrecognizing the disease in the setting of unrecognized endemic/epidemic character of the disease occurrence, making adults significant sources of transmission of *B. pertussis* to unvaccinated young infants. Adults most probably did not seek medical attention due to mild forms of illness.

Until the introduction of improved pertussis surveillance, pertussis was considered to be a childhood disease in unvaccinated children of the youngest age group. However, 19.2% (28/146) of cases in our study were detected in adults through improved surveillance. It is still lower than in some developed European countries [7, 10–13]. It can be the result of high seroprevalence due to unrecognized infections, less exposition to infection, insufficient sensitivity of clinical case definition, or the consequence of the current situation in regard to the cyclical nature of the disease [14].

CONCLUSION

Introduction of clinical case definitions proposed by GPI improved the quality of surveillance and enabled an insight in the distribution of pertussis in all age groups and at all levels of health care.

Sentinel surveillance provided better perception of pertussis incidence rates in the population of Novi Sad.

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Инциденција пертусиса у Новом Саду (Србија) пре и у току унапређеног надзора

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САЖЕТАК

Увод/Циљ У циљу унапређења надзора над пертусисом, нарочито код старије деце, адолесцената и одраслих, Глобална пертусис иницијатива (ГПИ) предложила је клиничке дефиниције пертусиса за три различите узрасне кохорте. Циљ овог истраживања је да упореди регистровано оптерећење пертусисом у новосадској популацији пре и после увођења унапређеног надзора у коме су коришћене клиничке дефиниције пертусиса предложене од ГПИ.

Методе Подаци о регистрованом оптерећењу пертусисом су добијени на основу извештаја из рутинског (непредострожног) надзора. Унапређен надзор (предострожни и болнички), у којем је сумња на пертусис постављана у складу са клиничким дефиницијама ГПИ, уведен је 16. септембра 2012. године, док у несентинелном надзору клиничка дефиниција пертусиса није уведена. За лабораторијску потврду дијагнозе су од свих суспектних случајева узети назофаингеални брис и/или узорак крви, а испитивање је, у зависности од трајања кашља и узраста пацијента, вршено молекуларном (ПЦР) или серолошком методом. За статистичку обраду података коришћен је *SPSS Statistics* 22.

Резултати Током анализираног периода пре увођења унапређеног надзора (око 12 година) на основу клиничке слике су пријављена само два случаја пертусиса, док је у двогодишњем периоду спровођења надзора регистровано 14 (сезона 2012/2013), односно 146 (сезона 2013/2014) случајева и доказане су сигнификантне разлике у распрострањености ове болести у популацији у зависности од врсте надзора и нивоа здравствене заштите.

Закључак Увођењем клиничких дефиниција предложених од ГПИ унапређен је квалитет надзора и сагледавање учесталости пертусиса у свим добним групама и на свим нивоима здравствене заштите.

Кључне речи: пертусис; надзор; епидемиологија

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Benefits of venom immunotherapy – How soon can they be expected

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SUMMARY

Introduction/Objective Allergic reactions to insect stings are medical emergencies that could be prevented by venom immunotherapy (VIT). The main purpose of VIT is to prevent fatal or life-threatening reactions. We aimed to show the rapidity with which patients experience the benefits of VIT and estimate the number of emergency treatments that are prevented.

Methods We reviewed the medical files of patients who started VIT between 2010 and 2014. We calculated the costs of treatment of the sting reactions, the costs of immunotherapy, and estimated the costs of prevented allergic reactions.

Results In a cohort of 514 patients (40.9% female, age 47.2 ± 14.4 years), the cost of treatment of the index sting reaction was 180.4 ± 166.8 euros. During VIT, 195 patients experienced 446 field stings. In 86.3% of patients, stings were well tolerated, and only one patient experienced a severe reaction (grade III, according to Mueller). A total of 20.4% of VIT treated patients were stung during the first year of VIT and 57% during five years of VIT. The expenditure for five years of VIT was 2,886 euros per patient, which corresponded to an average of 16 emergency treatments for systemic reactions.

Conclusion Emergency situations are prevented in a substantial number of venom-allergic patients and a beneficial effect was already observed during the first year of VIT.

Keywords: hymenoptera venom allergy; anaphylaxis; immunotherapy; emergency treatment; costs

INTRODUCTION

Up to 7.5% of the population report systemic allergic reactions (SAR) to honeybee, wasp, or hornet stings [1]. In frequently stung subjects, such as beekeepers, the prevalence of SAR could exceed 30% [2]. In total, 39.1% of reactions are mild (grade I], and 43.5% are moderate (grade II), according to the Ring and Messmer classification [3]. Patients of advanced age and those with concomitant cardiovascular diseases and elevated basal serum tryptase are prone to severe reactions [4]. After a person becomes allergic, allergic reactions are expected after further stings, and there is a tendency for repeated sting reactions to be as severe as the index reaction, with 10–15% being more severe [5, 6].

Allergic reactions to insect stings are medical emergencies. Patients require activation of emergency teams or are transported to emergency centers. In accordance with the European Academy of Allergy and Clinical Immunology anaphylaxis guidelines, patients who fulfil the criteria for anaphylaxis should be hospitalized overnight [7]. Some patients require treatment in an intensive care unit.

Up to 0.5 per one million people die per year from allergic reactions to hymenoptera venom [2, 8]. Venom-allergic patients have a decreased quality of life. Venom immunotherapy (VIT) is the therapy of choice for patients who have experienced a severe immunoglobulin E-mediated sting reaction of Mueller grade III (dyspnea) or IV (hypotension), particularly if there is a substantial risk of further stings, since VIT prevents serious allergic reactions to hymenoptera stings [8]. Increasing amounts of venom to which the patient is allergic are given subcutaneously, starting with less than 1 μg and then approximately doubling doses in intervals from 20 minutes to one week until reaching the maintenance dose of 100 μg (equivalent to two to 10 insect stings). Maintenance doses are applied every four to 12 weeks for three to five years. In addition to preventing life-threatening reactions, VIT significantly improves health-related quality of life scores [8].

There are very few studies on the cost effectiveness of VIT, and they focus exclusively on preventing fatal reactions [9, 10, 11].

We aimed to show the advantages of venom immunotherapy, specifically the rate at which patients experience benefit and the number of emergency treatments that are prevented. Additional objective was to estimate the costs of various therapeutic decisions.

METHODS

The study was performed at a tertiary institution as a part of research program P3-0360 financed by Slovenian Research Agency and approved by State Ethics Committee (number of approval 86/05/05).

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We reviewed the medical files of consecutive patients who started VIT from 2010 to the end of 2014. The diagnosis of venom allergy was made according to medical history and sensitisation to venom was assessed by skin tests, measurement of specific immunoglobulin E (Immulite system, Siemens, Munich, Germany) or basophil activation test. The severity of the index reaction was assessed according to Mueller grades (grade I – generalized urticaria; grade II – angioedema; grade III – dyspnoea; grade IV – cardiovascular collapse.

We calculated the expense of treatment for index sting reactions, which was an indication for VIT and the costs of immunotherapy. The costs were assessed according to the Slovenian health care insurance price list (Table 1). The costs of treatment for the index sting reaction were calculated for a subgroup of patients for whom the index reaction treatment data were available.

Table 1. The costs of treatment according to the Slovenian health care insurance price list

Activity/drug	Price (euros)
Emergency management at patient's home	208
Emergency management, primary care	65
Emergency management, secondary care	76
Hospitalization, 1 day	195
Hospitalization in intensive care unit, 1 day	503
Immunotherapy, outpatient visit	74
Maintenance dose of venom (100 µg)	25.5
Epinephrine 0.5 mg	1
Clemastine 2 mg	6
Methylprednisolone, i.v. 125 mg	10
Methylprednisolone tablet 64 mg	1
Antihistamine tablet	0.3
EpiPen epinephrine auto-injector	33.2

In the immunotherapy files, we searched for data on insect stings during VIT, the consequences of the stings, and their management. We calculated the number of prevented systemic allergic reactions: We assumed that if patients had not been treated with VIT, an allergic reaction similar to the index reaction would have occurred after each sting which was suffered during VIT and that the treatment would have been similar to the treatment of the index reaction, although it is known from epidemiological studies that up to 50% of patients don't experience any reaction after a subsequent sting, and in up to 15% of patients the reaction is more severe than the index reaction. We assumed that patients not treated with VIT would be equipped life-long with an epinephrine auto-injector, which should be refilled yearly.

The initial phase of immunotherapy was performed as one-day ultrarush immunotherapy with Venomenhal (HAL, Leiden, the Netherlands) or Alyostal (Stallergenes, Antony, Hauts-de-Seine, France,) venom. A maintenance dose of 100 µg was reached in three hours, using 10 mg of desloratadine as a premedication. Maintenance doses of 100 µg were given on days 3, 10, 24, and 45, and then monthly in the first year. Each year, the maintenance interval was prolonged for two weeks. The duration of immunotherapy was planned to be five years.

Statistics: The data are presented as the mean ± standard deviation. The differences between the groups were calculated using the Student's t-test and χ^2 test.

The life expectancy data were found on the web page of the Statistical Office of the Republic of Slovenia [12].

RESULTS

Patients

We included 514 patients (210 female, 40.9%). Their age at the beginning of VIT was 47.2 ± 14.4 years. The severity of the index reaction according to Mueller was as follows: grade I 0.4%; grade II 2.8%; grade III 26.8%; grade IV 70%. In all patients sensitisation to venom was confirmed.

Treatment of index reaction

The data on the treatment of the index reaction were available for 462 (89.9%) subjects. In total, 442 (95.7%) patients sought medical treatment. In 125 patients (36%), the treatment began at the site of the sting. Of the patients, 331 were treated at primary emergency care centers, and 161 at secondary emergency care centers; 105 were hospitalized in hospital wards, and six were hospitalized in intensive care units. In total, 20 patients did not receive medical intervention for an index sting. The cost of treatment for an index reaction was estimated at 180.4 ± 166.8 euros (190.8 ± 150.3 euros for honeybee allergy; 174.2 ± 176.6 euros for wasp allergy, $p > 0.05$).

Detailed information on the drugs used for treatment were available for 301 patients, as follows: the use of epinephrine was documented in 135 (44.9%) patients; parenteral antihistamines and steroids were used in 116 patients; 50 patients received peroral treatment only; we found no details on the drugs used for emergency treatment in 88 patients.

Efficacy of VIT

At the time of the analysis, 159 patients had been treated with VIT for up to one year, 75 for up to two years, 111 for up to three years, 83 for up to four years, and 86 had been treated for up to five years (Table 2). The venom used was from honeybees in 186 (36.2%) cases and from wasps in 328 cases.

In total, 195 patients experienced field stings during VIT; 68 (36.6% of the patients treated for honeybee stings) received honeybee stings, and 127 (38.7% of the patients

Table 2. Cohorts of patients according to duration of VIT and sting frequency

Duration of treatment	Number of patients	Number of patients stung
Up to 1 year	159	35 (22%)
Up to 2 years	75	22 (29.3%)
Up to 3 years	111	49 (44.1%)
Up to 4 years	83	42 (50.6%)
Up to 5 years	86	49 (57%)

treated for wasp stings) received wasp stings ($p > 0.05$). The total number of field stings was 446. The patients stung by honeybees were stung 2.9 ± 1.4 times, and the patients stung by wasps were stung 1.9 ± 1.7 times ($p > 0.05$). The proportion of patients who received a field sting during VIT is shown in Table 2. In total, 105 (20.4%) patients were stung already during the first year of immunotherapy.

In total, 27 (13.7%) patients reported systemic symptoms after receiving a field-sting during VIT; 18 reported only subjective symptoms, and six (3%) sought medical intervention. Only one reaction was severe (grade III, according to Mueller).

Cost of treatments

For the comparison of the costs of immunotherapy and the costs of prevented systemic reactions, the expenditures for VIT were calculated for an average duration of VIT (26 months), which consisted of one-day hospital immunotherapy plus 25 outpatient maintenance injections, for a total of 1,925 euros. The cost of the allergen was 662 euros per patient. In a group of 514 patients treated for an average of 26 months, the total costs were estimated to be 989,450 euros. During the same time, the patients experienced 446 field stings, of which only six were treated by medical professionals. The estimated cost of 440 prevented sting reactions was 79,388 euros.

To compare the costs of VIT with a lifelong supply of emergency epinephrine auto-injectors, the price for a complete five-year course of immunotherapy was calculated at 2,886 euros per patient, corresponding to 16 average emergency treatments of systemic reactions following unprotected hymenoptera insect stings. The cost of the allergen used for VIT is 992 E. The price of epinephrine auto-injectors in patients not treated with VIT was calculated as one auto-injector per year per patient. The average patient was born in 1968, and life expectancy was assumed to be 27 years for males and 33 years for females. An average patient would be prescribed 29.5 auto-injectors, costing 980.4 euros per patient. The additional cost of VIT over epinephrine auto-injectors was estimated at 1905.6 euros per patient.

DISCUSSION

We showed that more than one-half of the patients treated with venom immunotherapy for up to five years received an in-field insect sting while on maintenance immunotherapy, and 20.4% received a sting during the first year of immunotherapy.

Venom allergy is the most common cause of anaphylaxis [13]. Although the clinical presentation is dramatic and is frequently treated by emergency doctors, less than one half of patients are treated with epinephrine, as documented also in our analysis.

After an acute episode, a decision should be made to prevent further sting reactions. Avoidance measures are the cornerstone of prevention; however, these measures are sufficient in less than one half of patients – specifically, in those with low exposure to hymenoptera stings. Von Moos et al. [14] retrospectively analyzed the re-sting data of 96 bee venom-allergic and 95 vespid venom-allergic patients. They showed that the benefits of VIT are greater in subjects with higher exposure to further stings. In bee venom-allergic patients who lived in the vicinity of beehives, the median sting-free interval was 5.25 years compared to 10.75 years in subjects with less exposure. One half of vespid venom-allergic outdoor workers were re-stung within 3.75 years, compared to 7.5 years for indoor workers. Von Moos concluded that in highly exposed subjects, it is worth to offer VIT, even to patients with less severe allergic reactions because of the high probability of a re-sting. In our study, the risk of a re-sting was higher and it was equal in the bee- and wasp-allergic subjects.

Patients with severe reactions are equipped with epinephrine auto-injectors and/or are offered immunotherapy [15]. In addition to being life-threatening, an allergy to insect venom negatively affects the quality of life. Carrying an EpiPen as the sole treatment does not prevent deterioration of the quality of life [16]. It was shown that health-related quality of life is improved by VIT [8]. Moreover, in most patients, compliance in carrying an EpiPen is low, and the ability to correctly self-administer an EpiPen is poor; relying on self-treatment of severe allergic reactions is not a safe strategy [17]. Oude Elberink et al. [18] found that only 48% of patients with a severe venom allergy and who received an EpiPen were positive regarding their treatment. Of these patients, 68% would have preferred to be treated with VIT. On the other side, 91.5% of the VIT-treated patients were positive regarding their treatment, and 85% would select VIT again. We showed that the additional cost of VIT over having an emergency EpiPen is, at most, 1905.6 euros, not taking into account the costs of yearly medical visits and patient education for refilling a prescription for an EpiPen and possible emergency medical visits after insect stings in patients using only an EpiPen.

Focusing only on preventing fatal reactions, Hockenhull et al. [9] calculated that VIT combined with an adrenaline auto-injector and antihistamine compared with sting avoidance alone yields an incremental cost-effectiveness ratio (ICER) of £7,627,835 per quality-adjusted life years (QALY) gained. In the subgroup of patients at high risk of future stings (five stings per year), the VIT ICER is £23,868 per QALY gained. In the subgroup of patients whose quality of life improves because of anxiety reduction, VIT ICER is in the range of £25,767–27,504 per QALY gained.

In our study the calculated costs avoided by the VIT are the minimal estimate, since it is conceivable that at least some of the patients, if they were not on VIT, would progress to more severe and hence more costly reactions.

Alongside prevention of fatal outcomes, quality of life is also an important outcome measure when considering this type of treatment. In the majority of patients, VIT is effective after the maintenance dose is reached, as shown by Hunt et al. [19] and Goldberg and Confino-Cohen [20]. However, Koschel et al. [21] observed that some VIT-treated patients remained frightened of re-stings to the extent that the anxiety had a significant effect on the quality of

life (e.g., avoidance of outdoor activities). Oude Elberink et al. [16] performed a sting challenge, which was negative in 100 of 103 VIT-treated patients predominantly allergic to wasp venom. After a well-tolerated sting, 40 patients reported increased quality of life, as measured by the Vespid Allergy Quality of Life Questionnaire.

Not all patients who tolerate VIT injections are fully protected against insect stings [22]. A total of 16% of bee-allergic patients and 7.5% of wasp-allergic patients developed systemic reactions after stopping immunotherapy; however, most reactions were mild [23]. Some reactions are most probably psychogenic, resulting from fear, as patients frequently describe only subjective symptoms. Objective reactions might occur in VIT non-responders and in patients sensitized to minor venom epitopes, which are missing in commercial allergens used for VIT [24]. More severe systemic reactions could occur, particularly in patients with mastocytosis, thus mastocytosis has to be considered in insect allergic individuals and when confirmed patients should be offered epinephrine auto-injectors beside VIT [25].

CONCLUSION

We confirmed that emergency situations are prevented in a substantial number (over 20%) of venom-allergic patients already during the first year of VIT and that more than one half of treated patients benefit from VIT during a maintenance period of five years, for an additional cost of at most 1,905.6 euros per patient.

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Корист од имунотерапије отровом инсекта: када се може очекивати

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САЖЕТАК

Увод/Циљ Алергијске реакције на убод инсеката спадају у хитна медицинска стања која могу бити спречена применом специфичне имунотерапије отровом инсекта (ИОИ).
Основна сврха ИОИ је да спречи фатални исход и стања која непосредно угрожавају живот.
Циљ рада је био да укажемо на период примене ИОИ са којим оболели имају добит и утврдимо број хитних стања који су њиме спречени.

Методе Анализиране су историје болести лечених ИОИ од 2010. до 2014. године. Обрачунали смо трошкове лечења од реакција на убод, трошкове имунотерапије и спречених алергијских реакција.

Резултати У групи од 514 пацијената (40,9% жена, старости 47,2 ± 14,4 година) трошак лечења индексне реакције је био 180,4 ± 166,8 евра. Укупно 195 пацијената је доживело 446 убода током ИОИ, 86,3% су га добро толерисали, а само код једног се развио тежи облик реакције (III степен по Милеру). Укупно 20,4% су били убодени током прве године примања ИОИ, а 57,0% током пет година. Расход за пет година узимања ИОИ је био 2.886 евра по пацијенту, што је одговарало просеку од 16 хитних лечења за системске реакције.

Закључак Хитна стања су спречена код значајног броја пацијента алергичних на отров већ током прве године ИОИ.

Кључне речи: алергија на отров опнокрилаца; анафилакса; имунотерапија; хитно лечење; трошкови

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Caries risk assessment in pregnant women using Cariogram

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SUMMARY

Introduction/Objective "Cariogram" takes into account interactions between caries-related factors and expresses a graphic assessment of the caries risk.
The aim of this study was to evaluate the relationship between caries risk and different variables of Cariogram in pregnant women.

