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ЗА ЦЕЛОКУПНО ЛЕКАРСТВО Осн.1872

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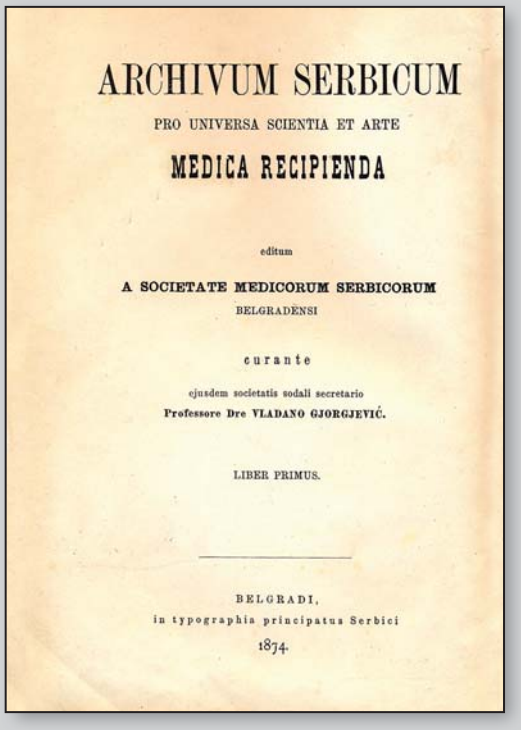

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1874.

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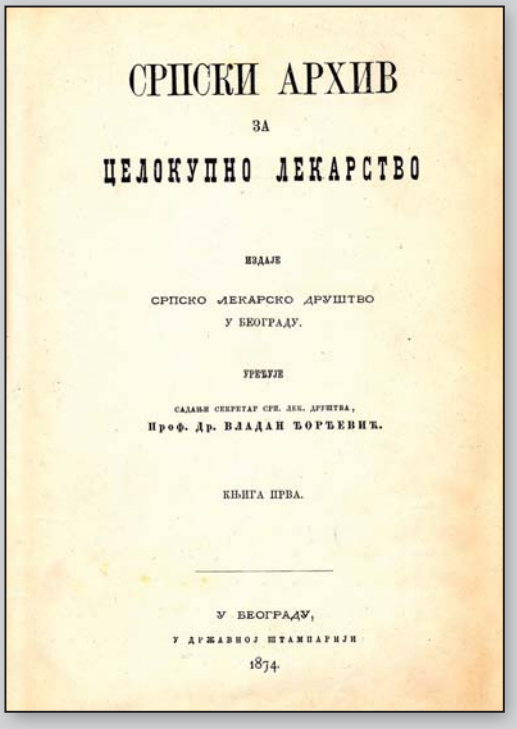

СРПСКИ АРХИВ

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.

Сви рукописи који се разматрају за штампање у „Српском архиву за целокупно лекарство“ не могу да се поднесу или да буду разматрани за публиковање на другим местима. Радови не смеју да буду претходно штампани на другим местима (делимично или у потпуности).

Приспели рукопис Уређивачки одбор шаље рецензентима ради стручне процене. Уколико рецензенти предложе измене или допуне, копија рецензије се доставља аутору с молбом да унесе тражене измене у текст рада или да аргументовано образложи своје неслагање с примедбама рецензента. Коначну одлуку о прихватању рада за штампу доноси главни и одговорни уредник.

За објављене радове се не исплаћује хонорар, а ауторска права се преносе на издавача. Рукописи и прилози се не враћају. За репродукцију или поновно објављивање неког сегмента рада публикованог у „Српском архиву“ неопходна је сагласност издавача.

Радови се штампају на енглеском језику са кратким садржајем на енглеском и српском језику, односно на српском језику, ћирилицом, са кратким садржајем на српском и енглеском језику.

Аутори прихватају потпуну одговорност за тачност целокупног садржаја рукописа. Материјал публикације представља мишљење аутора и није нужно одраз мишљења Српског лекарског друштва. С обзиром на брз напредак медицинске научне области, корисници треба да независно процењују информацију пре него што је користе или се на њу ослањају. Српско лекарско друштво, уредник или Уређивачки одбор „Српског архива за целокупно лекарство“ не прихватају било какву одговорност за наводе у радовима. Рекламни материјал треба да буде у складу с етичким (медицинским) и правним стандардима. Рекламни материјал укључен у овај часопис не гарантује квалитет или вредност оглашеног производа, односно тврдње произвођача.

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

Laboremus! 3. део: Путеви

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

„Лако је научити животињу, лако је научити простака, али је тешко научити онога ко је ненаучен већ постао учитељ другима".
Владика Николај Велимировић
(„Мисли о добру и злу")

Путеви

Данас, када су године као векови раније, не назадујете много ако стојите годину дана, али су други напредовали и 100 година су испред. И онда их је немогуће стићи. А ми смо стајали, из разних разлога много стајали. Ако сте желели и почели игру, морате је играти по правилима која важе за све, а на која немате утицаја. У тој утакмици са најбољима морате бити као они и још бољи. А они су модерни, користе сва техничка и технолошка средства да би били брзи, тачни, илустративни, динамични, продуктивни, експедитивни...

Информација и интернет

Развој информационих технологија (ИТ) направио је револуцију у свим областима, и то револуцију која још траје. Давно су заборављени (гушчја) пера и мастило, а још брже писаће машине. Могућности рачунара су невероватне. И морате их користити. Не морате знати како они раде, али морате знати шта вам могу пружити и како их искористити за оно што је вама потребно. Онај ко користи компјутер као писаћу машину је на нивоу тромесечног дактилографског курса у свему, а после годину дана и у својој научној области. Модернизација *CA* практично искључује све оне који још увек користе перо или писаћу машину.

Једно од открића ИТ је и интернет, који се вероватно може сврстати у 10 највећих светских открића. Предности интернета су бројне, а овом приликом истичем две: доступност информација и демократизација сазнања, а то је непосредно довело до губитка монопола на знање и нестајање ауторитета. Аутор ових редова се сећа колико је било потребно труда и времена да се дође до информације (чланка, књиге) пре 20 или 30 година. Због тога је интернет данас права благодет. Доступност информација је потпуна и, што је важно, она је доступна одмах. За писање књига је потребно око две године; ако се притом користе информације већ старе 1–2 године, јасно је да књига (уџбеник, монографија) може бити модерна и едукативна, али не и носилац најновијих сазнања. Најчешће је и тешко доступна. Часописи и чланци су најбржи и најдоступнији извор информација (научних, стручних, едукативних). Време од пријема чланка до одлуке о судбини чланка мери се у данима и мора бити мање од 45 дана. Проблем је и период од прихватања рада до његовог објављивања у штампаним издањима јер прође 1–2 године. То је главни разлог успеха електронских публикација, јер се информација после прихватања публикује за неколико дана.

Најбољи часописи публикују 5–10% понуђених радова. Најбољи су и имају велики број понуђених радова, а тада могу правити строжу селекцију да би остали најбољи. Експедитивни су и имају кратко време обраде рада, не губе време са лошим радовима, нити са поправкама и дорадама радова који би се могли објавити. Ако од пријема рада до одлуке прође годину дана и још годину дана до његовог објављивања, питање је колико је та информација актуелна после 2–3 године. *CA* публикује око 55% приспелих радова и улаже доста труда и времена у помагању ауторима да направе прихватљив рад. *CA* сада почиње са правим електронским публиковањем, максимално ће скратити време од пријема рада до одлуке о његовом прихватању или одбијању, прихваћене радове објављиваће одмах електронски, наставиће да помаже ауторима и све ће транспарентно изнети на свом сајту.

И у информатичкој ери све има и своју другу, тамну страну: много полуистина, дезинформација, манипулисања, непоштења. Зато у мору информација морате критички

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прихватати информације и тако избећи све замке прихватања неистина.

Губитак ауторитета се може посматрати кроз губитак монопола на скривано знање (своје и туђе), а прави научни ауторитети ће увек постојати јер су увек испред. Ауторитет међу једнакима се очигледно постаје на други начин. Не можете бити у врху ни у једној области и у исто време анахрони. Ти ауторитети морају схватити да их је време прегазило, да ће све њихово знање мукотрпно стицано годинама бити застарело за годину дана и да само морају размишљати да ли су заслужили да неко други брине о њиховом угледу. Титуломанија последњих година је последица губитка правих ауторитета и комплекса полуписмених, а бескомпромисних и агресивних. То је довело до инфлације и деградације титула, али све титуле испред имена не могу сакрити празнину после њих.

Језик

Научна истина је једна и универзална. Ако желите да се ваша истина чује, ако желите да будете део света, морате комуницирати са тим светом. Данас је језик комуникације енглески и морамо тако комуницирати јер не пишемо само за себе и због себе. Ми желимо да нас чују, јер нисмо аутистични. Јесмо ми најбољи и најпаметнији, али како нас свет види? Он може и без нас, а он нас прихвата по правилима за све и немогуће је наметнути своја правила. Ако нас свет не изолује, зашто бисмо ми тежили самоизолацији? Ако велике нације, као Немци и Кинези, могу публиковати на енглеском, зашто то не би могли и Срби? Да ли је то удар на српство и српски језик или одраз незрелог и недифинисаног националног бића? *CA* је давно прешао границе Србије и тешко се изборио да се и његова истина чује. На том пољу ће се борити и даље и стимулисаће ауторе да све форме чланака публикују на енглеском. *CA* ће публиковати искључиво на енглеском језику све оригиналне (научне и стручне) радове, метаанализе, приказе случајева, претходна и кратка саопштења. Само као компромис часопис ће објављивати на српском језику поједине форме појединих радова у целости, са апстрактом на енглеском.

Плагијаризам

Сви факултети и универзитети у Србији имају кодексе етике [1], правилнике и санкције за „неакадемско понашање у изради писаних радова“ [2], али на то нико не обраћа пажњу. Примери плагијаризма се налазе све чешће и на свим нивоима: од плагирања магистарских и докторских теза до плагирања научне информације. Много пута је указивано на ту појаву и у часописима [3] и у чувеним упутствима [3]. Плагијаризам јесте проблем часописа, али фалсификовање и фабриковање (измишљање) информације је још већи проблем институције плагијатора. Да ли су већи кривци од самих лопова они који им то дозвољавају? Прозивају се плагијатори, али зашто никада нису прозвани ментори или чланови комисија који су им то омогућили? Ко је крив нечињењем? Недавно, када је добио мали приказ случаја са 18 аутора, овај уредник се питао: Шта су аутори хтели тиме да кажу о часопису, о уреднику или сами о себи? Чланак је једноставно враћен да се смањи број аутора, јер чистачице са клинике не заслужују ауторство! Још се више питао: Зашто угледни професори, који су потписали сагласност да се рад публикује, нису опоменули младе ауторе да то тако не може? Да ли таква едукација није у њиховом опису посла? Лажно ауторство [3, 4] вишеструко умножава систем „ја тебе, ти мене стави у рад“, као да непосредна околина не зна ко су прави аутори. Проблем је лицемерство те исте околине.

Присвајање туђег и приказивање као својег је крађа. Али, плагијаризам није обична крађа: враћање украденог није и враћање на старо стање. Крађа је трајна. Опет прича о јабуци: ако вам неко украде јабуку (информацију), не може вам је вратити и остати без ње (украдене јабуке). Плагијаризам се мора посматрати као крађа најгоре врсте и мора се санкционисати. Плагијаризам је велики проблем *CA*, а плагијатори нису проблем часописа већ њихових институција, целокупне научне заједнице и друштва. *CA* ће се борити против сваког вида плагијаризма: забраном објављивања одређени временски период, обавештавањем научно-наставних већа факултета, универзитета, радних организација и других часописа. *CA* није у могућности да плагијатора изопшти из научне заједнице, али би то требало да уради њихово непосредно окружење. Да ли је оно спремно за то или се сви добро сналазимо у лажи и кањузи?

Међутим, како се у Србији борити против плагијаризма? Ако у Србији није лопов онај који краде, него онај којег ухвате. Ако је „најслаље украдено“! Ако се „најбоље прима и успева украдено“. Ако интелектуална својина вреди мало или ништа. Ако плагијатор постане академик. Како исправити генски поремећај који је условио сталну борбу против правила и сваког система? Поремећај који је настао после петовековног ропства и двовековног лошег вођства са масовним губицима у великим и „малим“ ратовима. Да ли су за поправку оваквих поремећаја опет потребни векови или се то једноставно мора сасећи? Када ће плагијаторима бити јасно да је такво дело криминално и неморално, а његов резултат „професионално самоубиство“ [3]?

На крају, лични савет младим ауторима: млади сте, имате времена и да научите како написати и саопштити своју истину, не треба да вас неко дописује у рад, јер ћете остати дужник. Сатисфакцију ћете имати само ако сте истински аутори. Не дописујте у рад шефове, довољно им је што су шефови. Не дописујте у рад неписмене, они су грамзиви, агресивни и нису „губили време“ на писање радова него су успостављали „контакте“ и били послушни, а бирократија ће управо њих довести вама за шефове. Онда ћете имати проблеме јер они знају да сте ви писмени, а они неписмени. Што је најгоре, знају и они да то знате и ви.

Реформе

Реформе *СА* су неминовне и то кроз модернизацију: техничку и суштинску. Оне су директно повезане јер техничка модернизација омогућава суштинску.

Технолошко прилагођавање је неминовно, редакцијски и уреднички поступак се мора максимално скратити, мора увести право електронско издање, повећати цитабилност радова, увести електронско уређивање часописа, увести нове врсте чланака (видео-чланке, видео-илустрације у чланцима, радове са *e*-презентацијом много слика, *How I Do It*, кратке форме чланака: слике из клиничке медицине, историје), а активним приступом уредништва публиковањем и: личних ставова, наручених коментара, *Pro et contra*, уводника, прегледних чланака, актуелних тема итд. Иако практично цео часопис има улогу у континуираној медицинској едукацији, поједини чланци ће бити посебно намењени таквој едукацији.

СА покреће и „*e*-библиотеку *СА*" на свом сајту и учиниће доступним публиковане књиге у издању *СА*, СЛД, као и старе и ретке књиге других издавача. Данас се са нешто новца може оформити добра библиотека, али се биоблиотеке посебно цене управо по тим старим и ретким књигама до којих се тешко долази. *СА* ће посебну пажњу усмерити на њих да би их спасао заборава, учинио доступним свим заинтересованим истраживачима, али и онемогућио даља оштећења тог блага.

Учићемо од најбољих и тежити ка њима. Нећемо се поредити са околином и пустићемо друге да нас оцењују, са што веће даљине – то боље. Има много критеријума за вредновање часописа и свима се може наћи мана. Али, неких критеријума мора бити и прихватићемо оне које већина прихвата. Пратићемо индексне базе и изворе: *Thomson Reuters Web of Knowledge℠* (*Web of Science®*, *JCR-Journal Citations Reports*), *SJR – SCImago Journal & Country Rank, Scopus* (*Elsevier*), *Medline* (*LinkOut*), *Google Scholar* и наравно *KoBSON* [4]. Пратићемо њихово вредновање као што су: *IF* (*Impact factor*), *SJR*, *CiteScore*, *h-index*, *Eigenfactor®* и друге [9]. У исто време ћемо сами пратити и анализирати свој рад и то врло транспарентно. Сви посматрани параметри ће бити јавни на сајту часописа као *Ranking* и *Metrics* и кроз овакве уводнике по одређеним темама.

Требало је много година рада да *СА* буде све мање информатор, билтен или службени гласник и да постане право научно и стручно гласило. Тај многогодишњи рад условио је да је *СА* последњих 70 година индексиран у највећој цитатној бази (*Index Medicus, Medline, PubMed*), а последњих 10 година у свим значајним цитатним базама.

Циљеви

Модернизовати форму часописа, створити повољне услове за ауторе, а потом ће зависити све од њих, тј. од квалитета радова које буду понудили. Уредништво сигурно неће само чекати. Прво ће јасно дефинисати уређивачку политику, а потом ће кроз „активно уређивање часописа" стимулисати и ауторе и себе.

Важно је да се нађемо на том „светом путу", а докле ћемо заједно стићи зависиће од нас, али и других, од оних који трче исту трку и од оних који нама стварају услове, одмажу или помажу.

Морамо ићи напред, напред и само напред.

Laboremus!

Српски архив за целокупно лекарство – *Vivat, crescat, floreat*!

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EDITORIAL / УВОДНИК

Homo homini lupus est

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Man's inhumanity to man of all kinds has been well documented throughout each era of recorded time [1]. Modern era and time of transition we live in brought with it new ways of inhumanity. In these times, living in Serbia, we are exposed to many kinds of violence. Acts of violence may originate from the state authorities via legislation aimed against particular groups, or from single perpetrators using intimidation, physical assault, or mobbing [2]. Some vulnerable social groups are particularly exposed to violence and they need special legal protection.

Ambroise Auguste Tardieu provided the first description of *child abuse* in contemporary medical literature when he reported 32 cases of cruelty to children in 1860. With specific reference to abusive head injuries to children, John Caffey in 1946 described six infants with multiple fractures in the long bones, who additionally had chronic subdural hematoma and no history of injury [2]. Child abuse is non-random physical and/or mental damage inflicted on a child, either willfully or through neglect, within the family or institutions, which causes injury and/or impaired development and which, in individual cases, may cause death [3]. The boundary between acceptable violence in the context of the so-called necessary educational measures by parents or as part of accepted tradition, and unacceptable violence leading to death or severe injury is not always clear [3]. Although the term *battered child* is often used to describe physical child abuse, the recent literature refers to *non-accidental injury* and *abusive* or *inflicted injury* [3]. There are many factors to consider when trying to distinguish accidental from non-accidental injury. Clinical findings and radiologic imaging studies not in keeping with the history and injuries of different ages are key indicators of inflicted trauma, especially in infants. The age and stage of development of the child, the timeliness of seeking treatment, other injuries of different ages, child's state of nutrition and cleanliness should be taken into consideration when separating inflicted from non-inflicted injuries [4]. Handicapped children are in particular danger [2]. Another factor that influences recognition of abusive injuries includes a physician's experience with child abuse and family violence [1].

The terms *intimate partner violence*, *intimate partner abuse* or *domestic abuse* describe physical, sexual or psychological harm originating from a current or former intimate partner or spouse and may happen among heterosexual and same-sex couples [2]. *Stalking* and nowadays *cyber-stalking* are often included among different types of intimate partner violence [2]. Older abused women face additional challenges, having grown up and married during a time when domestic abuse was tolerated or ignored, having lived with abuse for many years, which can lead to problems such as poor self-esteem, feeling the duty to take care of an ageing partner, or feeling afraid of living alone after being with someone for many years [2].

But we mustn't forget *school violence*: *school bullying* is primarily used to describe repeated harassment behavior in schools, and a new type of violent behavior among schoolchildren – *cyber mobbing* using mobile telephones, computers, the internet, and social networks such as Facebook [2]. Or *violence against homosexuals*, which originates from the state authorities through legislation or from single perpetrators tolerated by state authorities. Or *violence against the patients in mental hospitals*, which originates from the medical staff, in cases when monitoring of personnel in these institutions is poor. Or *violence against the elderly* as domestic violence or violence in nursing centers – including physical, sexual and psychological abuse, neglect, as well as financial exploitation and violation of civil rights [2].

In hurrying to catch up with the European Union, we have adopted modern European legislation easily, but we do not enforce these new laws consistently – now there is a great gap between reality and copied European legal norms.

There is a German saying: *Ohne Daten, keine Taten*. To act, one needs facts. The very first and key step in protection against violence is recognition of its existence in society, and after

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that diagnosis, which rests upon multidisciplinary efforts among clinicians, social workers, medicolegal death investigators and law enforcement agencies [2, 5, 6]. Collaborative efforts and funding by governmental and private sources support ongoing research to establish evidence-based markers for accurate diagnosis [2]. Finally, the last steps are protection of the abused persons, adequate punishment of perpetrators, and continuing education and prevention.

Sensationalistic headlines in tabloids are not the way to solve these social and medical problems. Each of us could be abused at a certain time in life. We need true action by the government and the virtuous political authority. We need deeds.

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Medicolegal characteristics of domestic violence

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SUMMARY

Introduction/Objective Domestic violence is a phenomenon as old as the history of human civilization, present in all cultures, epochs and social systems. Despite the fact that domestic violence represents a dangerous and unacceptable social phenomenon, as well as a significant medical problem, there are still no precise data on the prevalence of this phenomenon in our country.
This study aims to determine the elementary forensic characteristics of domestic violence that would represented the basis for future medical research in this field.

Methods A total of 4,593 records of forensic autopsy (n = 3,120) and clinical forensic medical examinations (n = 1,473) were analyzed in the 1996–2005 period in order to determine the cases of domestic violence.

Results The analysis encompassed 300 cases (6.5%) of clinically examined (n = 211; 70.3%) and autopsied (n = 89; 29.7%) victims of domestic violence. A statistically significant increase in domestic violence cases ($\chi^2 = 12.74$; p = 0.00036) was determined in the observed period. The victims were mostly females (78%), with the mean age of 45.8 years (min = 0.3; max = 85; SD = 17.7), married (45%), with personal income (74.4%), and urban residence (66.3%). The majority of abusers were males (89.3%). Intimate partner violence was present in 58.3% of the cases. Physical abuse was the most common form of violence (97.7%), while sexual violence (2.3%) and child abuse (4.3%) were rarely recorded.

Conclusion The results of this research indicate that forensic medicine can be of great help in designing appropriate standards for conducting clinical medical examination, preventive programs, and strategies in fighting domestic violence.

Keyword: domestic violence; forensic medicine; abuse; injury

INTRODUCTION

Domestic violence (DV) is a phenomenon as old as the history of human civilization, present in all cultures, epochs and social systems [1]. For this reason, the ubiquity and universality are essential characteristics of this phenomenon [2]. Until the late 1960s, DV had not drawn any particular attention of the society. The dominant opinion was that "a home is a man's fortress" and that violence within the family is a private matter. During the 1980s and 1990s, DV became more widely recognized and considered as one of the most under-reported crimes [2, 3]. In the coming years, DV has been seen not only as a dangerous and unacceptable social behavior produced and maintained by the cultural and social norms, but also a great burden on the health system at the global level [3, 4]. Numerous problems that affected Serbia over the past decades, including the long-lasting social and economic crisis, the general impoverishment of the population, an increase in unemployment, the inability to satisfy basic subsistence needs, the arrival of a vast number of refugees, and many other challenges, caused the dramatic rise in all forms of violence in our country, including DV [4, 5]. Despite this, there is a deficiency of exact data of DV incidence in Serbia both in the context of social and natural sciences [5].

The current study attempted to determine medicolegal characteristics related to the distribution, structure, nature, and consequences of DV, with the aim to achieve a better understanding of this phenomenon from a forensic perspective, which would represent the basis for future medical research of this phenomenon.

METHODS

This retrospective study conducted at the Institute of Forensic Medicine of the Faculty of Medicine, University of Niš, Serbia, by analysis of autopsy protocols and reports of DV victims who underwent clinical forensic examination in the 1996–2005 period. A total of 4,593 cases (3,120 autopsy reports and 1,473 clinical exams) were analyzed. The cases of DV victims (n = 300; 6.5%) were analyzed in the investigated sample.

The survey covered the territory of District of Niš (the second largest district in Serbia by size, with the area of 2,729 km² and 373,404 inhabitants) and surrounding areas of Southeastern Serbia (the area of 14,010 km² and a total of 1,551,268 inhabitants) [6].

Each clinical examination was preceded by obtaining the informed consent of the examined person about using their information for scientific research purposes, with absolute protection of their identity and privacy. The Ethics Committee of the Faculty of Medicine of the University of Niš approved research on human cadavers.

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Several items were analyzed in every case: the aspect of the victim, the aspect of the abuser(s), the characteristics of violence (form of violence, reason, time and place of violence act), as well as the forensic aspect of the victim's injuries (the type, topography, severity and outcome, weapon type and mechanism of harm). The results were statistically analyzed using SPSS Statistics for Windows, Version 17.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

The retrospective analysis of the records of the Niš Institute of Forensic Medicine between the years 1996 and 2005 revealed a total of 300 DV cases, which constituted 6.5% of all examined subjects (n = 4,593). Among the DV cases, clinically examined subjects and autopsied victims were represented in 70.3% (n = 211) and 29.7% (n = 89), respectively. In relation to the total sample, there was a statistically significant increase in DV cases ($\chi^2 = 12.74$; p = 0.00036), as shown in Figure 1. Clinical forensic examination was carried out at personal request of DV victims in 65.7% of the cases, while in 34.3% the examination was conducted at official order by investigating authorities (in all autopsied and in 4.6% of clinically examined victims).

Regarding the demographic characteristics of DV victims, it was found that 78% of the cases were females, and 22% were males. The mean age of victims was 45.8 years (min = 0.3; max = 85; SD = 17.7). The victims under the age of 18 years were represented in 4.3% of the cases (n = 13), as shown in Figure 2. More than one half of all victims were either formally married (45%) or lived in cohabitation, e.g. in an extramarital community (6.7%). Most victims (74.4%) had personal income (employed, retired, farmer), while victims without income (housewives, unemployed, and dependent persons) accounted for about one quarter of all cases (25.6%). Majority of the victims resided in the city (66.3%). The results showed an increase in the number of victims in urban areas, but not statistically significant ($\chi^2 = 0.335$; p = 0.56). In addition, there is a statistically insignificant negative trend of victims from the rural areas ($\chi^2 = 0.625$; p = 0.43).

The majority of abusers were males (89.3%), while women committed violence against family members in 10.7% of the cases. The most common reason for DV was quarrel and disagreement (56.7%). In only 11% of the cases abusers were under the influence of alcohol at the time of the act of violence, and the majority of them were found to be mentally competent (94.7%). Violence act mostly occurred in the residence of the victim (82%), in the afternoon and evening (a total of 59.3%), during the summer and autumn. The peak incidence was in September (11.7%). Regarding the relations between abuser and victim, the majority of abusers expressed violence within intimate partner relationships (58.3%), towards their current or former intimate partners (formally married, cohabitating, or after separation/divorce). Intimate partner violence (IPV) was committed by male abusers in 54.3% and by female abusers in 4% of the cases (Figure 3). After killing of a family member, male abusers committed suicide in 12 cases. Suicide followed intimate partner homicide in nine cases, and attempted suicide in one case. There was no suicide among female abusers.

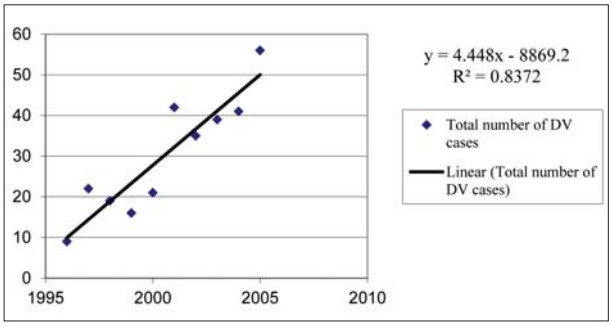

Figure 1. Trend of total number of domestic violence (DV) cases

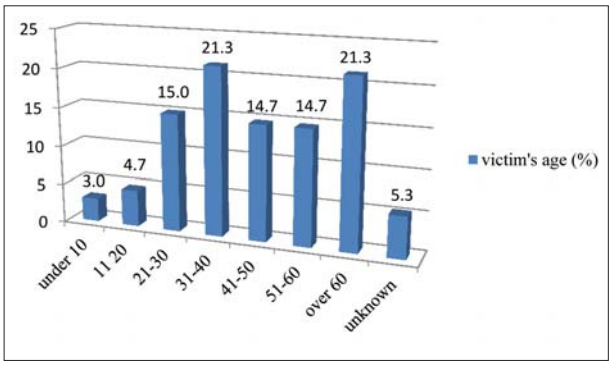

Figure 2. Distribution of domestic violence victims according to age

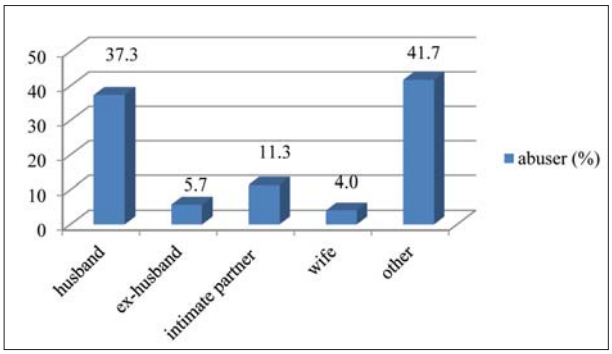

Figure 3. Incidence of abusers according to the relation with the victim

Continuous and long-lasting DV was present in 46% of the cases. The most common form of DV was physical violence (97.7%), while sexual violence was recorded only in 2.3% of the cases (all the victims were females, aged from 16 to 65 years). The psychological violence, which usually accompanied physical and sexual abuse, was not possible to investigate due to the lack of information in the study sample.

Physical abuse almost exclusively manifested by mechanical injuries (93.3%), while other types of injuries (e.g. asphyxia, thermal, chemical, etc.) were present to a much lesser extent (6.7%) (Figure 4). Blunt mechanical trauma caused 75.5% of all injuries, usually induced by blows with fists, feet, or various objects (wooden sticks, metal rods, hammers, agricultural tools, chairs, ashtrays, phones,

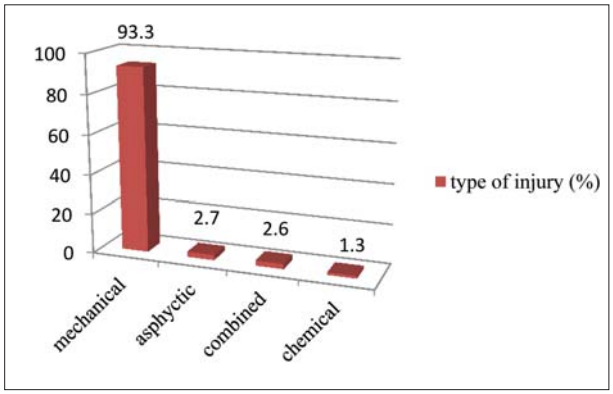

Figure 4. Incidence of injuries by type

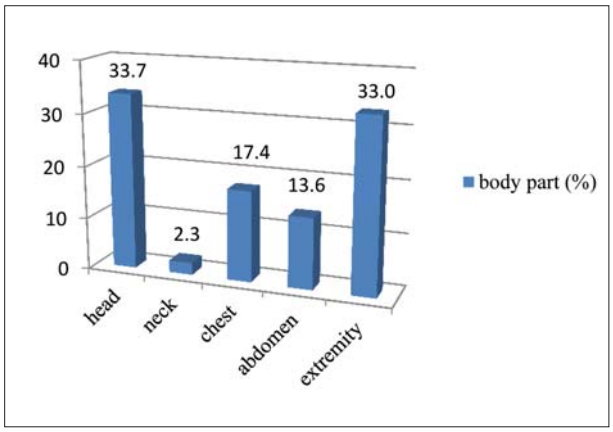

Figure 5. Topographic distribution of injuries

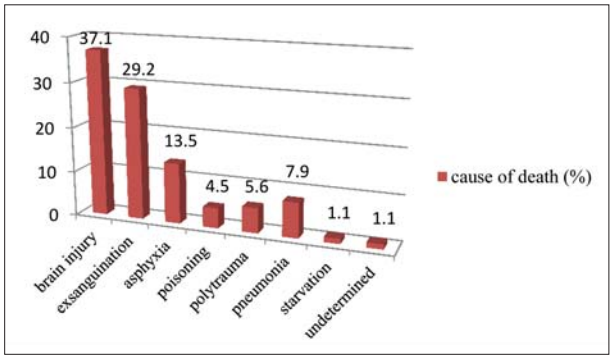

Figure 6. Incidence of causes of death in domestic violence victims

bricks, stones, straps, cables, ropes, etc.). Injuries inflicted by firearms and edged/pointed weapons were present in 10.7% and 10.4%, respectively. Most commonly encountered injury sites were the head (33.7%) and the extremities (33%) (Figure 5).

In the group of clinically examined victims (n = 211), the commonest were skin and underlying soft tissue injuries (hematoma, abrasion, contusion, laceration), and to a lesser extent bone fractures and dislocations, all inflicted by blunt objects. In this group, there were no injuries inflicted by firearms. In contrast to previous results, in the group of autopsied casualties (n = 89), the most frequent cause of death was a severe brain injury, chest and abdominal trauma, or multiple bodily injuries (polytrauma), inflicted by blunt or sharp objects and firearms (Figure 6). Regarding the severity of all mechanical injuries, minor bodily injuries were present in 65.9%, severe in 9.7%, serious life-threatening in 12.5%, and unconditionally fatal injuries in 11.8% of the cases.

DISCUSSION

DV represents any use of force, threats, or other forms of coercion sufficient to injure or endanger the physical and/or psychological integrity of the victim, which is committed by one family member against other person(s) with whom he/she lives or has lived with, or with whom is/was in an intimate relationship [7, 8]. Some feminist theorists advocate the view that apart from army during the war, family represents the most violent social institution with high chances of being killed, physically abused, punched, beaten, and slapped [1]. The results of our study are not so far from this standpoint.

Despite the fact that there is no systematic monitoring of DV in Serbia, the authorities have recognized this phenomenon as a separate entity, and have accordingly made significant steps in its disclosure and studying in different scientific fields [5]. The present study reveals some important points about DV in our community.

First, there is an obvious increase of DV cases within the studied group. According to the scientific data, it seems that the growing trend is not only a consequence of general rise in crime but also a result of active national strategy in the legislation of this offense [5, 8]. Patriarchal ideas about gender relations and parenting are still prevalent in our country. Those are the main reasons why DV had not been considered a serious form of violence for a long time, but a common and socially acceptable behavior [5, 7]. Our society has marginalized and ignored this phenomenon for decades. Until 2002, there were no adequate legal mechanisms to prevent and fight DV in Serbia [9]. Influence of positive legislation and greater individual sensitivity to this kind of violence has contributed to more frequent reporting, which is the condition that should be taken into consideration in the analysis of results [5].

The second important result of our study revealed overwhelming majority of female victims and male abusers, which corresponds to results of almost all previous studies conducted around the world [10–13]. A survey on male violence against women, carried out during 2011 by the Ministry of Labour and Social Development of the Republic of Serbia and funded by the United Nations, revealed that 54.2% of women suffer from some form of DV induced by men [10, 14]. This survey was based on a representative sample of 2,500 Serbian women between 18 and 75 years old.

Our findings about IPV, which includes violence towards current or former intimate partners showed the similar results as the research of Dixon and Graham-Kevan [13]: male abusers were violent towards their marital or extra-marital partners or ex-wives in 54.3% of cases. On the other side, female intimate partner abusers expressed violence exclusively towards their marital partners (4%), and never to the extramarital partners or ex-husbands.

The current study also confirmed the fact that the most severe forms of DV were related to IPV, especially to the marital violence [14, 15]. According to our results, IPV had a fatal outcome in 14% of the cases, out of which men conducted the violent act in 11.7%, and women in 2.3% of the cases. This research showed an interesting result that after the killing of a husband, there were no suicidal tendencies among female abusers. Unlike women, after taking the life of the wife, ex-wife, or intimate partner (n = 35), male abusers committed suicide in nine cases and attempted suicide in one case. Other authors obtained similar results in intimate partner homicide-suicide studies [16, 17]. In the light of the abovementioned results, it is necessary to undertake specific preventive measures directed at the most vulnerable population group – women in abusive intimate partner communities.

The third distinctive feature of DV relates to the small representation of children in the survey sample. Namely, minor victims (under the age of 18 years) were represented in 4.3% of the cases (n = 13). Among them, in four fatalities and nine non-fatal cases, the abusers were their biological parents. These results correspond with the findings of other researchers that also suggested the high number of under-reported cases of DV against children [18]. The explanation for this phenomenon lies in a child's total dependence on their abusive parents, who, logically, avoid self-reporting to the authorities [19, 20]. In our study, a non-violent parent (usually also a victim of the same abusive family member) has always reported DV against children. In accordance with these results, appropriate national strategies are required for the disclosure of DV and child protection [18, 19, 20].

The fourth characteristic result of this research refers to the small number of identified sexual violence cases (2.3%). Such finding almost certainly points to an "iceberg phenomenon," which indicates a high proportion of under-reported ("missed") cases [10, 11, 13]. The reason for such an outcome can be primarily explained by the fact that the marital rape was established in the Criminal Code of the Republic of Serbia as late as 2002 [9]. It means that the legislator did not recognize this form of violence as a criminal offense before this period. In addition to this, there is a deficiency of standardized protocols for medical examination not only for the DV victims but also for the rape victims. These circumstances greatly complicate professionals' dealing with victims. To be specific, rape in general, and particularly DV rape, is associated with a high degree of secondary victimization that occurs during the medical procedures, pre-investigation, and court proceedings, and additionally discourages victims in reporting the offense. For this reason, creating standardized medicolegal protocols for rape and DV victims can be of great help for society [21]. Determining the characteristics of injury on the victim's body and sampling of biological material as physical evidence that gave rise to the litigation is imperative in forensic detection of any crime, including DV offenses [21, 22]. Thus, comprehensive medical approach to work with victims imposes the necessity for forensic clinical examination in such cases.

The fifth important finding reveals physical violence as the dominant form of DV (97.7%). From medicolegal point of view, the severity of injuries directly correlated with the manner of medical treatment [23, 24]. In most cases of minor bodily injuries (65.9%), medication was required, but not necessary. Severe bodily injuries (9.7%) demanded particular medical assistance, while serious, life-threatening injuries (12.5%) always needed urgent and specialized medical care, as well as the obligatory hospitalization. Prompt and suitable medical help could not save victim's life in 11.8% cases of unconditionally fatal injuries. Similar results about physical injuries related to DV were reported in the scientific literature [24, 25], according to which DV represents one of the leading causes of injury in general population [5, 17].

CONCLUSION

The existence of numerous prejudices, conciliatory public attitude, and viewing DV as an acceptable behavior, significantly contribute to the high frequency and extent of this form of violence in our society. The results of this research on DV indicate that forensic medicine can be of great help not only for court proceedings, but also in the designing appropriate standards for conducting clinical medicolegal examination, prevention programs and strategies in fighting this phenomenon. Therefore, education and training of physicians of all specialties in recognizing the specific elements of DV abuse, as well as application of medical protocols to the treatment of DV victims, are necessary for a better understanding of the health hazards related to this field.

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Судско-медицинске карактеристике породичног насиља

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САЖЕТАК

Увод/Циљ Породично насиље је феномен стар колико и историја људске цивилизације, присутан у свим културама, епохама и социјалним системима. Упркос чињеници да насиље у породици представља опасно и неприхватљиво друштвено понашање, као и значајан медицински проблем, у нашој земљи и даље не постоје прецизни подаци о учесталости ове појаве.

Циљ овог истраживања је детерминација основних судско-медицинских карактеристика породичног насиља, које би представљале базу за будућа медицинска истраживања на овом пољу.

Методе У периоду 1996–2005. године анализирано је 4.593 протокола судско-медицинских обдукција (n = 3.120) и клиничких судско-медицинских прегледа (n = 1.473), у циљу евидентирања случајева породичног насиља.

Резултати У анализу је укључено 300 случајева (6,5%) клинички прегледних (n = 211; 70,3%) и обдукованих (n = 89; 29,7%) случајева породичног насиља. У посматраном периоду је утврђен статистички значајан пораст броја случајева породичног насиља (χ^2 = 12,74; p = 0,00036). Жртве су најчешће биле женског пола (78%), просечне старости 45,8 година (мин. = 0,3; макс. = 85, СД = 17,7), у браку (45%), са личним примањима (74,4%) и настањене у граду (66,3%). Највећи број насилника је био мушког пола (89,3%). Насиље између интимних партнера је било присутно у 58,3% случајева. Физичко злостављање је био најчешћи облик насиља (97,7%), док су сексуално насиље (2,3%) и злостављање деце (4,3%) били ретко заступљени.