Methods This study included 96 pregnant women. At baseline, data on general health, diet, oral hygiene, and fluoride exposure were obtained. DMFT (decayed, missing, and filled teeth) index was calculated by clinical examination. Saliva analyses included mutans streptococci and lactobacilli counts, buffer capacity, and secretion rate. Scores were entered and caries risk was assessed. The women were divided into five groups according to their Cariogram caries risk.

Results The results of the study showed that 29.17% (28) of the pregnant women had high caries risk, 21.88% (21) – medium, 17.71% (17) – low, 16.67% (16) – very high, and 14.58% (14) – very low caries risk. In an average caries risk profile of pregnant women, the dominant sector was "Bacteria" (18.85% of the risk structure profile), followed by "Diet" (17.97%), "Circumstances" (15.68%), and "Susceptibility" sector (14.65%).

Conclusion Cariogram shows that pregnant women in Banja Luka, Bosnia and Herzegovina, had 46.14% chance of avoiding caries in the future. The Cariogram model can successfully determine caries risk profiles for pregnant women.

Keywords: Cariogram; caries risk assessment; mutans streptococci; pregnant women

INTRODUCTION

Caries risk is the probability of a person to develop least certain sign of caries, reaching a given stage of the disease progression for specific period of time, on condition that the exposure to caries risk factors remains unchangeable during the period. Caries management by risk assessment is granted considerable attention [1–4].

In a view of the multifactorial nature of caries etiology and the fact that the course of the disease is determined by permutations and combinations of causal factors [1-4], the challenge is to develop a really effective model for predicting caries risk. Cariogram, a computer program, assesses an individual's caries risk profile and illustrates it graphically. Also, Cariogram offers recommendations for targeted preventive measures that should be implemented to avoid the formation of new caries lesions [5–9]. Cariogram has been used to assess the caries risk profile of schoolchildren, teenagers, orthodontic, and elderly patients [7, 10–14].

The aim of this study was to assess caries risk in pregnant women in Banja Luka, Bosnia and Herzegovina, and to evaluate the contribution of various risk factors among different caries risk groups.

METHODS

The study was conducted as a cross sectional study on a sample of 96 pregnant women from Banja Luka, Republic of Srpska, Bosnia and Herzegovina. The study sample was randomly selected and included only pregnant women who were a) in the last trimester of pregnancy, b) without risk in pregnancy, c) did not have any chronic disease, d) had not taken antibiotics or other drugs during pregnancy, e) signed an informed consent to participate in the research. The average age of the pregnant women was 27.4 years, ranging between 20 and 42. Ethical approval for the study was obtained from the Research Committee of the Faculty of Medicine, University of Banja Luka, Republic of Srpska, Bosnia and Herzegovina. The research has been conducted in full accordance with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. This study included a questionnaire, interview, clinical examination, saliva sampling, and assess of caries risk using Cariogram. Each of the patients completed a brief questionnaire on their general health, oral hygiene, dietary behavior, frequency of tooth brushing, the use of fluoridated toothpaste, and mouthwashes.

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The clinical examination comprised the DMFT (decayed, missing, and filled teeth) index and oral hygiene status. Clinical examinations were carried out in the morning, between meals by the same examiner following the WHO criteria [15]. Clearly visible lesions with cavities on tooth surfaces were classified as dental caries (i.e. d3-level cavities), whereas changes in transparency, initial enamel demineralization with intact surfaces and no cavitations were noted as intact teeth. The teeth were not professionally cleaned and no radiographs were taken. The patients did not take any food or drink (except water), did not wash their teeth, or chew gum at least one hour before the exam and saliva sampling. The plaque index was also evaluated, using Silness and Löe's scale. Plaque was assessed through collection from vestibular surfaces.

After the clinical examinations, the saliva tests (secretion rate, saliva's buffer capacity, *Lactobacillus* and mutans streptococci counts) were performed. All saliva tests were obtained from Orion Diagnostica, Espoo, Finland, and handled according to the instructions of the manufacturer. The patient chewed a sterile paraffin pellet for five minutes. The stimulated saliva was collected in a test tube graduated in milliliters, then the volume of stimulated whole saliva was read off and the result was expressed in ml/min. Buffer capacity was categorized as high, medium, or low using a Dentobuff® Strip (Orion Diagnostica). The salivary *Streptococcus mutans* were made with Dentocult® SM-Strip Mutans (Orion Diagnostica) by placing the vial with the sample carrier in an incubator at 37°C for 48 hours. For measuring *Lactobacillus* count, Dentocult LB® (Orion Diagnostica) was used, by placing the vial with the sample carrier in an incubator at 37°C for 96 hours. The density of colonies (*Streptococcus mutans* and *Lactobacillus)* was compared with a chart provided by the manufacturer.

The data from the clinical examinations and the questionnaire were entered into the Cariogram model. To create an individual risk profile, nine factors/variables are required to be entered into the Cariogram (Table 1). The Cariogram calculated the data and presented the result expressed as a pie chart, illustrating the "Chance of avoiding cavities" in the future. Sectors of the diagram are as follows: "Bacteria" (plaque amount and mutans streptococci level), "Diet" (*Lactobacillus* level and diet frequency), "Susceptibility" (fluoride program, saliva secretion, and saliva buffer capacity), and "Circumstances" (past caries experience and medical history) [16]. The chance to avoid caries was finally grouped in five risk categories: very low risk = 81–100% chance of avoiding caries, low risk = 61–80% chance of avoiding caries, medium risk = 41–60% chance of avoiding caries, high risk = 21–40% chance of avoiding caries, very high risk = 0–20% of avoiding caries.

Table 1. Caries related factors/parameters used at baseline for the Cariogram*

Factor	Information and data collected	Cariogram scores
Caries experience	Past caries experience at baseline, including cavities, fillings, and missing teeth due to caries	0: caries free 1: better than normal condition for ages 2: normal condition for ages 3: worse than normal condition for ages
Related diseases	General diseases or conditions associated with dental caries; medical history, medications; data from interviews and questionnaire results	0: no disease, healthy 1: general disease which can indirectly influence the caries process to a mild degree 2: general disease which can indirectly influence the caries process to a high degree
Diet, contents	*Lactobacillus* counts were used as a measure of cariogenic diet, using Dentocult test	0: $<10^3$ CFU/ml 1: 10^4–10^5 CFU/ml 2: 10^5 CFU/ml 3: $>10^6$ CFU/ml
Diet, frequency	Estimation of number of meals and snacks per day, mean for "normal days;" data from questionnaire results	0: maximum 3 meals per day 1: 4–5 meals per day 2: 6–7 meals per day 3: > 7 meals per day
Plaque amount	Estimation of hygiene according to Silness–Löe plaque index	0: PI < 0.4 (very good oral hygiene) 1: PI = 0.4–1.0 (good oral hygiene) 2: PI = 1.1–2.0 (poor oral hygiene) 3: PI > 2 (very poor oral hygiene)
Mutans streptococci	Estimation of levels of *Mutans streptococci* in saliva, using Strip mutans test (Dentocult)	0: $< 10^4$/ml 1: $10^4 – 10^5$/ml 2: $10^5 – 10^6$/ml 3: $> 10^6$/ml
Fluoride program	Estimation of the extent of fluoride available in the oral cavity	0: maximum fluoride program 1: fluoride supplements 2. only fluoride toothpaste
Saliva secretion	Estimation of flow rate of paraffin-stimulated saliva	0: > 0.7 ml/min. 1: 0.3–0.7 ml/min. 2: < 0.3 ml/min.
Saliva buffering capacity	Estimation of capacity of saliva to buffer acids, using Dentobuff test	0: pH > 5.5 (blue) 1: pH = 4.5–5.5 (green) 2: pH < 4.5 (yellow)

PI – Silness–Löe plaque index
* Taken from Caries Risk Evaluation, Department of Cariology, Malmö University

Statistics

All data were processed with the SPSS for Windows, Version 16.0 (SPSS Inc., Chicago, IL, USA) χ^2 test of contingency was used to compare differences between groups. Parametric ANOVA and Student's t-test for independent samples (if the difference variance observed characteristics were not statistically significant) and nonparametric Mann–Whitney test (if the difference in variance of the observed characteristics is statistically significant) were used to compare the mean values of the characteristics. P-values less than 0.05 were considered statistically significant.

RESULTS

The results of the study showed that 16 (16.67%) pregnant women had very high caries risk, 28 (29.17%) high, 21 (21.88%) medium, 17 (17.71%) low, and 14 (14.58%) had very low caries risk (Table 2).

Table 2 shows the odds ratios for different Cariogram groups. Pregnant women in the low risk group had a risk 1.2 times higher than that of pregnant women in the very low risk group. In the groups with a medium, high and very high risk, the corresponding values were 4.9, 7.0 and 7.7 times higher, respectively. These results are statistically significant.

In an average caries risk profile of pregnant women, the dominant sector was "Bacteria" (data about plaque amount and mutans streptococci level), with 18.85% of the risk structure profile. It was followed by "Diet" (data about *Lactobacillus* level and diet frequency) with 17.97%.

Table 2. Odds ratio values for different Cariogram groups in the logistic regression model

Caries risk assessed by Cariogram	Pregnant women		OR	95% CI	p-value*
	n	%			
Very high risk	16	16.67	7.7	1.16–51.17	0.020
High risk	28	29.17	7	1.45–33.7	
Medium risk	21	21.88	4.9	0.99–24.21	
Low risk	17	17.71	1.2	0.25–5.84	
Very low risk (reference value)	14	14.58	1		
Σ	96	100			

"Circumstances" sector (data about caries experience and medical history) was present with 15.68% in the Cariogram profile, and "Susceptibility" sector (data about fluoride program, saliva secretion, and buffering capacity) was present with 14.65%. In the groups of subjects with high and medium risk, the dominant sector was also "Bacteria" with 24.14% and 18.05%, respectively, followed by "Diet" (20.93% and 15.71%, respectively), "Circumstances" (16.79% and 15.19%, respectively) and "Susceptibility" (16.61% and 13.52%, respectively). In the group of pregnant women very high risk, the dominant sector was "Diet" (31.13%), followed by "Bacteria" (26.88%), "Susceptibility" (21.63%), and "Circumstances" (20.13%). In the groups of subjects with low and very low risk, the dominant sector was "Circumstances" (13.71% and 11.50%, respectively), than "Diet" (9.18% and 4.14%, respectively), "Susceptibility" (9% and 3%, respectively) and "Bacteria" (6.94% and 4.50%, respectively), as shown in Table 3.

The comparative assessment of study participants based on parameters used in the Cariogram model is shown in Table 4. The distribution of the patients within each Cariogram variable was significantly different ($p < 0.01$) for all factors considered. Majority of the participants (56.25%) in this study had caries experience worse than normal condition for ages. All participants in the group with the very high risk of caries had the highest *Lactobacillus* count (more than 10^6 CFU/ml), very high *Streptococcus mutans* count (more than 10^5 CFU/ml) and more than six meals per day. The maximum number of pregnant women with very low caries risk had very good and good oral hygiene (plaque index ≤ 1). Most of the pregnant women in all groups of risk were exposed to fluoride in the form of toothpastes only. The results show that most of the pregnant women (87.5%) had normal amount of saliva secretion (> 0.7 ml/min.). All pregnant women in the group of very low risk showed saliva secretion to be less than 0.5 ml/min. All of the participants in the group of very high risk had saliva pH of less than 4.5.

DISCUSSION

Over the past several decades, researchers have been looking for factors such as host, diet, microflora, past caries

Table 3. The average Cariograms of pregnant women

Sectors in Cariogram	Σ	Very high risk		High risk		Medium risk		Low risk		Very low risk	
		X	SD	X	SD	X	SD	X	SD	X	SD
Chance to avoid caries	46.14	9.88	2.36	27.86	5.95	46.43	5.13	71.06	5.84	93.43	6.42
		$p < 0.05$									
Diet	17.97	31.13	1.71	20.93	5.13	15.71	3.68	9.18	1.47	4.14	1.68
		$p < 0.05$ (except low and very low risk)									
Bacteria	18.85	26.88	3.4	24.14	3.01	18.05	3.57	6.94	3.07	4.5	1.29
		$p < 0.05$ (except high and very high; and also low and very low risk)									
Susceptibility	14.65	21.63	3.48	16.61	4.67	13.52	2.46	9	2.06	3	0.82
		$p < 0.05$ (except high and medium;and also low and very low risk)									
Circumstances	15.68	20.13	0.25	16.79	1.73	15.19	2.52	13.71	1.95	11.5	0.58
		$p < 0.05$ (except high and very high, high and medium, and low and very low risk)									

$\bar{x}$ – mean value

Table 4. Comparison of caries-related factors between groups of pregnant women

Factor	Score	Risk									
		Very high		High		Medium		Low		Very low	
		n	%	n	%	n	%	n	%	n	%
Caries experience	0	0	0	0	0	0	0	0	0	0	0
	1	0	0	0	0	9	34.62	9	34.62	8	30.77
	2	0	0	3	18.75	3	18.75	4	25	6	37.5
	3	16	29.63	25	46.3	9	16.67	4	7.41	0	0
	p-value	< 0.001									
Diet, contents	0	0	0	0	0	0	0	0	0	0	0
	1	0	0	0	0	0	0	0	0	13	100
	2	0	0	26	41.27	19	30.16	17	26.98	1	1.59
	3	16	80	2	10	2	10	0	0	0	0
	p-value	< 0.001									
Diet, frequency	0	0	0	2	10.53	0	0	12	63.16	5	26.32
	1	0	0	18	35.29	19	37.25	5	9.8	9	17.65
	2	2	16.67	8	66.67	2	16.67	0	0	0	0
	3	14	100	0	0	0	0	0	0	0	0
	p-value	< 0.001									
Plaque amount	0	0	0	0	0	0	0	7	38.89	11	61.11
	1	0	0	2	11.76	4	23.53	8	47.06	3	17.65
	2	10	21.28	21	44.68	14	29.79	2	4.26	0	0
	3	6	42.86	5	35.71	3	21.43	0	0	0	0
	p-value	< 0.001									
Streptococcus mutans	0	0	0	0	0	0	0	0	0	5	100
	1	0	0	0	0	5	16.13	17	54.84	9	29.03
	2	2	4.55	26	59.09	16	36.36	0	0	0	0
	3	14	87.5	2	12.5	0	0	0	0	0	0
	p-value	< 0.001									
Fluoride programme	0	0	0	0	0	0	0	0	0	0	0
	1	2	8.00	2	8	8	32	2	8	11	44
	2	14	20.59	26	38.24	13	19.12	15	22.06	0	0
	p-value	< 0.001									
Saliva secretion	0	12	14.29	25	29.76	19	22.62	14	16.67	14	16.67
	1	4	33.33	3	25	2	16.67	3	25.00	0	0
	2	0	0	0	0	0	0	0	0	0	0
	p-value	< 0.001									
Saliva buffering capacity	0	0	0	11	27.5	6	15	15	37.5	8	20
	1	0	0	9	39.13	7	30.43	1	4.35	6	26.09
	2	16	48.48	8	24.24	8	24.24	1	3.03	0	0
	p-value	< 0.001									

*$p < 0.05$, statistically significant

history, which would enable them to predict who would develop a carious lesion. An innovation in caries risk assessment has been the development of a computer program called Cariogram, which compared caries experience, related general diseases, diet content, diet frequency, amount of plaque, mutans streptococci counts, fluoride program, saliva secretion rate, saliva buffering capacity. This program is a prediction and a risk model, as it predicts who has a chance to develop the disease, identifies the risk factors, and based on that determines the appropriate intervention plan [16].

In previous studies, caries risk was assessed with Cariogram for different individuals of various communities, and different results were reported [12, 13, 17, 18, 19]. There is no data about caries risk assessments in pregnant women using Cariogram. Also, there is insufficient data about caries risk assessment in the population of Bosnia and Herzegovina. The present study is the first one where Cariogram has been applied on pregnant women for caries risk assessment.

In the present study, the majority of participants were at high risk of caries according to Cariogram. The number of patients in the very high caries risk group was found to be the highest in the Swedish elderly (55–75 years old) who had histories of multiple drug use and had no access to effective fluoride programs before the 1960s [14]. Another study performed in adults with several dental restorations in Saudi Arabia reported that the majority of participants had a high risk of caries [19]. Gökalp et al. [20] reported

that the prevalence rates of caries in children and adult populations in Turkey were high.

On the other hand, the number of patients in a low caries risk group was found to be the highest in Spanish dental students (18–19 years old) [17]. Celik et al. [21] reported that the number of Turkish adults (20–21 years old) had medium (33%) or low (24%) caries risk. Studies concerning Sardinian (7–9 years old) and Swedish (10–11 years old) schoolchildren also reported the highest number of patients in a low caries risk group [12, 22].

According to the 'opinion' of the Cariogram, the Laotian children (12–13 years old) demonstrated significantly higher caries risk than Swedish children. The average "chances of avoiding caries in the future" in the groups of Swedish and Laotian children were 69.2% and 37.3%, respectively [23].

The leading sector in pregnant women risk profile in this study was "Bacteria" (18.85%), followed by "Diet" (17.97%). A study in Sweden reported similar results [24]. Comparison of these results with those of Petersson et al. [24] showed that the leading sectors in Swedish children Cariogram were also "Bacteria" (9%) and "Diet" (8%). "Susceptibility" and "Diet" factors (23% and 20%, respectively) ranked first in the group of dental students (18–25 years old) from Minsk, Belarus [25]. The "Bacteria" factor was the most dominant sector for students in Valencia [17].

In this study, for all groups of participants, statistically significant correlations were found between the "frequency of food intake" and the risk of caries. If the frequency of food intake was higher, the risk was higher. Even 100% of pregnant women who consumed more than seven meals per day were in a group of very high risk. Petersson et al. [10] also confirmed that the frequency of food intake has a very important role in the risk assessment of Swedish children, especially for a high risk group.

In the present study a statistically significant correlation was found between caries risk and concentration of *Streptococcus mutans* in saliva. Other studies that used Cariogram for risk assessment in the Swedish elderly and children aged 10–11 years reported similar results. The largest increase in caries had over 60% of elderly who had more than 10^5 CFU/ml of saliva. As much as 94.5% of children (aged 10–11 years) who had more than10^5 CFU/ml saliva were at high risk [7, 14]. Günay et al. [26] reported in their study that 68.5% of pregnant women and 61.5% of children aged three years had concentrations of more than 10^5 CFU/ml of saliva. According to Köhler et al. [27], 80% of new mothers had concentrations of more than 10^5 CFU/ml of saliva. About 50% of tested pregnant women and young mothers had concentrations of more than 10^6 CFU /ml in saliva in researches in Finland [28, 29].

CONCLUSION

This study was performed with Cariogram in pregnant women in an effort to overcome data insufficiency of caries risk assessments in Bosnian populations. Cariogram shows that pregnant women in Banja Luka, Bosnia and Herzegovina, had a high risk of developing new caries lesions, with a 46.14% chance of avoiding caries in the future. The main risk Cariogram sectors were "Bacteria" and "Diet." The Cariogram model can successfully determine caries risk profiles for pregnant women. Further longitudinal studies in Bosnian populations are needed to assess caries risk in various age and risk groups.