Закључак Резултати овог истраживања упућују на то да судска медицина може бити од велике помоћи у пројектовању одговарајућих стандарда за обављање клиничких лекарских прегледа, као и превентивних програма и стратегија у борби против насиља у породици.

Кључне речи: породично насиље; форензичка медицина; злостављање; повреда

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Social, clinical, and radiological characteristics of physical abuse of children under three years of age hospitalized in a tertiary health institution

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SUMMARY

Introduction/Objective Child abuse is a significant public health problem in modern society. Many cases of violence against children remain undetected. Serbia has no official protocols for medical examination of abused children.

The aim of the study is an analysis of the social, clinical and radiological characteristics of physical abuse of children under three years of age that required hospital treatment.

Methods This retrospective study included 98 physically injured children admitted to the University Children's Hospital in the period from 2013 to 2015, with suspected physical abuse. In addition to the history of injuries, complete clinical examinations and standard laboratory analyses were performed in all children, as well as X-ray examination in children with apparent or suspected skeletal injury. Ultrasound examination and computerized tomography or magnetic resonance imaging were performed in selected patients. Final diagnosis of abuse was established by multidisciplinary assessment team. The children were divided into two groups – those with proven and those with suspected abuse.

Results Most of 98 children who were suspected of being abused (92%) were from one or both unemployed parents, 68% were male, 60% were first-born, and 44% younger than one year. Ninety-two percent of the children had skeletal fractures, 19% of whom had two or more fractures. The commonest fracture was a linear skull fracture, which was detected in 51% of the cases. Abuse was confirmed in only five of 98 suspected cases.

Conclusion Among the known social risk factors for abuse of children, the low economic status of the family was the most frequent one in our analyzed sample. The most common injury is a linear skull fracture. A national guideline for medical investigating of abused children is required.

Keywords: child abuse; children under three years; bone fractures

INTRODUCTION

Child abuse is a significant public health problem in modern society. Unfortunately, many cases of violence against children remain undetected [1, 2]. Several risk factors are associated with child abuse – parents younger than 20 years, lower socioeconomic status, separated parents, history of mental illness, alcohol and drug abuse. Abused children are more often male, unwanted children with developmental delay or chronic disease [1, 2, 3].

Fractures are the second most frequent manifestation of physical abuse, preceded only by skin lesions (bruises, contusions) [4, 5]. Fractures are usually multiple and may occur in any bone in the skeleton [4]. The evident cases of abuse are those that have occurred in the presence of witnesses or if there has been a confession. All other cases that raise the suspicion of abuse (age younger than 18 months, signs of fracture healing, unknown or inconsistent history of injury mechanism, and presence of other injuries) require material evidence specific to the identified injury [2, 4, 6–10].

Radiological investigations should include a high quality skeletal survey, while brain computed tomography (CT) and/or magnetic resonance imaging (MRI) are mandatory in children younger than two years and in older children with neurological signs/symptoms. The use of abdominal imaging, including ultrasonography (US), CT and/or MRI is debatable if the child has no symptoms [4, 6, 8, 9, 10].

In Serbia there are papers on forensic and psychiatric aspects of child abuse [11, 12], but with no mention of radiological investigations. Serbia does not have an official protocol that defines standards for performing skeletal surveys on children in whom physical abuse is suspected. The results of this study will highlight significant epidemiological factors associated with child abuse, and provide an overview of the radiological standards for diagnosis of child abuse in Serbia. Given the recent Europe-wide adoption of the Royal College of Radiology / Royal College

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of Pediatrics and Child Health (RCR/RCPCH) guidelines for investigating child abuse, results will also act as a baseline comparator for future similar studies [13, 14].

The aim of this study was to analyze the social, clinical, and radiological characteristics of physical abuse of children under three years of age that required hospital treatment.

METHODS

The data for this retrospective observational study were extracted from the medical records of 98 children younger than three years admitted to the University Children's Hospital in Belgrade in the period from 2013 to 2015 because of suspected physical abuse.

In addition to the history of injuries, complete clinical examination and standard laboratory analyses were performed in all children with suspected physical abuse, as well as X-ray examination in children with apparent or suspected skeletal injury. US, CT, or MRI examinations were performed in selected patients (Table 1 and 2). Final diagnosis of abuse was established by multidisciplinary assessment team. Children were divided into two groups – those with proven and those with suspected abuse. We defined the proven abuse cases with medical records agreed by the professionals involved, such as the signs of previously medically untreated fractures, unknown or inconsistent history of mechanism of injury in the presence of unexplained fractures on skeletal survey, the presence of injuries other than the presenting injury, especially if injuries were those specific for abuse, plus presence of at least two of the following criteria: admission of assault, presence of witnesses, involvement of police or social services, and legal outcome. We defined the suspected abuse as cases with inconsistent history of mechanism of injury, discrepancies between the extent of an injury and the reported mechanism of injury, estimated by a physician. Also, presence of the risk factors in parents that rise suspicion for child abuse were taken into account, such as parents younger than 20 years, lower socioeconomic status, separated parents, history of mental illness, alcohol and drug abuse.

Based on information provided by parents or the person who had brought the child to the hospital, we recorded the age, education, employment of parents or main occupation, marital status of parents, number of children and family members in total, guardianship of children, and mechanism of injury. In relation to findings from the physical examination, we recorded the presence of other visible non-skeletal injuries (bruises, lacerations, contusions, burns, abrasions, evidence of pinching) and presence of chronic disease or birth defect of children.

According to the radiographic signs of fracture, our patients were divided into the following three groups: patients with fractures described in the literature as highly specific for abuse (posterior and lateral rib fractures, metaphyseal fractures and long bone fractures in non-walking age, scapular fracture, spinous process fracture, multiple "eggshell" skull fractures, occipital impression fracture), patients with moderately specific fractures (multiple fractures, epiphyseal separation, vertebral body fracture, complex skull fracture), and patients with fractures of low specificity for abuse (clavicular fracture, oblique and spiral shaft fracture of long bones, linear skull fracture) [4].

Cases with skeletal dysplasia or other bone disease, as well as those with traffic trauma, were not included in this study.

Table 1. Skeletal surveys performed according to RCR/RCPCH guidelines

Radiographic projection	Number of children (n = 98)	
	Proven abuse	Suspected abuse
Skull (frontal and lateral)*	5	67
Thorax (AP)	5	38
Right and left oblique views of the chest	4	29
Abdomen (pelvis and hip) (AP)	0	2
Lumbosacral and cervical spine (lateral)	1	7
Both upper arms (AP)	0	7
Both forearms (AP)	1	14
Both femurs (AP)	1	11
Both lower legs (AP)	1	12
Hands (PA)	0	4
Feet (DP)	0	4
Follow-up survey	0	2

*Towne view – one child with proven abuse, four children with suspected abuse
RCR – Royal College of Radiologists; RCPCH – Royal College of Paediatrics and Child Health; AP – anteroposterior; PA – posteroanterior; DP – dorsal-plantar

Table 2. The frequency of use of additional diagnostic methods

Additional examination	Abdomen	Brain	Other*
Computed tomography (CT)	2	42	7
Magnetic resonance imaging (MRI)	0	19	1
Ultrasonography (US)	47	61	11

* CT of spine in five and upper leg in two cases, MRI of spine in one case, US of soft tissue in seven, and testicles in four cases

RESULTS

Most of 98 children who were suspected of being abused (92%) were from one or both unemployed parents, 68% were male, 60% the first-born and 44% younger than one year. Education beyond secondary school was obtained by 7% of parents, and 16% were up to 20 years old. Most parents (96%) were married. There were 40 children aged up to 12 months, 28 children aged 12 to 24 months, and 30 children aged 24 to 36 months. Skeletal fractures were found in 92% of the children, 19% of whom had two or more fractures (Table 3 and 4). The most common fracture was a linear skull fracture, which was detected in 51% of the cases. Additional injuries distant to the site of fracture were identified in 70% of the children. The physical abuse was undoubtedly proven in five (5%) out of 98 suspected cases, one in the age group of up to 12 months, one in the 12–24-month age group, and three cases in the 24–36 months age group, all of whom with craniocerebral injuries. In these cases, the perpetrator of abuse was dis-

Table 3. Distribution of fractures

Fracture type/site – number of fractures (n = 90)								
Skull			Long bone				Clavicle	Digit
Linear	Complex	Total	Diaphyseal	Metaphyseal	Epiphyseal	Total	3 (3%)	1 (1%)
46 (51%)	5 (6%)	51 (57%)	34 (38%)	0	1 (1%)	35 (39%)		

Table 4. Distribution of fractures by age

Fracture type/site Number of fractures (n = 90)	Age group		
	0–12 months	12–24 months	24–36 months
Skull	27 (77%)	16 (57%)	8 (30%)
Long bone	5 (14%)	11 (39%)	19 (70%)
Clavicle	3 (9)%	0 (/)	0 (/)
Digit	0 (/)	1 (4%)	0 (/)
Total	35 (100%)	28 (100)	27 (100%)

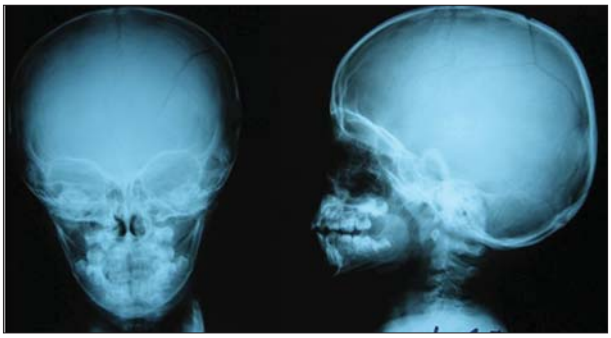

Figure 1. X-ray of the head of a 26-month-old boy injured with multilinear left fronto-parieto-occipital fracture

covered, and all of these children were hospitalized at the Department of Neurosurgery. Figure 1 shows the X-ray appearance of skull fractures in one of them.

DISCUSSION

Our results indicate that physical abuse of children younger than three years was undoubtedly confirmed in only 5% of 98 suspected cases. This is relatively low compared to other reports because there was no consistent approach to the investigation of these children [15–19].

According to our data, physical abuse is twofold more frequent in males. Numerous studies indicate that maltreatment of children most frequently occurs in families with lower economic status and education [1, 2, 3, 20], which was confirmed in our research. In our group of patients, only 3% of parents were educated beyond secondary school and only 7% of them were both employed. While the majority of parents were married, they were in their early twenties. Although there are scarce data in the literature related to birth order and child abuse, most cases in our study were first-born. All of these suggest that parental immaturity, lack of experience, and financial difficulties may be instrumental in the causation of abuse.

Unfortunately, we were unable to record data on the psychiatric disorders, confirmed use of alcohol and/or drugs or previous abuse in the families. This information should be included in future studies, since it will contribute to a more complete picture of the problem [20].

Large studies cite unknown or inconsistent history of mechanism of injury as a major indicator of abuse [1, 2, 3, 15–20]. In most of our cases, the mechanism of injury was either unknown or it was stated to be self-injury. Non-ambulant children are unable to self-inflict or independently sustain accidental injury. In older children, who walk and play independently, there is a greater probability of accidental injury, but they may also be abused and there is no single fracture that is an absolutely certain diagnostic sign of abuse. Therefore, the diagnostic dilemma of differentiating intentionally inflicted from accidental injury is always present.

The third major indicator of child abuse is the presence of visible soft tissue injuries (bruises, abrasions, lacerations), especially if they are present in several regions of the body in non-ambulant children or over non-bony sites (i. e. cheeks, buttocks or thighs), if they vary by date or have typical appearance suggestive for abuse (handprint, pinch, and tramline bruises, cord or belt buckle marks, bites) [1, 2, 3, 5, 15–20]. Upper lip frenulum tear and ear contusions are highly suggestive for child abuse. Burns and scald injuries should draw attention if they have appearance suggestive for intentional trauma (i.e. cigarette burns, immersion scald injuries with sharp demarcation and/or "stocking or glove" type distribution). Such lesions were observed in 70% of our patients with suspected and in all children with proven abuse.

Radiographs demonstrated at least one fracture in 92% of our patients. Unsuspected fractures were detected in 17%. This is slightly lower than in the study by Barber et al. [17], who reported that previously unsuspected fractures were noted on skeletal survey in 21% of their cases. However, it must be emphasized that their research referred only to infants and that they adhered to a standardized imaging protocol.

Almost 20% of our examined children had more than one fracture and this is an important clinical warning of possible physical abuse. This is similar to the findings of Karmazyn et al. [18], who proved multiple fractures in 18% of their cases. It is interesting that 91% of the patients had low-specificity and the remainder moderate-specificity fractures.

In the Barber's study, 14% of children had rib and 4.6% uncommon fractures, which can be considered highly specific [17]. Our lack of identification of high-specificity fractures may be mostly due to the low number of full skeletal surveys performed. Also, in only 2% of the cases there was a follow-up skeletal survey. This is even lower than the 14% and 8.5% reported by Sonik et al. [21] and Bennett et al. [22], respectively. Clearly, there is room for

significant improvement in the quality of imaging performed in Serbia.

In this study, 57% of the children had skull fracture and 39% of the children had long bone fracture. Skull fractures were the most common in the two younger age groups, with a note that the number of long bone fractures increased with age, presumably associated with the more active lifestyles in older children. Other authors identified more long bone fractures then skull fractures in their researches. Taitz et al. [15] verified long bone fractures in 65% and skull fractures in 24% of cases, Carty and Pierce [23] 62% and 27%, and Karmazyn et al. [18] 21% and 7% of cases.

Our study demonstrates that child abuse is a very serious problem that requires a multidisciplinary approach including police, court, and social services, as well as the creation of a national guideline for investigating these children. Following medical care, a safe permanent residence is required in order to protect the child from potentially repeated violence [20].

CONCLUSION

Child abuse is a significant public health problem in Serbia that requires immediate creation of a national guideline for medical investigation of these children and multidisciplinary approach for its solution. In most cases, the perpetrator of violence against children under three years of age remains unknown. Among the known social risk factors for the abuse of children, the most frequent one in the analyzed sample was low economic status of the family. More exposed to abuse in this age are males, firstborn, and those originating from parents with medium and low levels of education. Injuries in physically abused children at this age are very different. The most common and most serious injury is a linear skull fracture. Health professionals of all profiles should be aware of suggestive signs for child abuse. Child abuse prevention and early recognition should be emphasized, especially in suspected but not proven cases, in order to prevent further victim suffering.

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Социјалне, клиничке и радиолошке карактеристике физичког злостављања деце узраста до три године хоспитализоване у терцијарној здравственој установи

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САЖЕТАК

Увод/Циљ Злостављање деце је значајан јавноздравствени проблем савременог света. Многи случајеви насиља над децом остају неоткривени. Србија нема званичне протоколе за медицинско испитивање злостављане деце.

Циљ рада је анализа социјалних, клиничких и радиолошких карактеристика физичког злостављања деце узраста до три године која су хоспитално лечена.

Методе У ретроспективну опсервациону студију укључено је 98 физички повређене деце са сумњом на физичко злостављање, хоспитализоване на Универзитетској дечјој клиници у периоду 2013–2015. године. Код све деце су урађени анамнеза, клинички преглед, стандардне лабораторијске анализе, а радиографско испитивање је урађено код деце са очигледном или суспектном повредом скелета. Код појединe деце урађени су ултразвучни преглед, компјутеризована томографија и магнетна резонанца. Завршну дијагнозу злостављања је постављао мултидисциплинарни тим. Деца су подељена у две групе: са доказаним и суспектним физичким злостављањем.

Резултати Већина деце (92%) из породица су са једним или оба незапослена родитеља. Мушког пола је 68%, прворођених је 60% и 44% је млађе од годину дана. Прелом костију је имало 92% деце, од чега 19% два или више прелома. Најчешћи прелом је била линеарна фрактура лобање и то код 51% деце. Злостављање је потврђено само код пет од 98 сумњивих случајева.

Закључак У анализираном узорку низак економски статус породице је био најчешћи социјални фактор ризика за злостављање деце. Најчешћа повреда је линеарна фрактура лобање.

Кључне речи: злостављање деце; деца узраста до три године; преломи костију

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

The frequency of secondary glaucoma in patients with iridocorneal endothelial syndrome in correlation with the presence of uveal ectropion

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SUMMARY

Introduction/Objective Iridocorneal endothelial (ICE) syndrome incudes 3 clinical forms: progressive iris atrophy, Chandler's syndrome, and Cogan–Reese syndrome. It is characterized by various degrees of iris atrophy, corneal endothelial changes, uveal ectropion, corectopia, peripheral anterior synechiae (PAS) and secondary glaucoma.

The aim of the study was to illustrate forms of ICE syndrome, determine frequency of secondary glaucoma with emphasis on cases with uveal ectropion, analyze response to medicament treatment and the need for surgical treatment in intraocular pressure (IOP) control.

Methods Patients underwent slit lamp examination, applanation tonometry, gonioscopy, ophthalmoscopy, Humphrey visual field testing and Heidelberg retina tomography. Patients were divided into two groups: group I, without uveal ectropion (22 patients) and group II, with uveal ectropion (14 patients).

Results A total of 36 patients were examined in a 10-year period. The average age was 38 years, male to female ratio 1:2. Secondary glaucoma was confirmed in 26 (72.2%) patients, out of which 12 (54.5%) in group I and 14 (100%) in group II. PAS were more frequent in group II. In group I, mean initial IOP was 37 mmHg, and after medicament treatment 26 mmHg. Secondary glaucoma was controlled in 50% and remaining 50% underwent surgical treatment. In group II, mean initial IOP was 49 mmHg, and after medicament treatment 32 mmHg. All 14 patients (100%) underwent surgical treatment in order to achieve IOP control.

Conclusion ICE syndrome is a rare, progressive disease, with high incidence of secondary glaucoma, which is more frequent in cases with uveal ectropion. In these cases, medicament treatment is not effective and trabeculectomy with antimetabolite application is necessary.

Keywords: ICE syndrome; secondary glaucoma; uveal ectropion

INTRODUCTION

Iridocorneal endothelial (ICE) syndrome includes three clinical forms: progressive iris atrophy, Chandler's syndrome, and Cogan–Reese (iris nevus) syndrome. Common features of these entities include abnormality of corneal endothelium, iris changes, progressive closure of iridocorneal angle, and secondary glaucoma, in most instances – unilaterally [1].

Progressive iris atrophy was described by Harms [2] in 1903. He depicted extreme iris atrophy with full-thickness iris defects. Focal corneal endothelial changes have enhanced endothelial reflex in the form of "hammered silver." Iris atrophy usually develops in iris stroma and later in pigment epithelial layer, leading to the full thickness iris defects. When these defects occur in areas of iris stretching they are named "stretch holes" (Figure 1). Rarely, iris defects can be seen before the occurrence of corectopia and iris stretchning and are named "melting holes" (Figure 2). Cellular membrane composed of one layer of endothelial cells and a membrane similar to Descemet's membrane extends across the

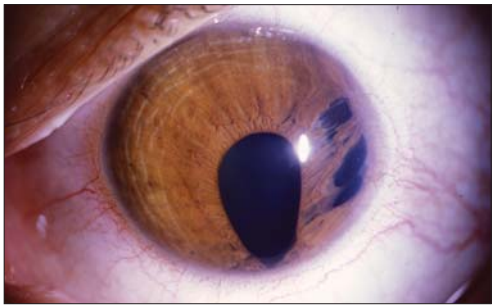

Figure 1. Progressive iris atrophy with corectopia and stretch holes, with uveal ectropion

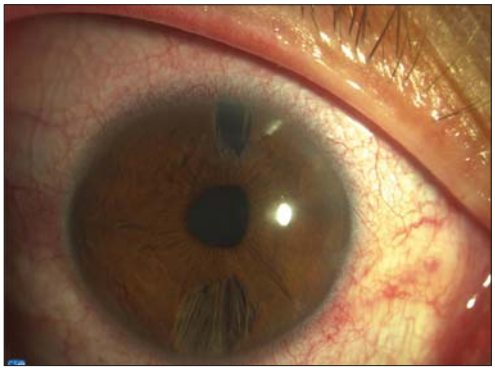

Figure 2. Progressive iris atrophy with iris atrophy, minor corectopia and melting holes, without uveal ectropion

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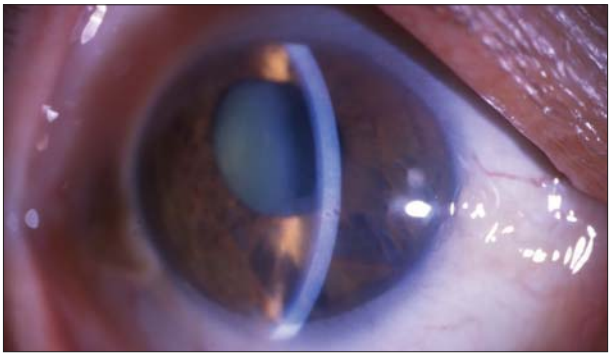

Figure 3. Chandler's syndrome with enhanced endothelial reflex, corneal edema, atrophy of iris stroma and corectopia, without uveal ectropion

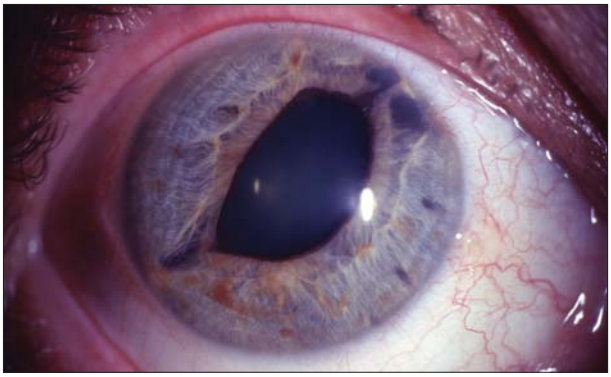

Figure 4. Chandler's syndrome with atrophy of iris stroma, corectopia and uveal ectropion

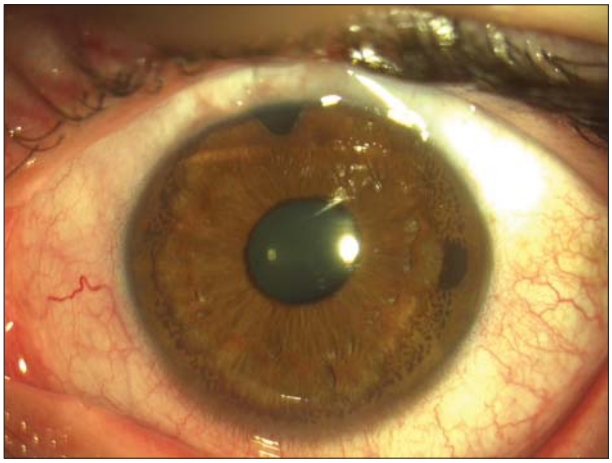

Figure 5. Cogan–Reese syndrome after trabeculectomy, without uveal ectropion

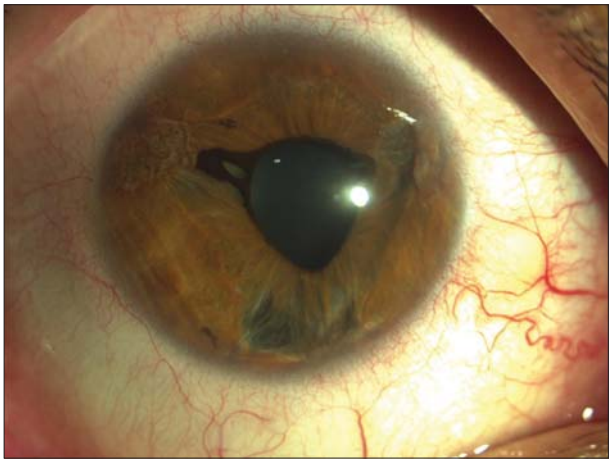

Figure 6. Cogan–Reese syndrome with uveal ectropion

iridocorneal angle towards iris root. Its retraction causes pulling of the iris and pupil distortion, with or without ectropion of the pigment epithelium (Figure 1 and 2).

The rise of intraocular pressure (IOP) is caused by the closure of iridocorneal angle with peripheral anterior synechiae (PAS). Although corectopia and pulling of the iris stroma exists, function of iris sphincter remains preserved for a long time. Posterior synechiae do not develop and lens remains clear. Blood vessels are rarely seen in the areas of iris atrophy. Hyphaema does not occur. Progression of the disease can be monitored using confocal microscopy [3], and ultrasound biomicroscopy [4].

Chandler's syndrome, described by Chandler [5] in 1956, is the most common form of ICE syndrome. The most significant changes are in corneal endothelium and manifest with enhanced endothelial reflex of "hammered silver" appearance (Figure 3). Specular microscopy shows endothelial cells with irregular nonhexagonal shape and variable size, number and density. This type of cells is pathognomonic for ICE syndrome and these cells are named ICE cells. Iris atrophy is mild and confined to superficial stroma, while pigment epithelial layer remains intact. In the majority of cases, the pupil remains round and centrally positioned. Rarely, the pupil can be irregular, slightly displaced towards the area with most prominent PAS, with or without ectropion of pigment epithelial layer (Figure 4).

In iridocorneal angle, PAS are present in a lesser extent and angle is rarely blocked. If IOP rise occurs, it is moderately increased and glaucoma has better clinical course. Patients usually complain of blurred vision and color circles around the source of light due to the corneal edema, which can be present without significant IOP rise. Each patient has a particular critical value of IOP at which corneal edema occurs. In the evolution of the disease, this critical value gradually decreases, and sometimes it can be below the normal values of IOP.

Cogan–Reese (iris naevus) syndrome was described by Cogan and Reese [6] in 1969. The syndrome consists of unilateral nodular pigmented lesions with or without uveal ectropion (Figure 5 and 6) or diffuse pigmented iris lesions histologically similar to naevi, with variable degree of iris atrophy, PAS, abnormal Descemet-like membrane and loss of normal iris architecture [7, 8]. Iris surface is smooth, without crypts and concentric folds [9]. Heterochromia is typical and affected eye is usually darker. Microscopically, nodules are seen as islands of elevated, dense, pigmented stromal tissue, surrounded at the base by endothelium and Descemet's membrane. Corneal endotheliopathy is present in some areas. In iridocorneal angle, wide PAS are present, similar to those in progressive iris atrophy. Iris atrophy is usually absent, and in cases where it exists, it is mild. Corectopia is often present and can be severe, as well as uveal ectropion.

Transitional forms between progressive iris atrophy and Chandler's syndrome have been described. In those cases

Table 1. Frequency of secondary glaucoma in different forms of iridocorneal endothelial (ICE) syndrome, respective to the presence of uveal ectropion

Clinical forms of ICE syndromes	No. of patients	No. (%) of patients without uveal ectropion	No. (%) of patients with uveal ectropion	Total No. (%) of patients with sec. glaucoma	No. (%) of patients with sec. glaucoma without uveal ectropion	No (%) of patients with sec. glaucoma with uveal ectropion
Progressive iris atrophy	12	7 (58.3)	5 (41.7)	9 (75)	4 (57.1)	5 (100)
Chandler's syndrome	14	8 (57.1)	6 (42.9)	10 (71.4)	4 (50)	6 (100)
Cogan–Reese syndrome	10	7 (70)	3 (30)	7 (70)	4 (57.1)	3 (100)
Total	36	22 (61.1)	14 (38.9)	26 (72.2)	12 (54.6)	14 (100)

anterior segment optical coherence tomography findings may be decisive in final diagnosis [10].

We performed a study to analyze prevalence of secondary glaucoma in eyes with ICE syndrome with uveal ectropion, compared to those without uveal ectropion. We also analyzed the efficacy of medicament treatment in IOP management and the need for surgical treatment in both groups of patients.

METHODS

We performed a prospective study of consecutive patients treated for ICE syndrome at Glaucoma Department of University Eye Clinic in Belgrade, Serbia. All patients underwent slit lamp examination, applanation tonometry, indirect gonioscopy, ophthalmoscopy, Humphrey visual field testing, and Heidelberg retina tomography (HRT) II. Iris specimens obtained after trabeculectomy were histologically analyzed. The patients were divided into the following two groups: group I, patients without uveal ectropion, and group II, patients with uveal ectropion.

The patients were clinically examined in six-month periods, while perimetry, photograph of the optic nerve head, HRT II, and indirect gonioscopy (that was used to monitor distribution and progression of PAS) were performed annually. If needed, anterior segment optical coherence tomography was performed. The difference in thickness between anterior limiting membrane and iris pigment epithelium was compared between the affected areas and healthy unaffected areas of the iris, and used to establish the diagnosis.

In order to control the secondary glaucoma, standard treatment included local administration of beta-blocking agents, carbonic anhydrase inhibitors, alfa-2 adrenergic agonists and prostaglandin agonists, as well as per oral use of carbonic anhydrase inhibitors. In cases in which goal IOP could not be achieved, surgical treatment was performed. Surgical intervention included trepanotrabeculectomy with intraoperative application of sponge soaked with mitomycin C, kept on the filtering site on the sclera for two minutes.

RESULTS

In a 10-year period, 36 patients with ICE syndrome were treated. Out of them, 12 patients had progressive iris atrophy, 14 Chandler's syndrome, and 10 Cogan–Reese syndrome. All the patients had monocular disease. Women (24 patients, 66.6%) were affected twice as often as men (12 patients, 33.3%). The average age of patients was 38 years. Out of 36 patients with ICE syndrome, secondary glaucoma was diagnosed in 26 (72.2%).

Group I comprised 22 patients (61.1%) without uveal ectropion, while group II consisted of 14 patients (38.9%) with uveal ectropion. In group I, secondary glaucoma was confirmed in 12 (54.5%) patients, while in group II this was the case in all 14 (100%) patients (Table 1). Out of 12 patients with progressive iris atrophy, secondary glaucoma was confirmed in nine (75%) of them. When patients with progressive iris atrophy were subdivided according to the presence of uveal ectropion, in group I, glaucoma was present in four (57.1%) out of seven patients, and in group II in all five patients (100%). Out of 14 patients with Chandler's syndrome, secondary glaucoma was confirmed in 10 (71.4%). When patients with Chandler's syndrome were subdivided according to the presence of uveal ectropion, in group I, glaucoma was present in four (50.0%) out of eight patients, and in group II in all six patients (100%). Out of 10 patients with Cogan–Reese syndrome, secondary glaucoma was confirmed in seven (70.0%) patients. When patients with Cogan–Reese syndrome were subdivided according to the presence of uveal ectropion, in group I, glaucoma was present in four (57.1%) out of seven patients, and in group II in all three patients (100%).

In group I, 12 (54.5%) out of 22 patients were diagnosed with secondary glaucoma. Mean IOP at baseline was 37 mmHg, and 26 mmHg after medicament treatment. Glaucoma was compensated in six (50%) patients, while in the remaining six, surgical treatment was necessary. After the procedure, glaucoma was compensated in four patients. Two patients were reoperated on and had neodymium-doped yttrium aluminium garnet (Nd:YAG) laser cyclophotocoagulation and cyclocrioanemization, after which IOP control was achieved.

In group II, all 14 (100%) patients with uveal ectropion had secondary glaucoma. Mean IOP at baseline was 49 mmHg, and after local medicament treatment it was 32 mmHg. Secondary glaucoma could not be controlled even with oral carbonic anhydrase inhibitors. All the patients underwent surgical procedure, after which target IOP was achieved in seven (50%) patients. In the remaining seven patients, additional surgical procedure was necessary (re-trabeculectomy with antimetabolite application) and was successful in four patients. In the remaining two patients, target IOP was reached after additional Nd:YAG laser cyclophotocoagulation or cyclocrioanemization. Enucleation had to be performed in one patient who had excruciating pain due to decompensated glaucoma.

Table 2. Iris changes in different clinical forms of ICE syndrome

Iris changes	Progressive iris atrophy		Chandler's syndrome		Cogan–Reese syndrome		Iris nevus syndrome	
	Without uveal ectropion	With uveal ectropion	Without uveal ectropion	With uveal ectropion	Without uveal ectropion	With uveal ectropion	Without uveal ectropion	With uveal ectropion
Uveal ectropion	7	5	8	6	5	2	2	1
Corectopia	4	5	4	6	4	2	2	1
Iris holes	7	5	0	0	0	0	0	0
Iris nodules or diffuse pigment lesion	0	0	0	0	4	3	2	1

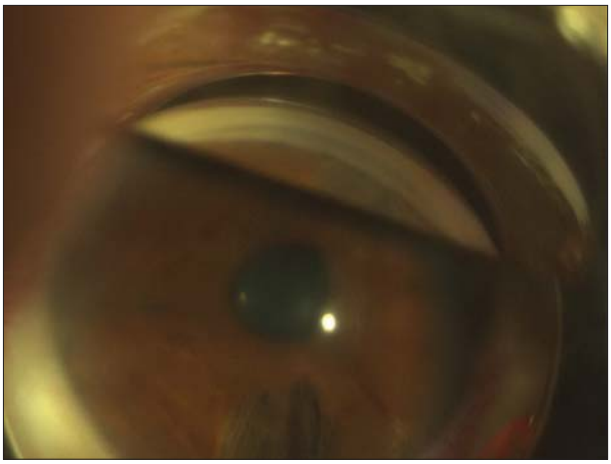

Figure 7. Gonioscopy in progressive iris atrophy without uveal ectropion

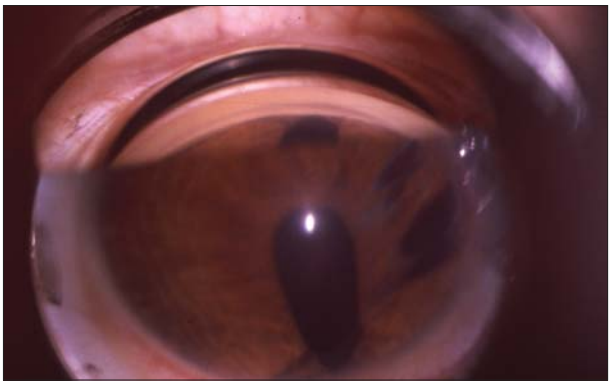

Figure 8. Gonioscopy in progressive iris atrophy with uveal ectropion

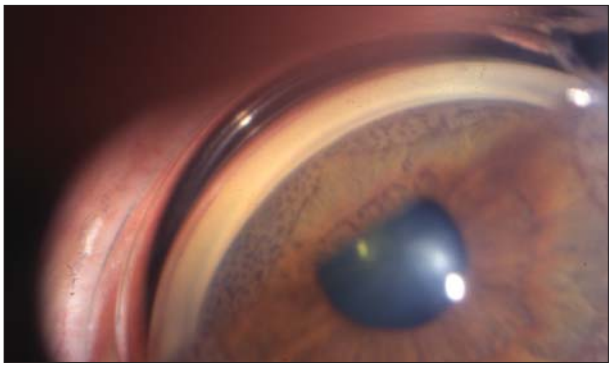

Figure 9. Gonioscopy in Cogan–Reese syndrome without uveal ectropion

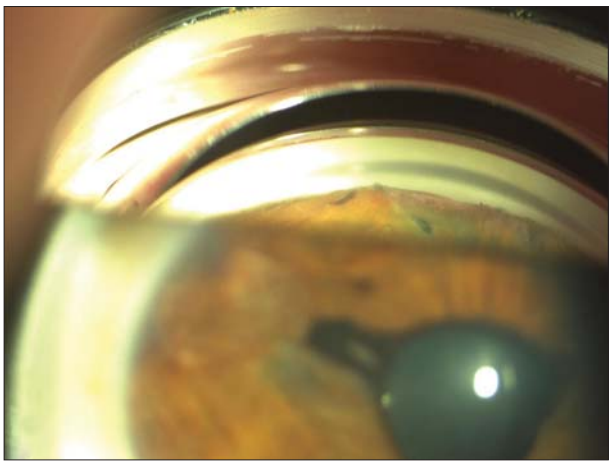

Figure 10. Gonioscopy in Cogan–Reese syndrome with uveal ectropion

Out of 36 patients with ICE syndrome, 28 (77.7%) had corectopia. Uveal ectropion was partially present (in one part of pupillary margin) in 12 patients, and completely present (in the whole circumference) in two patients. In group II, all 14 patients had uveal ectropion associated with corectopia, while in group I, the condition was present in14 out of 22 patients (Table 2).

PAS are the parameter that shows the progressive nature of the disease. Gonioscopy was performed in all cases on both eyes. In the healthy, unaffected eye, no PAS were found during the follow-up. However, in the eye with ICE syndrome, progression of the affected parts of iridocorneal angle with PAS was observed, especially in patients with progressive iris atrophy and uveal ectropion, in comparison to those without uveal ectropion (Figure 7 and 8). The progression of PAS was moderate in Cogan–Reese syndrome (Figure 9 and 10). In Chandler's syndrome, PAS were not frequent and didn't progress as quickly and anteriorly as in progressive iris atrophy and Cogan–Reese syndrome (Figure 11 and 12).

Progression of PAS in group I was moderate, and after five years of follow-up, only four patients had PAS in three quadrants. In contrast, in group II, PAS were present in three or more quadrants of iridocorneal angle in nine patients, and those patients had a progression of secondary glaucoma and reduced effect of filtering surgery. The course of glaucoma was more severe in patients with progressive iris atrophy and Cogan–Reese syndrome, which could be explained by the fact that PAS are more frequent and more quickly formed (Table 3 and 4).

Histological analysis of iris samples of patients with progressive iris atrophy and Chandler's syndrome showed various degrees of iris stromal atrophy, which depended

Figure 11. Gonioscopy in Chandler's syndrome without uveal ectropion

Figure 12. Gonioscopy in Chandler's syndrome with uveal ectropion

on the area of iris sampling and involvement. Pigment nodules in patients with Cogan–Reese syndrome, had histological ultrastructure similar to the iris stroma and were surrounded with cellular membrane. Microscopically, nodules appeared as islands of elevated, dense, pigmented stromal tissue, surrounded at their base with endothelium and Descemet's membrane.

DISCUSSION

Although term "essential iris atrophy" was initially used for this group of disorders, clinical and pathohistological studies showed that primary disorder was corneal endothelial abnormality, rather than iris pathology [11]. This is why Yanoff [9] suggested the term ICE syndrome for this spectrum of diseases in 1979. Since iris atrophy was not the basic disorder, the term 'progressive iris atrophy' was found more suitable.

ICE syndrome is a rare, acquired disease that affects one eye of middle-aged patients. It has higher incidence in females and has no genetic predisposition. Certain etiology of the disease is not known. Many possible factors such as congenital disorders, trauma, chronic inflammation, iris dystrophies, vascular insufficiency, and viral etiology were presumed [7, 12, 13]. For a long time, it was speculated that the disorder was caused by a congenital anomaly. However, very rare reports of family cases and histological evidence that endothelial and Descemet's membrane changes begin in the postnatal period do not support hereditary or congenital nature of the disease. In pathohistological specimens that were obtained after eight keratoplasties performed in eyes with ICE syndrome, Alvarodo et al. [14] found abnormal material in the posterior collagen layer of Descemet's membrane. These findings confirmed that ICE syndrome is an acquired rather than a congenital disorder, as all cases had normal pattern of membrane deposits. Shields [8] and Shields et al. [15] postulated that sudden appearance of abnormal posterior collagen layer is indirect evidence that an acute event has damaged the endothelium and that ICE syndrome is an acquired disorder, caused by the exogenous factor.

Findings made by Alvarodo et al. [14] and Rodrigues et al. [16] concerning lymphocyte infiltration in the endothelial layer indicate that the presence of chronic inflammation

Table 3. The distribution and progression of peripheral anterior synechiae in different forms of ICE syndrome without uveal ectropion during a five-year follow up

ICE sy. without uveal ectropion	Progressive iris atrophy					Chandler's syndrome					Cogan–Reese syndrome				
Presence of PAS	1st year	2nd year	3rd year	4th year	5th year	1st year	2nd year	3rd year	4th year	5th year	1st year	2nd year	3rd year	4th year	5th year
None	1	1	0	0	0	6	6	4	3	3	5	5	4	3	2
1 quadrant	5	4	4	3	2	2	2	3	4	2	2	2	1	2	2
2 quadrants	1	2	2	3	3	0	0	1	1	2	0	0	2	2	2
3 or 4 quadrants	0	0	1	1	2	0	0	0	0	1	0	0	0	0	1

Table 4. Distribution and progression of peripheral anterior synechiae in different forms of ICE syndrome with uveal ectropion during a five-year follow up

ICE sy. with uveal ectropion	Progressive iris atrophy					Chandler's syndrome					Cogan–Reese syndrome				
Presence of PAS	1st year	2nd year	3rd year	4th year	5th year	1st year	2nd year	3rd year	4th year	5th year	1st year	2nd year	3rd year	4th year	5th year
None	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0
1 quadrant	3	2	1	0	0	3	2	3	2	0	2	2	1	1	0
2 quadrants	1	2	2	3	1	2	3	2	3	3	1	1	2	1	1
3 or 4 quadrants	1	1	2	2	4	0	0	1	1	3	0	0	0	1	2

could support the theory of viral etiology of the disease. However, rare lymphocytes are also seen in the corneal endothelium of patients with posterior polymorphous dystrophy; hence, they could be considered normal "passenger" cells, traveling towards the endothelium. Although the cause of the corneal endothelial abnormalities in ICE syndrome is not known, strong evidence exists that this is the main pathological event that leads to other clinico-pathological manifestations. Polymerase chain reaction demonstrated the presence of herpes simplex viral DNA in significant percentage of corneas with ICE syndrome, which suggests that this disease could be of viral etiology [17].

Pathogenesis of ICE syndrome is complex, and the "membrane theory" of Campbell et al. [18] is widely accepted. Endothelial abnormality does not only cause corneal edema, it also leads to proliferation of cellular membrane that consists of a single layer of endothelial cells and a membrane similar to Descemet's membrane. According to this theory, contraction-retraction of the membrane causes the formation of PAS, iris changes, corectopia, ectropion of pigment layer, and secondary glaucoma. Iris pigment epithelium moves anteriorly due to the retraction of this membrane, covers the anterior surface of iris stroma, and causes pigment ectropion. Pigment ectropion can be present in all three clinical forms, although is most common in progressive iris atrophy and Cogan–Reese syndrome. It is always followed by corectopia, and those two findings are usually present in the quadrant with most prominent PAS. In the opposite quadrant, iris pulling is usually associated with iris thinning and, in some cases, iris holes. Along with membranous pulling of iris, other factors, such as secondary ischemia of the iris, are probably involved in the pathogenesis. In cases where the pupil is relatively central, its position and shape can be explained by the similar pulling forces from the opposite parts of iridocorneal angle by PAS.

Iris changes are the most significant clinical feature of progressive iris atrophy. At the onset of the disease, corneal endothelial changes are not so visible and are not associated with the development of corneal edema. Therefore, patients are asymptomatic for a long period of time. Symptoms occur after the change in pupil form or size, or after the onset of decompensated secondary glaucoma.

In Chandler's syndrome corneal endothelial abnormality is the predominant clinical characteristic. Consequent corneal edema typically occurs when IOP is moderately raised or even normal. Electron microscopy of the corneal endothelium shows both regular hexagonal cells, with visible interdigitations that correspond to pyknotic vesicles and sporadic microvilli in the periphery, and degenerated endothelial cells that vary in size (polimegatism) and shape (polimorphism) [14, 16]. Their borders form zipper-like multilayers, with inner indentations and sporadic warty protuberances. Later on, cells aggregate, move apart, and deplete Descemet's membrane, which leads to chronic corneal edema [19].

Contraction of the endothelial membrane over structures of anterior chamber angle and iris surface is most probably the cause of secondary glaucoma. Patel et al. [20] have examined iris samples after trabeculectomy and corneal samples after keratoplasty using electron microscopy and concluded that both proliferation and degeneration of corneal endothelium were present in eyes with progressive iris atrophy and Cogan–Reese syndrome, while in Chandler's syndrome those changes were not present.

Cogan–Reese syndrome has characteristic findings of nodular or diffuse pigment iris lesions and a variable degree of iris atrophy. Anterior border layer of iris is replaced with dense layer of melanocytes. Abnormal endothelial, glassy membrane in iridocorneal angle and on the anterior iris surface and confluent, peripheral PAS with secondary angle-closure glaucoma are characteristic for the disease. Corneal endotheliopathy is confined to certain areas of the cornea. Spreading of the corneal endothelium over iridocorneal angle and on the anterior iris surface with formation of new Descemet's membrane seems to be the basic event in this condition. Nevus cells can stimulate spreading of the corneal endothelium [21]. Also, it is assumed that cellular membrane surrounds and pulls parts of the stoma to form nodular iris lesions [6, 22].

Changes in the iridocorneal angle include the formation of PAS, which usually start from or under Schwalbe's line. Histological studies of iridocorneal angle confirm presence of cellular membrane, which consists of one layer of endothelial cells and membrane similar to the Descemet's membrane, which grows from the periphery of the cornea. Membrane covers the open iridocorneal angle, or can be associated with the synechial angle closure [18, 22]. The synechial angle closure is typically progressive, leading to the IOP rise. However, secondary glaucoma does not correlate with the degree of synechial angle closure [23]. Obstruction of aqueous drainage is caused by either covering of the trabecular meshwork with membrane or synechial closure of iridocorneal angle [24, 25].

Occurrence of secondary glaucoma in the course of the disease requires medicament antiglaucomatous treatment, which consists of local administration of beta blockers, carbonic anhydrase inhibitors, alpha-2 agonists, and prostaglandin analogues. Surgical interventions based on fistulising procedures, have good results in the beginning, but usually shortly after the procedure closure of fistule with proliferated tissue and endothelial membranes occurs [26, 27]. In most of the cases re-treatment is needed with obligatory intraoperative administration of antimetabolites or usage of drainage implants, in order to prevent or delay cicatricial closure of the surgical aperture in the limbus or in the filtering bleb [28, 29, 30].

Nd:YAG laser cyclophotocoagulation and cyclocryotherapy are the final option for achieving the IOP control and their effect is usually time-limited, due to the progressive nature of the disease.

CONCLUSION

Although ICE syndrome is a rare, acquired, benign, progressive disease, one must be cautious to timely diagnose and monitor secondary glaucoma, especially in cases with

uveal ectropion, since secondary glaucoma is twice as frequent in this group of patients. Secondary glaucoma is caused by wide and extensive angle closure with PAS and is characterized by poor response to medicament treatment. All cases with uveal ectropion required surgical treatment (trabeculectomy with antimetabolite administration), in order to prevent irreversible glaucomatous optic neuropathy.

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Заступљеност секундарног глаукома у иридокорнеалном ендотелијалном синдрому у зависности од присуства ектропијума увеје

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САЖЕТАК

Увод/Циљ Иридокорнеални ендотелијални (ИКЕ) синдром обухвата три клиничка облика: прогресивну атрофију дужице, Чандлеров синдром и Коган–Рисов синдром. Одликује се различитим степеном атрофије дужице, промена на ендотелу рожњаче, ектропијума увеје, коректопије, присуства периферних предњих синехија (ППС) и секундарног глаукома. Циљ рада је да прикаже форме ИКЕ синдрома, утврди учесталост секундарног глаукома, посебно са ектропијумом увеје, и испита одговор на медикаментну терапију и потребу за хируршким третманом у контроли интраокуларног притиска (ИОП).

Методе Прегледи болесника обухватали су биомикроскопски преглед, апланациону тонометрију, гониоскопију, офталмоскопију, компјутеризовану периметрију, Хеиделбергретина томографију. Болесници су подељени у две групе: група I – без ектропијума увеје (22 болесника) и група II – са ектропијумом увеје (14 болесника).

Резултати У десетогодишњем периоду праћено је 36 болесника. Просечна старост је износила 38 година, а однос мушког и женског пола био је 1 : 2. Секундарни глауком је потврђен код 26 (72,2%) болесника и то у групи I код 12 (54,5%), и у групи II код 14 (100%) болесника. ППС су чешће постојале у групи II. У групи I просечна почетна вредност ИОП-а износила је 37 *mmHg*, а након медикаментне терапије 26 *mmHg*. Секундарни глауком је компензован медикаментном терапијом код 50%, а код преосталих 50% је спроведен хируршки третман. У групи II просечна почетна вредност ИОП-а износила је 49 *mmHg*, а након медикаментне терапије 32 *mmHg*. Код свих 14 (100%) болесника спроведен је хируршки третман у контроли ИОП-а.

Закључак ИКЕ синдром је ретко прогресивно обољење. Учесталост секундарног глаукома је висока, и два пута већа код случајева са ектропијумом увеје. Код ових болесника медикаментна терапија је неефикасна, те је неопходан хируршки третман.

Кључне речи: ИКЕ синдром; секундарни глауком; ектропијум увеје

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Oral complications in irradiated head and neck cancer patients – 3D conformal radiotherapy planning vs. 3D conformal radiotherapy planning with magnetic resonance fusion

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SUMMARY

Introduction/Objective The incidence of radiation-induced side effects in patients with head and neck (H&N) cancer depends on the planning technique and the irradiation dose, as well as primary tumor location within the H&N region.

The aim of our research is to establish the incidence of side effects in patients with H&N cancer treated with conformal radiotherapy planning with computed tomography (CT) or computed tomography fusion with magnetic resonance imaging (CT-MRI fusion).

Methods Prospective analysis was performed on 40 patients with oropharynx carcinoma and on 40 patients with larynx carcinoma prospectively followed after radiotherapy. Forty patients with H&N cancer were irradiated by using 3D conformal radiotherapy planning with CT, while other 40 patients were treated using 3D conformal radiotherapy planning with CT-MRI fusion. In all cases standard fractionation was used at 2 Gy per day, five days a week.

Results Of the total of 80 patients treated, 52 patients (52/80; 65%) reported a side effect and the incidence of complications was higher in patients irradiated with 3D technique planning with CT (31/52; 60% for 3D CT vs. 21/52; 40% for 3D CT-MRI; p = 0.02). There were more complications in chemoradiotherapy group of patients than observed when only radiotherapy was used – 35/52 RT + HT vs. 17/52 RT (67%: 33% and p = 0.004).

Conclusion 3D radiotherapy technique planned solely on the basis of CT is related to high incidence of toxicity, which significantly affects the quality of life of irradiated patients. 3D conformal radiotherapy planned with CT-MRI fusion reduces the incidence of oral complications. Following the example of developed countries, this technique should be considered as a standard method for irradiating patients with H&N cancer. Planning technique with fusion technique using MR imaging is more suitable for delivering higher doses to the tumor with fewer side effects.

Keyword: radiotherapy; head and neck; oral complications; CT-MRI fusion in radiotherapy planning

INTRODUCTION

The treatment of tumors in head and neck (H&N) region most commonly combines the use of surgery, radiotherapy and chemotherapy with radiotherapy being applied in more than 50% of cases [1].

According to the data of Cancer Registry of the Oncology Institute of Vojvodina in Sremska Kamenica, a total of 500 people suffered from H&N cancer in 2010 in the province of Vojvodina in Serbia, which makes around 4–5% of all malignant tumors registered that year [2].

This data fits well with the data of the International Agency for Research on Cancer (IARC), where H&N cancers make up 5% of all malignant tumors [3].

The frequency of oral complications during radiotherapy is high and studies show the frequency to be up to 40% [3].

Radiation-induced changes can be divided into two groups, based on the usual time of their occurrence: early or acute side effects that are noted during or immediately after treatment, and late or chronic side effects, which develop months or years after the end of radiation therapy [4].

Xerostomia is the most frequent complication of irradiation in patients treated with conformal (3D) radiotherapy. About 64% of patients developed permanent xerostomia of a moderate to severe degree. The most pronounced changes are found in patients with laryngeal and oropharyngeal carcinoma due to its close proximity to major salivary glands. Irradiation changes the composition of saliva, leads to difficulties in maintaining oral hygiene and affects intake of cariogenic food and drinks [5, 6].

In addition to these, mucosal atrophy and fibrotic changes, radiation-related caries and bone necrosis occur as side effects of the treatment but are less frequent [7, 8].

With conventionally fractionated radical doses of radiotherapy, the first signs of mucositis usually appear already during the second week

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of the treatment and advance towards the end of the treatment from the enanthems to confluent forms of pseudomembranous mucositis [9]. The recovery starts within 2.5–3 weeks after the end of radiotherapy, and within one month the mucosa is healed in about 90–95% of patients [10].

In addition, xerostomia predisposes infections and development of dental caries and it affects speaking and swallowing [11].

Because of the lack of saliva, the probability for development of radiotherapy-related complications is increased. In the first two weeks following the beginning of radiotherapy and received cumulative radiation tumor dose even at 20 Gy, around 80% of salivary function is changed [12].

The increase in acidogenic and cariogenic bacteria in the mouth (*Streptococcus mutans*, *Lactobacillus* and *Candida* species) together with the decrease in non-cariogenic microorganisms (such as *Streptococcus sanguis*, *Neisseria* and *Fusobacterium*) increase the risk of development of oral complications [12].

Significant weight loss and deterioration of the patient's nutrition status tend to aggravate because of the pain while chewing and swallowing. Radiotherapy also incurs loss of appetite, nausea, and physical discomfort. Loss of taste occurs and progressively increases at the received radiation tumor dose of about 30 Gy [13].

The characteristics of modern radiotherapy planning are increasingly used fusion techniques such as positron emission tomography – computed tomography (PET CT) and magnetic resonance imaging (MRI) fusion. The adequate soft-tissue contrast of MRI allows the technique to have an increasing role in contouring the gross tumor volume, organs at risk, which leads to the decreasing incidence of treatment complications [14].

Frequency of complications of radiotherapy in patients with H&N cancer is about 35% [15, 16].

The aim of this research is to perceive the possibilities of lowering the radiotherapy-induced toxicity in patients with H&N carcinoma in a setting of a developing country. This aim can be reached by using the transition between three-dimensional (3D) conformal radiotherapy planning with computed tomography (3D CT) and 3D conformal radiotherapy planning with CT fusion MRI (3D CT-MRI). Acute side effects that are analyzed were xerostomia, mucositis, and dermatitis. On the other hand, dental caries was observed as a chronic complication of radiotherapy. Those side effects (acute and chronic) were monitored three times during radiotherapy:

(1) In the fifth week of radiotherapy, after the completion of the 25th fraction;
(2) 30 days following the completion of radiotherapy, and
(3) 90 days following the completion of radiotherapy.

Minimum two weeks prior to radiotherapy, initial (baseline) dental treatment (IDT) was performed, while medical examinations with documentation of complications and dental evaluation were done at every check-up. Radiotherapy complications were not monitored 90 days after the completion of radiotherapy.

METHODS

This investigation was carried out at the Oncology Institute of Vojvodina in Sremska Kamenica, Province of Vojvodina, Serbia in the period between January 2013 and October 2014. The study included patients with diagnosed H&N cancer treated with radiotherapy. Eighty prospective patients participated in the study, 40 of whom were diagnosed with laryngeal carcinoma and 40 with oropharynx carcinoma. The main aim of the study was to compare the two most commonly applied methods of radiotherapy planning, 3D with CT and 3D conformal with fusion CT-MRI, in relation to the incidence of the appearance of complications.

The study included patients over the age of 18 years for whose treatment radiotherapy was indicated by the oncology consultant team. All the patients had good general status, Eastern Cooperative Oncology Group (ECOG) Scale of Performance Status 0 or 1.

Patients who for any reason failed to complete the prescribed radiotherapy, as well as those whose general status was poor, such as ECOG 2 or more, were excluded from consideration.

Prior to the radiotherapy, the participants had IDT performed. It was performed at least two weeks prior to radiotherapy and all the tooth lesions were identified and repaired. Teeth that could not be repaired had to be extracted. Clinical examination and dental evaluation was performed five weeks into the radiotherapy (i.e. after the completion of the 25th fraction). The second examination was performed 30 days and the third 90 days following the completion of radiotherapy.

Radiation treatment

The patients were irradiated using two techniques: 3D conformal radiotherapy with CT and 3D conformal radiotherapy with fusion CT-MRI. The participants in the study were irradiated with a daily dose of 2 Gy, five days a week, with a curative radiotherapy tumor dose from 60 Gy to 70 Gy. Each patient was provided with a thermoplastic mask in order to immobilize the treated region and to deliver the radiation tumor dose more precisely.

3D conformal radiotherapy technique for oropharyngeal cancer includes tumor / tumor bed with margins of 2 cm and lymph node: N0 include levels II–IV and retropharyngeal lymph nodes (RPN), N1 include levels Ib–IV and RPN, N2–3 include Ib–V and RPN [17]. Radiation dose for adjuvant 3D radiotherapy for oropharyngeal cancer is 60 Gy (60 Gy to the preoperative tumor bed and 50 Gy to the lymph nodes with 2 Gy per day, five days a week).

For inoperable oropharyngeal cancer tumor radiation dose was 70 Gy (70 Gy on the tumor and involves lymph nodes and 50 Gy on elective lymph nodes with 2 Gy per day, five days a week) [18].

Curative 3D conformal radiotherapy technique for laryngeal cancer included primary tumor and any involved lymph nodes. Irradiation depended on laryngeal localization of the primary tumor. The levels that are included are

Table 1. Patient and tumor characteristics

Characteristics	3D-CT RT (total 40)	3D CT-MRI RT (total 40)	p
Age range (median)	54.7	52.9	
Gender (male:female)	31:9	28:12	0.61
ECOG (0:1)	26:14	24:16	0.82
Oropharynx	20	20	1
Supraglotic larynx	10	12	0.81
Glottic larynx	6	7	0.99
Subglottic larynx	4	1	0.36
T stage 1/2/3/4	3/8/17/12	8/12/15/5	0.11
N stage 0/1/2/3	7/13/16/4	12/20/7/1	0.04
RT	11	20	0.07
Concurrent RT + HT	29	20	0.07
Early stage T1–T2	3 + 8 = 11	8 + 12 = 20	0.07
Advanced stage T3–T4	17 + 12 = 29	15 + 5 = 20	0.07
Total dose 60 Gy	24	21	0.49
Total dose 70 Gy	16	19	0.49

ECOG – Eastern Cooperative Oncology Group; RT – radiotherapy; HT – chemotherapy

Table 2. Treatment-related toxicity

Toxicity	3D-CT RT (40 in total)	3D CT-MRI RT (40 in total)	p
Complications in total	31	21	0.02
Acute complications (1, 2, 3)	31	21	0.02
Acute complications RT vs. RT + HT	RT 10 RT + HT 21	RT 7; total = 17 RT + HT 14; total = 35	0.004
1. Xerostomia	27	13	0.002
Xerostomia (oropharyngeal/laryngeal)	16/11	8/5	
2. Mucositis	24	12	0.006
Mucositis (oropharyngeal/ laryngeal)	17/7	8/4	
3. Dermatitis	10	14	0.46
Dermatitis (oropharyngeal/laryngeal)	4/6	7/7	
Grade 0–2 RTOG scoring criteria	17	15	0.64
Grade 3–4 RTOG scoring criteria	14	6	0.03
Chronic complications (DC)	15	7	0.04
DC (larynx)	4	1	0.16
DC (oropharynx)	11	6	0.14
Total dose 60 Gy	17	15	0.64
Total dose 70 Gy	14	7	0.07
Corticosteroid therapy	35	17	0.004

RT – radiotherapy; HT – chemotherapy; RTOG – Radiation Therapy Oncology Group; DC – dental caries

II–IV, level VI with subglottic tumors, and level V if > 1 node is involved in that side of the neck.

Curative dose for oropharyngeal and laryngeal cancer was 60 Gy. For locally advanced tumor the radiation dose was escalated up to 70 Gy.

Indication for chemoradiotherapy included advanced stage of the disease (T3, T4) and positive surgical margin and extranodal capsular extension.

Every day, the patients themselves made notes of subjective difficulties with skin, oral pain and the sense of (insufficient) saliva. During the radiotherapy course, analgesic therapy was optionally included, as well as corticosteroid therapy when stronger pain appeared, and was left at the discretion of involved radiation oncologist.

A modified scale recommended by the Radiation Therapy Oncology Group (RTOG) was used for monitoring acute complications (xerostomia, mucositis, and dermatitis), specifically the part which relates to monitoring the complications of H&N region (RTOG acute radiation morbidity scoring criteria). In this classification, the acute complications are divided into a four-point scale [19]:

0 – no change;
1 – mild changes which demand no therapy;
2 – changes which demand symptomatic therapy and necessary analgesics;
3 – suffering which demands opioid analgesics, and
4 – changes which demand the termination of radiotherapy.

RESULTS

Results were statistically analyzed using χ^2 and Fischer exact probability tests. There were 80 participants in the study – 59 men and 21 women. The ratio 3:1 in favor of men fits into the general trend of the incidence of this disease, p = 0.61. Age ranged 18–65 years, with the median age at presentation of 54.7 years in 3D CT and 52.9 in 3D CT-MRI radiotherapy. All the patients had ECOG 0–1, p = 0.82 (Table 1).

The study involved 40 patients diagnosed with primary laryngeal carcinoma (22 patients with supraglottic localization tumor, 13 glottic, and 5 subglottic) and 40 with oropharynx carcinoma. The number of patients irradiated with 60 Gy and 70 Gy in both groups was nearly the same, p = 0.49 (Table 1). According to p > 0.05, we concluded that the groups were homogeneous concerning the age, gender, ECOG performance, and tumor dose.

Fewer patients had early stages of the disease (n = 31) while advanced stage disease was present in 49 patients. In advanced stages of the disease (T3 and T4), as well as with postoperative high risk (positive surgical margins and extranodal extension), concomitant radiotherapy and chemotherapy was used with 5-fluorouracil plus cisplatin (5FU/CDDP). Forty-nine patients with advanced stage of the disease were treated with chemoradiotherapy.

Twenty patients with laryngeal or oropharynx carcinoma were irradiated with 3D CT, the other group of 20 patients of both tumor localizations were treated with 3D CT-MRI conformal radiotherapy (2 Gy per fraction, 5 fractions a week, from 60 Gy to 70 Gy), p > 0.05 (Table 1).

Of the total number of 80 irradiated patients, 52 of them (65%) reported a side effect of radiation therapy. Thirty-one patient was irradiated by using 3D CT, while 21 patient were irradiated by using 3D CT-MRI, p = 0.02 (Table 2.). In this study p was < 0.05, which suggested that incidence of side effects in these groups was statistically significantly different.

Oropharyngeal mucositis developed in 36 out of 52 patients, being observed in 24 and 12 patients for the 3D

CT and 3D CT-MRI technique, respectively (p = 0.006). Xerostomia was present in 40/52 irradiated patients, in oropharyngeal cancer 3D CT vs. 3D CT-MRI = 16:8, in laryngeal cancer 11/5 (p = 0.002). According to the p, which was < 0.05, the incidence of complications was statistically higher in the group where radiotherapy was planned without fusion with MRI.

Skin changes on the face and neck during radiotherapy in the form of radiation dermatitis manifested in 24 patients, being observed in 10 and 14 patients for the 3D CT and 3D CT-MRI technique, respectively (p = 0.46) (Table 2). Based on the results of the p, there was no statistical difference in incidence of the radiation dermatitis (3D vs. 3D-MRI). The reason could be the number of the radiation fields which in conformal technique was 4–10 [20].

Dental caries (DC) was identified in 22 out of 80 patients who were irradiated (Figure 1). It was identified in three patients during the radiotherapy, in eight patients 30 days following the completion of radiotherapy, and in 11 patients 90 days after radiotherapy (Figure 2).

It was observed that radiation-related caries appeared in 17 patients treated for primary oropharynx carcinoma, and in only five patients treated for primary laryngeal carcinoma (Table 2). According to the dose volume histogram, the coverage of the planning target volume was homogeneous with 95% tumor dose.

Caries was identified in 15 patients treated with conventional 3D CT radiotherapy and in seven patients treated with 3D CT-MRI conformal radiotherapy, p = 0.04 (Table 2). Results in our study showed that incidence of dental caries was statistically more frequent if radiotherapy is planned only according to CT.

Regardless of the used treatment technique, acute complications were more common in chemoradiotherapy regimen (35/52 pts.) than in the application of radiotherapy alone (17/52 pts.), (p = 0.004). This implies that in chemoradiation the incidence of side effects was statistically significantly higher than in radiotherapy without concomitant chemotherapy.

Fifty-two patients out of the total number of 80 examined and irradiated patients received some form of corticosteroid therapy during the period of radiation, 35 (67%) for 3D CT vs. 17 (33%) for 3D CT-MRI, p = 0.004 (Table 2). The use of corticosteroid therapy in 3D conformal radiotherapy has been correlated with increased incidence of side effects.

Corticosteroid therapy was included as a symptomatic therapy with irradiated patients when non-steroidal analgesics could not eliminate the pain. Dexamethasone was usually used in the form of tablets with total daily dose of 1.5–3 mg per 24 hours or parenteral 4–12 mg per 24 hours.

Complications of radiation treatment grade 0, 1, and 2 which do not require interruption of radiotherapy or analgesics have the same incidence in both techniques, p = 0.64. Grades 3 and 4 have been statistically more frequent in patients treated with 3D CT technique, p = 0.03. There was no difference in incidence of side effects in either technique when the tumor dose was 60 Gy, while we noticed an increase in the 3D CT technique when the dose was 70 Gy, p = 0.07.

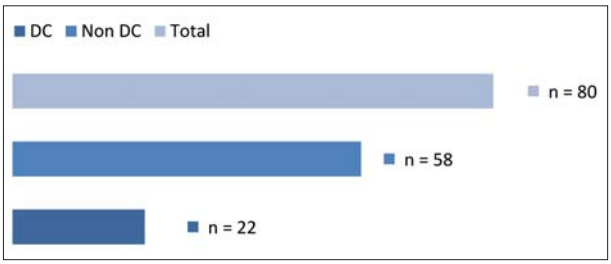

Figure 1. Distribution of dental caries (DC) in irradiated patients

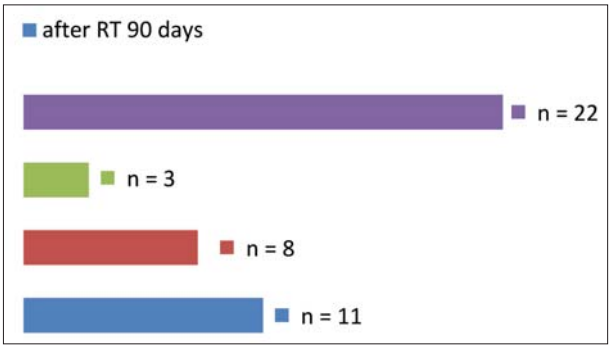

Figure 2. Distribution of dental caries at the first, second, and third patient medical examination and dental evaluation

DISCUSSION

Our study involved 80 patients of both genders. According to the results of the study the incidence of H&N cancer is higher in men than in women (59 M:21 W). The approximate ratio of 3:1 in favor of men corresponds to the general trend of incidence of this disease as shown in medical literature [21].

Complications of radiotherapy were observed in 65% of patients (52/80). In the literature, this percentage goes around 40%, and in our study it is higher, which can be explained by infrequent usage of planning radiotherapy based on computerized tomography with MRI fusion as well as fusion methods with PET-CT and intensity-modulated radiation therapy (IMRT) techniques [22].

Complications in the mucous membrane of the oral cavity in the form of radiation-related mucositis was registered in 36 out of 80 participants in the study, and approximately one half of the patients reported some kind of a problem. Although this complication was the most common, the manifestation of mucositis was mild (Grade 0–2: 32/52) and did not cause the interruption of radiotherapy [23]. Assessing our results we can conclude that there is statistically significant difference in the incidence of side effects if radiotherapy is planned using CT rather than using MRI fusion. This especially applies to the delivery of higher tumor dose of 70 Gy (3D CT : 3D CT-MR = 14:7; p = 0.07).

Xerostomia appeared in 40 patients, which is one half of the irradiated patients. Xerostomia is one of the most common symptoms in cancer patients [24]. Based on the results we concluded that for delivery of higher tumor dose

with fewer side effects, the optimal radiation technique is planning with fusion MRI.

These patients often suffered from skin changes and complications. These changes were seen in 24 patients and are mainly manifested in the form of Grade 0–1 with the presence of skin erythema. Dermatitis more often occurred in the 3D CT-MRI technique because there was a larger number of fields (3D CT : 3D CT-MRI = 10:14) (Table 2). The level of manifestation of acute radiation complications (Acute Radiation Morbidity Scoring Criteria) in the form of radiation-related dermatitis was classified into four categories [19].

All 49 patients with negative prognostic factors (RT:RT + HT = 31:49) received radiotherapy concomitant with chemotherapy. Side effects were reported by 35 patients (35/49; p = 0.004). According to this, the frequency of complications increase when chemotherapy is applied concomitant with radiotherapy.

In 22 out of a total of 80 patients dental caries was identified. As expected, radiation-related caries had higher incidence in patients treated for oropharyngeal (O) then in those treated for laryngeal (L) carcinoma (17:5 = O:L, p = 0.14). Participation of supraglottic localization was the most frequent of all laryngeal carcinoma in our study (22/40; 55%). The likely reason for this could be the size of irradiated area and localization which encompasses large salivary glands. It was also found that 15 patients irradiated with 3D CT technique developed radiation-related caries. Such high incidence (15/40; 37.5%) of dental caries can be linked to the use of 3D CT planning and execution technique, which is in many centers already proven as inferior and, hence, outdated. Different studies explain the impact of irradiation on the composition of teeth in a different way. Some claim that the immediate effect of irradiation is demineralization and damage of the prismatic structure of tooth, while others argue that the exposure to radiotherapy does not change tooth structure and composition [25, 26].

Data regarding this topic is scarce in literature. The phase III study which compared side effects of 2D, 3D radiotherapy techniques and IMRT, did not give any detail on dental management and complications [27]. Using newer radiation techniques, 3D CT-MRI fusion and IMRT, protection of critical organs became possible and decreased the incidence of early and late complications of the irradiated areas of H&N.

In their study, Walker et al. [17] have proven that doses above 60 Gy cause irreversible changes in the structure of the teeth and induced dental caries development. Dose of 30–60 Gy is likely related to salivary gland damage. Critical threshold seems to be at the ≥ 60 Gy level, and these findings suggest that 3D treatment planning process should be carefully done respecting this dose level. All the patients in the present study received radiation doses of 60 Gy or 70 Gy, depending on the type of radiotherapy – adjuvant vs. primary treatment in locally advanced H&N cancer.

Caries risk was reduced in patients receiving parotid-sparing radiotherapy, where salivary output is largely maintained [17].

Late radiotherapy-related complications, such as post irradiation caries that were analyzed 90 days after radiation treatment, occurred in about 27.5% of cases as mentioned earlier (22/80; 27.5%). Some studies have shown higher incidence of radiation-related caries (around 35%), mostly in patients which were irradiated for nasopharyngeal carcinoma [16]. The favorable results of this study can be explained by a small number of patients and localization of irradiated areas, as well as by avoidance of irradiation of large salivary glands. It is obvious that direct effect of irradiation may not be solely responsible for the occurrence of dental caries, and that other factors such as hyposalivation, xerostomia, mucositis, loss of taste, and diet change may be present. This clearly confirms multifactorial genesis of radiation-related caries [28].

New radiotherapy techniques make possible for the radiation dose to be localized to a smaller volume of mandible, which will certainly result in lower incidence of oral complications and radiation-related caries [29]. However, despite the expectations of lower incidence of oral complications, some authors claim that there is no great benefit to this. Ben-David et al. [30] claim that the daily intake of fluoride supplements up to 18 months following the completion of radiotherapy has a greater effect on reduction of oral complications.

Around 65% (52/80) of our patients received some form of corticosteroid therapy during radiation (Table 2). The combination of radiation-related complications with simultaneous application of corticosteroid therapy is very high in our patients and it is about 75%. Application of corticosteroid therapy during radiation and its impact on the increase of incidence of complication is not well understood in the literature [31]. Unreasonably frequent application of corticosteroid therapy is present and it can certainly affect the patient's immune system and the composition of saliva. It can also be related to a higher rate of oral complications during radiotherapy.

The pain is certainly not an absolute indication for the use of these medications, and the therapy should be directed to analgesic therapy and cancer pain therapy in the form of non-opioid and opioid analgesics as well as coanalgesics. Further recommendations for research would certainly include the effect of corticosteroid systematic therapy on the development of radiation-related complications.

Finally, it is worth saying that direct effect of radiation is not the sole factor in occurrence of oral complications and radiation-related caries. The complications that have been researched have a multifactorial genesis and depend on composition and quantity of saliva, bacterial colonization, age, dental hygiene and fluoride intake [32]. Minimizing the use of corticosteroid therapy has also been associated with the decrease in incidence of oral complications. Studies involving patients who were not irradiated also document the occurrence of secondary caries, which supports the finding that direct effect of radiotherapy is not the only causal factor in the occurrence of dental caries [21]. The use of IMRT can certainly reduce the incidence of radiotherapy complications in H&N cancer [32, 33].

CONCLUSION

3D radiotherapy planning techniques with computed tomography are associated with a high rate of toxicity, which affects patients' quality of life. In our study, 3D CT-MRI radiotherapy reduced the incidence of radiation-related oral complications. The experience of institutions of developed countries, based on fusion techniques in radiotherapy planning, shows successful decrease of side effect incidence. This benefit should be included in the clinical practice of radiotherapy planning in institutions of developing countries. Curative radiotherapy treatment methods should be planned with best available imaging techniques in the form of conformal techniques based on computerized tomography fusion with MRI alone or, whenever possible, using fusion with PET CT and/or IMRT.

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Оралне компликације радиолошке терапије код болесника са карциномом главе и врата – 3Д конформална радотерапија и 3Д конформална радиотерапија са МР фузијом

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САЖЕТАК

Увод/Циљ Учесталост нежељених ефеката зрачења код болесника са карциномом главе и врата зависи од технике планирања, спровођења радиотерапије и примарне локализације тумора.

Циљ нашег истраживања је да се утврди учесталост нежељених ефеката зрачне терапије код болесника са тумором главе и врата лечених 3Д конформалном радиотерапијом планираној само на основу КТ и 3Д конформалном терапијом планираној на основу фузије КТ са МР (КТ-МР).

Методе Проспективно је анализирано 40 болесника са карциномом орофаринкса и 40 болесника са карциномом ларинкса код којих је спроведена зрачна терапија. Двадесет болесника са карциномом орофаринкса и 20 болесника са карциномом ларинкса је зрачено 3Д конформалном техником на основу КТ, а још по 20 са карциномом орофаринкса и ларинкса фузијом КТ-МР. Код свих је примењена стандардна фракционација са 2 *Gy* дневно, пет дана седмично.

Резултати Од укупно 80 болесника лечених зрачењем, код 52 (52/80; 65%) забележени су нежељени ефекти зрачне терапије, а учесталост компликација је већа код примене 3Д КТ технике зрачења (31/52; 60% код 3Д КТ насупрот 21/52; 40% код 3Д КТ-МР; $p = 0,02$). Било је више компликација у групи болесника код којих је примењена хемоирадијација него код болесника лечених само радиотерапијом – 35/52 РТ+ХТ, а 17/52 РТ (67% : 33%; $p = 0,004$).

Закључак 3Д техника радиотерапије планирана само на основу КТ је повезана са високом стопом токсичности, које знатно утичу на квалитет живота зрачених болесника. 3Д конформална техника радиотерапије планирана фузијом КТ-МР смањује појаву оралних компликација. За примену виших туморских доза уз мању учесталост компликација је подеснија техника планирања са фузионисаном техником помоћу МР.

Кључне речи: радиотерапија; тумори главе и врата; оралне компликације; КТ-МР фузија у планирању радиотерапије

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Late vitamin K deficiency bleeding despite intramuscular prophylaxis at birth – Is there a need for additional supplementation?

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SUMMARY

Introduction/Objective Vitamin K deficiency is common in newborn infants and without prophylaxis there is a risk of vitamin K deficiency bleeding (VKDB). The most frequent prophylactic approach is an intramuscular (IM) injection of vitamin K_1 immediately after birth. Its efficiency to prevent late VKDB has been recently questioned by several reports.

Based on our experience, we discuss the need for additional vitamin K_1 supplementation after its IM administration at birth.

Methods We present a retrospective review of 12 infants, 11 with confirmed and one with probable late VKDB despite IM prophylaxis at birth, who were treated in the two largest tertiary care pediatric hospitals in Serbia during the last 15 years.

Results All the patients were exclusively breastfed. In 11 patients, daily weight gain was normal or increased, and one patient had failure to gain weight. Six infants were previously healthy, three infants received antibiotics prior to bleeding, and in two diarrhea and cholestasis, respectively, existed previously. An intracranial bleeding was documented in nine infants, four of whom died.

Conclusion Low content of phytomenadione in human milk could occasionally be attributed to late VKDB despite postnatal IM injection of vitamin K_1 in otherwise healthy, exclusively breastfed infants. This might be aggravated by transient disturbance of vitamin K turnover due to antibiotic use, acute diarrhea, or transient cholestasis. We suggest that an additional vitamin K_1 supplementation after postnatal IM prophylaxis could be justified in exclusively breastfed infants.

Keywords: vitamin K; late vitamin K deficiency bleeding; intramuscular prophylaxis

INTRODUCTION

Newborn infants are deficient in vitamin K due to its poor transplacental transport, delayed intestinal synthesis and low content in human milk [1, 2]. Therefore, vitamin K-dependent clotting factors (F II, VII, IX, X) express no more than 50% of activity attained in later life, and both prothrombin time (PT) and activated partial thrombin time (aPTT) are prolonged in comparison to adult values [3]. In some infants "physiological hypoprothrombinemia" leads to spontaneous or iatrogenic bleeding formerly known as hemorrhagic disease of the newborn (HDN), and lately more appropriately named vitamin K deficiency bleeding (VKDB). VKDB presents in three different forms – early, classic and late VKDB. Early VKDB is very rare and its occurrence with severe bleeding immediately after birth is mostly related to maternal intake of certain medications (anticonvulsive, antitubercular, and anticoagulant drugs). Without prophylaxis, classic form has incidence of 0.25–1.7% of all newborns, potentially presenting the most common acquired pediatric hemostatic disorder. Fortunately, in majority of cases there is only mild to moderate gastrointestinal, skin, or bleeding from umbilicus typically occurring during the first week of life. Late VKDB presents between the second and 26th week of life (the peak is between three and eight weeks), and without prophylaxis, incidence in the western world ranges from four to seven cases per 100,000 deliveries. Primary, or idiopathic late VKDB occurs in exclusively breastfed, otherwise healthy infants, while secondary form is a consequence of some pathological conditions which steadily disturb intestinal synthesis and/or absorption of vitamin K (biliary atresia, cystic fibrosis, celiac disease, alfa-1 antitrypsin deficiency, chronic diarrhea, etc.). Up to 60–80% of infants with the late VKDB have an intracranial hemorrhage, with a mortality rate of 14–24%, and nearly 50% of survivors have a permanent neurological impairment [1, 2].