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Процена ризика од каријеса код трудница употребом Кариограма

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САЖЕТАК

Увод/**Циљ** Кариограм програм процењује и графички илуструје ризик од каријеса узимајући у обзир интеракцију различитих фактора његовог настанка.
Циљ рада је био да се процени однос ризика од каријеса и разних параметара Кариограм програма код трудница.
Методе У студију је укључено 96 трудница. Узети су подаци о општем здрављу, исхрани, оралној хигијени и употреби флуорида. Након клиничког прегледа израчунат је КЕП (кариозни, естраховани и пломбирани зуби) индекс. Анализом пљувачке добијени су подаци о количини стимулисане пљувачке, пуферском капацитету пљувачке, степену колонизације *Streptococcus mutans*-а и лактобацила. Подаци су унесени у Кариограм програм и процењен је ризик од каријеса. Труднице су подељене у пет група по кариограму.
Резултати Са високим ризиком од каријеса је 29,17% (28) трудница, 21,88% (21) – са средњим, 17,71% (17) – ниским, 16,67% (16) – врло високим, а 14,58% (14) – врло ниским ризиком од каријеса. У просечном ризику од каријеса доминантан сектор је сектор „Бактерије" (18,85%), следе „Исхрана" (17,97%), „Околности" (15.68%) и „Осетљивост" (14,65%).
Закључак Кариограм програм је показао да су труднице у Бањој Луци имале 46,14% „шансе за избегавање каријеса у будућности". Кариограм модел може успешно одредити профил ризика од каријера за труднице.
Кључне речи: Кариограм; процена ризика од каријеса; *Streptococcus mutans*; труднице

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CASE REPORT / ПРИКАЗ БОЛЕСНИКА

Schwannoma of the upper lip – A case report and literature review

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SUMMARY
Introduction Schwannomas or neurilemmomas are well demarcated, benign neurogenic lesions arising by a fibroblastic proliferation of the nerve sheath cell (Schwann cell). They usually present as solitary encapsulated lesions with rare occurrence in the upper lip. Non-diagnosed or misdiagnosed schwannomas present a high risk for the tumor to continue growing and exerting pressure on surrounding nerves. These tumours based on their location could lead to facial weakness and paralysis, pressure in ears, tinnitus, hearing loss, balance loss, and could lead to a life-threatening situation.
Case Outline This case is a rare presentation of a schwannoma located in the upper lip of a 21-year-old male patient of Indian origin. The patient complained of a swelling in the mouth with a difficulty in keeping the mouth closed. The swelling was surgically excised and the patient healed completely.
Conclusion This case of occurrence of tumor on the upper lip points to the possibility of considering schwannoma as a possibility in the diagnosis of oral tumors in the future, as the location of the tumor was rare and had a high chance of misdiagnosis.
Keywords: schwannoma; neurinoma; neurilemmoma; oral lesion; head and neck tumors

INTRODUCTION

Schwannoma was first described by Vercay in 1910, who called it neurinoma [1]. But the term neurilemmoma was first coined by Stout in 1935 [1]. Neurilemmoma produces distinct patterns referred to as Antoni A and Antoni B [2]. It has a predilection for head, neck, and surface flexors of the upper and lower extremities [3], with 25–45% of all schwannomas occurring in the head and neck region, the tongue being the most common site [4]. Cranial nerves I and II are not sites for this tumour as they lack Schwann cells [5]. One percent of schwannomas occur in the intraoral region [6, 7]. Among the intraoral lesions, tongue is the most common site, with rare occurrence in the upper lip [7, 8].

CASE OUTLINE

A 21-year-old apparently healthy male reported to the Department of Oral and Maxillofacial Surgery, Government Dental College, Kottayam, India, with a two-year-old painless, slow-growing swelling on the inner aspect of the upper lip (Figure 1). He had history of trauma to the region seven years back, followed by root canal treatment and full crown restoration on teeth 21 and 22.

On examination it was an ovoid, firm, mobile mass approximately 3 × 2 cm in size, exhibiting a smooth, non-ulcerated, non-erythematous surface. The swelling was in the midline and extended to the labial vestibule (Figure 2). With a provisional diagnosis of traumatic fibroma, an excisional biopsy was performed under local anesthesia. The lesion was encapsulated and this facilitated the meticulous dissec-

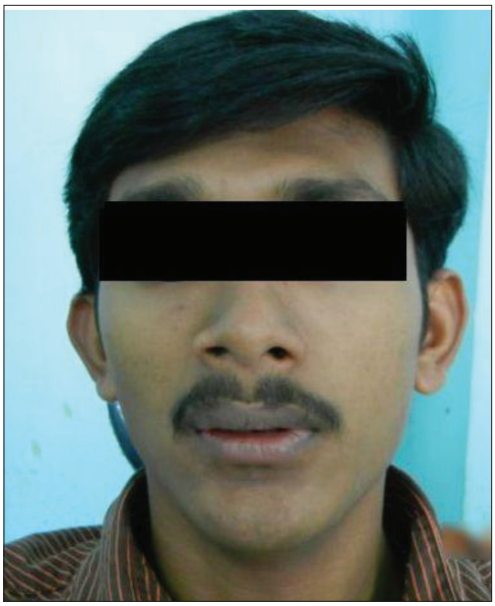

Figure 1. Preoperative view of the patient

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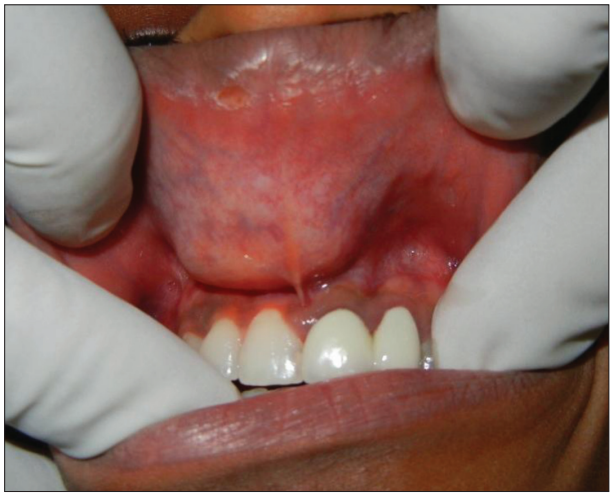

Figure 2. Preoperative – intraoral view

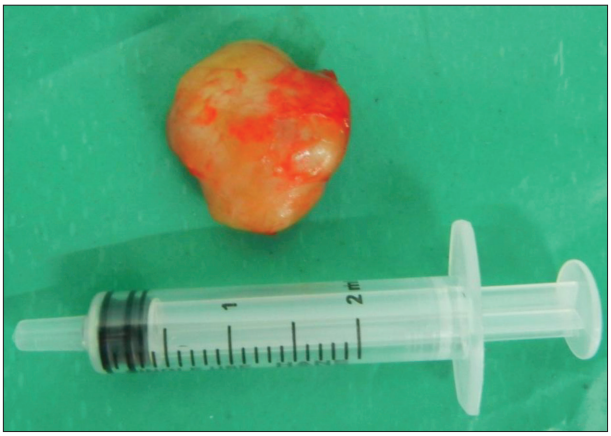

Figure 4. Photograph of excised gross specimen

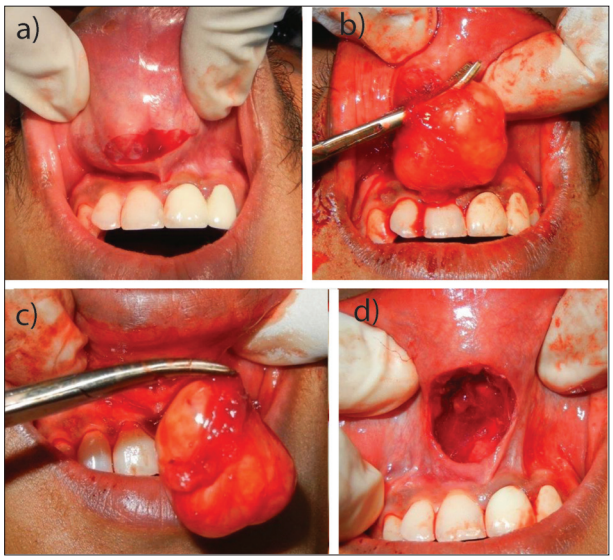

Figure 3. Intraoperative view: a) incision; b) meticulous dissection; c) excision of the lesion in toto; d) excised bed

tion. The surgical bed was thoroughly cleaned, hemostasis achieved, and wound closure was done (Figure 3).

The gross specimen was yellowish with a smooth, shiny surface (Figure 4). Upon histopathologic examination, both Antoni type A tissue, made up of cells with spindle shaped nuclei arranged in a palisading pattern, and Antoni Type B tissue, showing disorderly arranged cells and fibers, were seen (Figure 5). A diagnosis of schwannoma was made.

The postoperative period was uneventful and the patient is disease free after a year of follow-up.

DISCUSSION

Schwanoma is also known as neurilemmoma, neurinoma, lemmoma, and perineural fibroblastoma [9]. Tissue culture studies by Murray and Stout confirmed the Schwann cell origin when they cultivated the tumour in vitro. They usually present as solitary encapsulated slow growing lesions unless associated with neurofibromatosis. Despite the nerve tissue origin, they are painless. They cause pain only when they cause pain on adjacent nerves, rather than on the nerve of origin [9]. This case showed gradual increase in size and was otherwise asymptomatic. Frequency of lip lesions is comparatively less [8].Infraorbital nerve schwannomas can present as lip masses [10]. Rarely, multinodular neurilemmomas are also seen [11]. Central lesions which cause bony destruction can pres-

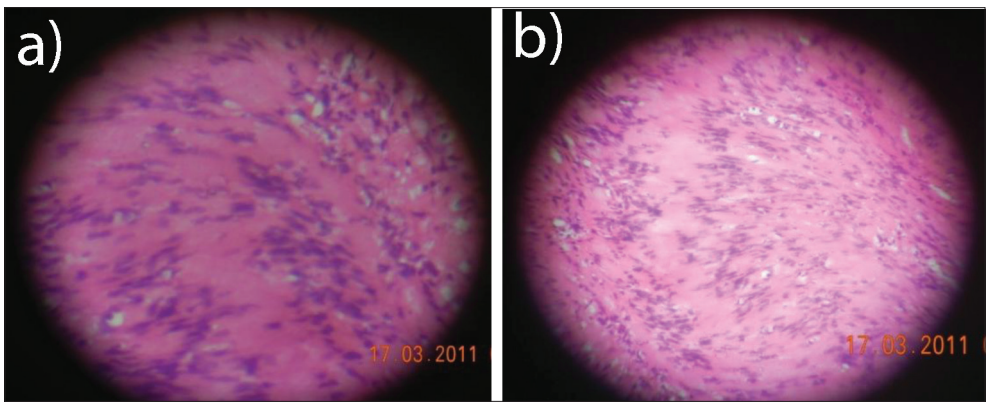

Figure 5. a) Photomicrograph showing Verocay body with palisaded arrangement of nuclei (H&E, × 45); b) photomicrograph showing Antoni type B tissue (H&E, × 10)

ent as unilocular or multinodular radiolucencies that are centered on the inferior alveolar nerve [2]. They may have a true capsule or a psuedocapsule made of fibrous connective tissue [4]. This lesion was encapsulated, which aided in complete removal.

Ultrasound scan with fine needle aspiration biopsy can be diagnostic in 30% and magnetic resonance imaging in 77% of cases [12]. Ultrasound scans show homogenous and hypo-echogenic findings and post-acoustic enhancement. Computed tomography scans show definitely marginated mass with homogenous soft tissue density. Magnetic resonance imaging scans demonstrate a homogenous lesion with low intermediate signal intensity on T1-weighted and high signal intensity on T2-weighted images [13]. This mass was not subject to any such investigation as we relied entirely on our clinical assessment.

Treatment of choice is surgical excision [14]. Recurrence is uncommon [4, 7, 14]. Malignant transformation of schwannomas is rare [7, 15]. Das Gupta and Brasfield [16] reported 8% incidence of malignant schwannomas in the head and neck region. Ghosh et al. [17] reported 13.9% incidence. A provisional diagnosis of traumatic fibroma was made based on the prior history of trauma. Minor salivary gland neoplasms and mesenchymal tumors can also be considered as possibilities.

Histopathology shows two types of tissue – Antoni type A and Antoni type B. The cells in Antoni Type A have elongated or spindle shaped nuclei aligned to form a characteristic palisading pattern. Intercellular fibers are arranged parallel to the nuclei, giving the impression of organoid swirls [4].Verocay bodies are central, acellular, eosinophilic bodies with reduplicated basement membrane and cytoplasmic processes. Antoni type B tissue shows oval nuclei and disordered cells and fibers with edema fluid and microcysts [9]. There is no myelin as no axis cylinders exist to induce myelin formation by the Schwann cells. Tumor cells also show diffuse positive immunohistochemical reaction for S-100 protein [9]. This lesion exhibited all the classic histopathologic features. If the nerve of origin is visualized, all attempts should be made to isolate it [18]. Here the nerve of origin could not be identified.

It is customary to submit all excised tissue for histopathologic analysis. This case report underscores the importance of the above tradition. Though lesions like the schwannoma are the exception rather than the norm, it is becoming of a prudent clinician to be on the lookout for such rare entities.

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Шваном горње усне – приказ болесника и преглед литературе

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САЖЕТАК

Увод Шваноми или неурилемоми су јасно ограничени, бенигни тумори нервног порекла који настају фибробластном пролиферацијом омотача нерава (Шванових ћелија). Обично се манифестују као солитарне инкапсулиране лезије, а ретко се развијају у горњој усни. Не постављање адекватне дијагнозе шванома представља велики ризик за даљи раст тумора и појаве притиска на суседне нерве. Ови тумори у зависности од локализације могу довести до слабости или парализе, осећаја притиска у уху, тинитуса, губитка слуха и равнотеже, као и по живот опасних стања.

Приказ болесника Овај случај представља ретку манифестацију шванома у горњој усни 21 године старог мушкарца индијског порекла. Пацијент се жалио на отицање у пределу усне дупље и тешкоће да спои усне. Тумор је хируршки уклоњен и рана је уредно зарасла.

Закључак Локализација шванома у горњој усни је изузетно ретка, али се мора разматрати у диференцијалној дијагнози тумора усне дупље јер лако може доћи до превида.

Кључне речи: шваном; неурином; неурилемом; орални тумор; глава и врат

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CASE REPORT / ПРИКАЗ БОЛЕСНИКА

Diagnostic imaging and biochemical findings of rare inherited X-linked adrenoleukodystrophy in a child

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SUMMARY
Introduction Adrenoleukodystrophy (ALD) is a rare genetic disease, caused by mutations in *ABCD1* gene located on the X chromosome (X-ALD), underdiagnosed worldwide.
Case Outline We present a clinical case of a six-year-old boy with childhood cerebral X-ALD. Magnetic resonance imaging of the patient's brain showed bilateral lesions similar to ALD in parietal-occipital lobes of the brain. Plasma very long chain fatty acids determination test showed an elevated level of C26 and C26/C22 ratio which confirmed the diagnosis of X-ALD.
Conclusion The key point of this clinical case report is to draw attention of physicians to the earliest possible recognition of X-ALD patterns, because an effective treatment can only be established for early-stage cerebral ALD.
Keywords: X-linked adrenoleukodystrophy; fatty acids; MRI

INTRODUCTION

Adrenoleukodystrophy is a rare genetic disease caused by mutations in the *ABCD1* gene, which maps to Xq28 chromosome, thus it is commonly called X-linked adrenoleukodystrophy (X-ALD). The occurrence of X-ALD is one in 20,000 to 50,000 individuals worldwide [1, 2]. *ABCD1* gene mutation causes the encoded ATP-binding cassette transporter protein inability to transfer CoA (coenzyme A)-activated very long chain fatty acids (VLCFA) into peroxisomes for their β-oxidation. Non-degraded VLCFA (carbon atoms ≥ C22) accumulate in tissues or body fluids and usually cause nervous system demyelination and adrenal insufficiency [2, 3]. Several phenotypes of X-ALD can be distinguished: childhood cerebral ALD, adolescent cerebral ALD, adult cerebral ALD, adrenomyeloneuropathy, Addison disease only, and women with X-ALD [2].

The aim of this study is to report a rare genetic disease caused by mutations in the *ABCD1* gene located on the X chromosome (X-ALD).

CASE REPORT

We present a case of childhood cerebral X-ALD in a six-year-old boy. The onset of the disease was observed at the age of five years. The first sign of the disease was an episodic lateral deviation of the right eye. Vomiting and febrile temperature appeared approximately at the same time. Symptomatic treatment was administered. Complaints of reduced hearing and eyesight appeared three months later. Furthermore, chronic bilateral neuritis of cochlear nerves was diagnosed by an otorhinolaryngologist and was treated with piracetam and vitamins B6 and B12. Piracetam (900 mg per day) was prescribed to prevent vertigo, and vitamins B6 and B12 were prescribed to treat neuritis. Blurred vision was corrected with glasses. There was no response to the treatment and the condition was slowly aggravating. The boy became hulky, complained of headaches in the forehead followed by sleep and articulation disorders. The patient was hospitalised in pediatric neurology unit because of complaint of blindness, nine months after the first symptom of the disease was recorded. On clinical examination the child's sight was diagnosed as abnormal, he could not appropriately respond to given orders or questions. Tendon reflexes and the Babinski sign were stronger on the left half of the lower limbs. Photoreaction on the left pupil was reduced, while there was no reaction observed on the right. Meningeal symptoms were negative. Other systems did not show any abnormalities. Blood count

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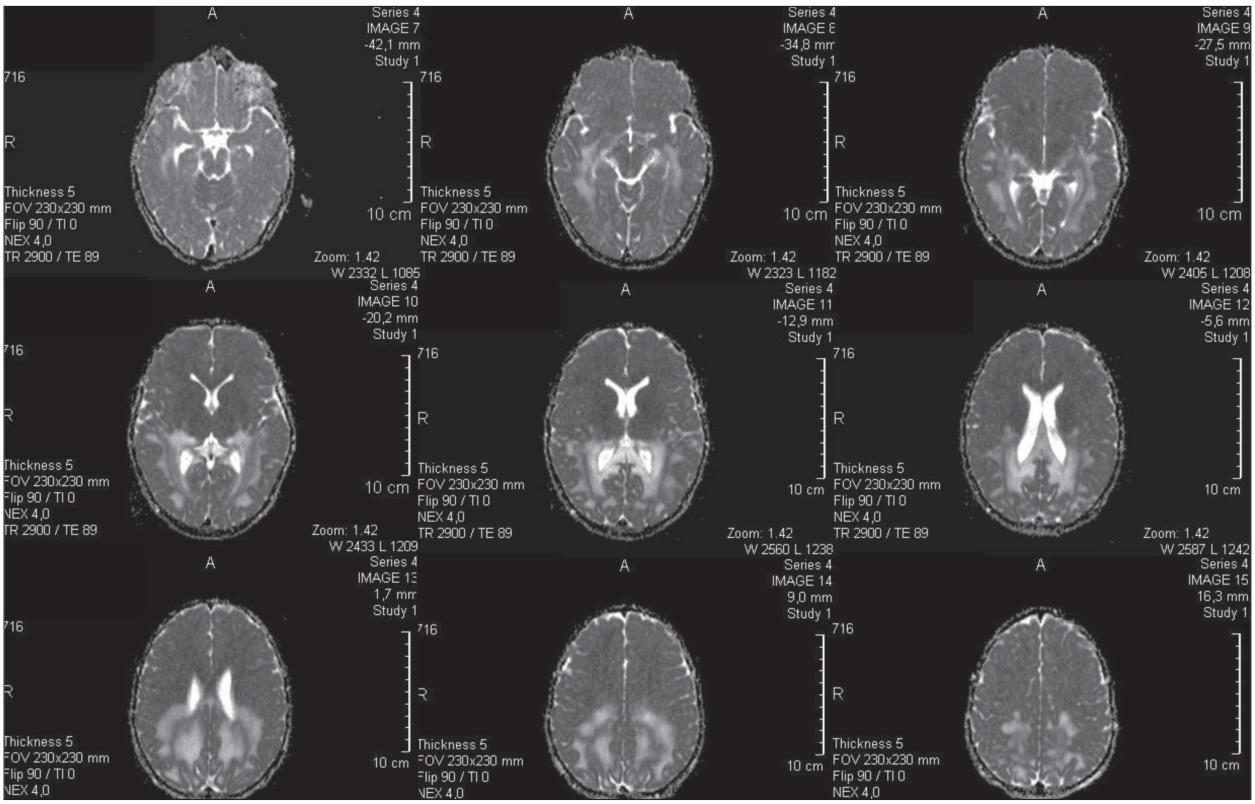

Figure 1. Brain MRI of the six-year-old child with X-linked adrenoleukodystrophy; bilateral symmetrical areas of hyperintense T2-weighted signals are noted in temporal, occipital, and parietal white matter of cerebrum; post-contrast study showed peripheral enhancement of these areas; similar hyperintense areas were found in pons and mesencephalon, corresponding to medial and lateral lemniscus

and chemistry, electrolytes, glucose levels were normal. Computer tomography (CT) with and without contrast was performed. There was a bilateral lesion similar to adrenoleukodystrophy in parietal-occipital lobes of the brain. After that, brain magnetic resonance imaging (MRI) and level of adrenocorticotropic hormone were performed. MRI showed bilateral symmetrical areas of hyperintense T2-weighted signals noted in temporal, occipital and parietal white matter of cerebrum. Post-contrast study showed peripheral enhancement of these areas. Similar hyperintense areas were seen in pons and mesencephalon corresponding to medial and lateral lemniscus (Figure 1). These classical symmetrical occipital white matter lesions were typical of ALD [4]. Adrenocorticotropic hormone was highly increased, so replacement therapy with hydrocortisone was recommended. Other instrumental examination involved BERA (brainstem-evoked response audiometry) and abdominal ultrasound, which were normal.

Plasma very long chain fatty acids (VLCFA) determination test confirmed the diagnosis of X-ALD. There was an elevated level of C26 and C26/C22 ratio in the VLCFA test (Table 1). The diagnosis was made according to the clinical presentation, MRI, and the fatty acid profile; however, *ABCDI1* gene mutations on the X chromosome were not tested.

Apart from the proband, his healthy two-year-old brother was also tested for the fatty acids profile. It was not elevated, even C22 was below the range (Table 2), thus the possibility of the onset of the disease in the younger sibling was excluded.

Table 1. X-ALD patient's and two-year-old healthy brother's profile of very long chain fatty acids

Long chain fatty acid	X-ALD patient	Healthy brother	Normal range
C22	41.3 µmol/l	36.3 µmol/l	41.9–119 µmol/l
C24	77.8 µmol/l	29.4 µmol/l	20.3–96.1 µmol/l
C26	3.34 µmol/l	0.36	0.18–1.06 µmol/l
C24/C22	1.883	0.812	0.39–1.38
C26/C22	0.08	0.01	0.002–0.021

C – number of carbon atoms of the fatty acid chain; C24/C22 – ratio of C24 to C22; C26/C22 – ratio of C26 to C22

Table 2. Two-year-old healthy brother's profile of very long chain fatty acids

Long chain fatty acid	Value	Normal range
C22	36.3 µmol/l	41.9–119 µmol/l
C24	29.4 µmol/l	20.3–96.1 µmol/l
C26	0.36	0.18–1.06 µmol/l
C24/C22	0.812	0.390–1.38
C26/C22	0.01	0.002–0.021

C – number of carbon atoms of the fatty acid chain; C24/C22 – ratio of C24 to C22; C26/C22 – ratio of C26 to C22

DISCUSSION

X-linked ALD leads to demyelination of the nervous system, adrenal insufficiency, and accumulation of long-chain fatty acids [2]. The clinical course in ALD is characterised by behavioural disorders, ataxia, visual loss, decreased hearing and epileptic seizures, followed by mental deterioration, psychosis, and death. Abnormal skin pigmentation and

other features of adrenal insufficiency may become apparent before neurological symptoms. The diagnosis of X-ALD is confirmed by analysing the plasma levels of VLCFAs or identifying aberrant mutations in the *ABCD1* gene [1].

X-linked ALD is a white matter disease, which can initially present with psychiatric symptoms and thus be misdiagnosed as a primary psychiatric disorder. Behavioural and emotional changes develop prior to progressive deterioration of vision, hearing, and motor functions. In our patient, the first symptoms were observed as episodic lateral deviation of the right eye. Within a few months, the child's cognitive abilities and speech deteriorated, and difficulty in walking developed accompanied with behaviour changes.

Many tests had been performed over approximately eleven months until the diagnosis of X-linked ALD was confirmed by VLCFA analysis. The main goal of this clinical case is to draw attention of doctors to recognize X-ALD as early as possible, because the only possible treatment is for the early-stage disease. It is the allogeneic hematopoietic cell transplantation that allows the stabilization of the disease [5].

The important point is that the patient has a two-year-old brother, whose fatty acids profile showed no abnormalities. Thus, the conclusion was that he would not develop a neurologic disease later on. Prior risk for the brother in current X-linked disorder to inherit the mutation was increased, since the mother is a possible carrier of the mutation. However, a *de novo* mutation cannot be excluded in the affected child, as well as gonadal mosaicism in the mother. Early diagnosis (presymptomatic), could be useful due to some new therapeutic methods, currently available.

Previously, the treatment of X-ALD was symptomatic – for example, steroid use for adrenal insufficiency and psychotropics for psychiatric symptoms. No clearly effective treatments are available, although Lorenzo's oil (4:1 glyceryl trioleate and glycerytrierucate) used before the age of six years may reduce the probability of developing neurological deficits in later life [4, 5]. A new mode of treatment for X-ALD is focused on hematopoietic cell transplantation, especially in the early-stage ALD. Good therapeutical outcomes are achieved only if the treatment is taken at an early stage of the disease. In addition, if the phase of demyelination has started, hematopoietic stem cell transplantation leads to the worsening of the disease. Also, the transplantation does not improve adrenal function. This shows the importance of the benefits of an early diagnosis. Also, *ABCD2*, which encodes ALDRP or ABCD2 proteins and which is the closest homolog of *ABCD1*, could be potentially used in gene therapy. Induced overexpression of *ABCD2* gene could be a possible treatment for X-ALD [6]. Recombinant adeno-associated virus serotype 9 (rAAV9) vectors were used for the delivery of the human *ABCD1* gene to the mouse central nervous system [7, 8]. Current article points to the importance of confirmation of the diagnosis by radiological and biochemical methods, which is beneficial for genetic prognosis, early treatment, and sibling testing.

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Морфолошки и биохемијски показатељи ретке наследне адренолеукодистрофије везане за *X*-хромозом код детета

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САЖЕТАК

Увод Адренолеукодистрофија (АЛД) ретка је наследна болест услед мутације гена *ABCD1* на хромозому *X* (*X*-АЛД).

Приказ болесника Приказан је случај шестогодишњег дечака са церебралном *X*-АЛД. Магнетна резонанца (МР) мозга је показала обостране лезије сличне АЛД у перијето-окципиталном режњу. Тест веома дугих ланаца масних киселина у плазми показао је повишен ниво *C26* и однос *C26/C22* и потврдио дијагнозу *X*-АЛД.

Закључак Ефикасно лечење *X*-АЛД могуће је у раној фази болести, када се дијагноза може поставити наведеним морфолошким и биохемијским показатељима.

Кључне речи: *X*-везана адренолеукодистрофија, масне киселине, МР

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CASE REPORT / ПРИКАЗ БОЛЕСНИКА

Two-stage surgical repair of type II acute aortic dissection and aortic coarctation in a 12-year-old child

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SUMMARY
Introduction Combination of acute aortic dissection associated with aortic coarctation in pediatric population is extremely rare. We are presenting a 12-year-old patient with these two conditions who was successfully treated with two-stage surgery.
Case Outline A boy with no trauma history was admitted for chest pain. The diagnosis of acute aortic dissection associated with aortic coarctation was established with echocardiography and computed tomography angiography. Emergent surgery was performed – excision of the ascending aorta aneurysm with supracoronary graft replacement and preservation of native aortic valve. Subsequently, through posterolateral left thoracotomy, the patient underwent end-to-end aortoplasty for coarctation repair.
Conclusion Two-stage surgery provides favorable outcome in this rare, life threatening condition in the pediatric age group. Native aortic valve was preserved and extra-anatomic bypass of aortic coarctation was avoided. Further monitoring of aortic valve is mandatory.
Keywords: ascending aorta aneurysm; pediatric; coarctation

INTRODUCTION

Rupture of dissecting aneurysm is an exceptionally rare, life-threatening condition in children and young adolescents [1]. Furthermore, the combination of acute aortic dissection associated with aortic coarctation in this age group is sparsely reported. Infrequent pediatric reports are mostly related to patients with Turner syndrome and other connective tissue disorders [1]. Co-existing aortic dissection and coarctation have been addressed in various ways. There are several previous reports of two-stage repair, as well as one-stage repair mainly using extra-anatomic ascending-to-descending aortic bypass. Hereby we present, to our knowledge, the youngest patient with this condition treated with staged procedures.

CASE REPORT

A 12-year-old boy with no previous medical history was admitted for distressing parasternal chest pain, accompanied with shortness of breath and dizziness. There was no trauma history. On clinical examination, radial pulses were present, whereas femoral pulses were barely palpable. A systolic murmur 2–3/6 was noted in the precordium. Electrocardiography indicated sinus rhythm with transitory ST elevation during the episodes of chest pain. Transthoracic echocardiography demonstrated hypertrophied left ventricle, trivial incompetence (+0.5/4) of bivelar aortic valve with annulus of 2.2 cm, and significant pericardial effusion. Mitral valve was dysplastic but competent. A posterior intimal flap was detected above the aortic valve with an aneurysmal dilatation of the ascending aorta (6 cm). Furthermore, the coarctation of the aortic isthmus was demonstrated with a gradient of 50 mmHg. A computed tomography scan showed identical findings with the intimal flap extending below the origin of the innominate artery (Figure 1).

With a diagnosis of type II aortic dissection and associated aortic coarctation, emergent surgery was performed through a median sternotomy. During routine anesthetic preparation, arterial pressure lines were placed in the right radial and left femoral artery. Upon pericardium

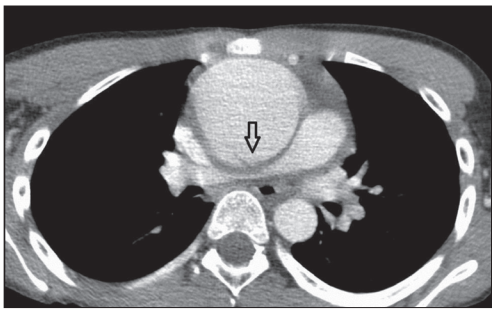

Figure 1. Computed tomography angiography: posterior intimal flap in aneurysmatic ascending aorta

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opening and draining of 600 ml of blood, grossly dilated, dissected aorta appeared. The aortic annulus was not dilated. The aneurysmatic change was above the sinotubular junction and stretched to 1.5 cm below the origin of innominate artery. Single arterial cannulation was performed high in the aortic arch, just below the innominate artery, the right atrium was cannulated with a two-stage venous cannula. Cardiopulmonary bypass was established with systemic cooling to 28°C. Pressure in the femoral artery was sufficient throughout the procedure (over 50 mmHg). The ascending aorta was highly cross-clamped, opened, and cardioplegia was infused in the coronary ostia.

The entry point of dissection was identified at the posterior aortic wall 2 cm above the right coronary ostium (Figure 2). The aortic valve was bicuspid, though it appeared as competent and anatomically normal. After excising the aneurysm, aortic valve was preserved and resuspended with a double layer of Teflon felt inside and outside the free margin of the proximal aorta. A 24 mm Dacron graft was anastomosed here, whereupon saline injection into the neo-aortic root demonstrated good aortic valve competence. The distal ascending aortic anastomosis was performed in the same manner with normal aortic wall just below the cross clamp. At the end of the procedure, after rewarming to 37°C, the patient was easily weaned off cardiopulmonary bypass.

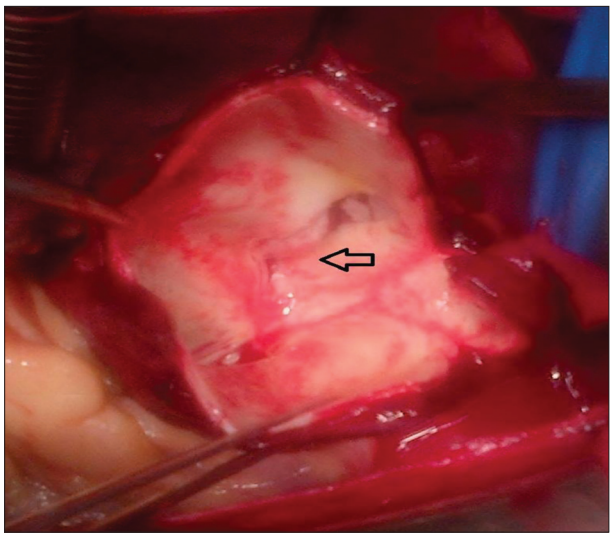

Figure 2. The entry point of dissection at the posterior aortic wall, 2 cm above the right coronary ostium

Postoperatively, he was extubated the following morning. Postoperative echocardiography demonstrated hypertrophied left ventricle with aortic valve insufficiency of +2.5/4, and mitral valve insufficiency of +1.5/4.

After achieving full recovery during the same hospitalization, the patient was operated on for aortic coarctation. Aortoplasty with coarctation resection and standard "end-to-end" anastomosis was performed through left posterolateral thoracotomy at the fourth intercostal space. Multiple collateral vessels of the descending aorta were observed.

Postoperatively, the patient was extubated five hours after the surgery. Intensive care unit stay was two days. The main postoperative complication was hypertension. He was discharged on enalapril and metoprolol, ten days after the second operation. Echocardiography demonstrated reduction of both mitral (+1/4) and aortic valve regurgitation (+2/4), good left ventricle function, and no residual gradient at the place of aortoplasty.

At 24-month-follow-up, the patient is asymptomatic, normotensive, still on metoprolol. Repeated echocardiography showed no further progression of aortic and mitral regurgitation, and no signs of left ventricle function deterioration.

DISCUSSION

The association between coarctation and aortic dissection has been described in early studies of the natural history of the aortic coarctation [2]. However, reports of this condition in pediatric population are extremely rare [3]. The usual onset of dissection is the adolescent period rather than childhood, and, to our knowledge, this is the youngest patient presented with this life-threatening condition. The most common predisposing factors in children are connective tissue disorders as Marfan's, Turner's, and type IV Ehlers–Danlos syndrome. These disorders usually have clear physical stigmata. None of those stigmata were present in the described case, our patient had been practicing water polo actively. Nevertheless, Hatzaras et al. [4] reported that grueling physical activity with severe emotional stress are clear precipitating factors of acute dissection. Furthermore, swimming has been reported to precipitate acute aortic dissection in the absence of any predisposing factors [5]. We can speculate that the mentioned factors, alongside with idiopathic dilatation and hypertension due to coarctation, were the main etiological factors for dissection.

The repair of aortic dissection in the presence of coarctation comprises a few difficulties: decision on the optimal timing and sequence of the surgical repair, optimal surgical exposure, and perfusion techniques. Several surgical options have been reported. Sampath et al. [6] first described staged approach with initial aortoplasty followed by dissection repair. On the other hand, there is an opinion that dissection repair as a lifesaving procedure takes precedence over coarctation repair. The first single-stage repair was described by Svensson [7] in 1994.

In the reported case we performed a two-stage strategy with initial repair of dissection. Clearly, primary coarctation repair was not an option because the patient required immediate repair of the acute dissection and relief of the cardiac tamponade. Furthermore, giving the extreme rarity of this condition, relatively low gradient over the coarctation, and unknown dissection duration time, we decided to proceed with the two-stage strategy as a safer alternative. We were able to maintain adequate blood flow on cardiopulmonary bypass through a single arterial cannula thanks to well-developed collateral vessels and relatively low gradient across coarctation. In the presence of nondilated aortic annulus, normal aortic sinuses, and functional bicuspid valve, we decided not to replace the valve.

Initial level of aortic and mitral regurgitation before coarctation repair raised numerous doubts about that decision. Fortunately, insufficiency of both mitral and aortic valve were reduced after the aortoplasty, and in the follow-up there has been no deterioration in the left ventricle function. We are aware that valve replacement will most likely prove necessary. In the meantime, the patient will hopefully complete growth and will be spared from problems related to anticoagulation therapy. One can speculate that valve-sparing aortic root implantation with a vascular graft would be a better solution, but this particular operation on bicuspid valve remains challenging [8]. The mechanical valve inserted into a composite graft is known for long-term durability, but young patients are exposed to a long-term risk of thromboembolism and oral anticoagulation. One clear advantage of two-stage repair is "end-to-end" aortoplasty, which is a far better solution for coarctation than extra-anatomical bypass grafting from the ascending to descending aorta as a standard technique in a single-stage procedures. One other option would be stenting of coarctation – nevertheless, regarding the patient's age, we chose to give priority to surgery.

In summary, we successfully performed a two-stage repair with the preservation of the aortic valve, replacement of the ascending aorta with Dacron graft and "end-to-end" aortoplasty for acute type II aortic dissection with coarctation. Further monitoring is mandatory for assessing the fate of the native aortic valve.

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Хируршко збрињавање у два акта 12-годишњег детета са дисекцијом анеуризме асцендентне аорте удружене са коарктацијом аорте

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САЖЕТАК

Увод Комбинација акутне аортне дисекције удружене са коарктацијом аорте је веома ретка у педијатријској популацији.
Приказ болесника Дечак стар 12 година, без анамнестичких података о трауми, примљен је због болова у грудном кошу. Дијагноза акутне дисекције анеуризме асцендентне аорте и аортне коарктације је постављена ехокардиографски и потврђена компјутеризованом томографијом**.** Учињен је хитан хируршки захват у виду ресекције анеуризме и супракоронарне уградње невалвулираног кондуита уз пре-зервацију нативне аортне валвуле. Након тога учињена је аортопластика из постеролатералне торакотомије ради збрињавања аортне коарктације.
Закључак Етапно двостепено лечење овог ретког животно угрожавајућег стања обезбеђује добар исход. Сачувана је нативна аортна валвула и избегнуто екстраанатомско премошћавање аортне коарктације. Неопходно је даље праћење функције аортне валвуле.
Кључне речи: анеуризма асцендентне аорте; деца; коарктација аорте

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CASE REPORT / ПРИКАЗ БОЛЕСНИКА

Gastric sarcoidosis

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SUMMARY

Introduction Sarcoidosis is a systemic disease with a 90% predilection for the lungs, but any organ can be involved. Gastric sarcoidosis may be a component of a generalized process, while isolated gastric sarcoidosis is very rare.