Initially, prevention of classic VKDB, intramuscular (IM) administration of vitamin K_1 (phytomenadione) to all newborn infants immediately after birth was introduced in the US more than 50 years ago [4]. Over time, this practice was adopted almost worldwide. Routine IM injection of vitamin K_1 (1 mg IM for all term newborn infants / 0.5 mg for preterm infants) was recommended in Serbia in 1995 [5]. Vitamin K

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prophylaxis at birth via IM route is obligatory, except in cases of parental refusal or their alternative choice of an oral mode. Both decisions should be stated in a written form.

Because of presumed but unproven association between IM prophylaxis and later greater risk of cancer, in the 1990s a shift from IM towards oral prophylaxis in some countries was accepted. However, it soon became evident that despite its efficiency against early and classic forms, a single oral dose of vitamin K_1 does not prevent late VKDB. Therefore, in order to increase the efficiency of oral regiment, several distinct strategies of prolonged oral supplementation of vitamin K have been implemented [6–9].

Unlike oral policy, a single IM injection of vitamin K_1 at birth has long been considered a "gold standard" and a reliable way to eradicate all forms of VKDB [2]. However, several reports of late VKDB occurring after IM prophylaxis called into question the traditional belief in the superiority of such an approach. In addition to some sporadic single cases [10, 11, 12], a case series of otherwise healthy infants with failure of postnatal IM injection of vitamin K_1 to prevent late VKDB has recently been reported from Turkey, Egypt, India and Albania [13–19]. Whilst these papers focus on intracranial hemorrhage as the main consequence of the late VKDB, we present the largest European group of patients with the late VKDB despite IM administration of vitamin K_1 at birth.

The aim of this study is to retrospectively review cases of late VKDB occurrence despite IM prophylaxis with vitamin K_1 given at birth. Based on our experience, we discuss a need and possible regimes of an additional vitamin K supplementation for exclusively breastfed infants during early infancy.

METHODS

Hospital files of patients with HDN/VKDB diagnoses or other unspecified neonatal hemorrhagic conditions (ICD-9 codes 776.0/776.3 and 269.0, respectively; ICD-10 code P53) treated between 2000 and 2015 in two largest tertiary care pediatric hospitals in Serbia – the Institute for Mother and Child Healthcare of Serbia (New Belgrade) and University Children's' Hospital (Belgrade) – were retrospectively reviewed. A confirmed case of late VKDB is defined by the following criteria: a) spontaneous or iatrogenic hemorrhage in an infant aged two to 26 weeks; b) PT prolonged ≥ 4 times over normal values, aPTT > 60 sec. and/or international normalized ratio (INR) > 4 control values; c) cessation of hemorrhage and normalization of PT and aPTT and/ or INR after the administration of vitamin K; d) normal both platelet count and fibrinogen levels. If criterion "c" is not satisfied, the case is classified as "probable" late VKDB. Coagulation was investigated on admission and 6–12 hours thereafter. Intracranial hemorrhage is documented by a computed tomography scan and/or nuclear magnetic resonance spectroscopy (NMR) imaging. All the patients were emergently treated with 1 mg/kg of vitamin K_1 intravenously. Fresh frozen plasma (10–15 ml/kg) was administered to patients with life-threatening bleeding.

RESULTS

Our research revealed 16 patients with a diagnosis of HDN/VKDB, treated in pediatric intensive care units of our hospitals during the previous 15 years. Of those, four patients who didn't receive vitamin K at birth were excluded from the final presentation: one newborn with a classic VKDB, whose parents refused vitamin K injection, and three patients with a late form, who were born in neighboring countries, without reliable data on postnatal prophylaxis. In the remaining 12 patients (10 males; two females), an IM injection of 1 mg of vitamin K_1 was administered and recorded in the discharge list from the maternity ward.

According to the data shown in Table 1, there were 10 male and two female infants, aging from 21 to 51 days (median age was 35 days). All our patients had a significantly prolonged PT and aPTT, as well as abnormal INR. The normalization of these tests after the vitamin K administration was documented in 11 cases. Patient No. 12 died soon after admission; as it was not possible to check coagulation tests after the administration of vitamin K, he was classified as a probable case. Except for patient No. 3, who was born at the 34th gestational week with body weight of 1,950 g, all others were born at term. There was a history of previous antibiotic use in three cases (patients No. 3, 4, and 6), while patient No. 8 had a history of two-day diarrhea before the bleeding. A female infant aged 49 days (patient No. 5) had prolonged indirect jaundice with a rise of total serum bilirubin level during the first month of life up to 204 μmol/l (direct fraction of 23 μmol/l). Upon hospital admission, conversion to direct hyperbilirubinemia indicating cholestasis was noted (total serum bilirubin level was 127 μmol/l; direct fraction was 43.6 μmol/l). Both the mother and the child had the same blood type, and there were no signs of hemolysis.

The platelet count, fibrinogen levels, as well as liver enzymes were within normal range in all our patients. It was documented in all cases that both FV and FVII expressed normal or increased clotting activity upon admission.

All our patients were solely breastfed. Their daily weight gain was calculated by dividing the difference between infants' weight on admission and birth weight with age in days. In 11 patients, including one born prematurely and one with intrauterine growth restriction, daily weight gain was in the 16.8–46.5 g range (mean 32.5 g; SD 11.2 g). In only one case (patient No. 10), daily weight gain was unsatisfactory, reaching 8.1 g. The intracranial bleeding was documented in nine infants (75% of patients). Four infants in the study group died, making an overall mortality of 25%.

DISCUSSION

The international definition of a confirmed late VKDB was fulfilled in 11 of our patients. An infant with extremely prolonged PT and APTT, who died immediately after admission, without the possibility to check the laboratory

Table 1. Relevant laboratory and clinical data on infants with late vitamin K deficiency bleeding despite intramuscular prophylaxis

Patient sex	Age (days)	PT (sec)		aPTT (sec)		INR		Localization of bleeding Outcome	Remarks
		(1)	(2)	(1)	(2)	(1)	(2)		
1 M	31	did not clot	11.6	did not clot	25.1	NC	0.8	Intracranial Recovery	-
2 M	35	74.0	11.4	63.0	24.6	4.1	0.9	Intracranial Recovery	-
3 M	34	98.7	14.5	70.7	28.1	10.6	1.1	Intracranial Died	Preterm, 15 days of antibiotic use
4 M	45	> 200	11.6	83.9	25.1	NC	0.9	Intracranial Died	2 days of antibiotic use
5 F	49	77.1	16.5	66.2	35.7	7.34	1.3	Intracranial Died	Prolonged jaundice with mild cholestasis
6 M	51	> 300	10.8	> 300	23.3	NC	0.9	Intracranial Recovery	15 days of antibiotic use
7 F	42	did not clot	14.8	> 300	28.2	NC	1.3	Hematoma after venipuncture Recovery	Intrauterine growth restriction
8 M	21	did not clot	14.9	did not clot	32.4	NC	1.3	Large hematoma after vaccination Recovery	2 days of antibiotic use
9 M	39	did not clot	11.4	did not clot	30.0	NC	0.9	Large hematoma after vaccination Recovery	-
10 M	37	119	10.6	139	30.2	14.6	1.0	Intracranial Recovery	-
11 M	36	> 200	9.7	83.9	28.7	NC	0.9	Intracranial Recovery	-
12 M	35	did not clot	ND	> 300	ND	NC	ND	Intracranial Died	Probable case of late VKDB

M – male; F – female; (1) – results before vitamin K therapy; (2) – results after vitamin K therapy; NC – not calculated; ND – not done; VKDB – vitamin K deficiency bleeding; PT – prothrombin time; aPTT – activated partial thrombin time; INR – international normalized ratio

testing, was classified as a probable case of the late VKDB [10, 20, 21]. Congenital as well as clotting disorders due to liver impairment were excluded in all cases.

Exclusive breastfeeding was the common factor for all our patients and the most of previously published cases of late VKDB occurring after IM prophylaxis with vitamin K at birth. Human milk contains 0.5–4 μg/l of phytomenadione, while the minimal daily requirements for vitamin K in infants from birth up to six months are 1.5 μg/l [1, 2, 21, 22]. Assuming the daily amounts of suckled milk of 0.5–0.8 l, the daily intake of vitamin K would be between 0.25 μg and 3.2 μg. Therefore, exclusively breastfed infants weighing 3–6 kg, which corresponds to the first six months of life, would not satisfy their total daily needs of ≈ 5–10 μg of phytomenadione even in the best case scenario.

Elaify et al. [17] documented that in infants given IM injection of vitamin K at birth, those suffering from intracranial bleeding due to late VKDB had significantly lower serum levels of phylloquinone than matched control group. They also showed that babies who bled more frequently used antibiotics or had acute diarrhea [17]. A large prospective British study revealed patients with association of biliary atresia and a severe late VKDB despite an IM administration of vitamin K at birth [10]. All aforementioned disorders interfere with intestinal synthesis and/or absorption of vitamin K, but without an adverse effect on the activity of vitamin K IM injection. This fact indirectly proves that effective prevention of late VKDB requires additional supply of phytomenadione from gastrointestinal tract. Hence, besides IM prophylaxis at birth, in healthy, solely breastfed infants, some oral supplementation of vitamin K is required thereafter. The US Nutritional Board of National Institute of Health estimates that if prophylactic dose of vitamin K was given as an IM injection at birth, 2 μg of vitamin K is an adequate daily intake during the first six months of life [23]. According to the previously calculated daily allowance of phytomenadione by human milk (0.25–3.2 μg), there are some exclusively breastfed healthy infants with possible insufficient vitamin K supply (< 2 μg per day) and consecutive risk of late VKDB in spite of previous IM prophylaxis.

Normal or even excessive weight gain was recorded in 11 of the 12 cases, so insufficient milk intake as a cause of lack of vitamin K could be excluded [24].

Six of our patients were healthy infants without any predisposing factor to the late VKDB. Out of the remaining five, three were treated with antibiotics during 2–15 days, while one had acute diarrhea. A seven-week-old infant with a transition of prolonged unconjugated hyperbilirubinemia to the cholestatic jaundice, which preceded a lethal intracranial hemorrhage, confirms that neonatal jaundice lasting for more than two to three weeks justifies the "yellow alert" [10]. We observed that in addition to secondary late VKDB due to serious pathological conditions, there is a subgroup of otherwise healthy exclusively breastfed infants with some transient risk factors which further deteriorate the vitamin K deficiency and increase the risk of late VKDB.

Male infants accounted for a large majority of our patients, corresponding to reported twofold-to-sevenfold

male predominance [13, 14, 15, 25]. Although this striking gender discrepancy is not yet clarified, results of previously reported investigation suggest that male infants may require more dietary phytomenadione than females with the same body weight [26].

Our hospitals, as tertiary referring pediatric institutions, cover a gravitating area with approximately 40,000 deliveries per year. For the entire period of 15 years, the total amounts to nearly 600,000 live births. Accordingly, 12 patients give an estimate of the rate of late VKDB of one case per 50,000 live births (two per 100,000 live births). Possible explanations for the two- to threefold higher incidence than in developed countries could be inadequate maternal diet with low intake of vitamins, and less critical use of antibiotics in infants [10, 27, 28, 29].

Frequent occurrence of intracranial bleeding and high mortality rate in our patients correspond with reported severity of late VKDB [1, 2]. A single case of failure of IM prophylaxis to prevent late VKDB, initiated the Italian Society of Neonatology to recommend 25 μg of vitamin K per day orally during the first three months of life for all breastfed infants, previously given an IM injection of vitamin K at birth [2, 11]. To date, no adverse effects even of higher intake of vitamin K by standard milk formula containing 50–60 μg/l have been reported [1, 2, 21, 22]. Unlike weekly "pharmacological" regime, the daily oral supplementation with low doses of phytomenadione is considered to be "physiological" because such approach maintains a constant serum level and efficiently compensates inadequate intake of vitamin K [30]. Therefore, after immediate postnatal IM dose, we recommend prolonged oral prophylaxis for all exclusively breastfed infants with daily intake of 25 μg of phytomenadione from the second to the 12th week of life. Prolonged oral prophylaxis, even with higher doses of phytomenadione, is strongly advised if parents choose the oral route as the way of prophylactic use of vitamin K at birth instead of the IM mode.

Except in cases of cholestasis, an oral intake of 25 μg of vitamin K efficiently prevented late VKDB [7, 8, 23]. The presence of cholestatic jaundice requires a more individualized approach. One option may be an increase of oral dose up to 150 μg of phytomenadione per day, which is recommended in Holland as a routine three-month policy after an initial oral prophylaxis at birth [8]. Some authors consider an additional IM dose of vitamin K [17]. The Danish regime of a three-month weekly oral supplementation with 1 mg of vitamin K is effective even in infants with biliary atresia [9]. In our country, there is no commercial oral vitamin K preparation containing the required dose. Off-label use of vitamin K glass ampules may be an alternative, but it is connected with problems such as parental resistance because of uncomfortable use. On the other hand, professional assistance makes the weekly oral doses a costly alternative [10]. Therefore, we consider that in cases of transient disturbance of vitamin K intestinal turnover, an additional parenteral dose of 1 mg should be given to infants on the daily oral intake of 25 μg of vitamin K. Such approach seems particularly justified if there is prolonged jaundice with any sign of cholestasis.

Like in a number of other cases, our recommendation is also an experts' opinion based on personal experience with severe and highly lethal late VKDB [6–9]. According to the facts that "oral vitamin K … has not been tested in randomized trials for its effect on either classic or late VKDB" [29], and that "the results regarding late HDN and prolonged oral prophylaxis are still inconclusive … due to lack of scientific evidence" [31], we suggest this recommendation despite its low level of evidence.

CONCLUSION

We hope that our experience will increase the awareness that despite an IM dose of vitamin K at birth there is still a risk of serious, potentially lethal late VKDB. At the moment, its occurrence could be attributed to an occasional extremely low content of phytomenadione in human milk. Therefore, an additional three-month daily oral supplementation with low doses of phytomenadione could be justified in solely breastfed infants in our country. In cases of transient disturbance of vitamin K turnover due to antibiotic use, acute diarrhea, or transient cholestasis, a more individualized approach, including additional parenteral dose of vitamin K, could be considered.

Final decision about vitamin K prophylaxis policy should be based on thorough assessment of overall circumstances, including incidence of late VKDB, availability and cost of vitamin K preparations.

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Касни облик крварења услед недостатка витамина К упркос примени интрамускуларне профилаксе на рођењу – да ли је потребна додатна надокнада?

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САЖЕТАК

Увод/Циљ Без одговарајуће профилаксе, недостатак витамина К код новорођене деце и одојчади може довести до хеморагијског синдрома познатог под називом крварење услед недостатка витамина К (КНВК). Најчешћа профилакса КНВК је интрамускуларна (ИМ) инјекција витамина K_1 одмах по рођењу. Новији извештаји о појави касног облика КНВК упркос интамускуларној профилакси по рођењу довели су у питање уверење у потпуну поузданост овог приступа. Циљ рада је да изнесе сопствена искустава и предлоге за надокнаду витамина К после ИМ примене на рођењу.

Методе Ретроспективно је анализано 12 новорођенчади и одојчади, лечених протеклих 15 година, 11 са доказаним и једно са вероватним касним обликом КНВК упркос ИМ профилакси витамином К1 на рођењу.

Резултати Сви болесници су били искључиво на природној исхрани. Пораст телесне масе код 11 је био нормалан или повећан и код једног недовољан. Пре појаве КНВК шест болесника је било здраво, три су претходно добијали антибиотике, један је имао акутни пролив и један холестазу. Интракранијално крварење доказано је код девет болесника, од којих су четири умрла.

Закључак Снижен садржај фитоменадиона у хуманом млеку може понекад резултирати КНВК код ексклузивно дојене здраве новорођенчади и младе одојчади. Пролазном недостатку витамина К додатно доприноси примена антибиотика, акутни пролив или холестаза. Зато је оправдано после профилаксе витамином K_1 на рођењу, код искључиво дојене деце, наставити додатни унос препарата фитоменадиона током прва три месеца.

Кључне речи: новорођенче; витамин К; крварење услед недостатка витамина К; профилакса

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Evaluation of adherence to calcium, vitamin D, and drugs for osteoporosis in patients with low bone mineral density

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SUMMARY

Introduction/Objective Osteoporosis is a systemic disease of bone tissue, which leads to an increase in bone fragility and higher risk of fractures.

The aim of the study was to determine adherence to calcium, vitamin D, and drugs for osteoporosis in patients with reduced bone mineral density, as well as to analyze reasons for low adherence.

Methods The study conducted in the Special Hospital for Rheumatic Diseases, Novi Sad, Serbia, involved 80 postmenopausal women with reduced bone mineral density measured by dual-energy X-ray absorptiometry. Each patient filled out a specially designed questionnaire. Assessment of adherence to calcium, vitamin D, and drugs for osteoporosis was done by the Morisky scale. In the statistical analysis we used the SPSS program v. 20.

Results All the patients were female; 67.5% had osteoporosis and 32.5% had osteopenia; 62.5% of women said that they use calcium supplementation, 81.3% vitamin D, and 62.3% drugs; 81.2% of women who used supplementation had low adherence to calcium, 82.8% low adherence to vitamin D, and 65.8% low adherence to drugs for osteoporosis. Adherence to medication for osteoporosis is better in relation to the adherence of vitamin D and calcium ($p < 0.05$, χ^2 test). The reasons for low adherence are mainly the cost of preparation and forgetfulness. Patients who received drugs intravenously had better adherence than patients who received drugs subcutaneously or orally.

Conclusion Adherence to vitamin D, calcium, and drugs for osteoporosis is presently low in investigated population and the understanding of the causes of low adherence is still insufficiently explored.

Keywords: osteoporosis; patient adherence; calcium; vitamin D

INTRODUCTION

Osteoporosis is a systemic disease characterized by low bone mass and microarchitectural deterioration of bone tissue, which leads to an increase in bone fragility and therefore a higher risk of fractures [1]. The World Health Organization definition of osteoporosis is based on the measurements of bone mineral density [2]. In the treatment of osteoporosis, calcium and vitamin D supplementation is used, as well as drugs with different mechanisms of action. Calcium and vitamin D are required for normal bone metabolism. Vitamin D deficiency leads to secondary hyperparathyroidism and bone resorption [3, 4]. Supplementation of vitamin D reduces bone fragility and increases bone mineral density. According to recommendations, a physician should initiate pharmacologic treatment after a patient has had hip or vertebral (clinical or asymptomatic) fractures, when T-score is ≤ -2.5 SD at the femoral neck, total hip, or lumbar spine by dual-energy X-ray absorptiometry, in postmenopausal women and men age 50 and older with low bone mass (T-score between -1.0 SD and -2.5 SD, osteopenia) at the femoral neck, total hip, or lumbar spine by dual-energy X-ray absorptiometry and a 10-year hip fracture probability ≥ 3% or a 10-year major osteoporosis-related fracture probability ≥ 20% based on the USA-adapted World Health Organization absolute fracture risk model (Fracture Risk Algorithm (FRAX®)) [5]. Drugs approved by the US Food and Drug Administration for the treatment of osteoporosis are bisphosphonates (alendronate, ibandronate, risedronate, and zoledronic acid), calcitonin, estrogen agonist/antagonist (raloxifene), estrogens and/or hormone therapy, tissue-selective estrogen complex (conjugated estrogens/bazedoxifene), parathyroid hormone 1-34 (teriparatide), and receptor activator of the nuclear factor kappa-B (RANK) ligand inhibitor (denosumab) [6, 7]. Bisphosphonates are prescribed most often for the treatment of osteoporosis. Patients can use them weekly, monthly, once in three months, and once per year. They are efficacious and well tolerated [8]. Estrogen/hormone therapy is approved by the Food and Drug Administration for the prevention of osteoporosis, relief of vasomotor symptoms, and vulvovaginal atrophy associated with menopause [9].

For optimal treatment it is necessary not only to recognize persons at risk, make ap-

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propriate diagnosis and treatment decisions, but also to ensure patient adherence. Adherence to medication is defined as the cooperation of the patient with the physician in relation to the dose, frequency, and timing of medication during the recommended period of treatment [10, 11, 12]. Medication adherence can be divided into three major components: persistence, initiation adherence, and execution adherence. Persistence is defined as the length of time a patient fills his/her prescriptions [13]. Initiation adherence gives answer at question does the patient start with the intended pharmacotherapy [13]. Execution adherence is the comparison between the prescribed drug dosing regimen and the real patient's drug-taking behavior and includes dose omissions (missed doses) and the so-called 'drug holidays' (three or more days without drug intake) [13]. There are objective and subjective ways of measuring adherence. Objective measures, including measurement of clinical outcomes, dose counts, and pharmacy records, electronic monitoring of medication administration (e.g. the Medication Event Monitoring System, MEMS), and drug concentrations, seemingly provide the best measure of a patient's medication-taking behaviour. Subjective measures of adherence include physician or family reports, patient interviews and self-report adherence scales. These measures have the potential to identify the specific reasons for a patient's non-adherence. Subjective measures can be relatively simple to use and are less expensive [14]. Morisky scale is the often used metric to assess adherence. This scale can evaluate reliably, easily and efficiently the cooperation of the patient with the physician [15, 16, 17]. With this scale, our study tried to analyze and evaluate the effects of therapy for osteoporosis in our patients, and compare them with the results in the related work, as there is no such data for our country.

The purpose of this pilot study was to determine the adherence to calcium, vitamin, D and drugs for osteoporosis in patients with reduced bone mineral density, as well as to analyze reasons for low adherence.

METHODS

This prospective cross-sectional study is a pilot project at the Special Hospital for Rheumatic Diseases in Novi Sad, including the sample of 80 postmenopausal women with low bone mineral density, measured by dual-energy X-ray absorptiometry. The women were treated with supplementation of calcium, vitamin D, and/or drugs for osteoporosis (bisphosphates or teriparatide). None of the women used hormone replacement therapy. All the patients signed informed consent to participate in this study. The study was approved by the Ethics Committee of the Special Hospital for Rheumatic Diseases in Novi Sad. Each patient filled out a specially designed questionnaire. The assessment of the adherence to calcium, vitamin D and drugs for osteoporosis was done by Morisky scale, which contains eight items. In the first seven questions the patient can answer with "yes" or "no," while in one last question the patient can answer choosing one of five options offered. The answers for the first seven questions are marked with 1 point for „yes" and 0 points for „no." In the five-options question, answers „never" and „rarely" scored 0 points, while other options („from time to time"/"sometimes"/"often"/"all the time") scored 1 point. A total score for each subject is obtained by adding up the points for all the questions. Score ≥ 3 was considered to be low adherence, 1–2 was medium adherence, and score 0 was high adherence.

IBM SPSS Statistics for Windows, version 20.0 (IBM Corp., Armonk, NY, USA) was used for statistical analysis in this study, as well as the measures of central tendency, the ANOVA test, and the χ^2 test.

RESULTS

Demographic data for all study subjects is presented in Table 1. All the patients were female. The average age for all subjects was 65.52 ± 8.29. Most of the subjects lived in the city and had secondary school education; 67.5% of them had osteoporosis with a duration of M 4.29 ± 3.36 years, and 32.5% had osteopenia with a duration of M 3.54 ± 2.42 years. The subjects had entered menopause with 35 years at the earliest, and with 55 years at the latest. The average entrance in the menopause was 47.5 years (M = 47.5).

Of all the women involved in this study, 62.5% used calcium supplementation. Using Morisky scale, our results showed that they had low adherence in 81.2% of the cases (Table 2). Vitamin D supplementation was used by 81.3% of the women, but adherence to vitamin D was also low in

Table 1. Patient characteristics

Variables		
Gender (frequency/percent)	Female	80/100
Age (M ± SD)		65.52 ± 8.29
Place of living (frequency/percent)	Urban	52/65
	Rural	28/35
Education (frequency/percent)	Primary school	23/28.8
	Secondary school	44/55
	University	13/16.3
Osteoporosis (frequency/percent)		54/67.5
Osteopenia (frequency/percent)		26/32.5
Duration of osteoporosis (M ± SD)		4.29 ± 3.36
Duration of osteopenia (M ± SD)		3.54 ± 2.42
The average age when entering menopause (M ± SD)		47.5 ± 4.8

M – arithmetic mean; SD – standard deviation

Table 2. Adherence to calcium, vitamin D, and drugs for osteoporosis

Adherence	Calcium		Vitamin D		Drugs for osteoporosis	
	Frequency	%	Frequency	%	Frequency	%
Medium adherence	9	18.8	11	17.2	13	34.2
Low adherence	39	81.2	53	82.8	25	65.8

$p < 0.05$

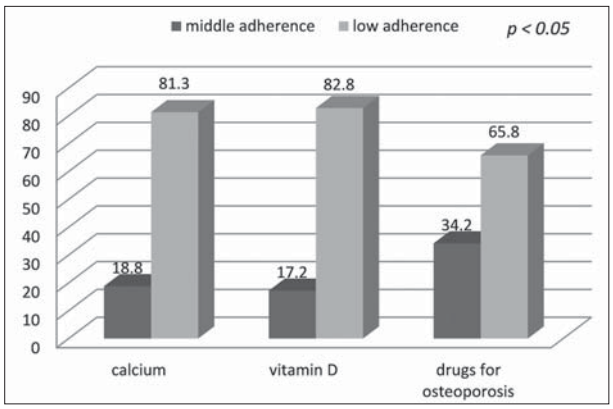

Figure 1. Comparison of adherence (%) to calcium, vitamin D, and drugs for osteoporosis

82.8% of them (Table 2). There was no high adherence in the study population. Some medication for osteoporosis was being taken by 62.3% of the women. Results of the adherence to drugs for osteoporosis showed that there was no high adherence that in the study population (Table 2). Most of the subjects had low adherence.

Comparison of adherence to calcium, vitamin D, and drugs for osteoporosis are shown in Figure 1. Adherence to drugs for osteoporosis was higher in comparison to vitamin D and calcium ($p < 0.05$, χ^2 test). However, there was no statistically significant difference in adherence to vitamin D and calcium ($p > 0.05$, χ^2 test).

Reasons for the low adherence to calcium, vitamin D, and medications for osteoporosis were also analyzed, based on the data obtained from questionnaires. The results showed that there was no statistically significant correlation between the level of adherence and the reasons for not taking calcium supplementation (likelihood ratio = 5.22, distribution function (df) = 3, p = 0.156), as shown in Table 3. Regarding the reasons for low adherence to vitamin D supplementation, the results showed that there was a statistically significant correlation (likelihood ratio = 8.20, df = 3, p = 0.042) with low adherence. The main reason for low adherence to vitamin D is the price of preparation (90.9% of all patients), as shown in Table 4. Results of low adherence and reasons for not taking medication showed that there is no statistically significant correlation between these factors (likelihood ratio = 7.33, df = 3, p = 0.063). Low adherence was present in patients who forgot to take the medicine (84.6%), and high adherence where the price of medication was problematic (66.7%), as shown in Table 5.

In relation to the drug application options and their effect on the adherence to drugs for osteoporosis, there was no statistically significant difference (likelihood ratio = 4.83, df = 2, p = 0.089). Middle level of adherence was present

Table 3. Reasons for low adherence to calcium

Adherence		Reasons				
		Forgetfulness	Price	Many other drugs	Low tolerance/ Side effects	Total
Medium adherence	Frequency	7	1	1	0	9
	%	33.3	8.3	14.3	0	20.5
Low adherence	Frequency	14	11	6	4	35
	%	66.7	91.7	85.7	100	79.5
Total	Frequency	21	12	7	4	44
	%	100	100	100	100	100

$\chi^2 = 4.41$, distribution function (df) = 3, p = 0.220;
likelihood ratio = 5.22, df = 3, p = 0.156

Table 4. Reasons for low adherence to vitamin D

Adherence		Reasons				
		Forgetfulness	Price	Many other drugs	Low tolerance/ Side effects	Total
Medium adherence	Frequency	5	1	4	1	11
	%	20	9.1	57.1	100	25
Low adherence	Frequency	20	10	3	0	33
	%	800	90.9	42.9	0	75
Total	Frequency	25	11	7	1	44
	%	100	100	100	100	100

$\chi^2 = 8.67$, df = 3, p = 0.034; likelihood ratio = 8.20, df = 3, p = 0.042

Table 5. Reasons for low adherence to drugs for osteoporosis

Adherence		Reasons				
		Forgetfulness	Price	Many other drugs	Low tolerance/Side effects	Total
Medium adherence	Frequency	2	2	1	1	6
	%	15.4	66.7	100	100	33.3
Low adherence	Frequency	11	1	0	0	12
	%	84.6	33.3	0	0	66.7
Total	Frequency	13	3	1	1	18
	%	100	100	100	100	100

$\chi^2 = 7.38$, df = 3, p = 0.061; likelihood ratio = 7.33, df = 3, p = 0.063

Table 6. Adherence to drugs for osteoporosis and drug consumption

Adherence		Drug administration			
		Oral	Intravenous	Subcutaneous	Total
Medium adherence	Frequency	10	2	1	13
	%	32.3	100	20	34.2
Low adherence	Frequency	21	0	4	25
	%	67.7	0	80	65.8
Total	Frequency	31	2	5	38
	%	100	100	100	100

$\chi^2 = 4.34$, df = 2, p = 0.114; likelihood ratio = 4.83, df = 2, p = 0.089

in all the participants (100%) who took medications for osteoporosis intravenously. Low adherence was present in most of the patients who took the medicine for osteoporosis through tablets and subcutaneously, as shown in Table 6.

DISCUSSION

Like in other chronic diseases, adherence to medications for osteoporosis is low. The reasons are numerous, but the most common are the fear of side effects, the cost of treatment and lack of motivation to take the drug for a disease that is clinically "silent." Lack of pain until fracture happens contributes to low adherence. Also, several social and economic factors are involved, especially in developing countries. Every physician has to think about these factors when he makes a treatment decision. Low adherence leads to poor treatment results, increased risk of fractures, and therefore increases treatment costs [18].

The most important characteristic of this study is that this is one of the first studies of adherence to calcium, vitamin D, and drugs for osteoporosis conducted in our country, the Republic of Serbia. Until now, all our knowledge on the subject relied on foreign studies. This study was conducted prospectively using the Morisky scale as a subjective measure of the adherence because it has benefits of being inexpensive, acceptable to patients, valid, reliable, has the ability to distinguish between different types of non-adherence, easy to administer, and able to provide information on attitudes and beliefs about medication [19]. The study was designed to be very close to common clinical practice.

Supplementation with calcium and vitamin D is required not just to treat patients with osteopenia, but also in patients with osteoporosis and continues after the start of osteoporosis treatment. The results of our study indicated a low adherence to calcium and slightly better adherence to vitamin D. Similar results were recorded also by the authors of a large ADVICE study [20]. This study, performed in the leading centers for the treatment of osteoporosis in Italy, analyzed adherence to calcium and vitamin D and factors affecting it. It was concluded that adherence was low, and it is necessary to increase it by frequent contact with the doctor, which showed an increase in the patients' motivation for the treatment [20]. In contrast, an observational study was carried out in three osteocenters in the Czech Republic, reported good adherence, but with a fixed combination of Ca – vitamin D, which was observed in 60% of persistent participants [21].

The main reason for low adherence to vitamin D in our study was the price of preparation. Daily use of drugs, for years, could be an economic burden for the patient. Results of one study conducted in Canada suggested that about 1 in 10 Canadians who receive a prescription report cost-related nonadherence [22]. Education of the patients was probably not the reason of low adherence, while all patients had at least medium level of education.

The number of options for the treatment of osteoporosis is increasing. The most commonly used medications are bisphosphonates, which can be given per week, per month, every three months, or per year. In addition to these medications there are also calcitonin, hormonal therapy, selective estrogen receptor modulators, and teriparatide. Despite having many options for the treatment, drug adherence is low. The assumption was that the estrogen hormone therapy would have better adherence since it eliminates the menopause symptoms [23]. However, studies do not confirm this. Contrary to our expectations, the present study confirmed that adherence to bisphosphonate therapy is better than adherence to hormones and calcitonin [23]. Our results align with the results of the meta-analysis of 24 observational studies on large populations, where it was confirmed that adherence to medications for osteoporosis is low [23]. Results from a recently conducted cohort in Bologna, Italy, which used administrative databases as a reliable source of data for "prescription continuity," showed that adherence to the fixed-dose combination (alendronate with cholecalciferol) was higher than that to plain alendronate throughout the follow-up period [24].

Also, our study considered the reasons for low adherence. It was found that the price did worry the patients, but it did not have a statistically significant effect on the adherence to drugs for osteoporosis, similar to other studies [25]. Our results indicate that patients often forget to take the medicine and that fear of the side effects or intolerance to the preparation was not the cause of low adherence, while, in contrast, other authors did conclude that the fear of side effects was a significant cause of low adherence [25, 26]. Also, in one study recently conducted by American authors it was concluded that barriers to prescription treatment include a preference for alternative, non-prescription treatments and not just a fear of possible side effects [26]. Another study also conducted in the USA reveals under-treatment of women diagnosed with osteoporosis. This study showed that in 41% of patients the physician did not recommend any treatment, and in 38%, the patient chose not to initiate the treatment. Among patients who did not initiate the recommended treatment, the predominant reason was concern over side effects, cost of medication, and pre-existing stomach or digestion problems [27].

The results of our study suggest that patients who received the drug intravenously had better adherence, in comparison with patients who received the drug orally or subcutaneously.

There were also some limitations in our study. We used just subjective measures for the adherence. Also, adherence to fixed combinations of Ca – Vitamin D, fixed doses of bisphosphonates – Vitamin D, and hormonal therapy was not explored. Nevertheless, we believe that the results provided by our survey contain valuable information for the adherence to calcium, vitamin D, and drugs for osteoporosis among Serbian population.

CONCLUSIONS

Adherence to vitamin D, calcium, and drugs for osteoporosis is presently low in investigated population and understanding of the causes of low adherence is still insufficiently explored. We believe that better patient education, more therapy possibilities, and frequent visits to the doctor can significantly help the patient to understand the importance of this problem and increase the adherence.

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Процена адхеренције калцијума, витамина Д и лекова за остеопорозу код болесника са сниженом коштаном густином

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САЖЕТАК

Увод/Циљ Остеопороза је системска болест коштаног ткива, која доводи до пораста фрагилности кости и тиме до већег ризика од прелома.

Циљ рада је процена адхеренције калцијума, витамина Д и лекова за остеопорозу код болесника са сниженом коштаном густином и анализа разлога за ниску адхеренцију.

Методе У ову студију је укључено 80 постменопаузалних жена са сниженом коштаном густином измереном двоструком апсорпциометријом *X* зрака. Сви болесници су попуњавали исти, специјално дизајнирани упитник. Процена адхеренције калцијума, витамина Д и лекова за остеопорозу је рађена скалом Мориски. Обрада података рађена је у програму *SPSS*, у верзији 20.

Резултати Сви испитаници су били женског пола. Остеопорозу је имало 67,5%, а 32,5% остеопенију. Суплементацију калцијума користило је 62,5% жена, 81,3% витамин Д, а 62,3% лекове. Ниску адхеренцију калцијума имало је 81,2% жена које користе суплементацију, 82,8% ниску адхеренцију витамина Д и 65,8% ниску адхеренцију на лекове. Адхеренција на лекове за остеопорозу је боља у односу на адхеренцију калцијума и витамина Д ($p < 0,05$, χ^2 тест). Болесници који примају лекове интравенски имају бољу адхеренцију него они који лек узимају субкутано или орално.

Закључци Адхеренција на витамин Д, калцијум и лекове за остеопорозу је ниска у испитиваној популацији, из нејасних разлога.

Кључне речи: остеопороза; адхеренција болесника; калцијум, витамин Д

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

C-reactive protein and procalcitonin as a predictive factors on appearance of postoperative complications after open appendectomy in children

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SUMMARY

Introduction/Objective Acute appendicitis is one of the most common surgical conditions in children that may be followed by inflammatory postoperative complications.
The aim of this study was to determine the association of the preoperative levels of C-reactive protein (CRP) and procalcitonin (PCT) and occurrence of inflammatory postoperative complications in children with appendicitis.

Methods Fifty-four patients were separated into two groups. The first group contained patients with uncomplicated appendicitis (UA) whereas the second group comprised patients with complicated appendicitis (CA). Clinical and laboratory parameters in preoperative period were used for prediction of complications after open appendectomy in children.

Results Patients with CA had significantly higher values of rectal temperature ($p < 0.05$), longer length of fever ($p < 0.001$), CRP ($p < 0.001$), PCT ($p < 0.001$), longer duration of stay at the intensive care unit (ICU) ($p < 0.001$), and prolonged hospitalization ($p < 0.001$) than the UA group. In the CA group, 41.93% had postoperative complications; these patients also had longer duration of fever ($p < 0.05$), higher level of CRP ($p < 0.05$), and prolonged hospitalization ($p < 0.01$) compared to patients in the CA group without complications. Preoperative cut-off values of CRP and PCT (75.8 mg/l and 0.36 ng/ml, respectively) pointed towards higher probability for development of postoperative complications. Rectal temperature and duration of fever had predictive influence in determination of postoperative complications in the CA group.

Conclusion The cut-off values of preoperative levels of CRP and PCT were able to discriminate the subset of patients with higher risk for postoperative complications. Rectal temperature and duration of fever had predictive influence on the occurrence of postoperative complications, while other clinical and laboratory parameters were not able to predict appearance of the complications after open appendectomy in children.

Keywords: appendicitis; children; C-reactive protein; procalcitonin; postoperative complications

INTRODUCTION

Acute appendicitis is among the most common urgent surgical conditions in children [1]. Preoperative diagnostics of acute appendicitis and estimation of severity of clinical picture still represent the clinical challenge in paediatric population due to similarities in laboratory analysis and clinical picture with other diseases [2]. Ideal laboratory parameter with the ability to estimate the severity and the course of appendicitis still remain unidentified [3]. Routine laboratory tests such as C-reactive protein (CRP) could not reliably discriminate the existence of appendicitis from the other conditions characterised with abdominal pain [4]. Recent studies have shown that procalcitonin (PCT) correlates significantly with the severity of inflammation in patients with appendicitis, being more accurate than parameters such as fever, number of leukocytes, or elevated sedimentation rate [5]. Levels of PCT could be several-fold higher in patients with an advanced form of appendicitis accompanied with bacterial infection [6]. For this reason, PCT is considered to be able to identify both more severe clinical forms of appendicitis and the existence inflammatory postoperative complications after appendectomy in children. The most common consequences of delaying the diagnosis of appendicitis or perforated appendicitis were wound to be infections, intra-abdominal abscesses, and small intestine obstruction during the postoperative period, which may increase the morbidity and mortality of hospitalized children.

The aim of the study is to estimate the significance of preoperative levels of CRP and PCT and to identify the cut-off values that could precisely predict the severity of clinical picture of acute appendicitis and the appearance of inflammatory postoperative complications in children.