Case Outline We describe a rare case of biopsy-proven gastric sarcoidosis in a 45-year-old woman with pulmonary sarcoidosis in remission, and highlight the importance of gastroscopy and biopsy to confirm the diagnosis. Her optimal response to anti-acid therapy required no alternate (glucocorticoid) therapy. We briefly review the clinical, diagnostic, and therapeutic aspects of gastric sarcoidosis.

Conclusion Glucocorticoids remain the cornerstone of the sarcoidosis treatment, although it has been insufficiently documented by clinical trials. The decision to treat sarcoid patients with systemic glucocorticoids is largely based upon the severity of symptoms. The anti-acid therapy may be an alternative in milder cases, as demonstrated in our patient.

Keywords: sarcoidosis; stomach diseases; granuloma

INTRODUCTION

Sarcoidosis is a systemic disorder of uncertain etiology, characterized by accumulation of mononuclear inflammatory cells, followed by the formation of non-caseating epithelioid granulomas at the site of involvement. Sarcoidosis is localized in the lungs in more than 90% of cases [1]. Gastrointestinal manifestations excluding liver involvement (which occur in 40–70% of cases) are a rare form of extrapulmonary sarcoidosis [2]. The stomach is the most commonly affected organ in gastrointestinal sarcoidosis. It is estimated that gastric sarcoidosis with the associated symptomatology is observed in less than 1% of patients with sarcoidosis [2]. However, it has been suggested that sarcoid granulomas may be observed in the stomach of as many as 10% of patients with pulmonary manifestation of this disease without causing any significant gastrointestinal complaints [3]. Only 1% of the cases of a systemic disease have symptomatic gastric involvement [3]. An autopsy study of patients with sarcoidosis, however, reported gastro-duodenal involvement in 4–10% of the cases [2, 3].

CASE REPORT

A 45-year-old woman with pulmonary sarcoidosis in remission developed epigastric pain. The diagnosis of pulmonary sarcoidosis (stage II) without extrapulmonary disease manifestations had been established ten years previously, when she, complaining on exertional dyspnea, underwent pulmonary and hilar lymph node biopsy revealing non-caseating granulomas. Her symptoms resolved with a six-month course of systemic glucocorticoids. Over the last five-month period, 10 years after the initial diagnosis of sarcoidosis, she developed intermittent abdominal pain exacerbated by food and associated with nausea, early satiety, anorexia, and a slight weight loss. She had been examined by a gastroenterologist. The physical examination was remarkable for mild tenderness in the epigastric region. Esophagogastroduodenoscopy was performed, revealing a mucosal erythema (Figure 1). Random biopsies were taken from different sections of the stomach.

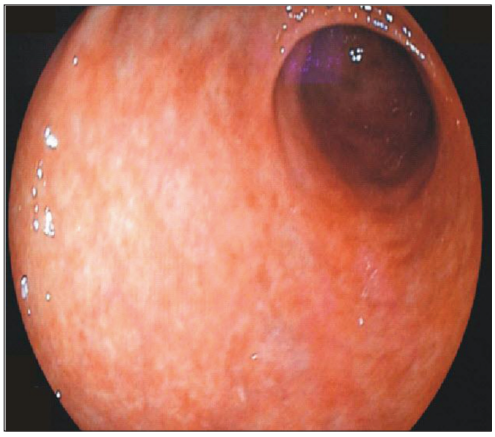

Figure 1. Upper gastrointestinal endoscopy showing mucosal erythema

The histological examination of the biopsy specimens from the gastric fundus and antrum

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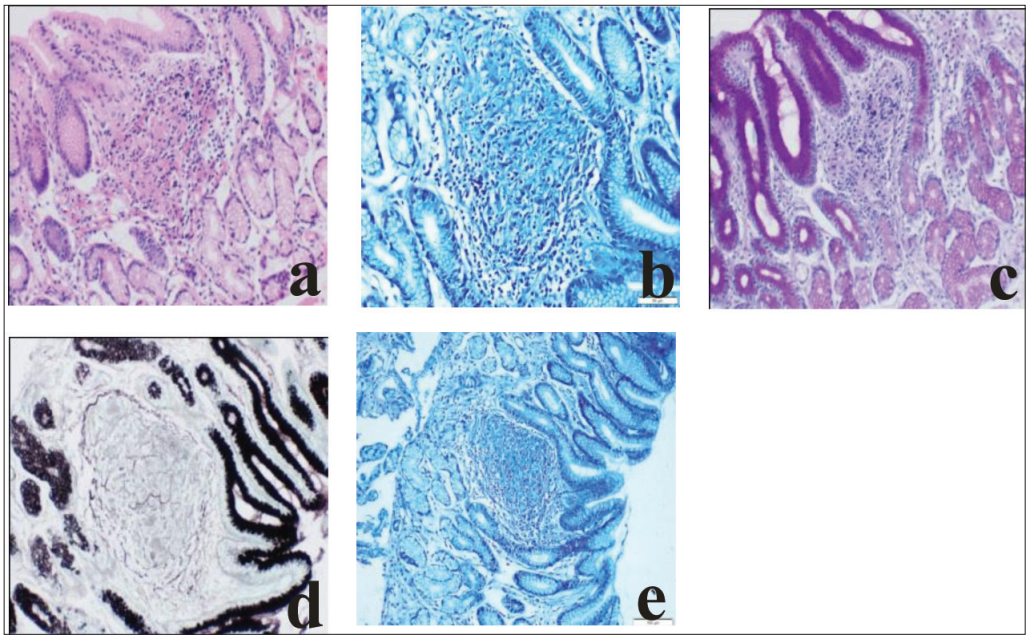

Figure 2. Sarcoid granuloma in the gastric mucosa: a) H&E, × 20; b) Ziehl–Neelsen, × 20; c) PAS, × 20; d) Grocott, × 20; e) Giemsa, × 10

showed non-necrotizing granulomas composed of epithelioid cells and lymphocytes (Figure 2). Biopsy samples were stained for *Helicobacter pylori* (Giemsa staining), acid-fast bacilli (Ziehl–Neelsen staining), and fungi (PAS and Grocott staining), and were all negative (Figure 2). The acid-fast staining and culture of the gastric aspirate were negative, as well as the serologic testing for *Treponema*. Other laboratory analyses in the differential diagnosis were negative as well.

After that, a total colonoscopy was performed providing normal findings. Ultrasound of the upper abdomen and abdominal computed tomography findings were also normal. The chest X-ray finding demonstrated no evidence of hilar lymphadenopathy or lung lesions. Laboratory studies at that time showed a normal complete blood count and no elevation in either the erythrocyte sedimentation rate, or the C-reactive protein (CRP) level. The serum and 24-hour urine calcium levels, as well as the serum angiotensin-converting enzyme (ACE) level were within reference ranges. The ophthalmologic evaluation did not reveal any changes characteristic of granulomatous inflammation.

With the former history of sarcoidosis, on the basis of the clinical history, physical examination, and histological findings, the diagnosis of gastric sarcoidosis was established.

A proton pump inhibitor was introduced and the patient had an alleviation of the symptoms in a few days. On controls, the patient was free of symptoms. She had routine follow-up gastroscopies and gastric mucosa biopsy sampling regularly performed in six-month intervals. Biopsy samples histopathology revealed persisting non-caseating epithelioid cell granulomas. After one year, histopathological analysis did not reveal any changes in the gastric mucosa (spontaneous or treatment-induced remission), and medicamentous therapy was suspended. No recurrence of symptoms occurred in two years of monitoring.

DISCUSSION

Among patients with sarcoidosis, the disease course is highly variable, and thus patients may present with a wide array of clinical manifestations and multiple non-specific symptoms. Fatigue is an integral part of the clinical picture of sarcoidosis [4]. Sarcoidosis can involve any organ, with pulmonary involvement being the most common one. Gastric sarcoidosis may be a component of a generalized process, while isolated gastric sarcoidosis is very rare. It mainly affects the antrum of the stomach [5, 6].

Gastric sarcoidosis may not cause any significant symptoms. The clinical manifestation of gastric sarcoidosis varies greatly and is obviously rather non-specific. Symptoms of gastric sarcoidosis are due to granulomatous inflammation and associated fibrosis of the gastric wall. Epigastric pain, emerging most commonly after meals, is the most common symptom. Other symptoms are nausea, vomiting, early satiety, anorexia, and weight loss [6, 7]. Complications are also various, and include a delayed gastric emptying, pyloric or duodenal obstruction, vitamin B12 deficiency, hematemesis and melena. Rarely, massive gastrointestinal bleeding occurs. The clinical symptoms of gastric sarcoidosis often lead to misdiagnoses of malignant or peptic disease [8].

Our case report is a warning that individuals with sarcoidosis in remission are still at risk of other, unrelated organ manifestations of the disease. In patients with a history of sarcoidosis, either active or in remission, presenting with gastrointestinal symptoms, the possibility of gastric sarcoidosis should be considered.

Gastroscopy, along with biopsy sampling, is essential in establishing the diagnosis of gastric sarcoidosis. In asymptomatic patients, the gastric mucosa may be normal and therefore unjustifiably disregarded during endoscopy

[3, 7]. The upper endoscopy with biopsy sampling is pivotal to establish the definitive diagnosis, even with the normal-looking mucosa. Mucosal ulcers with or without erythema, thickened mucosa, polypoid/nodular lesions (due to granulomas), diffuse infiltration of the mucosa (appearing as linitis plastica) and fibrosis may be seen as well. In gastric sarcoidosis, the following four principal categories of lesions have been distinguished: subclinical (usually asymptomatic and the most common), ulcerative, infiltrative, and polypous [9].

The diagnosis of gastric sarcoidosis may be established on the basis of the histopathological evaluation of biopsies collected during endoscopy. It should be remembered that endoscopic biopsies sometimes fail to identify sarcoid lesions, as the granulomas may be localized in the submucosa and deeper layers of the gastric wall, not just in the mucous membrane [9]. The diagnosis of gastric sarcoidosis is difficult without evidence of involvement of other organs. Gastric sarcoidosis can mimic other gastrointestinal diseases in presentation and its diagnosis requires a proper interpretation of the obtained biopsy samples as many other etiologies can present with non-caseating granulomas. In the differential diagnosis, other granulomatous gastritis should be considered, including Crohn's disease, Whipple's disease, tuberculosis, syphilis, reaction to malignancy (sarcoid reactions in cancer) or foreign body, peptic ulcer disease, gastric cancer, hypertrophic gastritis, histoplasmosis, lymphoma, Langerhans cell histiocytosis, and Ménétrier disease [9, 10]. Idiopathic granulomatous gastritis is diagnosed when none of the aforementioned conditions are identified [11]. However, the clinical relevance of this entity is questionable [11].

Hilar lymphadenopathy with/without lesions in the lungs may co-exist with, or precede the development of sarcoid lesions in the gastrointestinal tract [12, 13, 14]. This was the case in our patient. Isolated gastric sarcoidosis is very rare [9, 10].

Imaging studies are less useful, but barium studies are sometimes used to document ulcerations, stenosis, mucosal thickening, and loss of normal distensibility of the stomach. Abdominal computed tomography scan can be useful to assess the presence of hepatosplenomegaly or regional and retroperitoneal lymph nodes in the case of concomitant extragastric sarcoidosis [5]. Functional imaging with 8-F-fluorodeoxyglucose positron-emission tomography and computed tomography (FDG–PET–CT) have proved very sensitive to assess the inflammatory activity in sarcoidosis. Imaging studies are useful initially to assess the extent of the disease and guide the biopsy, or later to follow the response to the treatment. In gastric sarcoidosis, however, FDG–PET scan is limited by the variable physiologic uptake of the gastric mucosa [5]. Our patient refused FDG–PET–CT.

There are no specific laboratory features in gastric sarcoidosis. Inflammatory markers such as CRP, erythrocyte sedimentation rate, and gammaglobulins can be found elevated. The serum ACE elevation is neither sensitive nor specific for extrapulmonary sarcoidosis. Other laboratory tests are used only to assist in ruling out alternative diagnoses [6].

There are no available clinical trials on the therapeutic management of gastric sarcoidosis, which is therefore mostly derived from the experience in pulmonary sarcoidosis [5]. The treatment of gastric sarcoidosis depends on symptoms – that is on both the severity and the extent of the disease [5, 15, 16]. Asymptomatic patients do not need any specific therapy [16]. In a mild disease, the anti-acid therapy with proton pump inhibitors can be attempted, since a response has been reported in some cases [3]. The optimal duration of the treatment with proton pump inhibitors is unknown [5, 10, 12]. The treatment with proton pump inhibitors relieved digestive symptoms, although a control biopsy of the gastric mucosa revealed persistence of non-caseating granulomas. The role of glucocorticoids in the treatment of gastric sarcoidosis is unclear. Systemic glucocorticoids ameliorated symptoms and induced the clinical disease remission, demonstrating the usefulness of the drug in treating two thirds (66%) of patients with gastric sarcoidosis [17]. Clinical improvement is not always aligned with the resolution of pathological lesions [6]. Oral prednisolone 20–40 mg per day is recommended with a gradual tapering regimen [9]. The optimal duration is unknown, but a minimum of 6–12 months seems reasonable [7, 17]. Glucocorticoids have been used alone, or in combination with proton pump inhibitors [3]. Still, further studies aimed at assessing the efficacy of glucocorticoids and proton pump inhibitors in treating patients with gastric sarcoidosis are needed. Our patient, who had moderate symptoms and mild changes in the gastric mucosa and no significant other organ involvement, did not receive glucocorticoids because she achieved an optimal response to anti-acid therapy.

In cases of contraindicated corticosteroid treatment, corticosteroid-resistant symptoms or need for a corticosteroid-sparing therapy, alternative agents (such as methotrexate, azathioprine, infliximab, hydroxychloroquine or chlorambucil) have been used, alone or in combination with corticosteroids [5, 17].

A surgery might be useful in rare cases when there is a severe gastric lumen narrowing, pyloric or duodenal obstruction, or massive hemorrhage [18].

Patients with longstanding gastric sarcoidosis may involve various degrees of fibrosis of the gastric wall. Other patients recover spontaneously. Gastric cancer associated with gastric sarcoidosis has hardly been reported [18]. This is why repeated gastroscopies and biopsies of the gastric mucosa were performed in our patient.

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Саркоидоза желуца

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САЖЕТАК

Увод Саркоидоза је системско обољење које код 90% болесника захвата плућа, мада било који орган може бити захваћен овом болешћу. Саркоидоза желуца може бити саставни део генерализоване болести, а изолована саркоидоза желуца је врло ретка.

Приказ болесника Описали смо редак случај саркоидозе желуца потврђен биопсијом код болеснице старе 45 година са саркоидозом плућа у ремисији. Указали смо на значај гастроскопије и биопсије за потврду дијагнозе. Оптималан одговор на антацидну терапију није захтевао примену алтернативне (гликокортикоидне) терапије. Изнет је кратак преглед клиничких, дијагностичких и терапијских аспеката саркоидозе желуца.

Закључак Глукокортикоиди су основа терапије саркоидозе, што, међутим, није у потпуности доказано клиничким испитивањима. Одлука о лечењу саркоидозе применом системских гликокортикоида углавном се заснива на тежини симптома. Антацидна терапија може бити алтернатива код благе саркоидозе желуца, као што је приказано у нашем случају.

Кључне речи: саркоидоза; болести желуца; гранулом

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CURRENT TOPIC / АКТУЕЛНА ТЕМА
PERSONAL VIEW ARTICLE / ЛИЧНИ СТАВ

Anti-vaccinationists and their arguments in the Balkan countries that share the same language

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SUMMARY
The objective has been an analysis of anti-vaccination situation in the language-related Balkan countries. Mass and organized opposition to vaccination in this part of the world is a relatively recent phenomenon. It has been an offshoot of the respective ideas from the West, associated with New Ageism, postmodernism, and similar worldviews, but particularly beefed up by MMR (measles, mumps, and rubella) vaccine fear in the late 1990s. The four key local leaders from four countries have been selected to represent the whole diversity of the Balkan anti-vaccination scene. Each of them exerts his/her influence throughout the region. The result is that vaccination coverage has substantially decreased in many areas. Outbreaks of vaccine-preventable diseases that have to follow sooner or later would eventually, at least temporarily, bring a blow to the credibility of anti-vaccinationists. We already witnessed such a trend in Bosnia and Herzegovina where vaccines were not readily available during the Bosnian wars in 1990s. As a result, major epidemics of measles, mumps, and rubella recently took place all over the country. A dynamic balance between the influence of anti-vaccination movement and the incidence of diseases, characterized by an inverse relationship (the more damaging impact of vaccine opponents on public health, the more cases of diseases, and vice versa) has been a pattern that health services have to deal with.
Keywords: anti-vaccinationists; vaccination; the Balkans

INTRODUCTION

Resistance to mandatory vaccination was never a major issue in the countries that comprised the former Yugoslavia. Some uneasiness was observed among urban parents in 1947, when mandatory BCG vaccination of schoolchildren had been introduced. The reason for obstruction was a memory to the Luebeck tragedy in 1929/1930, when 72 babies died due to the use of a virulent strain of *B. tuberculosis* [1]. There were no riots (the system was too autocratic to allow any form of disobedience), but some parents tried to keep their children at home on the day of vaccination. Health authorities solved the problem by repeated unannounced visits to school premises.

The author's intention was to portrait key opponents to vaccination in the centrally positioned Balkan countries, their general views, attitudes to vaccination, motives for engagement, and arguments.

REGIONAL COOPERATION OF ANTI-VACCINATIONISTS

Anti-vaccination (antivax) movement is a recent phenomenon in the Balkan countries. It swept over from the West, sharing the same triggers and similar manifestations. The key issue has been a fear of MMR (measles, mumps, and rubella) vaccine after the well-known Lancet article [2]. New Ageism, postmodernistic hype with a relativistic attitude to any (including scientific) truth, and an enthusiastic embracement of "natural" products found a fertile soil in the Balkans. In addition, reliance on the internet as a source of information and a wide use of electronic social networking made anti-vaccinationists (antivaxers) much more efficient in imposing their messages to the public as compared to rigid and sluggish medical services.

The 1990s wars left emotional scars and fairly tense relations between the nations that once comprised Yugoslavia. An amazing fact, however, is an excellent cooperation of the regional antivaxers. Thus, in 2013, a right wing Serbian Cristian Orthodox site Vaseljenska TV (http://www.vaseljenska.com/) took over from its Croatian counterpart news on Bosnia that read, "Bosnian activist reveals dark secrets of UNICEF, WHO and Bill Gates ... and efforts needed for not forgetting 2,249 small graves" [3]. The implication was that all infant deaths (2,249) over a period were due to vaccination. The concerned Bosnian activist denied the wording, but maintained that some of the children might have been victims of vaccination (Ms. J. Savić – written communication). Witnessing to quite a number of such examples of efficient collaboration between nations, this author referred to the once most popular socialist slogan and gave a title to one of his lectures "Brotherhood–unity of antivaccinists" [4].