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METHODS

This retrospective clinical study encompassed 54 young patients with a diagnosis of acute appendicitis, with age ranging from three to 15 years, at the Clinic for Child Surgery and Orthopaedics, Clinical Centre of Niš. Written informed consent was obtained from parents of all the patients. The patients were divided into two groups: the first group consisted of 23 patients with uncomplicated appendicitis (UA) (phlegmonous appendicitis), while the second group comprised 31 patients with complicated appendicitis (CA). In this setting, appendicitis was considered complicated when localised or widespread peritonitis, abscess or appendix perforation were registered intraoperatively. A diagnosis of acute appendicitis was made, based on clinical, laboratory, and radiographic findings during the preoperative period. Postoperative pathohistological examination of appendix was used to determine the severity and the extent of appendix inflammation. The appearance of other inflammatory complications with special emphasis on wound infection, occurrence of postoperative abscess or intestinal obstruction, within 30 days after open appendectomy, was also analyzed in both groups of patients. Patients appendectomized due to other reasons as well as those that had a hematological disease or proved immune disturbances were excluded from the analysis. All the patients had undergone standard preoperative preparation and antibiotic prophylaxis.

The following parameters were analyzed: age, body weight, sex, length of preoperative observation, body temperature (axillar and rectal temperature), and duration of fever. White blood cell count (WBC) and percentage of neutrophils (Ne), levels of CRP and PCT were determined before the surgical intervention. After open appendectomy, the overall duration of hospital treatment as well as the duration of stay at the intensive care unit (ICU) was also followed.

Blood samples were taken from peripheral vein and hematology parameters were analyzed on the Ac·T diff™ hematology analyzer (Beckman Coulter, Brea, CA, USA). The levels of CRP were determined on Ilab 300 clinical chemistry analyser (Werfen, Barcelona, Spain) with the reference values range of 1–5 mg/l. The level of PCT was performed by the immunoluminometric method (LUMItest™, B·R·A·H·M·S Diagnostica, Berlin, Germany) using the Modular Analytics E170 analyser (Roche Diagnostics, Indianapolis, IN, USA). Values of PCT below 0.05 ng/ml were considered normal.

Statistical analysis

Descriptive statistics consisted of the number (n), percentage (%), arithmetic mean, and standard deviation. For parametric testing, Student's t-test or nonparametric Mann–Whitney test, as well as the χ^2 test or Fisher's exact test were used. The receiver operating characteristic curve (ROC) analysis was used to define sensitivity and specificity of laboratory parameters for measurement of their influence on the appearance of complications. In order to test whether clinical or laboratory parameters have predictive influence on appearance of postoperative complications after open appendectomy, we performed multivariate logistic regression. Values considered statistically significant had $p < 0.05$. Statistical analysis was performed using R: A language and environment for statistical computing (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

All the data were successfully collected according to the study protocol for the complete group of 54 patients. There was no difference between UA and CA groups, concerning the age of patients, their body weight, length of preoperative observation, and axillar body temperature values, as shown in Table 1. The CA group had higher values of rectal temperature compared to the UA group (38.37 ± 0.79°C vs. 37.79 ± 0.82°C, $p < 0.05$). The fever lasted longer in the CA group than in the UA group (4.33 ± 3.44 days vs. 1.56 ± 1.19 days, $p < 0.001$) (Table 1). There was no difference concerning the number of leukocytes or neutrophilia between the groups, although the higher number of leukocytes (17.94 ± 6.81 × 10^9/l vs. 14.42 ± 5.26 × 10^9/l), and more prominent neutrophilia (79.74% ± 8.36% vs. 76.65% ± 11.18%) were recorded in the CA group than in the UA group (Table1). The levels of CRP in the CA group were significantly higher than in the UA group of patients (69.49 ± 46.56 mg/l vs. 18.49 ± 24.15 mg/l, $p < 0.001$). Higher preoperative levels of PCT were also registered in the CA group than in the UA group patients (5.61 ± 9.68 ng/ml vs. 0.13 ± 0.11ng/ml, $p < 0.001$) (Table 1).

Inflammatory postoperative complications were found only in the CA group, while in the UA group no complications developed after the removal of the inflamed appendix

Table 1. Characteristics of clinical and laboratory parameters in patients with uncomplicated (UA) and complicated appendicitis (CA) groups

Parameters	UA (n = 23)	CA (n = 31)	p-value
Age (years)	10.26 ± 3.49	8.81 ± 3.69	p > 0.05
Body weight (kg)	37.52 ± 17.54	33.71 ± 14.6	p > 0.05
Boys – n (%)	15/23 (65.20%)	23/31 (74.20%)	p > 0.05
Girls – n (%)	8/23 (34.80%)	8/31 (25.8%)	p > 0.05
Length of preoperative observation (hours)	10.05 ± 10.75	8.10 ± 7.40	p > 0.05
Axillar temperature (°C)	37.35 ± 0.72	37.75 ± 0.72	p > 0.05
Rectal temperature (°C)	37.80± 0.82	38.37 ± 0.80	p < 0.05
Length of fever (days)	1.57± 1.20	4.33 ± 3.44	p < 0.001
WBC (× 10^9/l)	14.42 ± 5.26	17.94 ± 6.81	p > 0.05
Ne (%)	76.65 ± 11.18	79.74 ± 8.36	p > 0.05
CRP (mg/l)	18.49 ± 24.15	69.49 ± 46.56	p < 0.001
PCT (ng/ml)	0.13 ± 0.12	5.61 ± 9.68	p < 0.001
Postoperative complications (%)	0 (0%)	13/31 (41.90%)	p < 0.001
Duration of stay in the ICU (days)	0.48 ± 0.73	5.59 ± 4.08	p < 0.001
Overall hospital treatment (days)	7.21 ± 1.56	12.16 ± 5.77	p < 0.001

Ne – neutrophils; WBC – white blood cell count; CRP – C-reactive protein; PRC – procalcitonin; ICU – intensive care unit

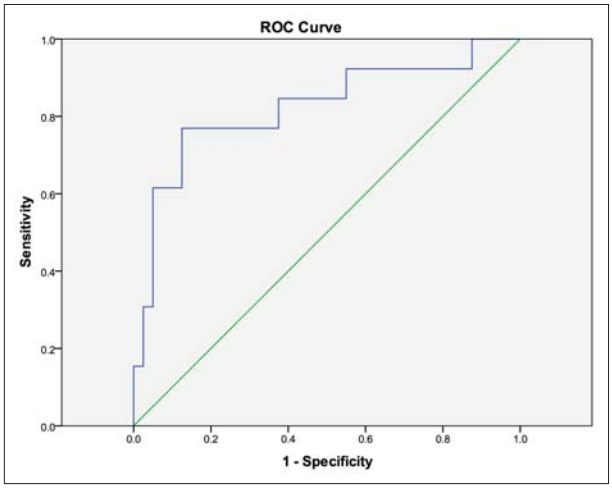

Figure 1. Receiver operating characteristic (ROC) curve for predicting inflammatory postoperative complications based on average CRP levels

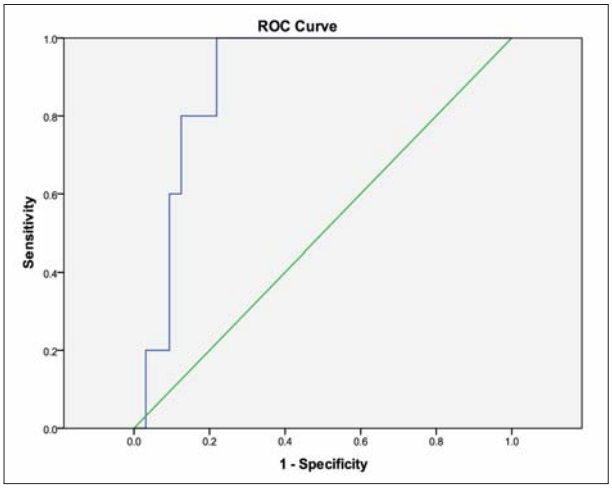

Figure 2. ROC curve for predicting inflammatory postoperative complications based on average PCT levels

($p < 0.001$). Duration of stay in the ICU was significantly prolonged in the CA group (5.59 ± 4.08 days) compared to the UA subgroup (0.48 ± 0.73 days) ($p < 0.001$). Overall hospital treatment was longer in patients with CA (12.16 ± 5.77 days) compared to the UA group (7.21 ± 1.56 days), $p < 0.001$ (Table 1).

The ROC analysis determined the cut-off values for CRP (75.8 mg/l) and PCT (0.36 ng/ml) (Figures 1 and 2). The ROC curve shape pointed out that CRP levels above the established cut-off values, with high sensitivity (76.9%) and specificity (87.5%), could predict occurrence of inflammatory postoperative complications in appendectomized children ($p = 0.001$) (Figure 1). Area under the ROC curve value of 0.823 with standard error of 0.076 and 95% confidence interval (0.674–0.973) points toward predictive impact of CRP levels for the development of postoperative complications.

The shape of ROC curve pointed that PCT values above the determined cut-off values are highly sensitive and specific predictors of appearance of inflammatory postoperative complications ($p = 0.006$) (Figure 2). Area under the curve of 0.888 with standard error of 0.055 and 95% confidence interval (0.780–0.995) points toward the positive predictive impact of PCT values, with sensitivity of 100% and specificity of 78.1%, on the postoperative complications occurrence.

Analysis of clinical and laboratorial parameters in patients with CA in regard to the development of postoperative complications detected that 18 (58.07%) patients have no postoperative complications; however, complications were detected in 13 patients (41.93%). Fever duration ($p < 0.05$), CRP level ($p < 0.05$), and the duration of hospitalization ($p < 0.01$) have significant influence for the development of postoperative complications in the CA group, i.e. CA patients without postoperative complications have shorter duration of fever, lower CRP levels, and shorter hospitalization time than patients with postoperative complications (Table 2). Multivariate analysis of clinical and laboratory parameters in patients with CA detected that preoperative rectal temperature ($p = 0.05$) and duration of fever ($p = 0.02$) had significant influence on the prediction of occurrence of postoperative complications (Table 3).

Table 2. Clinical and laboratory parameters of patients in complicated appendicitis group with postoperative complications

Parameters	No (n = 18)	Yes (n = 13)	p-value
Axillar temperature (°C)	37.74 ± 0.77	37.76 ± 0.67	p > 0.05
Rectal temperature (°C)	38.44 ± 0.88	38.28 ± 0.70	p > 0.05
Length of fever (days)	3 ± 1.37	6.08 ± 4.50	p < 0.05
WBC (× 10^9/l)	17.79 ± 7.84	18.13 ± 5.38	p > 0.05
Ne (%)	79.17 ± 8.28	80.50 ± 8.73	p > 0.05
CRP (mg/l)	55.60 ± 30.51	87.65 ± 58.03	p < 0.05
PCT (ng/ml)	5.03 ± 10.32	6.76 ± 9.28	p > 0.05
Duration of stay in ICU (days)	04.60 ± 1.97	7.00 ± 5.75	p > 0.05
Overall hospital treatment (days)	9.67 ± 3.53	15.62 ± 6.59	p < 0.01

Table 3. Multivariate analysis of clinical and laboratory parameters in prediction of postoperative complications in patients with complicated appendicitis

Omnibus tests of model coefficients	χ^2	Sig.	Model summary	-2 Log likelihood	Cox & Snell R^2	Nagelkerke R^2
	16.414	0.006		23.477	0.432	0.578
	B	S.E.	Wald	df	Sig.	Exp (B)
Axillar temperature	6.344	3.519	3.250	1	0.071	569.192
Rectal temperature	-6.405	3.264	3.850	1	0.050	0.002
Length of fever	0.935	.411	5.167	1	0.023	2.546
WBC	0.122	0.120	1.029	1	0.310	1.130
Ne	0.032	0.097	0.107	1	0.744	1.032
Constant	-2.370	33.992	0.005	1	0.944	0.093

Table 4. Multivariate analysis of CRP and PCT in prediction of postoperative complications in patients with complicated appendicitis

Omnibus tests of model coefficients	χ^2	Sig.	Model summary	-2 Log likelihood	Cox & Snell R^2	Nagelkerke R^2
	0.577	0.749		18.519	0.038	0.052
	B	S.E.	Wald	df	Sig.	Exp (B)
CRP	0.013	0.019	0.445	1	0.505	1.013
PCT	0.003	0.061	0.003	1	0.958	1.003
Constant	-1.457	1.224	1.418	1	0.234	0.233

Other parameters, including CRP and PCT, had no such predictive ability regarding the appearance of postoperative complications in the CA group (Table 4).

DISCUSSION

This study has shown that the rise of rectal temperature and prolonged fever during the hospital treatment, as well as high preoperative values of CRP and PCT, might be seen in children with complicated appendicitis. If the average preoperative values of CRP are above 75.8 mg/l and PCT values are higher than 0.36 ng/ml (high sensitivity for CRP amounts to 76.9%, and 100% for PCT), post-appendectomy inflammatory complications are expected. In the CA group of patients with postoperative complications, longer duration of fever and higher levels of CRP could be expected and consequently lead to the prolongation of hospital stay.

Laboratory parameters for the diagnosis of acute appendicitis traditionally rely on the number of leukocytes, CRP levels, and rectal temperature rise – all of which demonstrate low sensitivity and specificity for approximate confirmation of acute appendicitis [7]. In a study by Youatou Towo et al. [8], the rise of leukocytes and neutrophil percentage as well as the elevation of CRP and fibrinogen were detected in complicated forms of appendicitis. In contrast to previous authors, Eldar et al. [9] have shown that laboratory parameters have no significance in defining the severity of appendicitis. Our study suggests that elevation in the number of leukocytes and the rise of neutrophil percentage could not define the severity of acute appendicitis in children, based on the absence of difference between the CA and UA groups. The role of CRP in diagnosing appendicitis is problematic even nowadays [10, 11]. CRP is a plasma protein, whose level is raised in response to cytokines induced by tissue injury, infection, or inflammation [12]. In a study by Kim et al. [13], the CRP level was found to be a good predictor of complicated form of appendicitis. Another study found that CRP has a sensitivity of 83–90% for determining complicated forms of appendicitis in children (perforation and abscess formation) [12]. Contrary to this, some authors claim that specificity and sensitivity of biochemical markers might be improved with the clinical picture of patients [14]. Our study finds that CRP levels above 69.49 mg/l strongly point towards existence of complicated appendicitis in contrast to the uncomplicated form, where the levels of CRP tend to be lower than 18.49 mg/l. In this way this study shows that preoperative values of CRP could be a reliable predictor of complicated appendicitis in children. Kafetzis et al. [15] found that elevation of PCT above 0.5 ng/ml has sensitivity of 73% and specificity of 92.3% for defining the perforated appendicitis in children. Anielski et al. [10] found evidence that elevated PCT represents a biomarker of appendicitis, did not manage to define the cut-off values for various forms of appendicitis. Our study shows the existence of higher PCT values in children with complicated appendicitis (5.61 ng/ml), while PCT levels were considerably lower in uncomplicated forms (0.13 ng/ml), which might be useful in defining the severity of acute appendicitis in children. Our study finds that CRP and PCT are more useful than number of leukocytes and neutrophil percentage for identifying complicated forms of acute appendicitis in children.

In case of postoperative complications after appendectomy, elevated number of leukocytes could not reliably predict postoperative abscesses after appendectomy, since only 50% of cases have leukocytosis above 14×10^9/l [16]. A study that identifies CRP as a marker of postoperative complications after appendectomy found that CRP values above 100mg/l reliably predict appearance of postoperative complications [17]. Besides its diagnostic value, PCT was found to have prognostic impact able to identify post-appendectomy complications [18]. In case of preoperative levels of CRP higher than 3 mg/l and PCT values above 0.18 ng/ml, a higher percentage of complications might be expected. Therefore, the surgical intervention should be done sooner [19]. Preoperative values of CRP and PCT in our study have shown excellent predictive abilities for appearance of inflammatory postoperative complications in children after appendectomy. If the CRP and PCT are higher than their cut off values, 75.8 mg/l for CRP (sensitivity of 76.9% and specificity of 87.5%) and 0.36 ng/ml for PCT (sensitivity of 100% and specificity of 78.1%), respectively, the postoperative complications could be expected in 95% of patients after appendectomy.

The outcome of advanced appendicitis is difficult to determine. Ideally, a pathway for the treatment of advanced appendicitis would identify those at risk for developing a postoperative complication, having in mind that complications such as intra-abdominal abscess have the incidence of 5–28% [20, 21]. Other studies showed that the use of laboratory evaluation as discharge criteria in advanced appendicitis can help to identify a small subset of patients who are at an increased risk of developing an intra-abdominal abscess and prolonged hospitalization in the perforated appendicitis [16, 22]. In a recent study by Fike et al. [22] it was shown that the occurrence of an intra-abdominal abscess doubles the hospital stay and cost of perforated

appendicitis [22]. In our study, 41.93% of patients with postoperative complications in the CA group had longer duration of fever, higher level of CRP, and prolonged hospitalization compared to patients without postoperative complications in the CA group. In multivariate analysis, we detected that prolonged duration of fever and higher level of rectal temperature were significant predictors of occurrence of postoperative complications in the CA group. Also, Obinwa et al. [23] detected that preoperative pyrexia was the most discriminatory factor among other preoperative systematic inflammatory parameters in predicting postoperative complications. In our study, other clinical and laboratory parameters have no ability to predict postoperative complications. Determination of predictive abilities of preoperative risk factors, including PCT and CRP, was limited by the small number of patients with postoperative complications in the CA group (only 13 patients) in our pilot study. Therefore, further studies including more patients are needed to confirm certain risk factors for postoperative complications in pediatric patients with CA. Cut-off value of preoperative level of CRP and PCT discriminates the subset of patients at a higher risk for postoperative complications, and these inflammatory parameters should be monitored more closely after surgical treatment of CA. Early diagnosis of surgical complications in patients with complicated appendicitis is an ongoing challenge and there is a continuing quest for a more reliable prognostic biomarker or clinical scoring system.

CONCLUSION

This study has shown that higher values of CRP and PCT might be expected in children with complicated appendicitis. Cut-off values of CRP and PCT could discriminate the subset of patients with a higher risk for the development of postoperative complications. Patients with postoperative complications in the CA group are associated with longer duration of fever, higher level of CRP, and prolonged hospitalization; however, only rectal temperature and duration of fever had predictive significance regarding the occurrence of postoperative complications. Further studies with more patients are needed to detect influence of inflammatory parameters in predicting complications after open appendectomy in children.

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Ц-реактивни протеин и прокалцитонин као предиктивни фактори појаве постоперативних компликација након отворене апендектомије код деце

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САЖЕТАК

Увод/Циљ Акутни апендицитис је једно од најчешћих хируршких стања код деце, које може бити праћено појавом инфламаторних постоперативних компликација.

Циљ рада је одредити повезаност преоперативних вредности Ц-реактивног протеина (ЦРП) и прокалцитонина (ПКТ) на појаву инфламаторних постоперативних компликација код деце са апендицитисом.

Методе Анализирана су 54 болесника, који су подељени у групу са некомпликованим (НА) и компликованим апендицитисом (КА). Клинички и лабораторијски параметри у преоперативном периоду су коришћени за предикцију компликација након отворене апендектомије.

Резултати Болесници са КА су показали значајно више вредности ректалне температуре ($p < 0,05$), дужу фебрилност ($p < 0,001$), виши ниво ЦРП ($p < 0,001$) и ПКТ ($p < 0,001$), дужи боравак у Јединици интензивне неге ($p < 0,001$) и дужу хоспитализацију ($p < 0,001$) у односу на болеснике са НА. У групи деце са КА, 41,93% је развило постоперативне компликације и код њих је уочена дужа фебрилност ($p < 0,05$), виши ниво ЦРП ($p < 0,05$) и дужа хоспитализација ($p < 0,01$) у односу на болеснике КА групе без постоперативних компликација. Преоперативне *cut off* вредности ЦРП и ПКТ (75,8 *mg/l* и 0,36 *ng/ml*) показују ризичну групу за развој постоперативних компликација, док предиктивни утицај на појаву постоперативних компликација код КА групе имају само ректална температура и трајање фебрилности.

Закључак Преоперативне *cut off* вредности ЦРП и ПКТ дефинишу болеснике високог ризика за настанак постоперативних компликација. Ректална температура и трајање фебрилности имају предиктиван утицај на појаву компликација, док остали клинички и лабораторијски параметри нису у стању да предвиде појаву постоперативних компликација након отворене апендектомије код деце.

Кључне речи: апендицитис; деца; Ц-реактивни протеин; прокалцитонин; постоперативне компликације

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Factors associated with idiopathic adolescent scoliosis in female population – Preliminary results

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SUMMARY

Introduction/Objective Idiopathic scoliosis (IS) is an orthopedic condition of multifactorial origin. The aim of our study was to evaluate the factors that are associated with IS in female population and factors associated with varicose veins in females with IS.

Methods This retrospective-prospective cross-section study included 89 patients (the study group) and 87 controls. The following parameters were analyzed: body weight, body height, presence and the degree of varicose veins (the first, second, and third degree), and age (group in the range of 17–26 years, in the range of 27–36 years, and in the range of 37–46 years).

Results The study group has significantly lower body weight ($p = 0.046$), significantly higher proportion of varicose veins ($p < 0.001$) compared to controls, significantly lower proportion of patients aged 27–36 years ($p = 0.014$), and significantly higher proportion of patients aged 37–46 years ($p = 0.025$) compared to controls. There is significantly higher proportion of patients in the study group with the first degree of varicose veins ($p = 0.007$). There is weak positive correlation between body weight and body height in the group of patients without varicose veins ($R = 0.456$) and in the group with the second degree of varicose veins ($R = 0.291$), while for the group with the first degree of varicose veins there is moderate positive correlation ($R = 0.543$).

Conclusion Our preliminary findings point out that lower body weight and presence of varicose veins are significantly associated with IS. The group of patients with IS above 37 years of age tends to have significantly higher proportion of varicose veins.

Keywords: idiopathic scoliosis; varicose veins; age; body weight; body height.

INTRODUCTION

Idiopathic scoliosis (IS) is an orthopedic condition that is defined as a pathologic state of multifactorial origin, with a major relevance of those genetic and biomechanical factors that have impacts on the central nervous system, growth and metabolism [1, 2, 3]. The diagnosis is established on the base of clinical examination, radiographic imaging, and stereophotogrammetry. Assessment of patients with scoliosis includes medical history, clinical, physiatrist and neurological examination, and diagnostic tests [4]. Treatment of IS can be conservative and/or surgical.

Previous studies underscored that in certain hereditary pathological conditions, such as IS, the loss of integrity of the matrix proteins in the skin affects blood vessels, so that high incidence of varicose veins ensues [5, 6]. Conditions such as varicose veins and bone fragility are associated with changes in the strength of collagen and changes in metabolizing it [7]. In previous reports, there is insufficient information on the incidence of complications such as varicose veins in women affected with IS, although the theory of the defect in the synthesis of collagen and connective tissue suggests a higher incidence of these complications in the population of women treated for IS. It is unknown to what extent these complications are represented in the aforementioned population in relation to the population of women who are not treated for IS, and if it can even be promptly diagnosed and treated.

Therefore, the aim of our study was to determine the factors that are associated with IS in the female population and factors associated with varicose veins in females with IS.

METHODS

This study was designed as a retrospective-prospective cross-section study that investigated the incidence of varicose veins in the population of women with idiopathic scoliosis, treated at the Clinic for Physical Medicine and Rehabilitation "Dr Miroslav Zotović" in Banja Luka, Republic of Srpska, Bosnia and Herzegovina. The study group included 89 patients, while the control group comprised 87 participants. The

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inclusion criteria were female gender and signed informed consent of participation in the study. The exclusion criteria were secondary scoliosis and pregnancies. Patients from the control group were subjected to the same questions, tests, and examinations.

This study was approved by the relevant ethical committee (Ethics Committee of the Clinic for Physical Medicine and Rehabilitation "Dr Miroslav Zotović", Banja Luka) and followed by the adoption of the necessary documentation – the Notification for Respondents of the Study and Informed Consent. Prior inclusion in the study all participants were informed about study protocol and informed consent was obtained.

Further parameters were analyzed: body weight, body height, presence and degree of varicose veins, and age (groups in the age ranges of 17–26 years, 27–36 years, and 37–46 years).

The examination by the physical medicine specialist included the clinical examination and ultrasound examination of the state of the venous system and was presented as follows: normal state of the venous system (without any observed changes); medium stage expression of the venous disease (varicose veins confirmed by clinical inspection, but without trophic changes; the ultrasonic examination verified obstruction in the superficial system or in the perforated veins (C2–C3 according to Comprehensive Classification System for Chronic Venous Disorders – CEAP – classification)); and severe venous disease (trophic changes observed by inspection and ultrasonic examination verified obstruction of the deep venous system (C4–C6 according to CEAP classification)).

Statistical analysis

Categorical data were presented as whole numbers and percents, while continuous variables as median values with standard deviation and analyzed by SPSS Statistics for Windows, Version 17.0 (SPSS Inc., Chicago, IL, USA). Chi-squared test was used for statistical analysis of categorical data, while Mann–Whitney U-test and Student's t-test for independent samples for analysis of continuous variable. Pearson's correlation was performed to assess the correlation between body height and body weight in the group of patients with IS regarding the presence and the degree of varicose veins. Values with $p < 0.05$ were considered to be statistically significant. The minimal, statistically valid sample size was determined to be 86 subjects according to the Cohen tables.

RESULTS

In Table 1 we present distribution of evaluated parameters in evaluated groups of participants. The study group has significantly lower body weight ($p = 0.046$), and significantly higher proportion of varicose veins ($p < 0.001$) compared to controls (Table 1). The study group has significantly lower proportion of patients aged 27–36 years ($p = 0.014$) and significantly higher proportion of patients aged 37–46 years ($p = 0.025$) compared to controls (Table 1).

In the control group there is significantly higher proportion of participants without varicose veins ($p < 0.001$), and significantly higher proportion of patients in the study group with the first degree of varicose veins ($p = 0.007$) (Table 2).

There is non-significant difference for evaluated variables (age, body weight, and body height) in both the study and the control group with regard to the degree of varicose veins ($p > 0.05$) (Table 2).

In Figure 1, correlations between body height and body weight in patients with idiopathic scoliosis with regard to the presence and degree of varicose veins are presented. There is weak positive correlation between these two variables in the group of patients without varicose veins ($R = 0.456$) and the group with the second degree of varicose veins, ($R = 0.291$), while for the group with the first degree of varicose veins there is moderate positive correlation ($R = 0.543$) (Table 3).

DISCUSSION

This research is based on the theory that bone fragility is associated with changes in the strength of collagen and changes in its metabolism, where one of the crucial factors could be the quality of connective tissue [8]. Disruption in the synthesis of collagen is typical of varicose veins. Smooth muscle cells from varicose veins synthesize more collagen I,

Table 1. Distribution of evaluated parameters in the study group and the control group

Evaluated parameters			Study group (n = 89)	Control group (n = 87)	p-value
Age (years)		MV ± SD	30.9 ± 7.9	29.9 ± 6.7	0.367*
Body weight (kg)			63.6 ± 9.6	67.0 ± 12.7	*0.046**
Body height (cm)			168.6 ± 6.5	169.0 ± 6.2	0.677*
Varicose veins		n (%)	23 (25.8)	7 (8.0)	*0.001***
Age	17–26 years		31 (34.8)	28 (32.2)	0.710**
	27–36 years		26 (29.2)	41 (47.1)	*0.014***
	37–46 years		32 (36.0)	18 (20.7)	*0.025***

*Student's t-test;
**χ^2-test

Table 2. Distribution of varicose veins regarding the severity degree and participants' age between evaluated groups of participants

Varicose veins n (%)		Study group (n = 89)	Control group (n = 87)	p-value
None		66 (74.1)	80 (92.0)	*0.001**
First degree		17 (19.1)	5 (5.7)	*0.007**
Second degree		6 (6.8)	2 (2.3)	0.157*
Third degree		0 (0)	0 (0)	-
Participants with varicose veins				
Age	17–26 years	2 (8.7)	0 (0)	0.419*
	27–36 years	7 (30.4)	4 (57.0)	0.199*
	37–46 years	14 (60.9)	3 (43.0)	0.400*

*χ^2-test

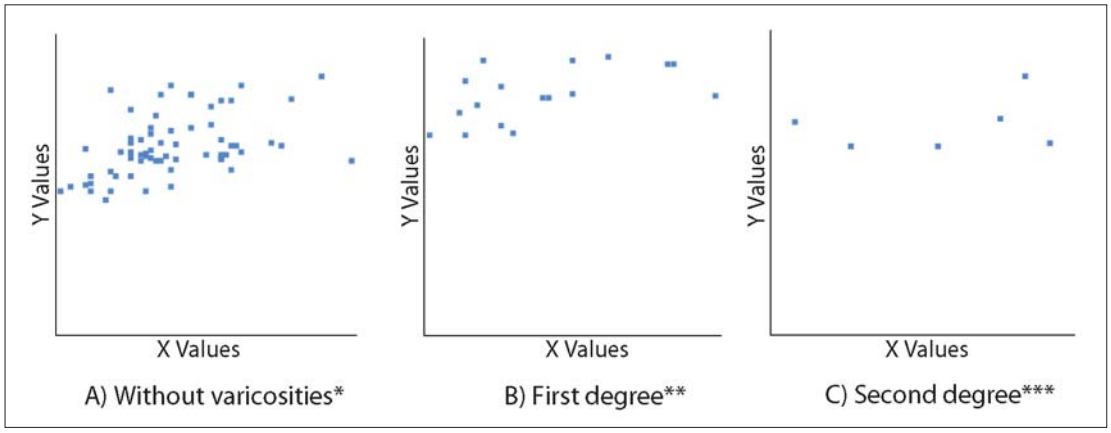

Figure 1. Correlations between body height and weight in the group of patients due to the presence of varicose veins and their degree of severity

*R = 0.456; **R = 0.543; ***R = 0.291; X values – body height; Y values – body weight

Table 3. Distribution of evaluated parameters in the group of participants with different degrees of varicose veins

Evaluated parameters	Study group		p-value*
	First degree	Second degree	
Age (years) (MV ± SD)	36.6 ± 5.1	34.3 ± 6.0	0.308
Body weight (kg) (MV ± SD)	64.9 ± 7.4	68.8 ± 8.9	0.646
Body height (cm) (MV ± SD)	169.4 ± 7.0	174.4 ± 8.2	0.219
Evaluated parameters	Control group		p-value**
	First degree	Second degree	
Age (years) (MV ± SD)	35.8 ± 3.0	36.0 ± 2.8	0.939
Body weight (kg) (MV ± SD)	83.7 ± 20.4	73.5 ± 10.6	0.546
Body height (cm) (MV ± SD)	170.2 ± 5.0	166.8 ± 7.8	0.469

*Mann–Whitney U-test;
**Student's t-test

less collagen III, and similar amounts of collagen V. This imbalance is a possible reason for the mechanical properties of the tissue, which, under these conditions, is of poorer quality [9, 10]. It should be stated that, in current literature, there are reports emphasizing defective synthesis of collagen and bad posture followed by the appearance of varicose veins; however, well-designed studies are still missing.

From the clinical point of view, we have noticed in the study that females with diagnosed IS had significantly lower weight. Even though there were non-significant differences in age between the two studied populations, age distribution frequencies demonstrated that it could be considered a significant factor associated with IS. Our results underline that females above 37 years of age have more frequent IS, while those between 27 and 36 years of age have significantly lower frequency of occurrence. Our findings are to the certain degree consistent with previous reports which stated that age is associated with a prevalence of idiopathic scoliosis [11]. Furthermore, we have pointed out that varicose veins are shown to be a significant factor associated with the presence of IS, where almost 25% of females with scoliosis had varicose veins, while less than 10% of patients in the control group had this condition. Bearing in mind that etiology of idiopathic adolescent scoliosis is multifactorial, including genetic predisposition, abnormalities of connective musculoskeletal tissues, it could be assumed that these individuals are more prone to develop varicose veins [12, 13, 14]. The complexity and multifactorial origin was underlined as well in the study Burwell and Dangerfield [15], where epigenetics was introduced as a concept in the evaluation of adolescent idiopathic scoliosis. They further stated that this type of scoliosis is associated with lower body mass index among other factors. Therefore, patients with IS should be screened for the presence of varicose veins and included into regular follow-up. Considering the degree of varicose vein presence, this study points out that the first degree is significantly more frequent in patients with IS, while the second degree, although frequent in the group of patients, was not significant. This observation could be explained to a certain degree by the assumption that other factors might influence the pathology of the second degree of varicose veins, thus influencing the frequency of occurrence in the population.

Our findings point to the fact that for patients with IS and the first degree of varicose veins there is greater correlation between height and weight, while for those without varicose veins and with the second degree of varicose veins there is lower correlation. Such findings are to the certain degree in line with our previous statement that other factors might play a certain role in the severity degree of varicose veins for patients with IS.

Also, it should be underlined that there are several limitations to this study. First, the study included small proportion of participants in the group of varicose veins. Second limitation refers particularly to the actual age of female participants where increase of age along with other factors might influence the frequency of varicose vein occurrence and the degree of such pathology. Therefore, further studies are needed on larger samples of patients and with longer follow-up observational periods.

CONCLUSION

Our findings demonstrate that lower body weight and presence of varicose veins are significantly associated with IS. The group of patients with IS above 37 years of age tends to have significantly higher proportion of varicose veins.

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Фактори који су повезани са идиопатском адолесцентном сколиозом у женској популацији – прелиминарни резултати

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САЖЕТАК

Увод/Циљ Идиопатска сколиоза (ИС) представља патолошки ентитет мултифакторијалног узрока.

Циљ рада је утврдити факторе који су повезани са ИС у женској популацији и факторе који су повезани са варикозним венама код испитаница са ИС.

Методе У ову ретроспективно-проспективну студију пресека укључено је 89 испитаница, док је у контролној групи било 87 испитаница. Анализирани су следећи параметри: телесна тежина, телесна висина, присуство и степен тежине (први, други и трећи степен) варикозних вена и године живота (група између 17 и 26, између 27 и 36 и између 37 и 46 година).

Резултати У студијској групи регистрована је значајно нижа телесна тежина ($p = 0,046$), значајно већа учесталост варикозних вена ($p < 0,001$) у поређењу са контролном, значајно нижа учесталост ових промена код болесника животне доби од 27 до 36 година ($p = 0,014$) и значајно веће присуство истих код болесника старије животне доби од 37 до 46 година ($p = 0,025$), у поређењу са контролном групом. У студијској групи је регистрована значајно већа учесталост болесника са проширеним венама првог степена ($p = 0,007$). Утврђено је да постоји блага позитивна корелација између телесне тежине и висине у групи болесника без варикозних вена ($p = 0,456$) и у групи болесника са варикозним венама другог степена ($p = 0,291$), док је у групи болесника са варикозним венама првог степена утврђена умерена позитивна корелација ($p = 0,543$).

Закључак Прелиминарни резултати ове студије показали су да су нижа телесна тежина и присуство проширених вена значајно повезани са ИС. Група испитаница са ИС преко 37 година је имала значајно чешће проширене вене у односу на контролу.

Кључне речи: идиопатска сколиоза; проширене вене; године живота; телесна тежина; телесна висина

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Pre-pregnancy body mass index and the risk of gestational diabetes mellitus

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SUMMARY

Introduction/Objective Not only do pre-pregnancy overweight or obesity increase the risk of adverse maternal and perinatal outcomes but they also lead to the development of gestational diabetes mellitus. The objective of this study was to estimate the prevalence of pre-pregnancy overweight and obesity in the Republic of Srpska and to investigate its association with hyperglycemia and risk of gestational diabetes mellitus.

Methods A cross-sectional study was carried out during the period from February to October 2012 among 555 pregnant women in gestational period from 24 to 28 weeks. The criterion for exclusion from the sample was previously diagnosed type 1 or type 2 diabetes.

Results Before pregnancy, 20.39% of participants had increased body mass index, while 4.04 % [95% confidence interval (CI); 2.62–6.13] were obese. Gestational diabetes mellitus was diagnosed in 10.91% (95% CI, 8.44–13.98) of them. The increase in body mass index by 1 increased the risk of gestational diabetes mellitus by 1.09 times [odds ratio (OR) = 1.09; 95% CI; 1.02–1.16]. Pregnant women who were overweight had a 4.88 times greater risk (OR = 4.88; 95% CI, 1.23–29.41) of developing gestational diabetes.

Conclusion Every fifth pregnant woman in this study was overweight or obese before pregnancy. The increase in body mass index by 1 increased the risk of gestational diabetes by 1.09 times (OR = 1.09; 95% CI; 1.02–1.16). Counselling is necessary for overweight and obese women planning pregnancy.

Keywords: pre-pregnancy body mass index; hyperglycemia; gestational diabetes mellitus

INTRODUCTION

Improvement of maternal, fetal and child health are key public health goals. Changes in public health trend have challenged the healthcare sector to provide optimal guidance to women before, during, and after pregnancy so that they can achieve healthy outcomes for both themselves and their newborns [1].

It has been shown that women being overweight or obese before pregnancy are at increased risk of adverse maternal and perinatal outcomes [2]. The Hyperglycemia Adverse Pregnancy Outcome (HAPO) study confirms that both obesity and maternal hyperglycemia alone are independently associated with adverse obstetrical outcomes, particularly abnormal fetal growth, newborn percent body fat and preeclampsia [3]. Pre-pregnancy overweight and obesity are also associated with gestational diabetes mellitus (GDM) development, as 65–75% of women with GDM are also overweight or obese [4].

Maternal overweight and obesity are the highest ranking modifiable risk factors. Raising of awareness and implementation of effective interventions for modifiable risk factors are priorities for stillbirth prevention [5]. Obesity prevalence has continued to grow, particularly in lower and middle-income countries. According to World Health Organization (WHO), in 2014, more than 1.9 billion adults 18 years and older were overweight. Of these, over 600 million were obese [6]. The prevalence of overweight and obesity in women older than 20 increased between 1980 and 2013 from 29.8% (29.3–30.2) to 38.0% (37.5–38.5) [7]. According to the 2010 Household Health Survey in the Republic of Srpska, one of two entities in Bosnia and Herzegovina (BiH), the obesity prevalence in women older than 18 is 22.7% [8].

The objective of this study is to estimate the prevalence of pre-pregnancy overweight and obesity among women in the Republic of Srpska (BiH) and to investigate its association with hyperglycemia and increased risk of GDM.

METHODS

Study design

The research was carried out in the form of a cross-sectional study during the period from February to October 2012 among pregnant women who had regular appointments with

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their gynecologists. The total sample consisted of 555 pregnant women in gestation period from 24 to 28 weeks. Data were collected by trained gynecologists and nurses from outpatient clinics whose selection was based on the Statistical Office analysis to ensure the equal presentation of all regions of the Republic of Srpska.

Ethical approval for the study was obtained from the Ethics Committee for Clinical Research of the Clinical Center of Banja Luka, and written informed consent was obtained from all the participants. The informative consent contained the basic information about the research, the explanation about the confidentiality of the information and to what end the information obtained in the research will be used [9, 10].

The information about the body mass before pregnancy was taken from the pregnancy medical records, or if the information was missing, it was taken from the pregnant women. The anthropometric measurements included height (cm) and weight (kg). Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters [11]. Classification of the nutritional status before pregnancy was done according to the WHO criteria [12]. Between 24 and 28 weeks of pregnancy, all the women underwent a 75-g oral glucose tolerance test (OGTT) in the morning, after fasting for 8–14 hours, according to WHO criteria [13]. Plasma glucose levels were taken before and one and two hours after the consumption of 75 g of glucose. Weight gain from pre-pregnancy to OGTT was estimated as gain in body weight. Plasma glucose was measured by a glucose oxidase method. GDM is defined according to the criteria of the American Diabetes Association (ADA) and its diagnosis is confirmed by one measurement of plasma glucose expressing values greater than > 5.1 mmol/l at start, > 10 mmol/l 1 hour or > 8.5 mmol/l two hours after the intake of 75 g of glucose [14].