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More recently, a major Serbian antivax group, Citizens' Initiative for Non-Mandatory Vaccination [5], organized meetings in Novi Sad and Belgrade, on May 26 and 27, 2016, respectively, and three key speakers were from Croatia (L. Gajski, Đ. Rušinović Sunara) and Slovenia (P. Verbič). Another invited Croat (S. Sladoljev) apologized for not being able to attend, and a chairperson of the most influential Bosnian antivax group (J. Savić) was duly present. Official medical organizations do not have such an intensive collaboration.

PROMINENT ANTIVAXERS

Any better known regional antivaxer builds up his/her reputation by "original" worldview of yet unknown health threats, covered international plots against human race, "neglected" individual rights, etc. A variety of approaches may be illustrated by presenting one example from each of the four neighboring countries.

The most influential antivaxer in the region is a general practitioner Slađana Velkov [6]. Though Macedonia-born, she used to spend most of her time in Serbia, spreading her "missionary" activities as far to the West as Slovenia. Velkov studied for 17–18 years, spent a short time as a doctor and then was left without the license. Her CV is greatly forged and her ideas are strikingly bizarre. Velkov believes in the spontaneous evolution (bacteria develop in our bodies), denies existence of AIDS, considers rabies a non-contagious disease, and claims that cancer, multiple sclerosis, and many other diseases should be treated by sunbathing (she also believes in a "theory" that the Sun is cold, though it transfers "vital orgone energy").

According to her, chemtrails contain biorobots that behave as parasites upon being inhaled. Furthermore, shoes are allegedly the most destructive invention in the human history since rubber soles somehow prevent crucial transfer of electrons. This blockade is important since diseases are caused by vibrations (common cold occurs at 58 Hz, candidiasis at 55 Hz, and cancer at 42 Hz).

Along with such a wide range of irrational statements, Velkov perceives vaccines as a major evil. Her assessments that vaccines are a biological weapon and the most dangerous practice in the history of classical medicine made her the antivax icon of the Balkans. Her destructive role against regional public health is reflected by the fact that the number of her followers reaches several dozen thousands on Facebook alone. Velkov's recent recommendation that autistic children should be treated with a toxic chlorine dioxide (one bottle of the solution costs €15–30) rose police interest [7, 8]. Since then, her activity outside of Macedonia appears to be less conspicuous.

Upon being sent to an early retirement three decades ago, Serbian dentist Todor Jovanović "invented" AIDS cure. The business developed extremely well, people rushed to his "pharmacy," and he extended indications for his allegedly miracle drugs, Todoxin and Torexin, to the prevention and cure of cancer and variety of other conditions [9, 10].

In a strange and logically hardly explainable way, absurdities that Todor has been claiming seem to only beef up his popularity. He allegedly made free of AIDS the basketball player Magic Johnson, King Zulu's grandchildren, as well as many nobilities. His imaginary successes ("96–98% cure rate") were demonstrated in a two-digit number of his private clinics scattered over three continents, including the East (Bethesda, MD, Washington, D.C.) and the West (Los Angeles, CA) Coast of the USA. This unlicensed dentist (of course, without USMLE and any chance to treat medical patients) impresses people by pretending to have been granted 100 million dollars by the US authorities.

From a common sense point of view, much more serious is his inclination to the conspiracy theories. Thus, eugenicists established an association for the reduction of the world population, and therefore "the cause of leprosy was composed only for well-defined unwanted populations." Furthermore, "the top management of Torlak" (a leading regional producer of vaccines and sera) developed a polio vaccine which caused breast cancer in 61% of women. Why did they do it? Well, they were "promised the Nobel Prize" if they "punished curious Serbs" (by the way, Torlak is a Serbian company). A shipment was somehow directed to Brazil, but Brazilians were clever enough to refuse mass vaccination.

Along the same mindset is this gifted story-teller's complaint for being deprived of Nobel Prize himself. It is true that he never published any contribution to science, but instead, he created "a distinct scheme of life development." Unfortunately, some evil people pushed his creation aside.

Inconsistency is one of his hallmarks. Thus, he "discovered" two mutually exclusive events – one that HIV does not exist, and the other one that Americans secretly introduced this virus to Africa in 1975, while being unable, due to some undisclosed reasons, to drop an atomic bomb.

As time passed by, Todor's popularity grew and even the fairly reputable regional daily Politika, the oldest in the Balkans, published an interview with him [11]. Statements he gave ("sleeping is a waste of time," all diseases are "absolutely" curable, etc.) made it clear that it was a covert paid advertisement. Generally speaking, his boasting is so far removed from reality that he would deserve only despise and pity, if his influence had not taken regional proportions. Many doctors are desperate when approached by terminally ill Todor's patients.

Ms. Jagoda Savić chairs the Association of the Parents of Severely Ill Children in Bosnia and Herzegovina [12]. She is a sociologist and tries to respect scientific standards. Her fight with the Bosnian medical community has two main aspects: a) too high expectations in terms of meticulously maintained documentation for a healthcare system of a developing country, and b) a suspicious attitude towards available evidence both at global and local levels.

She requested that all "infants' deaths without explanation" be reconsidered since 2000 onwards. Savić referred to SIDS, but actually had in mind children who developed severe unwanted manifestations following vaccination [13]. Her emphasis was in particular on 137 infants whose cause of death was labelled "unknown" or "unexplained" [14]. In

a rhetorical way, she points that "2,249 small graves appeal to the conscience of forensic pathologists and require an answer if a human life is for them a valuable orientation" [14]. Her Association offered free translation of all necessary documentation into English to the parents, as well as mailing this material to the US Vaccine Adverse Events Reporting System, or VAERS for short [13]. Within less than a month, 30 reports were sent to VAERS [15].

Some controversies were raised by Savić's "Report your own pediatrician" initiative [16], allegedly designed to identify departures from proper practice and thus to improve the services. All eight initially reported cases, both from the Federation of Bosnia and Herzegovina and the Republic of Srpska, were presented as victims of vaccination.

Savić bitterly opposed the practice of administering separately DTPr and HiB vaccines instead of pentavalent ones. She implied that many serious adverse reactions were associated with simultaneous application of two vaccines provided by different producers and requested clarification from GlaxoSmithKline, with a pending request prepared for Sanofi-Aventis [17]. It is apparently much more comfortable to administer a single pentavalent vaccine, but international experience implies that Savić's fear is not based on facts.

A major regional concern arose when two small children, from Lukavac and Doboj, Bosnia and Herzegovina, purportedly passed away due to vaccination [18]. A Belgrade's expert, whose comment was taken as the final truth, left room for such an interpretation in an email (J. Savić – written communication).

Among many other reasons for friction, the most bitter conflict between J. Savić and medical establishment (UNICEF Bosnia and Herzegovina, Prof. M. Zubčević, etc.) concerns subacute sclerosing panencephalitis (SSPE). Due to irregular and/or absent MMR vaccination during the stormy 1990s, Prof. Zubčević diagnosed 14 SSPE cases. On the other hand, J. Savić tries to demonstrate that at least some of these children developed SSPE due to vaccination, rather than after natural infection. Her doubts are not substantiated by the scientific literature. However, based on ELISA and genotyping testing, she took Bosnian authorities to court on March 26, 2016.

Dr. Djula Rusinović Sunara is an MSc in surgery, but her professional interest is medical law. She established and chaired the Croatian Association for Promotion of Patients' Rights. Sunara's points of view are presented in her book *Why Am I Involved in Politics*, where she develops the idea of agathocracy (rule for the common cause) [19]. She argues for two strategic tracks: a) promotion of human rights through patients' rights, thus building a better healthcare system, and b) promotion of family values by the affirmation of mothers and children.

In her biography, Sunara explains that in this book she "talks about the need to foster different kinds of democracy, since the existing ones are perceived as being full of corruption" [20]. She also revealed that in 2001 "she remained unemployed due to pervasive political corruption, direct discrimination, and her personal religious attitudes and non-party beliefs" [19, 20].

Sunara might have been a victim of political persecution, but her points of view are fairly radical indeed. One should appreciate her critics of an alienated healthcare system that applies both to Croatia as well as the whole region. On the other hand, her perceived mutually exclusive roles and duties of individual vs. community doctors are hardly acceptable. According to this rigid and confrontational division, an individual doctor is exclusively concerned with the interest of his/her patient and must not care about public health, while a community doctor may advise only his/her colleagues, but neither parents, nor politicians. Consequently, community doctors should not be allowed to suggest mandatory vaccination (Dj. R. Sunara – personal communication).

Sunara interprets the Convention on the Rights of the Child in her own way [21]. She ignores the responsibility of parents, community, and the government to protect children's rights, including the right to grow up healthy, reducing it instead only to the right to health care, that may be consumed or not. This standpoint departs from the ruling of the Constitutional Court of Croatia that the right of a child to health is above the right of parents to make a (wrong) choice [22].

She argues that health is a state, rather than a matter of rights, and that no reason, including community interest, may justify "sacrificing" the children. In her interpretation, "nowadays it would not be excusable to run Mengele-style experiments" and, consequently, "no one could prove the benefits of vaccination." She somehow misses the worldwide standard practice of vaccine safety and efficacy testing according to the highest ethical norms.

There are better known anti-vaccinationists in Croatia (L. Tomljanović, S. Sladoljev, L. Gajski), but Sunara appears to exert more powerful influence in the neighboring countries. The reason is her activity in international organizations for medical law (she served as a vice-president of one of such global bodies). From this position, she involves local medical law experts in antivax movement.

ANTIVAXERS' MOTIVES

A vast majority of people who do not vaccinate their children are victims of misinformation. They are overwhelmed by contradictory interpretation of data and do not know whom to trust. In Scandinavian countries, a parent who challenged a pediatrician's motives on professional or ethical grounds would be considered psychopathic. In this part of the world, however, people have been so frequently cheated and betrayed by officials and countrymen alike, that they do not take as granted anyone's sincerity any more.

The role of community leaders, above all medical doctors, is critical at this point. They are the ones who dictate vaccination policy. Some of them, pediatricians in particular, are not enthusiastic about vaccination because the risk of epidemics of vaccine-preventable diseases has been, at least until recently, fairly low (except in Bosnia and Herzegovina). They have never seen a case of poliomyelitis,

tetanus of a newborn, diphtheria, and similar conditions. Instead, they, as well as parents, are annoyed by occasional postvaccinal reactions of children. Doctors' lives are easier if they simply do not encourage parents to bring their children for vaccination. This policy may pay off when mass immunity exceeds 90–95%. However, since there are more and more such "smart" doctors, population immunity goes down and dire consequences of such a shortsighted approach may be ahead.

Doctors who had experienced some problems with their children's health frequently blame vaccination for their ordeal. In such a case, arguments are irrelevant. An otolaryngology professor from Zagreb was "99% convinced" that a vaccine caused his daughter's encephalitis. He was shown his own notes that excluded any possible cause–effect relationship. He admitted that handwriting was his, but commented that what he wrote at the time of his daughter's hospitalization was apparently wrong. A professor of pediatrics from Belgrade strongly believes that MMR vaccine has been responsible for his son's autism. As a pulmonologist, he favors pneumococcus vaccine, but resists to acknowledge plenty of studies on the lack of association between MMR vaccine and autism.

Manipulators may be easily recognized because they offer their "miracle" medicaments instead of vaccines. Close to them are sociopaths. Some have strange preconceived ideas, while others do their best to escape anonymity by any means. The last group is a mixture of religious fanatics, people obsessed with rigidly perceived human rights and freedoms, xenophobes, followers of conspiracy theories, etc.

It is frequently difficult to classify an antivaxer into a single group. Thus, an associate professor of pediatrics from Belgrade had a preconceived idea that diphtheria vaccine led to leukemia [23]. The whole concept was so baseless that it experienced a devastating critic [24] and was never seriously considered. The author, however, kept repeating the same absurdities in low-esteemed media over a period of years [25]. Media attention pushed him further, even to claim that most viruses, including measles and influenza, are beneficial, with an "argument" that God would not sent us influenza virus each year if it were something harmful [25, 26].

ANTIVAXERS' ARGUMENTS

The most widespread and stubbornly maintained urban legend is that MMR vaccine leads to autism. Scientific arguments on the lack of any causal relation between the two events simply do not touch the skeptics' mind frame [27].

Another traditionally used argument is that vaccines contain mercury. Toxic mercury compounds, such as methyl mercury, are than considered, instead of much less toxic ethyl mercury that used to be added to formulas in the past. There is nowadays hardly any example in the whole region that infants and toddlers are exposed to any amount of mercury.

People are also frightened by a variety of other substances, as a rule present in minute, negligible concentrations, sometimes lower than in human milk or other food that infants consume. Manipulation with statistical data is antivaxers' favorite means of impressing people. Thus, absolute numbers are used to compare vaccinated and non-vaccinated children affected in an outbreak, ignoring the fact that most children (in some examples over 95%) have been vaccinated. If a proper approach were applied, it would be obvious that the proportion of diseased children in the two groups differed 10–20 times, i.e., that vaccination had an impressive protective effect.

Another way to frighten parents is to refer to the US passive surveillance data (VAERS). It is a system where any event that followed vaccination is reported, either related to this intervention or not. This data repository only serves for sorting out if there is any cause–effect relationship in each individual case (overwhelmingly, it is absent).

VAERS is sometimes used (and exploited by antivaxers) as a data source for listing possible unwanted events in instructions for using vaccines. This way, pharmaceutical companies are on safe ground, because they could not be blamed for hiding any side effect, even if it is non-existent or might appear in less than one in a million cases.

Suggestions that childhood contagious diseases should not be prevented because they provide better immunity as compared to vaccines could only be seriously considered if such diseases were not leading to human suffering, permanent impairment, or even death. This line of reasoning leads some opponents of vaccination close to the condemned theory of racial hygiene.

It is nowadays a hype to argue in favor of free choice as a human right. It may be an argument for non-contagious conditions, but for effective prevention of infectious diseases it is mandatory to achieve a high level of herd immunity. Antivaxers raise a question: "How can my unvaccinated child jeopardize other (assumingly vaccinated) children in a community?" Firstly, some children have been spared of vaccination due to medical reasons (permanent contraindications), and secondly, a vaccine may not provide 100% protection. An adequate post-vaccinal immunity may not develop at all or induced resistance may wane quicker than expected.

In this presentation we ignore suspicions that vaccines are a biological weapon designed to reduce human population, means for introduction of chips into humans in order to subjugate them, and similar absurdities offensive to common sense. A recent phenomenon that should be mentioned, however, is Vladimir Putin's role in revealing putative Western plots by use of vaccination [28].

THE FUTURE OF THE ANTIVAX MOVEMENT

Anti-vaccinationists exist almost as long as the vaccination [29]. Their influence fades away after major epidemics. This process is going on nowadays in the West, after reduced comprehensiveness of vaccination in the previous decade and consequent outbreaks of measles and whooping cough. The same sequence of events may be expected in the Balkans.

CONCLUSION

Mass opposition to vaccination has taken place only recently and almost simultaneously in the Central Balkan countries. Values that local anti-vaccinationists argue for, their arguments and motives, resemble the agenda of their Western counterparts. Once established, the movement against vaccination will not fade away. However, epidemics of vaccine-preventable disease will, at least temporarily, reduce their influence on the general population.

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Антивакциналисти и њихови аргументи у балканским земљама које повезује исти језик

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САЖЕТАК

Циљ рада је анализа антивакциналне ситуације у језички повезаним балканским земљама. У том делу света је масовно и организовано противљење вакцинацији релативно скорашњи феномен. Последица је ширења одговарајућих идеја са запада, повезаних са „њуејџизмом", постмодернизмом и сличним погледима на свет, а нарочито оснажених страхом од ММР (мале богиње, заушке, рубеола) вакцине од краја 1990-их. Одабране су и детаљније приказане четири водеће локалне личности из четири земље, како би репрезентовале сву разноликост балканске антивакциналне позорнице. Свака од њих врши свој утицај у целом региону. Резултат је знатни пад обухвата вакцинацијом у многим подручјима. Епидемије вакцинама спречивих болести, које пре или касније морају да уследе, бар привремено ће задати ударац антивакциналистима. Већ смо сведоци таквог тренда у Босни и Херцеговини, где вакцине нису биле лако доступне 1990-их током грађанског рата. Последице се огледају у великим епидемијама малих богиња, заушака и рубеоле које су захватиле ову земљу. Динамична равнотежа међуутицаја антивакциналног покрета и инциденције оболевања, оличена у обрнутој сразмери (што је веће штетно дејство противника вакцинације на народно здравље, више је оболевања), представља проблем са којим здравствена служба мора да се суочава.

Кључне речи: антивакциналисти; вакцинација; Балкан

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CURRENT TOPIC / АКТУЕЛНА ТЕМА
INVITED COMMENTARY / КОМЕНТАР ПО ПОЗИВУ

Anti-vaccinationists

In Online First issue of the *Serbian Archives of Medicine*, Radovanović [1] reviewed the anti-vaccination movement in the Balkan region. The anti-vaccinationists in the region were especially active since the 1990s and their ability to spread misinformation about "harmful, toxic and lethal" effects of vaccines has increased in the past decade.

Although the anti-vaccination movement has been present since the first vaccines were introduced, little of the old quasi-arguments or statements about harmful effects of vaccines has changed. In the United Kingdom, the Vaccination Act of 1840 provided free vaccination for the poor and outlawed "inoculation," which at that time meant "variolation," inoculation of smallpox material [2]. The Vaccination Act of 1853 made vaccination compulsory for all infants in the first three months of life and made defaulting parents liable to a fine or imprisonment. However, the founding of Anti-Vaccination League in the same year provided the first established movement against immunization. Then, a large number of anti-vaccination tracts, books, and journals such as the *Anti-Vaccinator* (founded in 1869), the *National Anti-Compulsory Vaccination Reporter* (1874), and the *Vaccination Inquirer* (1879) were published [3]. As a consequence, the majority of the population began to refuse vaccination. In 1872, vaccination rates in Stockholm decreased to 40%, and in 1874 a major epidemic affected the city and led to widespread vaccination and an end to further epidemics. Anti-vaccination activities in the United States led to a decrease of immunization rates and subsequently to epidemics of small pox.

These 19th century readings can be easily compared with modern anti-vaccine web-based statements on the internet. Rogers and Pilgrim gave probably the best definition: anti-vaccination movement encompasses a wide range of individuals, from a few who express conspiracy theories, to educated, well informed consumers of health care, who often have a complex rationale for their beliefs, related to a "mixture of world views held about the environment, healing, holism... and a critical reading of the scientific and alternative literature" [4, 5].

In this issue, an author concluded that epidemics of vaccine-preventable disease will, at least temporarily, reduce the influence of the anti-vaccination movement on the general population [1].

Today, the key role against anti-vaccination is played by the primary-care pediatrician, who can reassure parents that vaccines are safe and effective, and that usual adverse events are mild/transient and common, while serious events are extremely rare (e.g. anaphylaxis after immunization 1/1,000,000 of vaccine doses). Pediatricians could also reassure parents that almost all statements about vaccines found on the internet are not scientific or proved by medical, controlled studies. It may be useful to remind parents that stories of people whose children suffered serious diseases that could have been prevented by vaccination also exist on the internet.