Statistical methods

All statistical analyses were conducted using SPSS Statistics for Windows, Version 17 (SPSS Inc., Chicago, IL, USA). Baseline demographic characteristics were summarized using frequencies and percentages for categorical characteristics, and mean ± SD for continuous variables. Symmetric 95% confidence intervals (95% CI) were calculated for frequency. Spearman rank correlation was used for testing the association between an age group and a pre-pregnancy BMI. The evolution of glycemic levels was evaluated by the Friedman repeated measure test (one-way ANOVA test) in each group. The categorical variables were compared using Fisher's exact χ^2 test where appropriate, and for continuous variables using the Student's t-test. We used Mantel–Haenszel χ^2 test to test for association between an age group (ordinal categorical variable) and the diagnosis of GDM. Finally, we used binary logistic regression to analyze whether GDM could be predicted by both pre-pregnancy BMI and an age group, while controlling for the effect of other variables. P values lower than 0.05 in two-tailed tests were considered to be significant.

RESULTS

The characteristics of the screened sample are shown in Table 1. The sample included 555 pregnant women from the Republic of Srpska in the gestation period from 24 to 28 weeks. The highest percentage of pregnant women, [36.27% (95% CI; 32.32–40.30)] was in the 25–29 years age group. A majority of participants (60%) resided in urban areas. For 6.49% of the participants there was no information related to the nutritional status before pregnancy. The highest percentage of pregnant women [70.96% (95% CI; 66.91–74.70)] were in normal BMI range before pregnancy, whereas 20.39% of the them had increased BMI, and 4.04% (CI 95%; 2.62–6.13) were obese, while 8.65% were underweight (Table 1).

Overall, age correlated positively with the BMI score r_s (520) = 0.188; $p < 0.001$, Table 2. In particular, it was obvious that obesity before pregnancy was more frequent among the older participants. Although we had only 10.44% of participants older than 35, they comprised almost a half of all obese participants in our sample (9/21, 42.85%).

According to the methodology applied in the research, plasma glucose values were assessed between the 24th and 28th week of gestation [14]. Fasting plasma glucose values and OGTT were measured in 89.2% of participants. Women with higher BMI scores before pregnancy tend-

Table 1. Baseline characteristics of the study population screened for gestational diabetes mellitus

Characteristics		Number (n)	Percentage (%)	95% CI
Age group (years)	< 25	165	29.73	26.07–33.66
	25–29	201	36.27	32.32–40.30
	30–34	130	123.42	20.09–27.13
	35–39	50	9.01	6.88–11.70
	> 40	9	1.62	0.81–3.10
Body mass index (kg/m^2) category*	< 18.50	45	8.65	6.82–10.91
	18.50–24.99	368	70.96	66.91–74.70
	25–29.99	85	16.35	13.41–19.78
	> 30	21	4.04	2.62–6.13
Place of living	Rural area	222	40	36.01–44.13
	Urban area	333	60	55.87–63.99

*Note: information about pre-pregnancy nutritional status for 36 examinees was missing

Table 2. Nutritional status related to age category in women screened for gestational diabetes mellitus

Prepregnacy BMI group* (kg/m²)	Total		Age group (years)									
			< 25		25–29		30–34		35–39		> 40	
	n	%	n	%	n	%	n	%	n	%	n	%
< 18.5	45	8.65	22	48.89	16	35.56	5	11.11	2	4.44	0	0
18.5–24.99	368	70.96	106	28.73	141	38.48	92	24.93	25	6.78	4	1.09
25–29.99	85	16.35	22	25.88	25	29.41	20	23.53	14	16.47	4	4.71
> 30	21	4.04	5	23.81	4	19.05	3	14.29	8	38.09	1	4.76
Total	519	93.69	155	27.92	187	33.69	120	21.62	49	8.82	9	1.62

Note: information about pre-pregnancy BMI for 36 examinees was missing;
*p < 0.001, value derived from Spearman range of correlation

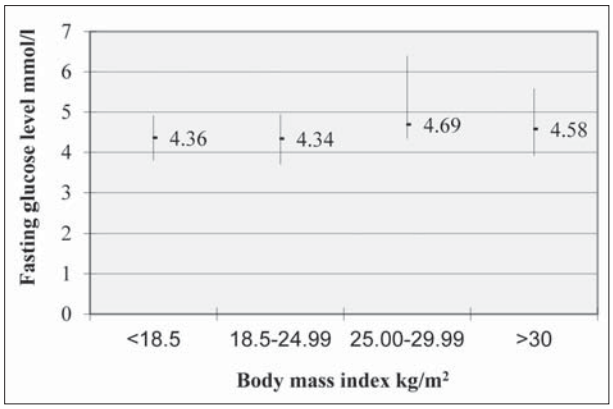

Figure 1. Distribution of fasting glucose level related to the pre-pregnancy BMI in women screened for gestational diabetes mellitus

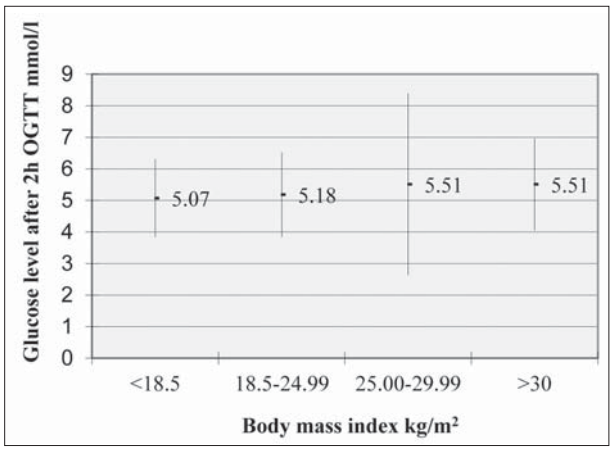

Figure 2. Distribution of postload glycemia values two hours after OGTT compared to BMI before pregnancy, in women screened for gestational diabetes mellitus

ed to have higher plasma glucose values both at fasting [r_s (446) = 0.14, p = 0.002] and two hours after OGTT (r_s (462) = 0.11, p = 0.014). Figure 1 illustrates that plasma glucose levels were higher in pregnant women which were overweight or obese before pregnancy [F (3.461) = 3.221, p = 0.023].

The highest mean value of plasma glucose two hours after OGTT with 75 g of glucose (5.51 mmol/l) was established in pregnant women who were overweight or obese before pregnancy. However, no significant statistical correlation of plasma glucose values with the nutritional status was established [F (3.461) = 1.102, p = 0.348]. The greatest

Table 3. Maternal characteristics stratified by gestational diabetes mellitus diagnosed by the American Diabetes Association criteria

Characteristics		Non-GDM 441 (89.1)	GDM 54 (10.9)	p-value
Age group, year	< 25	143 (32.4)	7 (13)	< 0.001*
	25–29	164 (37.2)	16 (29.6)	
	30–34	98 (22.2)	16 (29.6)	
	35–39	31 (7.0)	11 (20.4)	
	> 40	5 (1.1)	4 (7.4)	
Pre-pregnancy BMI Group (kg/m²)	< 18.50	39 (8.8)	2 (3.7)	0.056†
	18.50–24.99	297 (67.3)	33 (61.1)	
	25–29.99	66 (15)	10 (18.5)	
	> 30	16 (3.6)	4 (7.4)	
Pre-pregnancy BMI (kg/m²)		22.51 ± 3.67	24.10 ± 4.97	0.006§
Weight gain		8.22 ± 4.49	8.27 ± 4.27	0.949§

GDM – gestational diabetes mellitus;
Note: information about the nutritional state before pregnancy was missing for 28 examinees;
*χ^2-test, †Fisher's exact test, §Student's t-test

dispersion of measured glycemia values two hours after OGTT was established in pregnant women who were overweight (M = 5.51 ± 2.88 mmol/l), as shown in Figure 2.

GDM was diagnosed in 10.91% (95% CI; 8.44–13.98) of participants according to the ADA criteria for diagnosing gestational diabetes mellitus. We observed that with the increase of pregnant woman's age, the frequency of GDM increased as well: (χ^2 (1) = 24.81, p < 0.001), as shown in Table 3. By applying the ADA criteria for diagnosing gestational diabetes for the measured values of glycemia and by analyzing the prevalence of GDM related to the pre-pregnancy BMI group for the participants who had information about their nutritional status before pregnancy, we found a positive trend of GDM with increasing BMI scores (Wald z = 7.07, p = 0.001).

The increase of BMI by 1 increased the risk of GDM occurrence by 1.09 times [odds ratio (OR) = 1.09; 95% CI; 1.02–1.16]. Nevertheless, the effect of BMI became slightly smaller and statistically insignificant (Wald z = 2.28, p = 0.131, OR = 1.06) when it was controlled for age in a multiple logistic regression. In contrast, the effect of age remained significant (Wald z = 19.98, p = 0.001). For instance, compared to women younger than 25, the women who were between 35 and 39 years of age had 8.32 times greater risk of developing diabetes, while the risk increased up to 18.67 times for those who were older than 40. Pregnant women who were overweight had a 4.88 times greater risk (OR = 4.88; 95% CI, 1.23–29.41) of developing GDM.

DISCUSSION

The results of our research showed a statistically significant correlation between the increased pre-pregnancy BMI and the presence of GDM and a significant positive association between age and the presence of GDM. According to the ADA criteria for diagnosing gestational diabetes, the prevalence of GDM was 10.91% [14].

Pre-pregnancy overweight and obesity have many adverse pregnancy outcomes, including those related to hyperglycemia and the risk of developing GDM [3]. According to the results of our research, 16.35% of pregnant women were overweight, while 4.04% of the participants were obese before pregnancy. Hence, every fifth pregnant woman in the Republic of Srpska was overweight or obese before pregnancy. Statistically significant linear increase of pre-pregnancy BMI was evident in women over the age of 35. According to the WHO, the prevalence of overweight and obesity has been increasing in middle income countries to which Bosnia and Herzegovina also belongs [6, 15]. The prevalence of obesity is increasing, especially in women at the generative age. In France, the obesity prevalence increased from 5.2% to 11% over the period from 1997 to 2006 in women aging between 20 and 39 [16]. In America, according to the data from Pregnancy Risk Assessment Monitoring System (PRAMS), one in five women was obese when they became pregnant, which presents the increase of the obesity prevalence by 70% compared to the previous decade [17].

A positive relation was established between the nutritional status before pregnancy and the mean values of fasting and postload plasma glucose levels. The results showed an association between fasting and postload plasma glucose levels and adverse pregnancy outcomes, even in the range previously considered normal [18]. The Monash Medical Center (Clayton, Australia) research [19] also found the correlation between the increased BMI and glycemia during pregnancy. It was established that pre-pregnancy BMI higher than or equal to 35 kg/m^2 was the third independent risk factor in the development of gestational diabetes (after already diagnosed GDM registered in previous pregnancies and older age). The results of our study are in concordance with previous studies [19, 20, 21], meaning that the advance maternal age implies higher risk factor for GDM. Similarly to our research, French study of Pre and Early Post Natal Determinants of the Child's Development and Health (EDEN study) [22] also showed that BMI before pregnancy is independently associated with increased plasma glucose level, i.e. gestational diabetes. The risk for the gestational diabetes in EDEN study increased with the increase of BMI before pregnancy [22]. Pregnant women from the research done in the Republic of Srpska, who were obese before pregnancy, had a 4.88 times higher risk of developing GDM. Results of meta-analysis of 20 studies show that the risk of developing GDM was about two, four ,and eight times higher among overweight, obese or severely obese compared with normal-weight women at the beginning of their pregnancies, respectively [23].

Meta-analysis of the observation studies including several electronic databases and research published from 1977 to 2000 showed that the risk of gestational diabetes is positively associated with the pre-pregnancy BMI [22]. For every 1 kg/m^2 increase in BMI, the prevalence of GDM increased by 0.92% [24], which is similar to the results from the research done in the Republic of Srpska, where every 1 kg/m^2 increase in BMI increased the risk for developing GD by 1.09%. Our research did not establish any correlation between weight gain during pregnancy and the prevalence of gestational diabetes. Meta-analysis of the research done in England from 1990 to 2007 showed inconsistent results related to the maternal weight gain during pregnancy and the risk of GDM [25]. According to the EDEN study, the association between the risk of gestational diabetes and maternal gestational weight gain was positive and significant only when the pre pregnancy BMI was increased [20].

We are fully aware of the limitation of our study in that the information about pre-pregnancy BMI was taken from pregnancy medical records or during interviews with mother if the information was not in the medical record.

CONCLUSION

Overweight and obesity are largely preventable. Based on the results of our research done in the Republic of Srpska, it can be concluded that it is necessary to counsel women on the importance of obtaining normal weight before pregnancy. It is necessary that the creators of healthcare policies and public healthcare institutions intervene with the aim of providing non-obesogenic environments**,** the education related to healthy diet before and during pregnancy, and the importance of physical activity. The abovementioned activities can result in the reduction of overweight and obesity prevalence and to reduced risk for developing gestational diabetes.

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Индекс телесне масе пре трудноће као чинилац ризика за настанак гестацијског дијабетес мелитуса

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САЖЕТАК

Увод/Циљ Повећана телесна маса и гојазност пре трудноће повећавају ризик за настанак компликација код мајке и плода у току трудноће, у току и после порођаја, а доводе се у везу са појавом гестацијског дијабетес мелитуса (ГДМ). Циљ истраживања био је утврдити учесталост повећане телесне масе и гојазности пре трудноће код жена и њихову повезаност са појавом повећане хипергликемије и ГДМ-а.

Методе Студија пресека спроведена је од фебруара до октобра 2012. године са 555 трудница гестације 24–28 недеља. Критеријум за искључење из испитивања био је раније дијагностикован дијабетес мелитус тип 1 или тип 2.

Резултати Повећан индекс телесне масе (ИТМ) пре трудноће имало је 20,39% испитаница, од којих је 4,04% (*CI* 95%; 2,62–6,13) било гојазно. ГДМ је дијагностикован код 10,91% (*CI* 95%; 8,44–13,98) испитаница. Повећање ИТМ-а за један повећавало је ризик за појаву ГДМ-а 1,09 пута (*OR* = 1,09; *CI* 95%; 1,02–1,16). Труднице које су имале прекомерну телесну масу пре трудноће имале су 4,88 пута већи ризик (*OR* = 4,88; *CI* 95%; 1,23–29,41) за развој ГДМ-а.

Закључак Свака пета испитаница имала је прекомерну телесну масу или гојазност пре трудноће. Повећање ИТМ-а за један повећавало је ризик за појаву ГДМ-а 1,09 пута. Неопходно је саветовање жена са прекомерном телесном масом и гојазношћу које планирају трудноћу.

Кључне речи: индекс телесне масе пре трудноће; хипергликемија; гестацијски дијабетес мелитус

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Characteristics of chronic obstructive pulmonary disease patients with depressive disorder

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SUMMARY

Introduction/Objective The origin of depressive disorder in chronic obstructive pulmonary disease (COPD) patients is still not completely known and probably is caused by various factors.
The aim of this study is to establish the most important characteristics of COPD patients who have depressive disorder.

Methods Eighty-nine COPD patients and 65 demographically-matched referents without COPD were included. All the patients underwent lung function examination, and gas exchange, nutritional status, dyspnoea level by the modified Medical Research Council (mMRC) scale and exercise tolerance were also assessed, as well as depressive disorder by Hospital Anxiety and Depression Scale (HADS) and Geriatrics Depression Scale (GDS) and quality of life by St. George's Respiratory Questionnaire (SGRQ).

Results Depressive disorder has been found in 30.3% of COPD patients evaluated by HADS and 25.3% of COPD patients evaluated by GDS. When COPD subjects were stratified by forced expiratory volume in 1 second (FEV_1) categorization, all subgroups were more likely to have depressive disorder, according to HADS and GDS, relative to referents with the odds ratio highest (3: 95% confidence interval 1.6–4.9) among those with the FEV_1 < 30%. COPD patients with depressive disorder (HADS) compared to non-depressed patients had (differences in mean values) higher intensity of smoking [6.9 (0.5–10.1)], lower body mass index [-4.9 (-7.2–5.4)], lower value of FEV_1% [-8.3 (-16.3–1.2)] higher value of total lung capacity (%) [17.8 (2.3–28.4)], higher mMRC score (1.07 (-1–3.0), and higher SGRQ – giving a total score of 32.9 (24.1–40.3).

Conclusion Evaluation of depressive disorder should be considered in every patient with COPD, especially in patients with greater degree of airflow limitation and lung hyperinflation, dyspnoea level and malnourished.

Keywords: COPD; depressive disorder; quality of life

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is a severe treatment-resistant pulmonary disease with varying impact on the patient's general physical condition, functioning, and quality of life. It is already known that dyspnoea and poor exercise tolerance are frequently observed in patients with COPD [1]. Aside from dyspnoea, mechanisms responsible for depressive disorder in COPD patients are still not completely known and are probably multifactorial [2]. Influence of aging, smoking and hypoxemia on brain function, most likely contribute to the development of depression. The depressive symptoms in COPD are predominantly irritation, tearfulness, thoughtfulness, anxiety, and too much worry. Therefore, it is difficult to recognize depressive disorders in patients with COPD, because symptoms and signs of depression are incorporated in primary pulmonary disease.

Different questionnaires are used to estimate depressive disorder in COPD. However, these questionnaires are rather screening than diagnostic tools. In older patients these questionnaires can be less precise, because they contain items exploring somatic state in which patients can answer positive because of aging process, and not for depression existence, which can lead to overestimated prevalence of depression in COPD.

The aim of this study was to analyze characteristics of COPD patients who have depressive disorder and to establish potential influence of depression to quality of life in COPD patients.

METHODS

This study included 89 patients with COPD who were treated at Pulmonary Clinic at the Clinical Centre of Kragujevac, Serbia, from 2013 to 2015. We aimed to recruit 65 control subjects without COPD, who were matched to COPD subjects by age and sex. The protocol was approved by the institutional ethics committee and informed consent was signed by every

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patient before inclusion into this study. The patients were evaluated in the stable phase of the disease (respiratory symptoms and therapy not changed at least four weeks before the examination).

Patients were excluded if they had any of the conditions where suboptimal lung function results are likely (chest or abdominal pain of any cause, dementia or confusion, etc.) [3], who could not perform the six-minute walking test (6-MWT), if they had a myocardial infarction four months before the beginning of the study, if they had unstabile angina pectoris or congestive heart insufficiency (New York Heart Association Functional Classification, classes III and IV). Evaluation of lung function was performed by spirometry (Master Screen Pneumo, Jaeger, Würzburg, Germany) and body plethysmography (Master Screen Body, Jaeger). Measurements followed the criteria of the European Respiratory Society and the American Thoracic Society for spirometric standardization and procedures [3]. Forced expiratory volume in 1 second (FEV_1) was expressed as a percentage of predicted values from the European Community for Steel and Coal [4]. The patients were divided according to lung function into three groups: Group I with $FEV_1 \geq 50\%$, Group II with $30\% \leq FEV_1 < 50\%$, and Group III with $FEV_1 < 30\%$ of predicted values. Evaluation of gas exchange was done by arterial gas analyzer (GEM Premier 3000, Instrumentation Laboratory Company, Bedford, MA, USA). Dyspnoea level was determined by the Modified Medical Research Council (mMRC) scale. Exercise tolerance was determined by the 6-MWT. Nutritional status was estimated by the body mass index (BMI). For evaluation of depressive disorder we used Hospital Anxiety and Depression Scale (HADS) and Geriatrics Depression Score (GDS) [5, 6]. HADS contains seven questions for depression and anxiety, where borderline disorder exists if the score is 8–10, and depressive disorder if probable if the score is ≥ 11. The GDS score 10–19 is graded as mild and 20–30 as serious. For evaluation of quality of life we used St. George's Respiratory Questionnaire (SGRQ).

In this study we used descriptive statistics – arithmetic means, standard deviations (SD), percentages. For comparison of arithmetic's means in two independent groups of patients we used the independent t-test, and ANOVA, as appropriate. To compare differences of measured variables in the same patients at the beginning and the end of the study, a paired T-test was used. The correlation between two numeric features was tested by Pearson's coefficient of correlation. We used multivariate logistic regression to determine the adjusted odds ratio (OR).

RESULTS

A total of 89 COPD patients and 65 control subjects without COPD, who were analyzed between 2013 and 2015, satisfied the eligibility criteria for inclusion into the study. By design, patients with and without COPD were similar in age, sex, BMI (Table 1). Among the 89 patients, 27 (30.3%) were found to be depressed according to the HADS score, and 23 (25.8%) by GDS (Table 1). Compared with referents, patients with COPD had a greater prevalence of depressive disorder ($p < 0.001$ for all).

Table 1. Characteristics of COPD patients and referents

Variables	COPD patients (n = 89)	Referents (n = 65)	p
Age, (yr) mean ± SD	62.6 ± 9.1	64.5 ± 8.4	0.46
Male sex, n (%)	62 (69.6)	46 (70.7)	0.68
Never smoked, n (%)	9 (10.1)	38 (58.4)	
Ex-smokers, n (%)	38 (42.7)	11 (17)	
Current smokers, n (%)	42 (47.2)	16 (24.6)	
BMI mean ± SD, kg/m^2	27.6 ± 7.8	26.4 ± 6.9	0.59
Depressive simptoms (HADS), n (%)	27 (30.3)	8 (12.3)	< 0.001
Depressive simptoms (GDS), n (%)	23 (25.8)	6 (9.2)	< 0.001

HADS – Hospital Anxiety and Depression Scale; GDS – Geriatrics Depression Scale

Table 2. Airflow limitation and depression scores in COPD patients

Depression score	$FEV_1 \geq 50\%$ (n = 27)	$30\% \leq FEV_1 < 50\%$ (n = 35)	$FEV_1 < 30\%$ (n = 27)	p
GDS (mean ± SD)	9.16 ± 6.27	14.0 ± 8.08	15.33 ± 4.86	0.048
HADS-D (mean ± SD)	5.58 ± 4.05	9.75 ± 5.01	10.26 ± 4.63	0.028

FEV_1 – forced expiratory volume in 1 second

Table 3. Prevalence and odds of depression (HADS) in patients with COPD compared to referents

COPD patients	n	Depressive symptoms (HADS-D ≥ 11)	OR (95% CI)
$FEV_1 \geq 50\%$	27	5 (18.5%)	1.8 (1.1–3.4)
$30\% \leq FEV_1 < 50\%$	35	9 (25.7%)	2.1 (1.2–3.8)
$FEV_1 < 30\%$	27	13 (48.1%)	3.0 (1.6–4.9)
Referents	65	8 (12.3%)	1.0 (referent)

Table 4. Prevalence and odds of depression (GDS) in patients with COPD compared to referents

COPD patients	n	Depressive symptoms (GDS ≥ 10)	OR (95% CI)
$FEV_1 \geq 50\%$	27	4 (14.8%)	1.7 (0.9–3.6)
$30\% \leq FEV_1 < 50\%$	35	8 (22.8%)	1.9 (1.1–4.3)
$FEV_1 < 30\%$	27	11 (40.7%)	2.8 (1.5–6.0)
Referents	65	6 (9.2%)	1.0 (referent)

We analyzed the presence of depressive disorder using GDS and HADS in patients with different airflow limitation. The values of both scores differed significantly among the groups (Table 2). In the group with $FEV_1 < 30\%$ we observed the highest average values for both scores. In this group the mean HADS depression score was 10.2 (SD 4.6) and GDS score was 15.3 (SD 4.8) (Table 2).

In addition, when COPD subjects were stratified by FEV_1 categorization, all subgroups were more likely to have depressive disorder, according to HADS, relative to referents, with the OR highest (3.0, 95% CI 1.6–4.9) among those with the $FEV_1 < 30\%$ (Table 3). Similar results were obtained in the group with the $FEV_1 < 30\%$ using GDS (OR 2.8, 95% CI 1.5–6.0) (Table 4). The results are from multivariate logistic regression adjusted for age, sex, body mass index, and smoking status (Table 3 and 4).

We divided the COPD patients into two groups based on HADS depression score. Table 5 shows that depressed

Table 5. HADS scores and COPD patient characteristics

Subject characteristics	Nondepressive disorder (HADS ≤ 10) n = 62	Depressive disorder (HADS ≥ 11) (n = 27)	Difference* in means or proportions (95% CI)
Age, yr (mean ± SD)	64.1 ± 8.1	63.2 ± 9.2	-0.8 (-0.5–4.1)
BMI (kg/m^2), (mean ± SD)	26.2 ± 4.3	21.3 ± 3.8	-4.9 (-7.2–5.4)†
Cumulative smoking, pack-years (mean ± SD)	30.2 ± 8.4	37.1 ± 9.6	6.9 (0.5–10.1)
FEV_1, % of predicted value (mean ± SD)	42.5 ± 10.1	34.2 ± 7.4	-8.3 (-16.3–1.2)†
TLC, % of predicted value (mean ± SD)	101.2 ± 15.4	118.1 ± 19.6	17.8 (2.3–28.4)†
PaO_2 (Kpa), (mean ± SD)	8.5 ± 2.2	8.1 ± 1.9	-0.4 (-0.8–1.2)
mMRC, (mean ± SD)	1 ± 1	2.07 ± 0.75	1.07 (-1–3)†
6MWT (m), (mean ± SD)	402 ± 40.1	346.2 ± 59.6	-56 (-124–26)†
SGRQ total and subscore (mean ± SD)			
SGRQ total score	43.3 ± 16.7	76.6 ± 10.5	32.9 (24.1–40.3)†
SGRQ symptom	36.2 ± 9.4	69.4 ± 7.3	33.2 (25.3–38.9)†
SGRQ acitivity	49.8 ± 11.5	81.7 ± 8.4	32.4 (23.9–39.6)†
SGRQ impact	38.3 ± 18.9	78.9 ± 10.4	40.6 (21.7–47.3)†
Long-term oxygen therapy, n (%)	8 (12.9%)	6 (22.2%)	9.3% (2.1–11.5)†

*Differences (95% CI) in mean values (for continuous variables) and in proportions (for categorical variables) between patients with and without psychological disorders;
†p-value < 0.05; unpaired two-tailed t-test was used for continuous variables (compare two means); χ^2-test was used for categorical variables (compare percentages)

patients with depressive disorder had lower FEV_1, higher TLC expressed in percentage of predicted values, lower BMI, had more severe dyspnea and a shorter 6-MWT compared to non-depressed patients. Finally, they had worse HRQL (higher SGRQ total score and all subscores) compared to COPD patients without depressive disorder (Table 5).

DISCUSSION

The main finding of this study is that depressive disorder identified in patients with stable COPD was significantly associated with lower FEV_1, higher TLC expressed in percentage of predicted values, lower BMI, more severe dyspnea, shorter 6-MWT and worse HRQL. Compared with referents, the patients with COPD had greater prevalence of depressive disorder. In the group with FEV_1 < 30% we observed the highest average values for both scores for assessment of depression. When COPD subjects were stratified by FEV_1 categorization, all the subgroups were more likely to have depression relative to referents adjusted for age, sex, body-mass index, and smoking status.

In our study, the incidence of depressive disorder in COPD patients was 30.3% according to HADS. Data from clinical research show that in clinically stable patients with COPD, prevalence of depression which requires medical treatment varies in the 10–57% range [7, 8]. Such large difference between depression frequencies in patients with COPD can be explained by differences in size of examined population, especially the difference between methodology and instruments and borderline scores used for depression evaluation. We showed a higher prevalence of depressive disorder in COPD patients, according to HADS, compared to the control group, although there were no significant differences in age between the groups. The questionnaires for the assessment of depression in older people may be less accurate because they contain somatic compartments which can exist as part of the aging process, which may overestimate the prevalence of depression. Geriatric Depression Scale is specifically designed to overcome these limitations. Using this score, we have also found a higher frequency of depressive disorder (25.8%) compared to the control group, which could indicate that depression is not a consequence of age but of COPD itself.

Smoking increases the risk and severity of COPD, makes daily activities effortful and stressful, and increases the risk of depression in patients with COPD [9]. Smoking and depression have a bidirectional interaction. Depressed individuals are more likely to smoke, display higher risk to commence smoking, and find smoking cessation more difficult. Conversely, smokers are more likely to be depressed, which could be caused by the activation of nicotinic acetylcholine receptors, or direct inflammatory effects of smoking [10]. When COPD subjects in our study were stratified by FEV_1 categorization, all subgroups of COPD patients were more likely to have depressive disorder relative to referents adjusted for smoking status. In a study conducted by Negi et al. [11], there is no statistically significant relationship between the occurrence of depression and smoking status in COPD patients.

We found the highest frequency and scores for depression evaluation in COPD patients who had severe airflow limitation. Explanation for significant depressive disorder in more advanced stages in COPD can be expressive dyspnoea, decreased physical activity, worse exercise tolerance, frequent exacerbations which can lead to further physical activity decrease, social isolation, fear, and depression [12]. In a study by Tse et al [13], only the exacerbation frequencies in prior year and dyspnea level remained significant independent predictors for depression in COPD patients.This study has shown that the COPD phenotype of frequent exacerbator represents a significant independent predictor for depression in COPD patients.

In patients with advanced COPD, respiratory failure is common and treated with long-term oxygen therapy

(LTOT). The influence of LTOT on depression symptoms in patients with COPD is less known. Balbo et al. [14] found that LTOT can decrease movements and social communications in patients with COPD, which can aggravate depression. In our study, the percentage of patients who have used LTOT was significantly higher in the group with depressive disorder (22.2%).

In the present study, group of COPD patients with depressive disorder had lower average value of BMI compared to non-depressed COPD patients. Malnutrition shows to be connected with muscle mass loss, leads to worse exercise tolerance, and possible social isolation and depressive disorders. Study by Negi et al. [11] showed that the incidence of depressive disorder is associated with lower BMI. On the other hand, Ghoddusi et al. [15] had shown that higher depression score according to HADS is connected with higher BMI in patients with COPD. It is considered that several factors contribute to psychiatric disorders in obese patients, such as social status, degree of obesity and negative perception about own body.

We found that depressed COPD patients had more severe dyspnea compared to non-depressed patients. The cause and relationship between psychological factors and dyspnea is, however, unclear. There are indications that dyspnea may induce psychiatric disorders while other studies indicate that psychological illnesses intensify with the subjective sensation of dyspnea [16].

In our study, depressed COPD patients, according to HADS, tolerated physical effort significantly worse compared to non-depressed ones. Kim et al. [17] found that depression had greater prognostic significance than FEV_1 value on the level of decrease of physical activity in patients with COPD.

It is considered that depressive symptoms are correlated with psychical, physical, and social functioning which determines the quality of life [18]. We have found significant worsening in every area of the quality of life in depressed COPD patients and the highest score was noted in the area connected with physical activities (SGRQ-subscore activity). Depressive symptoms, controlling for COPD severity as well as sociodemographics and comorbidities, were strongly associated with worse respiratory-specific quality of life and worse overall physical quality of life, as reported by Omachi et al. [18]. In a study by Blakemore et al. [19], depression predicts the quality of life in COPD patients, but this longitudinal analysis did not show the cause and effective relationships between depression and future quality of life.

There is evidence that treating depression in COPD patients improves the quality of life [20]. Although this might include antidepressive pharmacotherapy, interventions such as pulmonary rehabilitation, which often includes psychosocial support, may also improve the mood and reduce depressive symptoms. Because of the strong association between depressive symptoms and the quality of life, further studies regarding effective methods of treating depression in COPD appear clearly warranted.

Several study limitations must be considered. There were significantly more smokers in the group with COPD compared to the control group. The study did not consider the impact of comorbidity in patients with COPD in the development of depressive disorder.

CONCLUSION

Depressive disorder is very common in COPD patients. It is not easy to diagnose depressive disorder in COPD patients because of the overlapping symptoms between COPD and depression. However, the seven-item HADS depression subscale and GDS appear to be useful screening tools. Although the respiratory disorder is the dominant somatic problem, emotional response on COPD significantly correlates to poor quality of life. Evaluation of depressive disorder should be considered in every patient with COPD, especially in patients with greater degree of airflow limitation and lung hyperinflation, dyspnoea level, and malnourished patients.

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Карактеристике болесника са хроничном опструктивном болешћу плућа и депресивним поремећајем

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САЖЕТАК

Увод/Циљ Порекло депресивног поремећаја код болесника са хроничном опструктивном болешћу плућа (ХОБП) још увек није потпуно познато и вероватно је узроковано различитим факторима.

Циљ ове студије је да се утврде најважније карактеристике болесника са ХОБП који имају депресивни поремећај.

Методе У студију је укључено 89 болесника са ХОБП и 65 особа без ХОБП сличне старосне доби, као контролна група. Код свих са ХОБП урађено је испитивање плућне функције, процењен нутритивни статус, степен диспноје помоћу скале *mMRC*, постојање депресивног поремећаја помоћу Болничке скале за процену анксиозности и депресије (БСАД) и Геријатријске скале за процену депресије (ГСД). Квалитет живота је процењен помоћу респираторног упитника болнице „Свети Ђорђе" (РУСЂ).

Резултати Депресивни поремећај је имало 30,3% болесника са ХОБП на основу БСАД и 25,3% болесника на основу ГСД. Када смо болеснике са ХОБП поделили према тежини на основу вредности форсираног експиријумског волумена у првој секунди ($ФЕВ_1$), ризик за развој депресивног поремећаја, на основу БСАД и ГСД, био је значајно већи у свим групама са ХОБП у односу на контролну групу. Највећи ризик је био у групи са $ФЕВ_1$ < 30% (*OR* 3,0; *CI* 95%; 1,6–4,9). Болесници са ХОБП са депресивним поремећајем (БСАД) имали су у односу на остале болеснике са ХОБП (разлика у средњим вредностима) значајно већи интензитет пушења (6,9 (0,5–10,1)), мањи индекс телесне масе (-4,9 (-7,2–5,4)), мањи $ФЕВ_1$% (-8,3 (-16,3–1,2)), већи тотални плућни капацитет (%) (17,8 (2,3–28,4)), већи *mMRC* скор (1.07 (-1–3,0)) и укупни скор РУСЂ 32,9 (24,1–40,3).

Закључак Процену депресивног поремећаја треба урадити код свих болесника са ХОБП, а посебно код оних са тежом бронхопструкцијом и хиперинфлацијом плућа, већим степеном диспнеје и неухрањених.

Кључне речи: ХОБП; депресивни поремећај; квалитет живота

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

The phenotypic and genotypic characterization of vancomycin-resistant enterococci in outpatients' urine culture

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SUMMARY

Introduction/Objective In the era of emerging antibacterial resistance, the major burden of resistant strains is on hospitalized patients. Although community factors are also important in the spread of resistance, less attention has been paid to non-healthcare settings.
The aim of the study is to determine the prevalence of vancomycin-resistant enterococci (VRE) in the outpatient's urine culture and to perform phenotypic and genotypic characterization of VRE strains.

Methods During an 18-month period, a total of 5,164 *Enterococcus* spp. strains were isolated from urine and identified by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. Antimicrobial susceptibility testing was performed by disk diffusion method and by gradient test for glycopeptide-resistant strains. Genotypic characterization of VRE strains was done by multiplex polymerase chain reaction for the detection of the vancomycin resistance genes.

Results Among the isolated enterococci, 5,060 (98%) were *E. faecalis* and 104 (2%) were *E. faecium*. *E. faecalis* strains were susceptible to all tested antibiotics except norfloxacin (33% of strains were resistant), while *E. faecium* showed high level of resistance to most of the tested agents (91.3% to ampicillin, 77% to norfloxacin, and 75% to nitrofurantoin), and 26% of strains were resistant to vancomycin and teicoplanin. *VanA* gene was detected in all vancomycin resistant *E. faecium* (VRE*fm)* strains.

Conclusion A high proportion of VRE*fm* was noticed among outpatients in our country. All analyzed VRE*fm* strains belonged to *vanA* genotype. Future surveillance studies of VRE are needed to follow up on this baseline study to monitor any possible changes in abundance and genotype of VRE in this population group.

Keywords: VRE; urine; outpatients

INTRODUCTION

Enterococcus spp. are not generally regarded as highly virulent bacterial pathogens. These bacteria are part of normal intestinal flora of both humans and animals, and can cause vast majority of human infections, such as: urinary tract infections, bacteremia, endocarditis and, less frequently, infections of other sites (wounds, bones, meninges, etc.). *Enterococcus faecalis* is the most common isolated species of *Enterococcus* spp., but in the last couple of decades, *Enterococcus faecium* has caused a substantial proportion of enterococcal infections, especially in hospital settings [1, 2].

Enterococci have emerged as important nosocomial pathogens. The major reason for this is the trend of increasing antimicrobial resistance seen in these organisms [2]. One of the main reasons why these organisms have survived in the hospital environment is their intrinsic resistance to commonly used antibiotics and, perhaps more importantly, their ability to acquire resistance to all currently available antibiotics, either by mutation or by receipt of foreign genetic material through the transfer of plasmids and transposons [3]. In the past decade, antibiotic resistance has been increasingly identified in the community. Although community factors are also important in the spread of resistance, less attention has been paid to non-healthcare settings [4]. Community-acquired infections account for the majority of prescribed antibiotics, very often wide-spectrum antibiotic therapy, which increases the rate of multidrug-resistant bacteria, e.g. multidrug-resistant enterococci strains isolated from urine culture [5, 6, 7]. The emergence of vancomycin-resistant enterococci (VRE) in the community has emphasized the non-existence of boundaries between hospitals, between people and animals, between countries, and probably between continents [8].

The aim of the study is to determine the prevalence of vancomycin-resistant enterococci (VRE) in outpatients' urine culture and to perform phenotypic and genotypic characterization of VRE strains.

METHODS

Bacterial strains

From February 2014 to July 2015, a total of 53,348 urine samples were analyzed in our

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laboratory. In accordance with European guideline recommendations [9], 5,164 clinically significant enterococci strains were included in this study. If there was more than one sample per patient, only the first isolated strain was included. Identification was performed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) (Vitek MS®, bioMerieux, Marcy-l'Étoile, France).

Antimicrobial susceptibility testing

The antimicrobial susceptibility testing was done by Kirby–Bauer disk diffusion method using the following disks (Bio-Rad Laboratories, Inc., Hercules, CA, USA): ampicillin (2 μg), norfloxacin (10 μg), nitrofurantoin (100 μg), vancomycin (5 μg), and teicoplanin (30 μg). Minimum inhibitory concentration (MIC) in glycopeptides resistant strains was determined by gradient test (E-test, bioMerieux) for vancomycin and teicoplanin. The results were interpreted and quality control was done in accordance with the European Committee on Antimicrobial Susceptibility Testing (EUCAST) recommendations from 2015 [10, 11].

Multiplex polymerase chain reaction

Genotypic identification and determination of glycopeptide resistance genotype was done by multiplex polymerase chain reaction (PCR). For identification, detection of the genes encoding D-alanine–D-alanine ligases specific for *E. faecium* ($ddl_{E.\ faecium}$) and for *E. faecalis* ($ddl_{E.\ faecalis}$) was performed. For detection of the vancomycin resistance genes, the attempt was made to identify the commonest ones, i.e., *vanA*, *vanB*, and *vanC* genotypes (*vanC1* gene or *vanC2/C3* gene) [12, 13]. *E. faecium* BM4147 (*vanA* positive strain) was used as a positive control strain.

The PCR conditions and the primers used for the genotypic characterization of vancomycin resistant strains were as previously described [14–17]. The following pairs of primers were used: for $ddl_{E.\ faecium}$F (5'-GCAAGGCTTCTTAGAGA-3'), $ddl_{E.\ faecium}$R (5'-CATCGTGTAAGCTAACTTC-3'), $ddl_{E.\ faecalis}$F (5'-ATCAAGTACAGTTAGTCTT-3'), $ddl_{E.\ faecalis}$R (5'-ACGATTCAAAGCTAACTG-3'), *vanA*F (5'-GGAAAACGACAATTGCTATT-3'), *vanA*R (5'-GTACAATGCGGCCGTTA-3'), *VanB*F (5'-ACTGGCCTACATTCTTACA-3'), *VanB*R (5'-AGCGTTTAGTTCTTCCGT-3'), *vanC1*F (5'-TCTCCAGAATACTCAGTGT-3'), *vanC1*R (5'-ACATGGCAACCAACATAAG-3'), *vanC2/C3*F (5'-CCTCAAAAGGGATCACTAA-3'), *vanC2/C3*R (5'-TCTTGATAGGATAAGCCGA-3').

Statistical analysis

The data obtained in this study were analyzed in the SPSS statistical program (PASW statistics for Windows, Version 18.0, SPSS Inc., Chicago, IL, USA) using methods of descriptive statistics and χ^2 test.

RESULTS

Among isolated enterococci, 5,060 (98%) strains were *E. faecalis* and 104 (2%) strains were *E. faecium*.

E. faecalis strains were susceptible to all tested antibacterial agents, except 33% of strains that were resistant to norfloxacin, which is used for fluoroquinolones resistance screening according to EUCAST recommendations (Table 1). Among tested *E. faecium* strains, 91.3% were resistant to ampicillin, 77% to norfloxacin, 75% to nitrofurantoin and 26% to vancomycin and teicoplanin, respectively (Table 1).

MIC for vancomycin among all detected vancomycin resistant *E. faecium* (VRE*fm*) strains was higher than 256 μg/ml and for teicoplanin it was in the 8–256 μg/ml range (Figure 1).

Out of 27 strains of VRE*fm* subjected to multiplex PCR for detecting vancomycin resistance genes, all strains were found to possess the *vanA* gene (Figure 2).

Table 1. Antibiotic susceptibility of enterococci isolated from the urine of outpatients

Antibiotics	*Enterococcus faecalis* (n = 5,060)		*Enterococcus faecium* (n = 104)	
	Susceptible	Resistant	Susceptible	Resistant
Ampicillin	5,060 (100%)	0 (0%)	9 (8.7%)	95 (91.3%)
Nitrofurantoin	5,060 (100%)	0 (0%)	26 (25%)	78 (75%)
Norfloxacin	3,390 (67%)	1,670 (33%)	24 (23%)	80 (77%)
Vancomycin	5,060 (100%)	0 (0%)	77 (74%)	27 (26%)
Teicoplanin	5,060 (100%)	0 (0%)	77 (74%)	27 (26%)

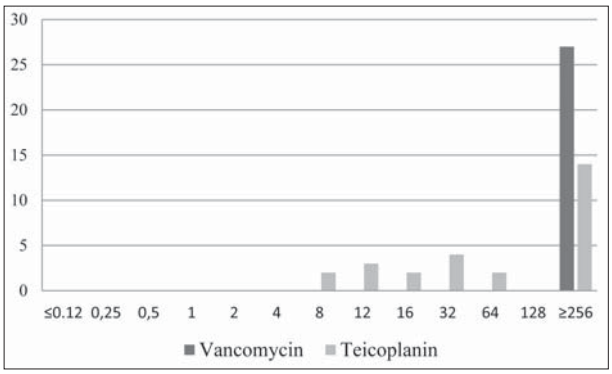

Figure 1. Glycopeptides MIC Distribution for VRE*fm* strains

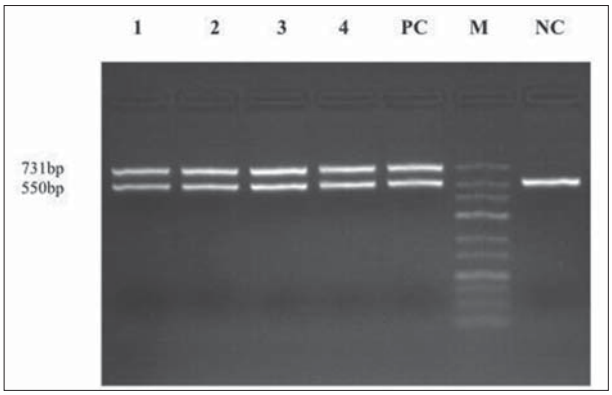

Figure 2. Gel electrophoresis of amplified products by PCR for vancomycin resistance genes; M – Gene Ruler Low range DNA Ladder (Thermo Scientific); PC – positive control for *vanA* gene; NC – negative control *VanA* gene; lines 1–4 positive for *vanA* gene (731 bp), $ddl_{E.\ faecium}$, D-alanine–D-alanine ligases *E.faecium* (550 bp)

Incidence of *vanA* gene was significantly higher in all strains of VRE*fm* compared to *vanB* and *vanC* genotypes ($p < 0.001$).

DISCUSSION

For accurate interpretation of antimicrobial resistance data, especially for glycopeptides, precise species identification is necessary. When interpreting the MIC/disk diffusion results, it is important to ensure that isolate is not *E. casselifalvus* or *E. gallinarum*, species that possess intrinsic resistance to glycopeptides. Furthermore, despite the number of studies on antibiotic-resistance in enterococci from Serbian clinical settings, there were no data about prevalence of VRE in the outpatients' settings in our country [18, 19, 20]. The results of the Central Asian and Eastern European Surveillance of Antimicrobial Resistance (CAESAR network) [7] showed high level of resistance to aminopenicillins among *E. faecalis* and *E. faecium* (41% and 94%, respectively). This result may reflect problems with species identification (comprising *E. faecium*, which is commonly resistant to aminopenicillins), rather than true high resistance in *E. faecalis*. On the other hand, high level of VRE*fm* among invasive isolates in Serbia (75%) may indicate difficulty in distinguishing *E. faecium* from *E. casseliflavus* and *E. gallinarum*. The application of the latest methods for identification such as MALDI-TOF MS or molecular methods, overcomes this problem [21]. Therefore, MALDI-TOF MS, as the most reliable phenotypic method for bacterial identification, was used in this study.

Antimicrobial resistance data in our study indicates overall significant level of multidrug-resistant *E. faecium* among enterococci in outpatients' urine culture, with 26% of VRE*fm* strains. Comparing the results with other studies [22, 23], where no VRE*fm* was detected, it can be concluded that increasing antibiotics resistance in community settings is a current trend.

Two principal phenotypes of acquired inducible vancomycin resistance have been described, VanA and VanB, encoded by two distinct gene clusters, the *vanA* and *vanB* clusters, respectively, which are carried on transposons Tn*1546* and Tn*1547*, respectively. The VanA phenotype confers high-level resistance to both vancomycin and teicoplanin, while the VanB phenotype confers only moderate to high-level resistance to vancomycin. A third type of vancomycin resistance, termed VanC, has been known for many years to be natural (intrinsic) vancomycin resistance found in the motile enterococci (*E. casseliflavus*, *E. gallinarum*, and *E. flavescens*). VanC confers only low-level resistance to vancomycin [24, 25]. Compared to other phenotypes, the VanA is the most common in European countries [26, 27]. Our results confirmed this fact: 100% of VRE*fm* strains belong toVanA phenotype.

To the best of our knowledge, this is the first molecular study on VRE strains among outpatients in our country. In accordance with phenotyping results, all strains were positive for *vanA* and negative for *vanB, vanC1*, and *vanC2/C3* genes as evidenced by PCR. Genotypic results in various studies show similar results. Libisch et al. [18] reported that the *vanA* gene was the dominant gene among invasive isolates in Serbia. Similar results were obtained for hospitalized patients in Turkey [27]. As per Werner et al. [28], the *vanA* and *vanB* resistance genotypes are by far the most prevalent in Europe. The reservoir for *vanA* and *vanB* type resistance in humans is *E. faecium*, which shows an enhanced capacity to disseminate in the nosocomial setting and are thus called epidemic or hospital-acquired. These clones of *E. faecium* are mostly ampicillin-resistant, partly high-level ciprofloxacin-resistant. In our study, *E. faecium* strains were 91.3% resistant to ampicillin and 77% resistant to quinolones. This may suggest that probably majority of our strains originated from nosocomial settings, but this requires further investigations.

CONCLUSION

A high proportion of VRE was noted among outpatients. All analyzed VRE strains belonged to *Enterococcus faecium* species associated with *vanA* genotype. Future surveillance studies of VRE are needed to follow up on this baseline study to monitor any possible changes in abundance and genotype of VRE in this population group.

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Фенотипска и генотипска карактеризација ентерокока резистентних на ванкомицин, изолованих из урина ванболничких болесника

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САЖЕТАК

Увод/Циљ У ери антимикробне резистенције највећи број резистентних сојева потиче од хоспитализованих болесника. Иако су ванболнички фактори важни у ширењу резистенције, мање пажње се поклања амбулантним болесницима. Циљ рада је одређивање учесталости енетерокока резистентних на ванкомицин (*VRE*) у уринокултури ванболничких болесника и њихова фенотипска и генотипска карактеризација.

Метода Током периода од 18 месеци укупно 5.164 ентерокока је изоловано из урина и идентификовано методом *MALDI-TOF MS*. Осетљивост на антимикробне агенсе је одређена диск-дифузионом методом и *E*-тестом за сојеве резистентне на гликопептидне антибиотике. Генотипизација *VRE* сојева је извршена мултиплекс *PCR* методом.

Резултати Међу изолованим ентерококама, *E. faecalis* чини 98% сојева, осетљивих на већину испитиваних антибиотика изузев норфлоксацина (33% сојева је било резистентно), док *E. faecium* чини 2% сојева, који показују висок ниво резистенције на већину тестираних антибиотика (91.3% сојева је било резистентно на ампицилин, 77% на норфлоксацин и 75% на нитрофурантоин), док је 26% сојева резистентно на ванкомицин и теикопланин. Код свих сојева *E. faecium* резистентних на ванкомицин (*VREfm*) утврђено је присуство *VanA* гена.

Закључак Међу ванболничким болесницима у нашој земљи утврђен је висок степен учесталости *VREfm*, што указује на неопходност сталног праћења антимикробне резистенције и у овој популационој групи.

Кључне речи: *VRE*; урин; ванболнички болесници

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Risk factors of metabolic syndrome among food suppliers

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SUMMARY

Introduction/Objective As a risk factor for chronic diseases, metabolic syndrome (MS) is increasing at an alarming rate. The prevalence of MS varies according to lifestyle and occupation in different populations. The present study aimed to determine the prevalence of MS and its components in food suppliers.

Methods A total of 112 food suppliers were randomly selected from all around the city. Data collection tools included demographic, physical activity, and food frequency questionnaires. Body composition was measured using Bio-Electrical Body Analyzer. A sample of 5 ml of fasting blood was taken from participants to assess lipid profile, blood sugar, insulin, and liver enzymes. The data were analyzed using χ^2, Kolmogorov–Smirnov and ANOVA tests.

Results Participants' mean BMI was 27.1 ± 3.9 kg/m^2, 43.6% were overweight, and 26.4% were obese. Consumption of vegetables was less and of meats more than recommended amounts. The prevalence of MS was 45.5% (51 people), which increased with aging ($p = 0.02$). Among factors causing MS, the most common one was waist-to-hip ratio (WHR) > 0.09 (72.7%), followed by high triglyceride and low HDL.

Conclusion In this study, the prevalence of MS among food suppliers was higher than the world average and than prevalence in other countries. WHR (or obesity) was found to be the most important risk factor for MS. To reduce the risk of MS, changing dietary consumption habits and increased physical activity are recommended to persons with high risk and sedentary occupations.

Keywords: metabolic syndrome; food suppliers; body mass index; risk factors

INTRODUCTION

Metabolic syndrome (MS) is a series of metabolic disorders and cardiovascular diseases and type II diabetes risk factors, including central obesity, insulin resistance, lipid disorders, and hypertension. MS increases the risk of cardiovascular diseases and type II diabetes two-fold and five-fold, respectively [1]. Several factors can affect the incidence of MS, including genetic and environmental factors such as lifestyle, regular exercise, diet, and smoking [2]. The prevalence of MS is increasing at an alarming rate.

Other studies have shown that job can also affect the incidence of MS [3]. A number of recent studies have shown that the prevalence of obesity and MS is different in different occupation groups. The prevalence of MS was reported to be 15% among administration employees of oil industry, 17.5% in bank clerks, and 56.6% among firefighters [3, 4, 5]. In the Unites States, the prevalence of MS among food serving workers or food suppliers and those in transportation has been reported higher compared to other occupations [3].

An interesting point recently addressed in some studies was easy access to prepared food outside home, which further increases its consumption [6]. To taste and look better, more fat is added to the food in restaurants and fast food outlets. Thus, their consumption is associated with adverse health risks, including increased risk of overweight, obesity, diabetes, insulin resistance, low quality diet, and MS [7]. In recent years, consumption of foods with high trifluoroacetic acid content has adversely affected people's health. Adverse effects of this fat in plasma lipoproteins increase low-density lipoproteins (LDL), and decrease lipoprotein and high-density lipoproteins (HDL) [6].

Therefore, studying people who routinely deal with industrial and ready-made foods can produce important and interesting results. Considering varying prevalence according to jobs, and also since no study has yet been conducted in the country to assess the prevalence of MS and diet among people working in food supply, this study aims to determine the prevalence of MS and its components among such workers.

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METHODS

This cross-sectional study was conducted in 2015 on 112 all-male workers in the 30–65 years age range working in patisseries, sandwich shops, restaurants, pizza and doughnut outlets, lamb liver kebab shops, and lamb head and offal cookeries. Participants were randomly selected from among those with more than three years' experience in their current jobs.

Study questionnaires included demographic, physical activity, and Food Frequency Questionnaire (FFQ). Demographic questionnaire contained questions on age, education, work history, smoking, and daily, weekly, and monthly frequency of consumption of fried and barbequed foods.

Participants' normal food intake was assessed using FFQ, whose validity and reliability had been confirmed in some local studies [8]. FFQ contains a list of 168 food items and standard portion size. Amounts of foods, as recommended portion size, were converted into daily units. According to Food Guide Pyramid recommended by the Ministry of Health, recommended number of units per day for each food group was as follows: bread and cereals 6–11 units, fruits 2–4, vegetables 3–5, meats and pulses 2–3, milk and dairy products 2–3, and miscellaneous little.

To assess the participants' level of physical activity, International Physical Activity Questionnaire (IPAQ), whose validity and reliability had been confirmed in Iran [9], was used, and participants were classified according to the Total Met scores based on instructions provided by this questionnaire, so that over the previous seven days, Met-min/week less than 600 meant low physical activity, Met-min/week equal to 600 moderate physical activity, and Met-min/week reaching 3000 meant intense physical activity.

The participants' body composition was measured using Avis 333 Body Analyzer system (Jawon Medical Co., Ltd., Gyeongsan-si, South Korea) in terms of weight, height, body fat mass , percentage body fat (PBF), soft lean mass (SLM), total body water , body mass index (BMI), body impedance, body protein, minerals, lean body mass, and waist-to-hip ratio (WHR). Height was measured using tape measure in standing position by the wall, without shoes, and with shoulders heels and buttocks touching the wall, with 1 cm precision. WHR for normal and obese upper body are defined by the WHO (WHR > 0.9 for men). According to the World Health Organization (WHO) criteria, BMI ≥ 30 is considered obese, and 25 < BMI < 29.9 overweight . Systolic and diastolic blood pressures were measured using a a calibrated digital brachial sphygmomanometer (Omron, Kyoto, Japan).

With prior knowledge of participants, and to assess blood factors such as fasting blood sugar and lipid profile including triglycerides (TG), LDL, HDL, total cholesterol, and insulin and liver enzymes (alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase), 5 ml of fasting blood was drawn from each participant, and, after serum separation, kept frozen at -40°C; the samples were then sent to the laboratory under the same conditions. RA1000-RAXT autoanalyzer (Technicon Corporation, Tarrytown, NY, USA) was used to measure fasting blood sugar, RA-XT and standard kits from Pars Company to measure lipid profiles using photometric method and a Monobind kit. Insulin level measured using ELISA reader, and a Biosystems Company kit. RA-1000 autoanalyzer was used to measure liver enzymes.

Insulin resistance (HOMA-IR) and β-cell function (HOMA-%β) calculated by applying following formulas (for the conversion of fasting glucose units from mg/dl to mmol/l, the number was multiplied by 18 [10]:

HOMA-IR = [FPI (mIU / L) × FPG (mmol/L)] / 22.5

HOMA-%β = [20 × FPI (mU/L)) / (FPG (mmol/L) - 3.5]

MS was defined according to the criteria of the third report of the National Cholesterol Education Program / Adult Training Program (NCEP / ATP III) in 2005 [11].

According to the current ATPIII criteria, the presence of three of the following five criteria is necessary for MS to be consideerd:

1. TG ≥ 150 mg/dl or receiving medication for high TG;
2. HDL cholesterol < 40 mg/dl in men and less than 50 in women;
3. Blood pressure ≥ 130/85 mmHg or receiving medicinal treatment for hypertension;
4. Fasting plasma glucose (FPG) ≥ 100 mg/dl or receiving medication for high FPG;
5. WHR of more than 0.9 cm in men and more than 0.85 cm in women.

Data is analyzed using χ^2 test to determine the relationship between the prevalence of MS and age groups, Kolmogorov–Smirnov test to verify normal distribution of data, ANOVA to compare mean values in three age groups, and Tukey's post hoc for comparison of pairs in SPSS for Windows, Version 16.0 (SPSS Inc., Chicago, IL, USA). A value of $p < 0.05$ is considered significant.

RESULTS

This study was conducted on 112 all-male food suppliers with a mean age of 43.4 ± 9.1 years, of whom only 18 (16.1%) had university education and the rest had high school diploma or below, and 69 (61.6%) had more than 10 years of work experience. Eighty (71.4%) participants had low to moderate physical activity, and the rest were highly active. Thirty (26.9%) reported daily smoking or hookah use.

Among participants, mean weight and BMI were 80.8 ± 13.5 kg and 27.1 ± 3.9 kg/m^2, respectively. Mean WHR, PBF, and SLM were 0.9 ± 0.06, 26.1 ± 4.9% and 54.6 ± 7.6 kg, respectively.

Mean protein and minerals were found to be 11.9 ± 1.6 kg and 4.6 ± 0.7 kg, respectively. Mean systolic and diastolic blood pressures were 125 ± 17.2 mmHg and 81.5 ± 10.8 mmHg, respectively. Mean TG and total cholesterol were 177 ± 6.2 mg/dl and 196.1 ± 35.9 mg/dl, respectively, and fasting blood sugar was 81.2 ± 15.7 mg/dl (Table 1).

Mean consumption of meats and protein products was found to be 4.97 ± 1.4 units/day, which was higher than recommendation level, and mean vegetable consumption

Table 1. Study variables in participating food suppliers

Variables		Mean ± SD	Normal range	Max.	Min.
Lipid profile	TG (mg/dl)	177 ± 6.2	< 200	490	70
	TC (mg/dl)	196.1 ± 35.9	< 200	307	145
	LDL (mg/dl)	170.7 ± 20.1	< 130	149	70
	HDL (mg/dl)	40.1 ± 7.5	> 35	65	30
Blood sugar	FBS (mg/dl)	81.2 ± 15.7	70–110	200	66
Insulin	(μIU/ml)	4.2 ± 3.1	0.7–9	15	0.3
HOMA-IR	Unit	0.81 ± 0.60	≤ 2.5	2.89	0.07
HOMA-β	Unit	16.35 ± 15.23	-	67.95	-2.30
Liver enzymes	AST (U/L)	24.9 ± 9.8	< 45	48	4
	ALT (U/L)	25.2 ± 9.8	< 45	47	6
	ALK-P (U/L)	180.6 ± 57.5	40–306	317	82
Blood pressure	Systolic (mmHg)	125 ± 17.2	120	175	90
	Diastolic (mmHg)	81.5 ± 10.8	80	113	58

TG – triglycerides; TC – total cholesterol; FBS – fasting blood sugar; HOMA-IR – insulin resistance index; HOMA-β – beta-cell function index

Table 2. Daily consumption of food groups in food suppliers

Food groups (servings/day)	Mean ± SD	Daily recommendation
Bread and cereals	7.47 ± 3.61	6–11
Meats	4.97 ± 1.4	2–3
Dairy products	4.07 ± 2.93	2–3
Fruits	2.91 ± 1.30	2–4
Vegetables	2.46 ± 1.15	3–5

Table 3. Consumption of food by subjects according to the type of cooking

Fried n (%)	Grilled or steamed n (%)	Use frequency
11 (9.8)	10 (8.9)	Daily
28 (25)	12 (10.7)	3–5 days per week
53 (47.3)	32 (28.6)	1–2 days per week
15 (13.4)	43 (38.4)	1–2 times per month
5 (4.5)	15 (13.4)	Never

was 2.46 ± 1.15 units/day, which was less than recommended amount (Table 2).

Elevated level of HOMA-IR (2.5 ≤ HOMA-IR) and insulin resistance ($p < 0.05$) was found in 2.7% of the participant. Significant positive correlation was observed between TG and HOMA-IR ($p < 0.05$). However, negative correlation was found between BMI and WHR with HOMA-B ($p = 0.04$ and $p = 0.05$, respectively).

In this study, 25% of participants consumed fried foods three to five days per week, 10.7% used kebabs or steamed food, and 9.8% consumed fried foods every day (Table 3).

According to BMI classification, 48 (43.6%) participants were overweight, and 29 (26.4%) were obese. The prevalence of MS was found to be 45.5% (51 participants). Among factors causing MS, the most common was WHR > 0.9 cm, found in 80 (72.7%) participants, followed by TG > 150 mg/dl and HDL < 40 mg/dl (Figure 1). In body composition components, WHR showed a significant relationship with MS ($p = 0.003$). The prevalence of MS was 30.4% in age group < 40 years, 51.4% in the 40–49 age group, and 61.3% in the ≥ 50 years age group, and the difference between age groups was statistically significant ($p = 0.02$).

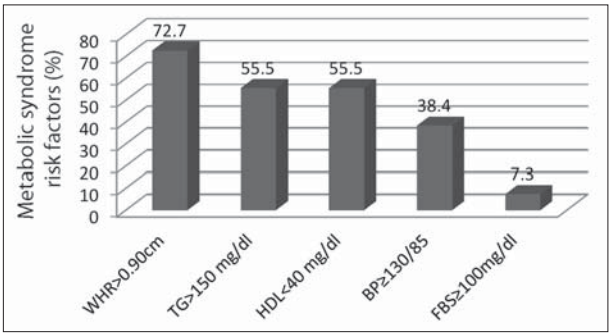

Figure 1. Frequency of MS risk factors in food suppliers

The prevalence of MS showed an increase with increasing consumption of fried foods ($p = 0.06$), but showed no significant relationship with other cooking methods ($p = 0.16$).

DISCUSSION

According to the literature, very few studies have reported high prevalence of MS in food suppliers. In the present study, the prevalence of MS was found to be more than 40%, which was relatively high compared to other studies conducted in Iran. Prevalence of MS from 22.5% to 55.6% has been reported in various studies in Iran [4, 12, 13]. In a study conducted on firefighters in Tabriz, the prevalence of MS was higher compared to the present study, which was believed to be due to their stressful job. However, it may have been due to the difference in study population, since occupation was not considered in these studies.

In other countries, different prevalence rates have been reported for MS for different occupations, including 7.5% in radiologists and 17.5% in bank clerks in Brazil. Low prevalence was reported in European countries, but higher prevalence was reported in China compared to Iran, which may have been due to the older study population [3, 14, 15, 16]. Compared to various countries in the world, Iran has a high prevalence of MS, which may be due to occupation of participants in the present study, who were all shopkeepers with little daily physical activity. More importantly, because of their jobs, participants had easier access to ready-made and high-fat foods (or generally foods that do not comply with principles of healthy diet). In the United States, the prevalence of MS among food serving workers or food suppliers and those in transportation has been reported higher compared to other occupations [7].

As well as the difference in the prevalence of MS reported in various studies, factors affecting MS have also been reported differently. In the USA, the most common reported risk factor was abdominal obesity and low HDL-C, and in China, the most common factor was high blood pressure [17, 18]. In another study, mean BP, BMI, WHR, and TG were significantly higher in participants with MS compared to those without it, but their HDL-C was lower [19]. In the present study, the most common risk factor was WHR > 0.9 cm, followed by high TG and low HDL-C, which may have been due to a lack of physical activity

and greater accumulation of fat around the waistline. High starch consumption in the form of bread and rice can also increase TG and abdominal obesity.

People with wrong food habits more frequently suffer obesity. Thus, perhaps one of the causes of obesity is improper dietary pattern. In this study, consumption of meats and foods from the miscellaneous group was high, and consumption of vegetables and fruits was low. More extensive studies investigating the relationship between dietary pattern and MS, including a study on Korean adults, have shown that consumption of fruits and dairy products is associated with reduced risk of MS [20]. A study by Bodor et al. [21] in New Orleans showed that easier access to ready-made foods in restaurants and food outlets (especially in workers), increased the risk of obesity in these people.

Using healthy methods of cooking such as boiling, grilling, and steam cooking was less common among the studied subjects. High consumption of fried foods may increase the risk of overweight, obesity, and their resulting with cardiovascular diseases is very common. Trans-fatty acids production during frying may increase the risk of cancer and coronary heart diseases [21, 22].

A study conducted in the USA showed that high consumption of restaurant and buffet foods increased the risk of obesity, indicating a relationship between easy access to local restaurants and diet and high BMI [23]. In a longitudinal study, Wright et al. [24] reported that consumption of restaurant food once a week significantly increased likelihood of overweight compared to those that did not consume restaurant food at all. Moreover, buying food for the family from restaurants once a week also increased mean percentage of body fat and incidence of cardiovascular diseases [24]. Frequent consumption of ready-made foods by adults is associated with increased BMI and body weight and increased body weight affects insulin resistance over time and metabolic outcomes [25, 26]. Comparison of the present study results to other studies showed the undeniable effect of frequent consumption of ready-made and restaurant foods on increased BMI, body fat, and incidence of chronic diseases.

Obese people have significantly higher levels of TG, glucose, and systolic and diastolic blood pressures compared to normal-weight people, and obesity is significantly related to their food habits. Furthermore, the relationship of BMI and food habits with cardiovascular risk factors has been demonstrated [25]. Thus, given the effect of lifestyle (including physical activity) and especially food habits, special attention should be paid to people's diet, especially in people working in food outlets and have to have their meals at work. A healthy lifestyle with a balanced diet, consumption of more fruits and vegetables, adequate physical activity, regular aerobic exercise, keeping the right weight, and weight loss is the best strategy for preventing obesity and MS, which should be observed by most people.

CONCLUSION

The present study shows high prevalence of MS among workers in food supply industry, and the prevalence increased with aging. In the study population, obesity is considered a risk factor for incidence of MS, and WHR was found to be the most common risk factor. Another notable result is low consumption of fruits and vegetables and high consumption of meats in participants due to easier access to meat. A high prevalence of MS is an important predictor of cardiovascular diseases. Thus, workers in food outlets should receive appropriate dietary recommendations, and periodic medical examination and health check.

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Фактори ризика метаболичког синдрома код снабдевача храном

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САЖЕТАК

Увод/Циљ Метаболички синдром (МС) као фактор ризика хроничних болести је у алармантном порасту. Учесталост МС-а варира зависно од начина живота и врсте занимања. Циљ овог рада је да се одреди учесталост МС-а и његових компоненти код снабдевача храном.

Методе Од свих снабдевача храном у граду случајним узорком је издвојено 112. Путем упитника прикупљени су демографски подаци и подаци о физичкој активности и врсти исхране. Грађа тела је одређена уређајем *Bio-Electrical Body Analyzer*. Од испитаника је узето по 5 *ml* крви ради одређивања липодног профила, шећера, инсулина и ензима јетре у крви. Подаци су анализирани χ^2 тестом, Колмогоров–Смирновљевим и Фишеровим тестом.

Резултати Индекс телесне масе испитаника је 27,1 ± 3,9 kg/m^2, 43,6% испитаника има повећану телесну масу, а 26,4% чине гојазни. У исхрани је поврће коришћено мање, а месо више од препоручених количина. Учесталост метаболичког синдрома је 45,5% (51 испитаник) и расте са старењем (p = 0,02). Најчешћи узроци метаболичког синдрома су однос струк–кукови (ОСК), потом висока триглицеридемија и ниска *HDL*-холестеролемија.

Закључак У овој студији учесталост МС-а код снабдевача храном је већа од светског просека. ОСК (или гојазност) најважнији је фактор ризика за МС. Особама са високим ризиком и седантерним послом, ради умањивања ризика МС-а, препоручује се промена начина исхране и повећање физичке активности.

Кључне речи: метаболички синдром; индекс телесне масе; фактори ризика; снабдевачи храном

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CASE REPORT / ПРИКАЗ БОЛЕСНИКА

Coincidence of retinitis pigmentosa and pseudoexfoliative glaucoma

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SUMMARY
Introduction This is an observational case report presenting retinitis pigmentosa associated with pseudoexfoliative glaucoma.
Case outline A 69-year-old man presented with retinitis pigmentosa. On examination, pseudoexfoliative material was detected on anterior segment structures, and intraocular pressure was 26 mmHg in the right and 24 mmHg in the left eye. The patient was commenced on topical antiglaucomatous therapy (timolol + dorzolamide twice daily, latanoprost once in the evening) to both eyes.
Conclusion To the best of our knowledge, this is the first reported case of retinitis pigmentosa associated with pseudoexfoliative glaucoma. Although rare, retinitis pigmentosa and glaucoma can occur in the same eye.
Keywords: glaucoma; pseudoexfoliation; retinitis pigmentosa; intraocular pressure

INTRODUCTION

Retinitis pigmentosa (RP) is a group of inherited disorders in which abnormalities of the photoreceptors (rods and cones) or the retinal pigment epithelium lead to progressive visual loss. RP can be associated with a wide variety of ocular and systemic disorders: Weill–Marchesani syndrome, ectopia lentis, Fuchs' heterochromic cyclitis [1–4]. Rarely, RP can be associated with various forms of glaucoma [5]. To the best of our knowledge, association of RP and pseudoexfoliative glaucoma (PXFG) has not yet been reported.

CASE REPORT

A 69-years-old man was referred to our glaucoma clinic for a consultation. He had a history of RP since his young age (teenage years). On examination, best-corrected visual acuity was 0.50/60 in the right eye, and hand movements in the left. Goldman applanation tonometry revealed intraocular pressure (IOP) of 24 mmHg in the right (RE) and 26 mmHg in the left eye (LE). Central corneal thickness (Palm Scan AP 2000, ophthalmic ultrasound, Micro Medical Devices Inc., Calabasas, CA, USA) was 556 μm in the right eye and 559 μm in the left. Pseudoexfoliative material was present on pupillary margin and anterior capsule of lens, bilaterally (Figure 1 and 2).

Gonioscopy demonstrated wide-open angles bilaterally, and heavily pigmented trabecular meshwork. Fundoscopy showed optic disc asymmetry with cup:disc ratios being 0.4 RE and 0.8 LE (Figure 3 and 4).

Standard automated perimetry was not possible due to poor visual acuity.

DISCUSSION

A diagnosis of PXFG was made, and the patient was commenced on topical antiglaucomatous therapy (timolol + dorzolamide twice daily,

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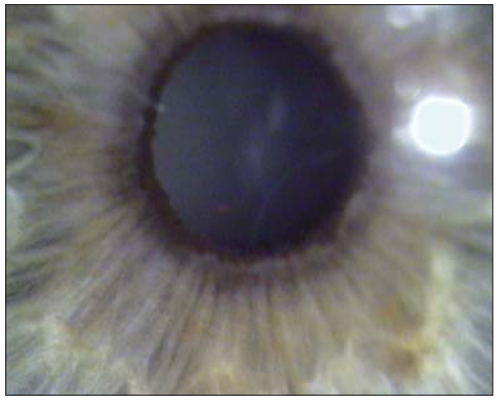

Figure 1. Slit lamp photography of the right eye

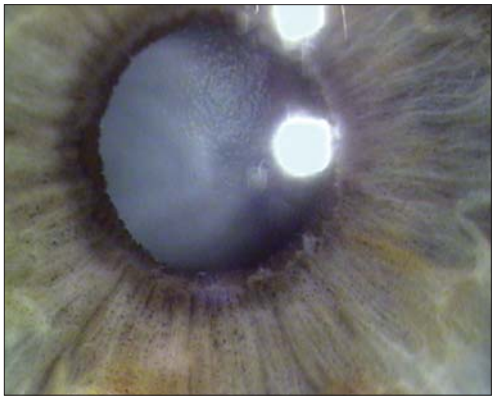

Figure 2. Slit lamp photography of the left eye

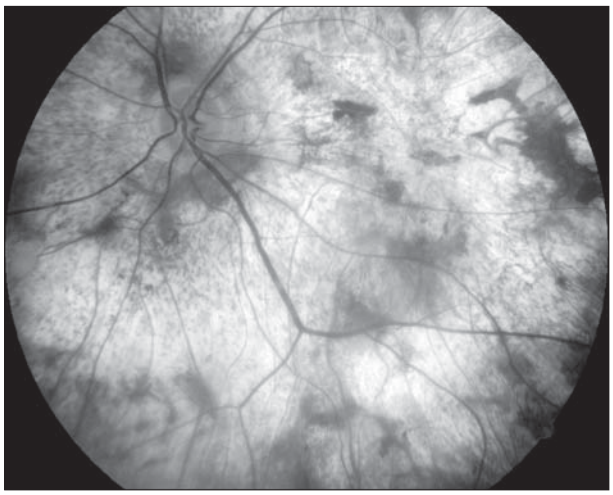

Figure 3. Fundus photography of the right eye

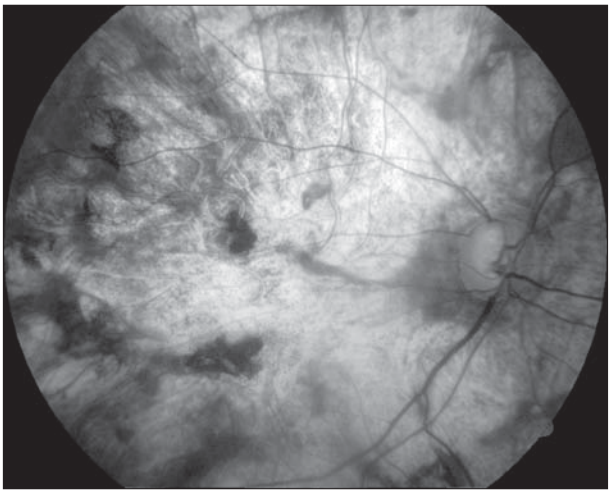

Figure 4. Fundus photography of the left eye

latanoprost once in the evening) to both eyes. The rationale for such an aggressive antiglaucoma therapy was poor visual acuity in both eyes. After three days, IOP had decreased to 16 mmHg (the right eye) and 18 mmHg (the left eye). Since satisfactory IOP reduction was accomplished with medication, no further therapeutical steps were taken (laser treatment or surgery).

RP is an inherited bilateral condition. Most cases are familial, inherited in a variety of ways, including dominant, recessive, and sex-linked recessive. Some cases are sporadic and lack family history of the disease, like the case we are presenting. According to available literature, the prevalence of primary open angle glaucoma in patients with RP ranges 2–12% [6]. Literature review shows association of RP with chronic angle-closure glaucoma, acute angle-closure glaucoma, and pigmentary glaucoma [7], but this is the first time that RP accompanied by PXFG is reported. In cases of RP associated with advanced glaucoma, we must emphasize the need for making an early glaucoma diagnosis, and almost aggressive glaucoma treatment in spite of poor visual acuity, as further deterioration of the visual field can significantly affect the quality of life of our patients.

To the best of our knowledge, this is the first reported case of retinitis pigmentosa associated with pseudoexfoliative glaucoma. Although rare, retinitis pigmentosa and glaucoma can occur in the same eye.

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Коинциденција пигментне ретинопатије и капсуларног глаукома

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САЖЕТАК

Увод Приказујемо случај ретинопатије пигментозе удруженог са капсуларним глаукомом.

Приказ болесника При прегледу мушкараца старости 69 година са пигментном ретинопатијом откривен је псеудоексфолијативни материјал на структурама предњег сегмента ока, а интраокуларни притисак је био 26 *mmHg* на десном и 24 *mmHg* на левом оку. Болеснику је прописана одговарајућа локална антиглаукоматозна терапија (тимолол + дорзоламид капи два пута дневно, латанопрост капи једном увече) у оба ока.

Закључак Према нашим сазнањима, ово је први случај пигментне ретинопатије удружен са капсуларним глаукомом. Иако ретко, глауком и пигментна ретинопатија могу бити присутни у истом оку.

Кључне речи: глауком; псеудоексфолијације; ретинопатија пигментоза; интраокуларни притисак

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CASE REPORT / ПРИКАЗ БОЛЕСНИКА

Dorzolamide in management of cystoid macular edema in a patient with retinitis pigmentosa sine pigmento

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SUMMARY

Introduction Retinitis pigmentosa (RP) is a group of inherited retinal dystrophies caused by mutations in various genes. The disease leads to progressive photoreceptors loss (rods predominantly) and retinal pigment epithelium alteration. RP can lead to blindness in the advanced stages of the disease, when the central retina is involved, mostly due to the presence of cystoid macular edema (CME). Several therapeutic approaches for CME in RP patients have been attempted but responses have been variable.

Case outline A 51-year-old man was referred due to progressive six-month-long blurring of vision in both eyes. The patient underwent complete ophthalmological examination at baseline. Based on the clinical presentation of mottled mid periphery of the retina and characteristic tubular visual field loss, hence typical fluorescein angiography and optical coherence tomography (OCT) findings, the patient was diagnosed as bilateral retinitis pigmentosa sine pigmento with CME. In an attempt to control the edema, treatment was started with dorzolamide, instilled three times daily in each eye, which resulted in reduction of macular edema in a one-month-period, as documented by OCT. This effect was further monitored for five months and was stable.

Conclusion In the presented case, we investigate the six-month therapeutic efficacy of dorzolamide for dealing with the CME secondary to RP. Topical carbonic anhydrase inhibitors are considered as the first option for treatment of CME in RP patients, due to their high efficacy and safety**.**

Keywords: retinitis pigmentosa sine pigmento; cystoid macular edema; topical carbonic anhydrase inhibitors; dorzolamide

INTRODUCTION

Retinitis pigmentosa (RP) is a group of inherited retinal dystrophies caused by mutations in various genes [1]. This disease leads to gradual and progressive loss of photoreceptors (predominantly rods) and alteration of retinal pigment epithelium [2]. RP can occur in sporadic form without any familial history, or it can be inherited as a dominant and recessive autosomal or X-linked disease [3]. The symptomatology consists of night blindness and gradual loss of visual fields [4]. This condition can lead to blindness in the advanced stages of the disease, when the central retina is involved, mostly because of the presence of cystoid macular edema (CME) [5]. The exact CME etiology is not well understood, but it is proposed that the retinal pigment epithelium pump dysfunction and/or compromise of the blood-retinal barrier bring the fluid to accumulate in cystoid spaces within the retina [6, 7]. Cystoid macular edema may cause blurred vision or reduced visual acuity and finally atrophic foveal changes with permanent loss of visual function. Thus, it is necessary to find an optimal and effective treatment for it [7]. Several therapeutic protocols have been considered, such as systemic or topical carbonic anhydrase inhibitors (CAI) (acetazolamide and dorzolamide, respectively), systemic or intravitreal corticosteroids (triamcinolone, dexamethasone), laser photocoagulation and pars plana vitrectomy but responses have been variable [6, 8–12].