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PRO MEMORIA

Forty years of the Academy of Medical Sciences of the Serbian Medical Society

Recently, a gathering in tribute to forty years of the Academy of Medical Sciences (AMS) as a constitutive part of the Serbian Medical Society (SMS) was held at the Serbian Academy of Sciences and Arts. This was an opportunity to review its establishment, basic principles, and results (Figure 1).

The AMS is an integral part of the SMS, and was officially established in 1976. The SMS was founded in 1872 as one of the first medical societies in Europe. For 145 years now it has been serving as the basic professional society in medicine and dentistry for generations of medical practitioners. It has been a place for extensive communication, exchange of experience in practice, and new scientific contributions over the years, which all contributed to the development of healthcare services and medical standards in Serbia. After the Second World War, gradually, with the increased number of medical doctors educated in Serbia and new health services, the number of specialized sections in the SMS grew, in order to deal with the ever-increasing amount of information in medical practice and science. The idea that an academy of medical sciences should be formed was discussed at a broad level in medical centers and the Association of Medical Societies in the former Yugoslavia in 1960s, but a united academy was never formed. Within the SMS, an initiative council was formed to establish basic principles for the Medical Academy (MA). At the assembly of the SMS held on December 15, 1976, the MA was officially formed [1]. The first members were introduced and elected according to the SMS Statutory decision and its high criteria. In 1977, as a substructure of the MA, scientific groups were formed and named Scientific Group for Surgery, Scientific Group for Internal Medicine, and Scientific Group for Preventive Medicine. Multidisciplinary Scientific Group was formed in 1994, and later, in 1999, it was linked to the Scientific Group for Preventive Medicine, forming the Scientific Group for Preventive and Multidisciplinary Medicine. In the same year, at the Assembly of the MA, new constitutive parts were formed in Vojvodina and Niš. The Medical Academy changed its name to Academy of Medical Sciences of the Serbian Medical Society in 2000 [2, 3].

Figure 1. Logo of the Academy of Medical Sciences

Members of the AMS may be regular, associate, honorary, and foreign. Election criteria are similar to those for the Serbian Academy of Sciences and Arts. The overall number of members at present is 298, out of which 132 members are regular, 79 associate, 61 honorary, and 26 foreign, which is around 1% of the total number of SMS members (Figure 2).

The basic principles of AMS activities are determined in Article 10 of the SMS Statutory proposals as stated below:

The Academy shall perform the following tasks and works in realization of professional and scientific research work of the Society:

- It shall monitor and encourage the development of scientific work of basic, developmental and applied research in all fields of scientific-research work that are of interest for medicine and dentistry;
- It shall perform continuous education of physicians from its scope of work;
- It shall popularize and help the application of the scientific work results within the medicine and dentistry group;
- In the scope of its scientific programs, it shall make and propose the standards for accomplishment of the newest achievements in the field of medicine and dentistry;
- It shall perform the public influence in the field of science promotion and the adoption of new standards of general interest;

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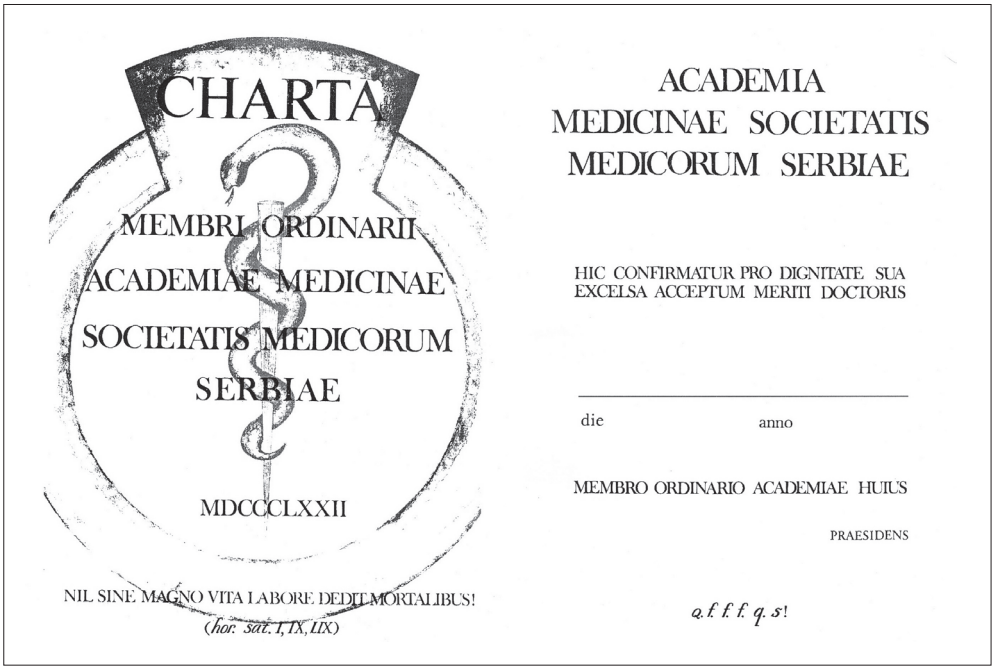

Figure 2. Diploma of the Academy of Medical Sciences

– In the scope of its activity it shall establish the cooperation with other relevant institutions and authorities to which it shall propose standards for introduction of the scientific research results into practice;
– It shall make proposals for harmonization of educational curricula, scientific and professional training in medicine and dentistry;
– It shall cooperate with other agencies and institutions at home and abroad to facilitate faster and easier implementation of the scientific and research results into practice
– It shall achieve affiliation with other related academies in the region and abroad.

The activities of the AMS and operational issues are closely regulated by the Rule of Procedure accepted by the Assembly of the AMS and approved by the Presidency of the SMS.

During the past 40 years, the activities of the AMS included the organization of 858 scientific and professional events, which encouraged the development of scientific research, its implementation in practice, and education in every area of medicine.

The AMS is also engaged in publishing activities and it possesses a library of 484 books and 81 special issues which were published by the AMS or donated by its members. Special publishing activities include editing books with content of presentations held at scientific conferences. These editions are evaluated by the AMS Editorial Board, as well as by independent reviewers.

Annual information on the entire scope of activities, members, retorts, and plans for the next year has been regularly published since 2001. This appears to be useful for keeping all the members well informed. The website about the AMS is incorporated into the web presentation of the SMS. Activities of the AMS and its bodies are public and open to public media.

The aim of AMS activities has been continuously achieved by the enthusiastic approach of its members over the years. We are looking forward to continuous contribution of the AMS to the basic values of the SMS, not only as a professional but also a cultural value of the Serbian society.

Professor Pavle Milenković, MD, PhD
President of the AMS

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ПИСМО УРЕДНИКУ / LETTER TO THE EDITOR

Antivakcinalisti i njihovi argumenti u balkanskim zemljama

Poštovani uredniče,

U elektronskom izdanju našeg časopisa „Srpski arhiv za celokupno lekarstvo“ sa interesovanjem sam pročitao priloge o antivakcinalistima uglednih prof. Zorana Radovanovića [1] i prof. Srđana Pašića [2]. Baveći se mnogo godina ovom temom, želim da dam svoj komentar.

Cilj autora je bio da predstavi ključne antivakcinaliste Centralnog Balkana, njihove stavove u odnosu na imunizaciju, motive i argumente. Kako su navedeni stavovi i ponašanja dostupni opštoj javnosti, predstavljaju odgovarajuću osnovu za jednu opisnu analizu. Podaci, stavovi i „argumenti“ koji se analiziraju dostupni su preko sajtova na internetu, snimaka sa Jutjuba, medijski su objavljeni kroz razne tekstove i intervjue, a koriste se podaci i iz direktne komunikacije. Citirane su bizarne preporuke koje su bile dostupne preko društvenih mreža. Ukazano je na netačnosti, brojna besmislena objašnjenja i iracionalnosti, uključujući i izjave nekih od antivakcinalista da su „izlečili“ neke poznate licnosti od AIDSa odnosno da je potrebno „lečiti“ vakcinisane sa hlor-dioksidom ... Oslikavanjem ličnosti antivakcinalista u jednom slučaju prikazana je i oslonjenost na teorije zavere kada je uz niz drugih preterivanja, navodno menadžment poznatog domaćeg proizvođača vakcina „trebalo da dobije Nobelovu nagradu za proizvodnju vakcine protiv dečje paralize koja je izazivala rak dojke kod 61% žena“ da bi se kaznili „radoznali Srbi“.

Posebno su prikazana uticajna udruženja i njihovi predsednici koja imaju antivakcinalne stavove i aktivno se bore protiv aktuelnih stavova naučne medicine o imunizaciji, kao i protiv obavezne imunizacije. Predsednik „Udruženja roditelja teško bolesne dece BiH“ predstavljena je kao osoba koja pokušava da, kao sociolog, u okviru naučnih standarda uđe u sukob sa medicinskom zajednicom, ali se ukazuje na to da ima preterana očekivanja o vrlo pedantno čuvanoj medicinskoj dokumentaciji za jednu zemlju u razvoju, kao i da ispoljava veliku sumnjičavost u odnosu na dostupne dokaze bilo na lokalnom ili globalnom nivou. Na retorički način ona tvrdi da je potrebno da se svi slučajevi neobjašnjene smrti novorođenčadi u periodu posle 2000. godine preispitaju i „stavlja ih na savest forenzičkim patolozima postavljajući pitanje da li ljudski život za njih predstavlja vrednost“. Njeno udruženje nudilo je pomoć u prijavljivanju neželjenih reakcija posle vakcinacije, pa je pokrenula inicijativu „Prijavi svog pedijatra“, nakon čega su navodno osam prijavljenih slučajeva bile žrtve vakcinacije. Najogorčeniji sukob bio je u odnosu na pojavu subakutnog sklerozirajućeg panencefalitisa (SSPE) kod 14 pacijenata, posle teških 90-ih godina, kada je snabdevenost vakcinama bila značajno poremećena, a ovo udruženje i njen predsednik povezivalo je ove slučajeve sa vakcinacijom.

Predsednica Hrvatskog udruženja za promociju prava pacijenata je kao hirurg poseban interes profesionalno razvila u odnosu na medicinske zakone. Svoje stavove prikazuje u knjizi „Zašto sam u politici?“ Vladavinu dobrog vidi kroz promovisanje ljudskih prava i prava pacijenata gradeći tako bolji zdravstveni sistem kao i kroz promovisanje vrednosti porodice i afirmaciju majke i deteta. Njeni stavovi vezani za javno zdravlje i Konvenciju o pravu deteta su da „nikakav razlog uključujući i interes zajednice ne može da opravda ‘žrtvovanje’ deteta i ’eksperimente po ugledu na Mengeleove oglede’“ jer „niko ne može da potvrdi koristi od vakcinacije“. Takvi stavovi su suprotni od viđenja vladajućeg Ustavnog suda Hrvatske. Njena shvatanja su da se lekari dele na lične i one koji rade za zajednicu odnosno javno zdravlje, smatrajući da lekari u javnom zdravlju mogu samo da savetuju i preporučuju imunizaciju lekarima koji rade sa pacijentima, a ne roditeljima. U Hrvatskoj postoje i drugi antivakcinalisti, ali je uticaj ovog društva i njene predsednice najveći.

Naglašen je poguban uticaj koje su sve navedene aktivnosti imale po javno zdravlje i kolika je štetnost po društvo njihov uticaj koji se vidi kroz više desetina hiljada pratilaca na društvenim mrežama. Aktivnosti navedenih antivakcinalista i njihovi stavovi povezuju se sa padom obuhvata imunizacijom u regionu i povećanom incidencijom obolevanja od bolesti koje se mogu sprečiti vakcinacijom. Ukazujući na dobru saradnju koju predstavnici antivakcinalnog pokreta održavaju u regionu, pomenut

je i organizovan skup „Građanske inicijative za neobaveznu imunizaciju“ u Beogradu i Novom Sadu 26. i 27. maja 2016, na koji su pozvani istaknuti antivakcinalisti iz Hrvatske, Slovenije i Bosne i Hercegovine.

Među „argumentima“ antivakcinalista dominantna je tvrdoglava urbana legenda da MMR vakcina izaziva autizam uprkos brojnim naučnim dokazima koji to osporavaju, zatim da vakcine sadrže živu kao i niz drugih navodno štetnih supstanci. Manipulacija statističkim podacima je najomiljeniji način pokušaja impresioniranja sagovornika direknim poređenjem obolevanja kod vakcinisanih i nevakcinisanih u apsolutnim brojevima, ne uzimajući u obzir obuhvaćenost imunizacijom. Drugi način zastrašivanja je korišćenje podataka iz VAERS (*Vaccine Adverse Event Reporting System*) pasivnog nadzora, pri čemu se lista registrovanih neželjenih događaja vremenski povezanih sa vakcinacijom koristi kao „dokaz“ o neželjenim reakcijama uzročno-posledično povezanih sa vakcinacijom.

Masovno oponiranje vakcinaciji se simultano pojavilo u zemljama istog govornog područja na Balkanu. Iste vrednosti, argumente i motive ovi antivakcinalisti imaju kao i njihovi partneri sa Zapada. Jednom ustanovljen pokret neće lako da izbledi. Međutim, epidemije bolesti koje se sprečavaju vakcinacijama smanjiće uticaj ovog pokreta na opštu populaciju.

Rad i njegovo publikovanje daju značajan doprinos u rasvetljavanju antivakcinalnog pokreta. Rad obrađuje aktuelnu temu i predstavlja jedinstveno i značajno istorijsko svedočenje vezano za pojavu antivakcinalnih stavova i njihovih protagonista sa istog govornog područja zemalja Centralnog Balkana.

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Пре подношења рукописа Уредништву часописа „Српски архив за целокупно лекарство“ (СА) сви аутори треба да прочитају Упутство за ауторе (*Instructions for Authors*), где ће пронаћи све потребне информације о писању и припреми рада у складу са стандардима часописа. Веома је важно да аутори припреме рад према датим пропозицијама, јер уколико рукопис не буде усклађен с овим захтевима, Уредништво ће одложити или одбити његово публиковање. Радови објављени у СА се не хонорарашу. За чланке који ће се објавити у СА, самом понудом рада Српском архиву сви аутори рада преносе своја ауторска права на издавача часописа – Српско лекарско друштво.

ОПШТА УПУТСТВА. СА објављује радове који до сада нису нигде објављени, у целости или делом, нити прихваћени за објављивање. СА објављује радове на енглеском и српском језику. Због боље доступности и веће цитираности препоручује се ауторима да радове свих облика предају на енглеском језику. У СА се објављују следеће категорије радова: уводници, оригинални (научни и стручни) радови, метаанализе, прегледни радови, претходна и кратка саопштења, прикази болесника и случајева, слике из клиничке медицине, видео-чланци, радови за праксу, актуелне теме, радови из историје медицине и језика медицине, лични ставови, наручени коментари, писма уреднику, прикази књига и други прилози. Оригинални радови, претходна и кратка саопштења и прикази болесника и случајева публикују се искључиво на енглеском језику, а остале врсте радова се могу публиковати и на српском језику само по одлуци Уредништва. Радови се увек достављају са сажетком на енглеском и српском језику (у склопу самог рукописа). Текст рада куцати у програму за обраду текста *Word*, фонтом *Times New Roman* и величином слова 12 тачака (12 *pt*). Све четири маргине подесити на 25 *mm*, величину странице на формат А4, а текст куцати с двоструким проредом, левим поравнањем и увлачењем сваког пасуса за 10 *mm*, без дељења речи (хифенације). Не користити табулаторе и узастопне празне карактере (спејсове) ради поравнања текста, већ алатке за контролу поравнања на лењиру и *Toolbars*. За прелазак на нову страну документа не користити низ „ентера“, већ искључиво опцију *Page Break*. После сваког знака интерпункције ставити само један празан карактер. Ако се у тексту користе специјални знаци (симболи), користити фонт *Symbol*. Подаци о коришћеној литератури у тексту означавају се арапским бројевима у угластим заградама – нпр. [1, 2], и то редоследом којим се појављују у тексту. Странице нумерисати редом у доњем десном углу, почев од насловне стране.

При писању текста на енглеском језику треба се придржавати језичког стандарда *American English* и користити кратке и јасне реченице. За називе лекова користити искључиво генеричка имена. Уређаји (апарати) се означавају фабричким називима, а име и место произвођача треба навести у облим заградама. Уколико се у тексту користе ознаке које су спој слова и бројева, прецизно написати број који се јавља у суперскрипту или супскрипту (нпр. ^{99}Tc, *IL*-6, O_2, $Б_{12}$, *CD*8). Уколико се нешто уобичајено пише курзивом (*italic*), тако се и наводи, нпр. гени (*BRCA1*).

Уколико је рад део магистарске тезе, односно докторске дисертације, или је урађен у оквиру научног пројекта, то треба посебно назначити у Напомени на крају текста. Такође, уколико је рад претходно саопштен на неком стручном састанку, навести званичан назив скупа, место и време одржавања, да ли је рад и како публикован (нпр. исти или другачији наслов или сажетак).

КЛИНИЧКА ИСТРАЖИВАЊА. Клиничка истраживања се дефинишу као истраживања утицаја једног или више средстава или мера на исход здравља. Регистарски број истраживања се наводи у последњем реду сажетка.

ЕТИЧКА САГЛАСНОСТ. Рукописи о истраживањима на људима треба да садрже изјаву у виду писаног пристанка испитиваних особа у складу с Хелсиншком декларацијом и одобрење надлежног етичког одбора да се истраживање може извести и да је оно у складу с правним стандардима. Експериментална истраживања на хуманом материјалу и испитивања вршена на животињама треба да садрже изјаву етичког одбора установе и треба да су у сагласности с правним стандардима.

ИЗЈАВА О СУКОБУ ИНТЕРЕСА. Уз рукопис се прилаже потписана изјава у оквиру обрасца *Submission Letter* којом се аутори изјашњавају о сваком могућем сукобу интереса или његовом одсуству. За додатне информације о различитим врстама сукоба интереса посетити интернет-страницу Светског удружења уредника медицинских часописа (*World Association of Medical Editors – WAME; http://www.wame.org*) под називом „Политика изјаве о сукобу интереса“.

АУТОРСТВО. Све особе које су наведене као аутори рада треба да се квалификују за ауторство. Сваки аутор треба да је учествовао довољно у раду на рукопису како би могао да преузме одговорност за целокупан текст и резултате изнесене у раду. Ауторство се заснива само на: битном доприносу концепцији рада, добијању резултата или анализи и тумачењу резултата; планирању рукописа или његовој критичкој ревизији од знатног интелектуалног значаја; завршном дотеривању верзије рукописа који се припрема за штампање.

Аутори треба да приложе опис доприноса појединачно за сваког коаутора у оквиру обрасца *Submission Letter*. Финансирање, сакупљање података или генерално надгледање истраживачке групе сами по себи не могу оправдати ауторство. Сви други који су допринели изради рада, а који нису аутори рукописа, требало

би да буду наведени у Захвалници с описом њиховог доприноса раду, наравно, уз писани пристанак.

НАСЛОВНА СТРАНА. На првој страници рукописа треба навести следеће: наслов рада без скраћеница; предлог кратког наслова рада, пуна имена и презимена аутора (без титула) индексирана бројевима; званичан назив установа у којима аутори раде, место и државу (редоследом који одговара индексираним бројевима аутора); на дну странице навести име и презиме, адресу за контакт, број телефона, факса и имејл адресу аутора задуженог за кореспонденцију.

САЖЕТАК. Уз оригинални рад, претходно и кратко саопштење, метаанализу, преглед литературе, приказ случаја (болесника), рад из историје медицине, актуелну тему, рад за рубрику језик медицине и рад за праксу, на другој по реду страници документа треба приложити сажетак рада обима 100–250 речи. За оригиналне радове, претходно и кратко саопштење, метаанализе и прегледне радове, сажетак треба да има следећу структуру: Увод/Циљ, Методе, Резултати, Закључак; сваки од наведених сегмената писати као посебан пасус који почиње болдованом речи. Навести најважније резултате (нумеричке вредности) статистичке анализе и ниво значајности. Закључак не сме бити уопштен, већ мора бити директно повезан са резултатима рада. За приказе болесника сажетак треба да има следеће делове: Увод (у последњој реченици навести циљ), Приказ болесника, Закључак; сегменте такође писати као посебан пасус који почиње болдованом речи. За остале типове радова сажетак нема посебну структуру.

КЉУЧНЕ РЕЧИ. Испод Сажетка навести од три до шест кључних речи или израза. Не треба да се понављају речи из наслова, а кључне речи треба да буду релевантне или описне. У избору кључних речи користити *Medical Subject Headings – MeSH (http://www.nlm.nih.gov/mesh)*.

ПРЕВОД НА СРПСКИ ЈЕЗИК. На трећој по реду страници документа приложити наслов рада на српском језику, пуна имена и презимена аутора (без титула) индексирана бројевима, званичан назив установа у којима аутори раде, место и државу. На следећој – четвртој по реду – страници документа приложити сажетак (100–250 речи) с кључним речима (3–6), и то за радове у којима је обавезан сажетак на енглеском језику. Превод појмова из стране литературе треба да буде у духу српског језика. Све стране речи или синтагме за које постоји одговарајуће име у нашем језику заменити тим називом.

Уколико је рад у целости на српском језику (нпр. рад из историје медицине, језика медицине и др.), потребно је превести називе прилога (табела, графикона, слика, схема) уколико их има, целокупни текст у њима и легенду на енглески језик. Сажетке и радове који су у целости на српском језику аутори из Србије треба да пишу ћирилицом.

СТРУКТУРА РАДА. Сви поднаслови се пишу великим масним словима (болд). Оригинални рад, метаанализа, претходно и кратко саопштење обавезно треба да имају следеће поднаслове: Увод (Циљ рада навести као последњи пасус Увода), Методе рада, Резултати, Дискусија, Закључак, Литература. Преглед литературе чине: Увод, одговарајући поднаслови, Закључак, Литература. Првоименовани аутор метаанализе и прегледног рада мора да наведе бар пет аутоцитата (као аутор или коаутор) радова публикованих у часописима с рецензијом. Коаутори, уколико их има, морају да наведу бар један аутоцитат радова такође публикованих у часописима с рецензијом. Приказ случаја или болесника чине: Увод (Циљ рада навести као последњи пасус Увода), Приказ болесника, Дискусија, Литература. Не треба користити имена болесника, иницијале, нити бројеве историја болести, нарочито у илустрацијама. Прикази болесника не смеју имати више од пет аутора.

Прилоге (табеле, графиконе, слике итд.) поставити на крај рукописа, а у самом телу текста јасно назначити место које се односи на дати прилог. Крајња позиција прилога биће одређена у току припреме рада за публиковање.

СКРАЋЕНИЦЕ. Користити само када је неопходно, и то за веома дугачке називе хемијских једињења, односно називе који су као скраћенице већ препознатљиви (стандардне скраћенице, као нпр. ДНК, сида, ХИВ, АТП). За сваку скраћеницу пун термин треба навести при првом навођењу у тексту, сем ако није стандардна јединица мере. Не користити скраћенице у наслову. Избегавати коришћење скраћеница у сажетку, али ако су неопходне, сваку скраћеницу објаснити при првом навођењу у тексту.

ДЕЦИМАЛНИ БРОЈЕВИ. У тексту рада на енглеском језику, у табелама, на графиконима и другим прилозима децималне бројеве писати са тачком (нпр. 12.5 ± 3.8), а у тексту на српском језику са зарезом (нпр. 12,5 ± 3,8). Кад год је то могуће, број заокружити на једну децималу.

ЈЕДИНИЦЕ МЕРА. Дужину, висину, тежину и запремину изражавати у метричким јединицама (метар – *m*, килограм (грам) – *kg* (*g*), литар – *l*) или њиховим деловима. Температуру изражавати у степенима Целзијуса (°C), количину супстанце у молима (*mol*), а притисак крви у милиметрима живиног стуба (*mm Hg*). Све резултате хематолошких, клиничких и биохемијских мерења наводити у метричком систему према Међународном систему јединица (*SI*).

ОБИМ РАДОВА. Целокупни рукопис рада – који чине насловна страна, сажетак, текст рада, списак литературе, сви прилози, односно потписи за њих и легенда (табеле, слике, графикони, схеме, цртежи), насловна страна и сажетак на српском језику – мора износити за оригинални рад, претходно и кратко саопштење, рад из

историје медицине и преглед литературе до 5.000 речи, а за приказ болесника, рад за праксу, едукативни чланак и рад за рубрику „Језик медицине“ до 3.000 речи; радови за остале рубрике могу имати највише 1.500 речи.
Видео-радови могу трајати 5–7 минута и бити у формату *avi, mp4*(*flv*). У првом кадру филма мора се навести: у наднаслову Српски архив за целокупно лекарство, наслов рада, презимена и иницијали имена и средњег слова свих аутора рада (не филма), година израде. У другом кадру мора бити уснимљен текст рада у виду апстракта до 350 речи. У последњем кадру филма могу се навести имена техничког особља (режија, сниматељ, светло, тон, фотографија и сл.). Уз видео-радове доставити: посебно текст у виду апстракта (до 350 речи), једну фотографију као илустрацију приказа, изјаву потписану од свег техничког особља да се одричу ауторских права у корист аутора рада.

ПРИЛОЗИ РАДУ су табеле, слике (фотографије, цртежи, схеме, графикони) и видео-прилози.

ТАБЕЛЕ. Свака табела треба да буде сама по себи лако разумљива. Наслов треба откуцати изнад табеле, а објашњења испод ње. Табеле се означавају арапским бројевима према редоследу навођења у тексту. Табеле цртати искључиво у програму *Word*, кроз мени *Table–Insert–Table*, уз дефинисање тачног броја колона и редова који ће чинити мрежу табеле. Десним кликом на мишу – помоћу опција *Merge Cells* и *Split Cells* – спајати, односно делити ћелије. Куцати фонтом *Times New Roman*, величином слова 12 *pt*, с једноструким проредом и без увлачења текста. Коришћене скраћенице у табели треба објаснити у легенди испод табеле.

Уколико је рукопис на српском језику, приложити називе табела и легенду на оба језика. Такође, у једну табелу, у оквиру исте ћелије, унети и текст на српском и текст на енглеском језику (никако не правити две табеле са два језика!).

СЛИКЕ. Слике су сви облици графичких прилога и као „слике“ у СА се објављују фотографије, цртежи, схеме и графикони. Слике означавају се арапским бројевима према редоследу навођења у тексту. Примају се искључиво дигиталне фотографије (црно-беле или у боји) резолуције најмање 300 *dpi* и формата записа *tiff* или *jpg* (мале, мутне и слике лошег квалитета неће се прихватати за штампање!). Уколико аутори не поседују или нису у могућности да доставе дигиталне фотографије, онда оригиналне слике треба скенирати у резолуцији 300 *dpi* и у оригиналној величини. Уколико је рад неопходно илустровати са више слика, у раду ће их бити објављено неколико, а остале ће бити у е-верзији чланка као *PowerPoint* презентација (свака слика мора бити нумерисана и имати легенду).
Видео-прилози (илустрације рада) могу трајати 1–3 минута и бити у формату *avi, mp4*(*flv*). Уз видео доставити посебно слику која би била илустрација видеоприказа у *e*-издању и објављена у штампаном издању.
Уколико је рукопис на српском језику, приложити називе слика и легенду на оба језика.

Слике се у свесци могу штампати у боји, али додатне трошкове штампе сносе аутори.

ГРАФИКОНИ. Графикони треба да буду урађени и достављени у програму *Excel*, да би се виделе пратеће вредности распоређене по ћелијама. Исте графиконе прекопирати и у *Word*-ов документ, где се графикони означавају арапским бројевима према редоследу навођења у тексту. Сви подаци на графикону куцају се у фонту *Times New Roman*. Коришћене скраћенице на графикону треба објаснити у легенди испод графикона. У штампаној верзији чланка вероватније је да графикон неће бити штампан у боји, те је боље избегавати коришћење боја у графиконима, или их користити различитог интензитета.

Уколико је рукопис на српском језику, приложити називе графикона и легенду на оба језика.

СХЕМЕ (ЦРТЕЖИ). Цртежи и схеме се достављају у *jpg* или *tiff* формату. Схеме се могу цртати и у програму *CorelDraw* или *Adobe Illustrator* (програми за рад са векторима, кривама). Сви подаци на схеми куцају се у фонту *Times New Roman*, величина слова 10 *pt*. Коришћене скраћенице на схеми треба објаснити у легенди испод схеме.

Уколико је рукопис на српском језику, приложити називе схема и легенду на оба језика.

ЗАХВАЛНИЦА. Навести све сараднике који су допринели стварању рада а не испуњавају мерила за ауторство, као што су особе које обезбеђују техничку помоћ, помоћ у писању рада или руководе одељењем које обезбеђује општу подршку. Финансијска и материјална помоћ, у облику спонзорства, стипендија, поклона, опреме, лекова и друго, треба такође да буде наведена.

ЛИТЕРАТУРА. Списак референци је одговорност аутора, а цитирани чланци треба да буду лако приступачни читаоцима часописа. Стога уз сваку референцу обавезно треба навести *DOI* број чланка (јединствену ниску карактера која му је додељена) и *PMID* број уколико је чланак индексиран у бази *PubMed/MEDLINE*.

Референце нумерисати редним арапским бројевима према редоследу навођења у тексту. Број референци не би требало да буде већи од 30, осим у прегледу литературе, у којем је дозвољено да их буде до 50, а у метаанализи до 100. Број цитираних оригиналних радова мора бити најмање 80% од укупног броја референци, односно број цитираних књига, поглавља у књигама и прегледних чланака мањи од 20%. Уколико се домаће монографске публикације и чланци могу уврстити у референце, аутори су дужни да их цитирају. Већина цитираних научних чланака не би требало да

буде старија од пет година. Није дозвољено цитирање апстраката. Уколико је битно коментарисати резултате који су публиковани само у виду апстракта, неопходно је то навести у самом тексту рада. Референце чланака који су прихваћени за штампу, али још нису објављени, треба означити са *in press* и приложити доказ о прихватању рада за објављивање.

Референце се цитирају према Ванкуверском стилу (униформисаним захтевима за рукописе који се предају биомедицинским часописима), који је успоставио Међународни комитет уредника медицинских часописа (*http://www.icmje.org*), чији формат користе *U.S. National Library of Medicine* и базе научних публикација. Примери навођења публикација (чланака, књига и других монографија, електронског, необјављеног и другог објављеног материјала) могу се пронаћи на интернет-страници *http://www.nlm.nih.gov/bsd/uniform_requirements.html*. Приликом навођења литературе веома је важно придржавати се поменутог стандарда, јер је то један од најбитнијих фактора за индексирање приликом класификације научних часописа.

ПРОПРАТНО ПИСМО (*SUBMISSION LETTER*). Уз рукопис обавезно приложити образац који су потписали сви аутори, а који садржи: 1) изјаву да рад претходно није публикован и да није истовремено поднет за објављивање у неком другом часопису, 2) изјаву да су рукопис прочитали и одобрили сви аутори који испуњавају мерила ауторства, и 3) контакт податке свих аутора у раду (адресе, имејл адресе, телефоне итд.). Бланко образац треба преузети са интернет-странице часописа (*http://www.srpskiarhiv.rs*).

Такође је потребно доставити копије свих дозвола за: репродуковање претходно објављеног материјала, употребу илустрација и објављивање информација о познатим људима или именовање људи који су допринели изради рада.

ЧЛАНАРИНА, ПРЕТПЛАТА И НАКНАДА ЗА ОБРАДУ ЧЛАНКА. Да би рад био објављен у часопису *Српски архив за целокупно лекарство*, сви аутори који су лекари или стоматолози из Србије морају бити чланови Српског лекарског друштва (у складу са чланом 6. Статута Друштва) за годину у којој се рад предаје Уредништву. Сви домаћи аутори такође морају бити претплаћени на часопис или измирити накнаду за обраду чланака (*article processing charge*) за годину у којој се рад предаје Уредништву, у износу од 3.000 динара. Аутори и коаутори из иностранства су у обавези да плате накнаду за обраду чланака (*article processing charge*) у износу од 35 евра. Уплата у једној календарској години обухвата и све наредне, евентуалне чланке, послате на разматрање у тој години. Сви аутори који плате ову накнаду могу, уколико то желе, да примају штампано издање часописа. Треба напоменути да ова уплата није гаранција да ће рад бити прихваћен и објављен у *Српском архиву за целокупно лекарство*. Обавеза плаћања накнаде за обраду чланка не односи се на студенте основних студија и на претплатнике на часопис.

Установе (правна лица) не могу преко своје претплате да испуне овај услов аутора (физичког лица). Уз рукопис рада треба доставити копије уплатница за чланарину и претплату / накнаду за обраду чланка, као доказ о уплатама, уколико издавач нема евиденцију о томе. Часопис прихвата донације од спонзора који сносе део трошкова или трошкове у целини оних аутора који нису у могућности да измире накнаду за обраду чланка (у таквим случајевима потребно је часопису ставити на увид оправданост таквог спонзорства).

Додатне информације о чланарини и претплати могу се добити путем имејла (*office@srpskiarhiv.rs*) и на интернет-страници часописа *http://srpskiarhiv.rs/en/subscription/).*

СЛАЊЕ РУКОПИСА. Рукопис рада и сви прилози уз рад могу се доставити имејлом (*office@srpskiarhiv.rs*), електронски преко система за пријављивање на интернет-страници часописа (*http://www.srpskiarhiv.rs*), препорученом пошиљком или лично, доласком у Уредништво. Уколико се рад шаље поштом или доноси у Уредништво, рукопис се доставља одштампан у три примерка и нарезан на *CD* (снимљени материјал треба да је истоветан оном на папиру).

НАПОМЕНА. Рад који не испуњава услове овог упутства не може бити упућен на рецензију и биће враћен ауторима да га допуне и исправе. Придржавањем упутства за припрему рада знатно ће се скратити време целокупног процеса до објављивања рада у часопису, што ће позитивно утицати на квалитет чланака и редовност излажења часописа.

За све додатне информације, молимо да се обратите на доленаведене адресе и број телефона.

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When writing text in English, linguistic standard American English should be observed. Write short and clear sentences. Generic names should be exclusively used for the names of drugs. Devices (apparatuses, instruments) are termed by trade names, while their name and place of production should be indicated in the brackets. If a letter-number combination is used, the number should be precisely designated in superscript or subscript (i.e., ^{99}Tc, IL-6, O_2, B_{12}, CD8). If something is commonly written in italics, such as genes (e.g. *BRCA1*), it should be written in this manner in the paper as well.

If a paper is a part of a master's or doctoral thesis, or a research project, that should be designated in a separate note at the end of the text. Also, if the article was previously presented at any scientific meeting, the name, venue and time of the meeting should be stated, as well as the manner in which the paper had been published (e.g. changed title or abstract).

CLINICAL TRIALS. Clinical trial is defined as any research related to one or more health related interventions in order to evaluate the effects on health outcomes. The trial registration number should be included as the last line of the Summary.

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The authors should enclose the description of contribution to the article of every co-author individually (within the Submission Letter). Funding, collection of data or general supervision of the research group alone cannot justify authorship. All other individuals having contributed to the preparation of the article should be mentioned in the *Acknowledgment* section, with description of their contribution to the paper, with their written consent.

TITLE PAGE. The first page of the manuscript (cover sheet) should include the following: title of the paper without any abbreviations; suggested running title; each author's full names and family names (no titles), indexed by numbers; official name, place and country of the institu-

tion in which authors work (in order corresponding to the indexed numbers of the authors); at the bottom of the page: name and family name, address, phone and fax number, and e-mail address of a corresponding author.

SUMMARY. Along with the original article, preliminary and short communication, meta-analysis, review article, case report, article on history of medicine, current topic article, article for language of medicine and article for practitioners, the summary not exceeding 100–250 words should be typed on the second page of the manuscript. In original articles, the summary should have the following structure: Introduction/Objective, Methods, Results, Conclusion. Each segment should be typed in a separate paragraph using boldface. The most significant results (numerical values), statistical analysis and level of significance are to be included. The conclusion must not be generalized, it needs to point directly to the results of the study. In case reports, the summary should consist of the following: Introduction (final sentence is to state the objective), Case Outline (Outline of Cases), Conclusion. Each segment should be typed in a separate paragraph using boldface. In other types of papers, the summary has no special outline.

KEYWORDS. Below the summary, 3 to 6 keywords or phrases should be typed. The keywords need not repeat words in the title and should be relevant or descriptive. *Medical Subject Headings – MeSH* (*http://www.nlm.nih.gov/mesh*) are to be used for selection of the keywords.

TRANSLATION INTO SERBIAN. The third page of the manuscript should include: title of the paper in the Serbian language; each author's full name and family name (no titles), indexed by numbers; official name, place and country of the institution in which authors work. On the fourth page of the manuscript the summary (100–250 words) and keywords (3–6) should be typed, but this refers only to papers in which a summary and keywords are compulsory. The terms taken from foreign literature should be translated into comprehensible Serbian. All foreign words or syntagms that have a corresponding term in Serbian should be replaced by that term.

If an article is entirely in Serbian (e.g. article on history of medicine, article for "Language of medicine", etc.), captions and legends of all enclosures (tables, graphs, photographs, schemes) – if any – should be translated into English as well.

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LENGTH OF PAPER. The entire text of the manuscript – title page, summary, the whole text, list of references, all enclosures including captions and legends (tables, photographs, graphs, schemes, sketches), title page and summary in Serbian – must not exceed 5,000 words for original articles, preliminary and short communications, review articles and articles on history of medicine, and 3,000 words for case reports, articles for practitioners, educational articles and articles for "Language of medicine"; for any other section maximum is 1,500 words.

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If the manuscript is entirely in the Serbian language, tables and corresponding legend should be both in Serbian and English. Also, the table cells should contain text in both languages (do not create two separate tables with a single language!).

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ACKNOWLEDGMENT. List all those individuals having contributed to preparation of the article but having not met the criteria of authorship, such as individuals providing technical assistance, assistance in writing the paper or running the department securing general support. Financial aid and all other support in the form of sponsorship, grants, donations of equipment and medications, etc., should be mentioned too.

REFERENCES. The reference list is the responsibility of the authors. Cited articles should be readily accessible to the journals readership. Therefore, following each reference, its DOI number and PMID number (if the article is indexed for MEDLINE/PubMed) should be typed.

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