We have the opportunity to report a case of cystoid macular edema in a patient with RP sine pigmento treated with topical CAI (dorzolamide). The therapeutic response was monitored by visual acuity assessment, central visual filed program (10-2 program) on Humphrey field analyzer, and by measuring central macular thickness on optical coherence tomography (OCT).

CASE REPORT

A 51-year-old man was referred to our clinic due to six-month long progressive blurring of vision in both eyes. He had a history of hypertension, with no family history of diabetes, glaucoma and RP. He reported he was an ex-smoker. The patient underwent complete ophthalmological examination at baseline including visual acuity assessment (measured by Snellen chart), applanation tonometry, and slit lamp examination, indirect ophthalmoscopy with 90D lens and immunological and biochemical investigation.

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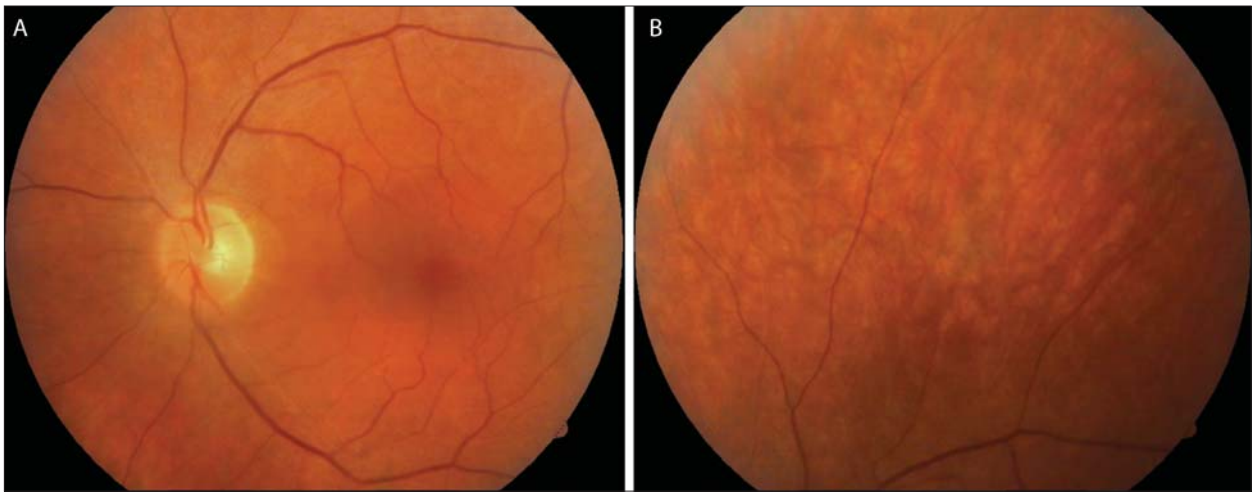

Figure 1. Fundus photo of the left eye (A: posterior pole; B: mid-periphery) with visible arteriolar narrowing and sheathing; note that there are no typical pigmentary clumps nor significant pallor of the optic nerve head

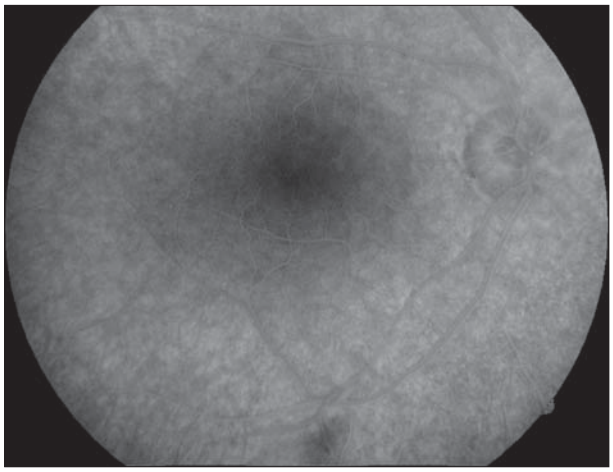

Figure 2. Fluorescein angiography of the right eye after the injection of the dye (posterior pole); note the unusual degree of choroidal hyperfluorescence surrounding darker macular area, indicating abnormality of retinal pigment epithelial layer

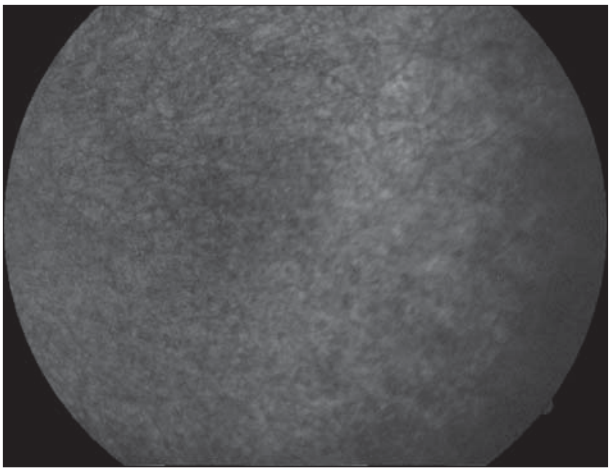

Figure 3. Abnormal fluorescein angiography of the right eye (mid-periphery); note diffuse mottled hyperfluorescence observed during the most of the period of the dye transit through the retina vessels which correspond to the retinal pigment epithelial changes

Upon examination, best corrected visual acuity (BCVA) was 0.9 in the right eye and 0.7 in the left eye. He had normal color vision and no relative afferent pupillary defect. Fundoscopy of both eyes revealed very discrete mid- and far-peripheral mottled retina, arteriolar narrowing, and sheathing. There were no typical pigmentary changes or clumps, nor significant pallor of the optic nerve heads (Figure 1). The intraocular pressure by applanation tonometry was within the normal range (10 mmHg). All investigations at presentation which included complete blood count, erythrocyte sedimentation rate, C-reactive protein, prothrombin and partial thromboplastin time, blood sugar level, renal and liver function tests, rheumatoid factor, and autoantibody profile were within normal range. The visual field tests, fluorescein angiography (FA), OCT, and dark adaptation testing were performed. Fluorescein angiography showed an unusual degree of choroidal hyperfluorescence which surrounded darker central macular zone (Figure 2), and diffuse mid and far periphery mottled hyperfluorescence extended to the equatorial region (Figure 3). Fluorescein angiography did not show any macular edema. Thinned/disappeared retinal layers including the photoreceptor layer/junction between inner and outer segment of photoreceptors were shown by OCT in the parafoveolar region. OCT also detected the subclinical CME (Figure 5a). Automated white-on-white threshold perimetry showed constricted peripheral visual field in both eyes (Figure 4). Dark adaptation testing showed prolonged rod dark adaptation in both eyes. We also performed magnetic resonance imaging of the brain with contrast which was unremarkable. Based on the clinical presentation of discrete mottled mid periphery of the retina and characteristic tubar visual field loss, hence typical FA and OCT findings, the patient was diagnosed with bilateral retinitis pigmentosa sine pigmento and cystoid macular edema. The patient was consulted about therapeutic options for CME. In an attempt to decrease the edema, treatment was started with instillation of dorzolamide three times daily in each eye. As a result of a one-month treatment, the reduction of macular edema was documented by OCT (Figure 5b), while BCVA improved to 1.0 in the right eye and 0.9 in the left eye. The patient was also seen five months later, and the improvement was

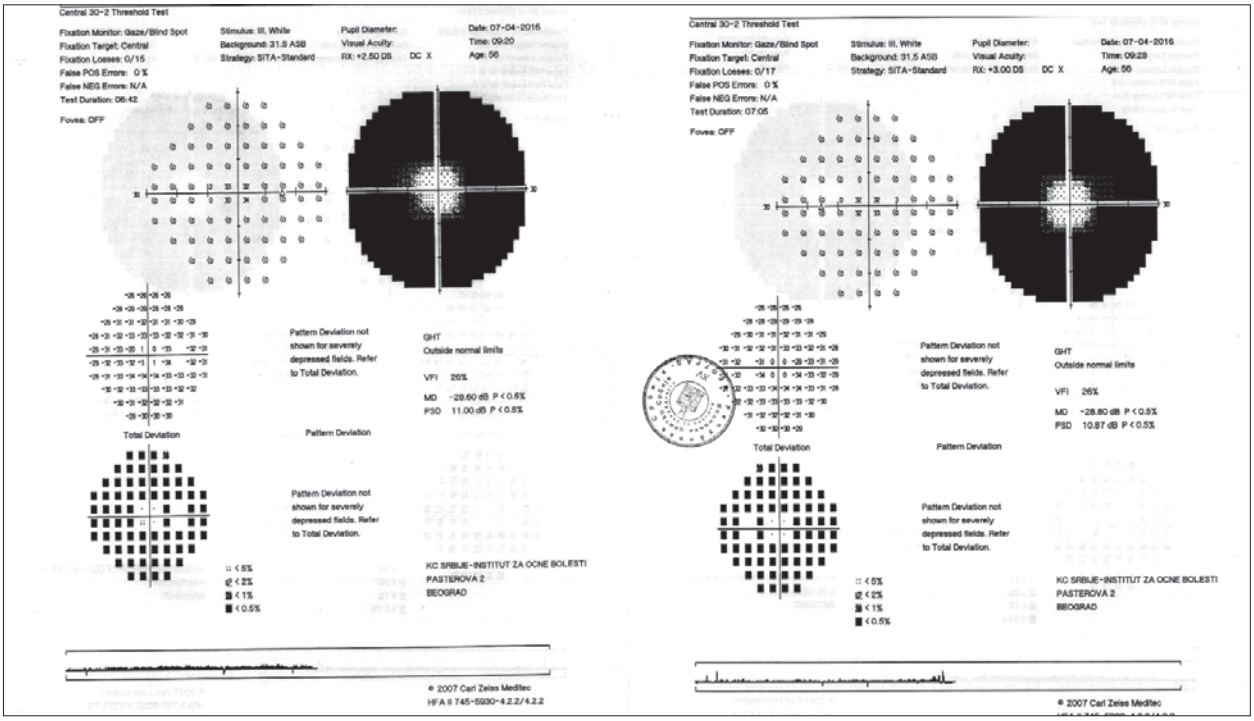

Figure 4. Automated white-on-white threshold perimetry showing constricted peripheral visual field in both eyes

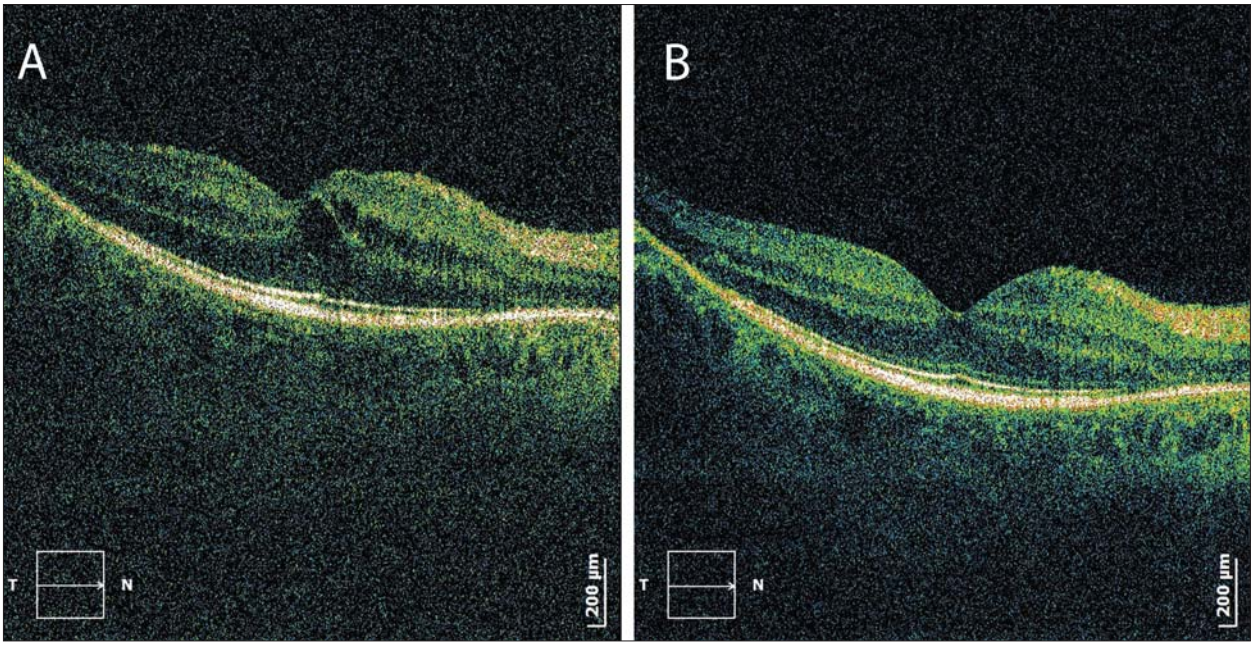

Figure 5. A: pretreatment optical coherence tomography (OCT) of the right eye, showing cystoid macular edema (CME); B: OCT of the same eye one month after the treatment, showing resolved CME

maintained with BCVA unchanged (1.0 for the right eye, 0.9 for the left one). The patient did not receive any other kind of therapy for his condition.

DISCUSSION

In the current paper, we showed the therapeutic effects of a topical CAI for the management of CME in patients with RP over a six-month period. Retinitis pigmentosa is retinal dystrophy characterized by night blindness, constricted visual fields, pigmented clumps of the retina, and photoreceptor cell dysfunction and loss [13]. The long-term prognosis is unfavorable, as there is a final loss in central vision because of direct involvement of the macula from the photoreceptors loss and/or maculopathy [14]. The diagnosis of retinitis pigmentosa sine pigmento purely from retinal findings is more challenging than in the typical cases. The retinal pigment epithelial defects could be very subtle and discrete and therefore could easily be overlooked. The vascular attenuation is also not always obvious, and sometimes retinal vascular occlusion could

be considered and initially suspected. On the other hand, optic disc pallor may be discreet, partial, or even absent, as in our case [15]. Although the subjective symptomatology is typical for RP, such as night blindness and reduced visual field in its early stages, it could be completely obscured as it affects only the peripheral fundus [16]. Without this typical clinical finding, advances in imaging and testing could help in setting the diagnosis of RP sine pigmento, such as visual field and dark adaptation testing, FA, OCT, and conventional or full-field electroretinography.

CME is an uncommon complication of RP, occurring in 10–20% of patients [9]. Currently, there are no uniform guidelines how to treat this condition. The responses to the treatment seem to be individually variable. There are several therapeutic approaches for CME in RP, such as systemic or intravitreal corticosteroids, grid laser photocoagulation, systemic or topical carbonic anhydrase inhibitors [9], and in the recent years some authors found the inhibitors of the vascular endothelial growth factor (VEGF) to be effective [17]. In any case, many practitioners believe that carbonic anhydrase inhibitors are the mainstay of treatment [9]. These drugs stimulate the pumping mechanism of the retinal pigment epithelium [18]. Unfortunately, severe side effects could appear with oral administration of CAI (drowsiness, confusion, allergic reactions, paresthesias, myelosuppression, renal calculi, loss of potassium, or, with extended use, hyperchloremic metabolic acidosis). That is the reason why this treatment is not advised for any prolonged period of time. On the other hand, topical CAI is free from the adverse effects that are related to systemic administration and thus appropriate for prolonged use [19].

Ikeda et al. [7] found the dorzolamide to be an effective and safe treatment option for CME in RP patients, and consider it as the first treatment choice for CME. If the therapeutic efficacy is not sufficient, i.e. when CME does not completely resolve within six months, additional or change of the treatment could be required. They also believed that other therapeutic approaches such as intravitreal injections of corticosteroids, inhibitors of VEGF or vitrectomy are not standard therapy because of their potentially severe complications and because of the fact that safer and still effective alternative exists in the form of topical CAI [7].

Pacella et al. [19] believed that although topical CAI are less potent than systemic ones, because of their lacking in systemic adverse effects it could be a reasonable option for the treatment of this condition, particularly if it is necessary to be used continuously. The reduction of CME commonly results in visual acuity improvement [20]. However, Chung et al. [18] found that, occasionally, improvement in visual acuity did not match the degree of edema resolution shown by OCT. This limitation of treatment could be explained by irreparable functional impairment within the fovea, arising from either chronic macular edema or photoreceptor cell dysfunction. Moustafa and Moschos [17] reported only 10% improvement in BCVA after the treatment, despite the significant resolution of macular edema in OCT scan. They deem that reasonable explanation is that CME is only one factor that affects the vision, while atrophy of the retina (particularly of the photoreceptors) also has impact on the visual function. Although our patient had anatomical and functional improvement after the treatment with topical CAI, the attention must be paid to the pre-intervention anatomical changes of the retina (photoreceptors and retinal pigment epithelium band shown on OCT), which have prognostic significance for the efficacy of the treatment. Thus, timely diagnosis and prompt treatment of CME in patients with RP are necessary before permanent photoreceptor loss occurs [16].

In the presented case we investigate the six-month therapeutic efficacy of dorzolamide for management of the cystoid macular edema secondary to RP. Our patient showed an anatomical and functional improvement after topical CAI. There are various therapeutic options for this condition, but because of their safety and efficacy, topical CAI are considered as the first treatment choice by many authors. Treatment of CME should be rapid and effective before structural changes of photoreceptors occur.

NOTE

The paper was presented at the 16th EURETINA Congress – Copenhagen 2016 and published electronically with identical title in the form of an apstract.

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Дорзоламид у лечењу цистоидног едема макуле код болесника са *Retinopathia pigmentosa sine pigmento*

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САЖЕТАК

Увод Пигментна ретинопатија (ПР) јесте група дистрофија ретине проузрокованих мутацијама различитих гена. Она доводи до прогресивног губитка фоторецептора (посебно штапића) и промена у ретиналном пигментном епителу. ПР може да доведе до слепила у узнапредовалим случајевима болести, када је захваћен централни део ретине, најчешће присуством цистоидног макуларног едема (ЦМЕ). Постоји неколико опција у лечењу ЦМЕ код болесника са ПР, иако су одговори на лечење различити.

Приказ болесника Мушкарац стар 51 годину је прегледан због прогресивног замагљења вида на оба ока уназад шест месеци. Болесник је комплетно офталмолошки прегледан. На основу клиничког налаза ишаране средње периферије ретине и карактеристичног тубарног налаза видног поља, потом карактеристичних налаза флуоресцеинске ангиографије и оптичке кохерентне томографије, постављена је дијагноза билатералне *Retinopathia pigmentosa sine pigmento* са цистоидним едемом макуле. Започет је третман дорзоламидом у оба ока три пута на дан, у жељи да се контролише едем макуле. После месец дана примене дошло је до смањења едема макуле, што је доказано и оптичком кохерентном томографијом. Овај налаз је потом праћен још пет месеци и није било знакова рецидива.

Закључак У приказаном случају смо пратили шестомесечни ефекат дорзоламида у лечењу цистоидног едема макуле код ПР. Локални инхибитори угљене анхидразе сматрају се третманом првог избора за ЦМЕ код ПР због високе ефикасности и безбедности.

Кључне речи: *Retinopathia pigmentosa sine pigmento*; цистоидни едем макуле; локални инхибитор угљене анхидразе; дорзоламид

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CASE REPORT / ПРИКАЗ БОЛЕСНИКА

Partial resection of the splenic cyst using radiofrequency ablation system

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SUMMARY
Introduction This paper presents a case of a patient with a benign splenic cyst, which was removed by way of partial resection of the spleen.
Case outline The patient's benign cyst in the lower pole of the spleen was excised using single Cool-tip™ radiofrequency ablation electrode (Cool-tip RF Ablation System, Covidien™, Dublin, Ireland). More than half of the spleen was excised without setting stitches to the splenic parenchyma and without any other hemostyptics.
This way, the function of the spleen was preserved, which was proven with scintigraphy and computed tomography two years after the intervention.
Conclusion Radiofrequency ablation system with internally cooled needles can be used successfully and without any consequences to the organ, especially in case of large benign splenic cysts, when it is necessary to preserve the function of the spleen.
Keywords: spleen; partial splenectomy; radiofrequency ablation

INTRODUCTION

Preservation of the spleen and its function is paramount for resistance to infections and prevention of overwhelming post-splenectomy infection [1, 2]. Spleen preservation is imperative in all cases of spleen surgery that allow it. This includes traumas and other pathomorphologies of the spleen, namely tumours, metastatic changes, and hypersplenism [3, 4, 5]. Splenic cysts occur in approximately 0.07% of the cases and are usually asymptomatic until their growth starts putting pressure on the surrounding organs [6, 7]. They are usually benign, but cysts of other etiology must be excluded prior to surgery. This primarily means hydatid cysts. In case of elective surgery of benign cysts, one must preserve the function of the spleen and attempt partial resection. Partial resection of the spleen is carried out using various surgical techniques [8, 9]. As of 2003, it is possible to use radiofrequency (RF) ablation system in the so called bloodless partial splenectomy [10].

CASE REPORT

A 34-year-old Caucasian woman reported to the doctor due to vague symptomatology in the upper abdomen. The ultrasound and computed tomography of the abdomen revealed a large 10.5 cm cyst in the lower pole of the spleen, covering 50% of the organ (Figure 1). Elective surgery was proposed to the patient. During the preoperative treatment, the patient was tested for carcinoembryonic antigen and carbohydrate antigen (CA 19-9) tumour markers. Hydatid disease was excluded with the serological test. During the preoperative treatment, the patient received antibiotic prophylaxis and low-molecular-weight heparin.

The upper abdomen is accessed by way of left-side paracostal laparotomy. The spleen is mobilized toward the midline, by cutting the splenophrenic and splenocolic ligaments. After exposing the entire organ without clamping arterial or venous blood vessels, a series of ablation-induced coagulation necroses are made on the splenic parenchyma by a single Cool-tip™ RF ablation electrode (Cool-tip RF Ablation System, Covidien™, Dublin, Ireland), which is then cut with a scalpel, without placing a single stitch to the remaining splenic parenchyma (Figure 2). This method ensures resection of the entire lower pole of the spleen that contains the cyst. A drainage tube is positioned

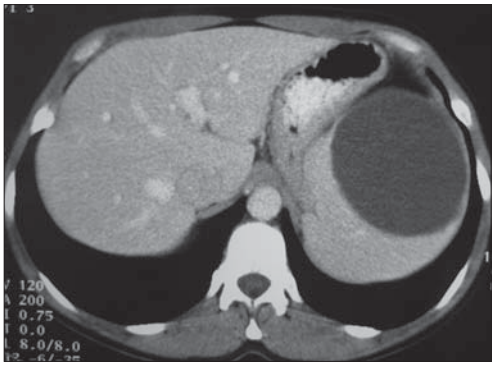

Figure 1. Preoperative computed tomography with the cyst in the spleen

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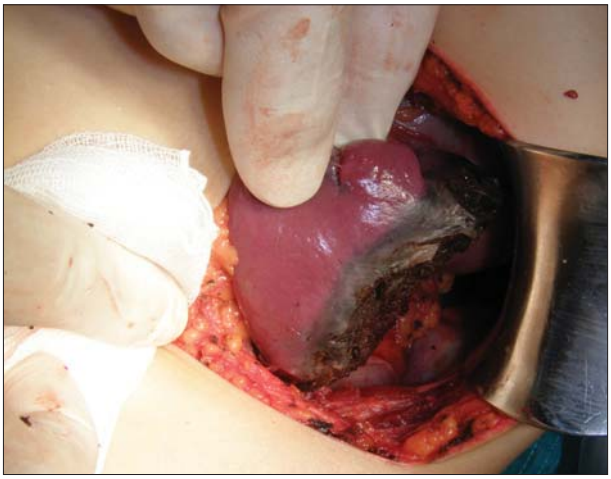

Figure 2. Spleen surface after radiofrequency ablation

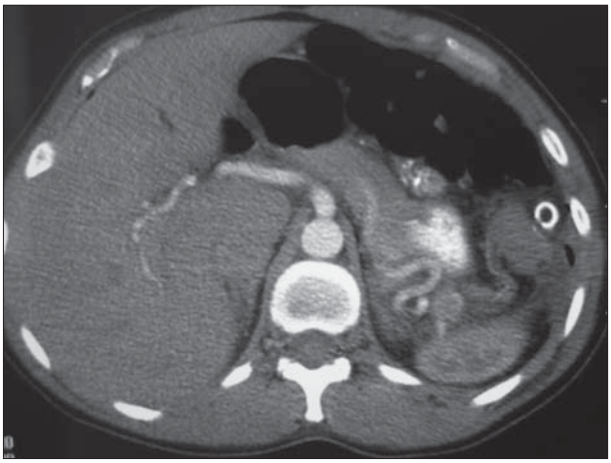

Figure 3. Computed tomography two days after the operation

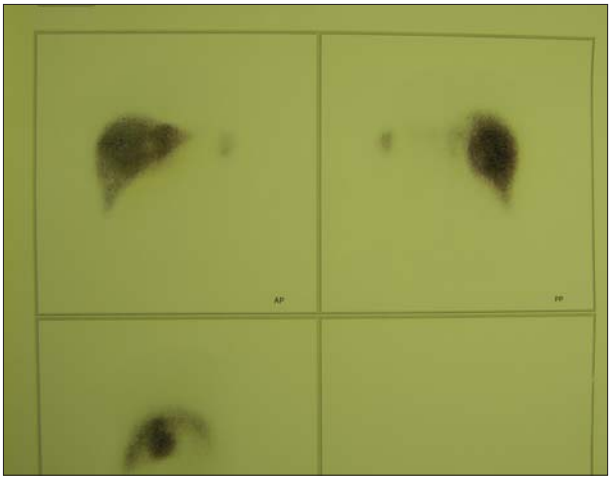

Figure 4. Spleen scintigraphy one month after the intervention

in the subphrenic space, and the operative wound is closed by anatomic layers. There were no complications, namely bleeding, neither during nor after the intervention. A follow-up computed tomography scan was performed two days after the operation in order to observe the blood vessels and ascertain the vitality of the splenic tissue (Figure 3).

On the fourth postoperative day, the drainage tube was removed and the patient discharged with normal vital parameters and on oral nutrition. Spleen scintigraphy with

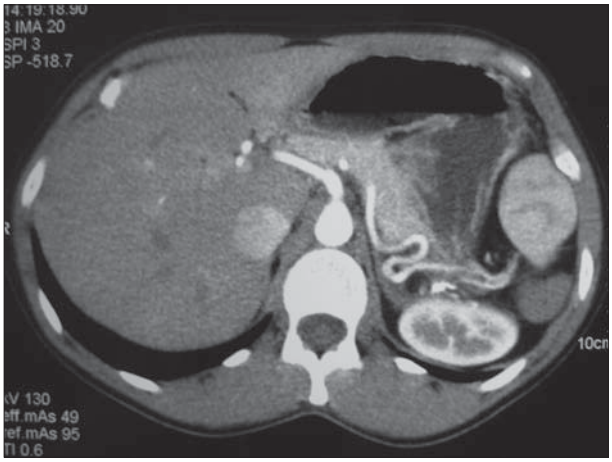

Figure 5. Computed tomography two years after the operation

labelled red blood cells was performed one month after the intervention and revealed preserved function of the somewhat smaller spleen (Figure 4). Computed tomography two years after the operation also showed normal findings (Figure 5). The operation was performed at the Clinic for Thoracic Surgery, Institute for Pulmonary Diseases in Sremska Kamenica, Serbia.

DISCUSSION

Surgical techniques for preserving the function of the spleen were developed during the 1980s and 1990s [11, 12, 13]. These were particularly significant for pediatric traumas, when non-operative treatment was recommended in case of blunt traumas in children, and later in adults as well [14, 15]. These techniques always implied the mobilization of the spleen and selective ligating of arterial blood vessels, as well as the use of absorbable sutures or specially designed nets made of the same absorbable material [16, 17, 18]. Radiofrequency ablation and its use in spleen surgery have been known since first reports in 2003 by Habib et al. [10]. This technique has been widely used in liver surgery [19]. A series of coagulation necroses induced on the splenic parenchyma results in a completely avascular resection surface after cutting the splenic tissue. This is achieved with internally cooled needles with ablation sphere of approximately 3 cm. The application of RF ablation system on one part of the splenic parenchyma does not damage the function of the remaining part of the spleen after a partial resection, which was proven with scintigraphy. In particular, this technology could be implemented during laparoscopic partial resection of benign splenic cysts, provided safe access is ensured for the electrode through the anterior or anterolateral abdominal wall. We believe that this is possible, since the internally cooled needle remains cool during the emission of RF waves.

The use of RF ablation system with internally cooled needles can be used successfully and without any consequences to the organ, especially in case of large benign splenic cysts, when it is necessary to preserve the function of the spleen.

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Парцијална ресекција слезине коришћењем радиофреквентног аблационог система

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САЖЕТАК

Увод Циљ рада је да прикаже болесницу са бенигном цистом слезине која је уклоњена парцијалном ресекцијом слезине.

Приказ болесника Код болеснице са бенигном цистом доњег пола слезине помоћу једне *cool-tip*™ електроде за радиофреквентну аблацију (*Cool-tip RF Ablation System, Covidien*™, Даблин, Ирска) одстрањено је више од половине слезине без постављања шавова на паренхим слезине и коришћења других хемостатских метода. На овај начин презервирана је функција слезине која је доказана сцинтиграфски и компјутеризованом томографијом две године након интервенције.

Закључак Употреба радиофреквентног аблационог система са интерно хлађеним иглама може се применити успешно и без последица по орган код великих бенигних циста слезине.

Кључне речи: слезина; парцијална спленектомија; радиофреквентна аблација

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CASE REPORT / ПРИКАЗ БОЛЕСНИКА

Necrotizing soft tissue infection in pregnancy

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SUMMARY

Introduction Necrotizing soft tissue infection (NSTI) is a life-threatening condition, characterized by widely spread necrosis of skin, subcutaneous fat, fascia and muscles. Treatment involves surgical debridement and broad-spectrum antimicrobial therapy. Mortality is still high due to diagnostic delays. NSTI is rare in general population, there are even less literature data of this condition in pregnancy. Timely diagnosis and therapy is crucial for outcome of these patients. Clinicians should have in mind NSTI in patients with perianal infections, especially in cases where immunosuppressive role of pregnancy is present.

Case outline We present a case of a 21-year-old pregnant woman with NSTI spreading from perianal region. The patient was admitted to hospital in the 31st week of otherwise healthy twin pregnancy one day after incision of perianal abscess. At admission she was examined by a gynecologist; vital signs were stable, laboratory results showed the presence of infection. She was referred for another surgical procedure and broad-spectrum antibiotics were prescribed. The next morning the patient complained of intense abdominal pain. Clinical exam revealed only discrete redness of the skin tender on palpation, crepitating**.** She was immediately referred to surgery. Intraoperative findings revealed massive soft tissue infection spreading up to the chest wall. Wide skin incisions and debridement were performed. The patient developed septic shock and after initial resuscitation gynecologist confirmed intrauterine death of twins and indicated labor induction. Over the next few days the patient's general condition improved. On several occasions the wounds were aggressively debrided under general anesthesia, which left the patient with large abdominal wall defect. Twenty-three days after the initial operation, the defect was reconstructed with partial-thickness skin grafts, providing satisfactory results.

Conclusion Diagnosis and outcome of NSTI are challenging for many reasons. Course of the disease is rapid and hidden. Chances of survival depend on early recognition and prompt treatment.

Keywords: necrotizing soft tissue infection; necrotizing fasciitis; pregnancy

INTRODUCTION

Necrotizing soft tissue infection (NSTI) is a life-threatening condition, characterized by widely spread necrosis of skin, subcutaneous fat tissue, fascia and muscle [1]. In literature, it is also often referred to as necrotizing fasciitis (NF), or Fournier's gangrene, which is only one of the forms of necrotizing infection of soft tissues. Its diagnosis and outcome are challenging for many reasons. The course of the disease is rapid and hidden, and its rarity further complicates diagnosis and onset of treatment. Even in cases with optimal treatment, morbidity and mortality can be as high as 35% [2]. Of around 28 million patients in the NIS database (Nationwide Inpatient Sample of the Healthcare Cost and Utilization Project) in the US only, 0.04% were identified as having a NSTI [3]. Chances of survival depend on early recognition and prompt treatment. Because of the importance of early diagnosis, primary care physicians need to maintain high index of suspicion for these infections and should be aware of possible presenting features [4]. There are less data in literature on NSTI in pregnancy. Timely diagnosis and therapy is crucial for the outcome. Clinicians should have in mind NSTI in patients with perianal infections, especially in cases where immunosuppressive role of pregnancy is present.

CASE REPORT

A 21-year-old woman was admitted to hospital in the 31st week of otherwise healthy spontaneously conceived twin pregnancy, which was regularly checked, according to the patient. Past medical and family history was unremarkable. She was referred from the regional hospital one day following incision of perianal abscess at the right side. Complains started seven days prior to admission, described as discomfort and edema around the anus. She denied any recent trauma. Antibiotics were prescribed, but since there was no improvement, after four days she was hospitalized in the regional hospital for perianal abscess. An incision was performed under local anesthesia.

At admission, obstetrics ultrasonography examination confirmed living intrauterine fetuses of 31weeks gestational age. The patient's vital signs were stable, body temperature was normal, laboratory results showed following

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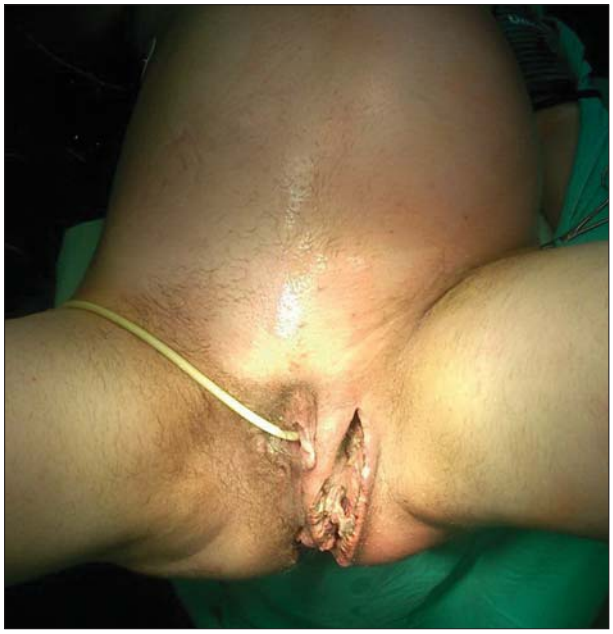

Figure 1. Clinical presentation prior to surgery

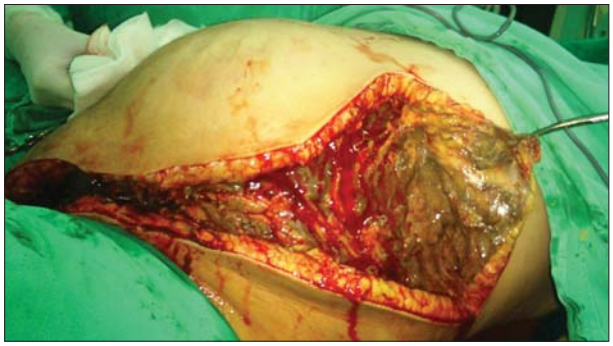

Figure 2. Intraoperative finding

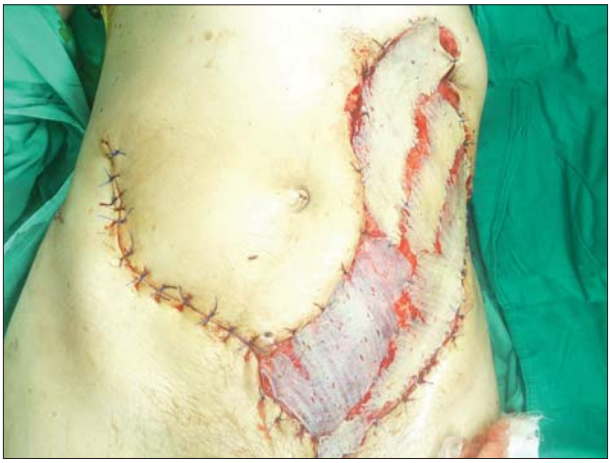

Figure 3. Abdominal wall reconstruction with partial-thickness graft

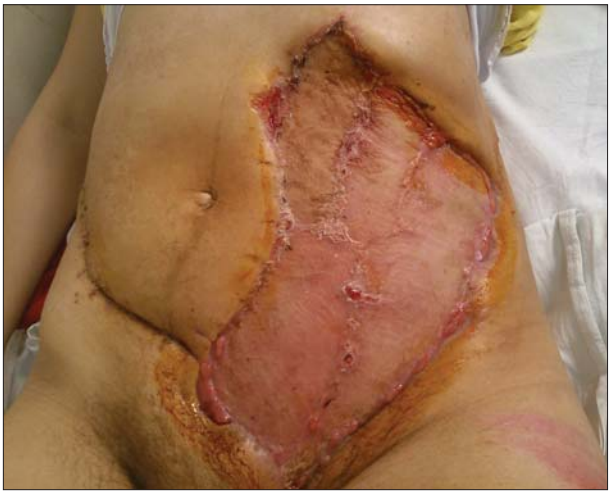

Figure 4. Abdominal wall at hospital discharge

abnormalities: white blood cell count 8.5, red blood cell count 2.88, hemoglobin 75 g/l, hematocrit (HCT) 26.9%, C-reactive protein (CRP) 294.2 mg/l, total protein 43.7 g/l, albumin 17.4 g/l. Clinical examination revealed an incision on the right side of the anus with secretion of small amounts of pus, with signs of cellulitis on the left side spreading up toward the left vulva area. Due to unsatisfactory clinical finding, the patient was referred to another surgical procedure the same day. After incision, antibiotics were prescribed (meropenem 1 g / 8 hours and ampicillin 1 g / 8 hours), as well as paracetamol and fluids in consultation with gynecologist. During the morning round, the patient complained on intense abdominal pain. Body temperature was still normal. Clinical examination revealed only discrete redness of the skin which was tender on palpation and crepitating (Figure 1). She was immediately referred to surgery, since NSTI was suspected. Intraoperative findings revealed massive soft tissue infection spreading from left perineal region up to the chest wall, predominantly on the left side (Figure 2). Wide skin incisions and excisions followed by necrectomy and debridement to the anterior abdominal wall were performed. Septic shock developed immediately, requiring mechanical ventilation in the postoperative course. The patient was hypotensive (80/40 mmHg), with heart rate of 128 beats/min., and arterial blood gas confirmed metabolic acidosis. Laboratory results showed hemoglobin to be 62 g/l, HCT 18%, total protein 35.1 g/l, albumin 12.5 g/l, CRP 206.7 mg/l, while body temperature rose to 38.6°C. After initial resuscitation, one day after admission, the gynecologist confirmed intrauterine death of twins based on ultrasonography and indicated labor induction. Over the next few days the patient was treated in the intensive care unit with broad-spectrum antibiotics, and her general condition improved. Wound dressing was performed at least twice a day. Wound cultures identified *Acinetobacter* spp. and *Enterococcus faecalis*, and antibiotic therapy was modified accordingly. On the seventh postoperative day, mechanical ventilation was no longer needed. On several occasions the wounds were aggressively debrided under general anesthesia, which left the patient with a large abdominal wall defect. After stabilization, she was referred to the Clinic for Plastic and Reconstructive Surgery for the further treatment. Twenty-three days after the initial operation, the skin defect was reconstructed with partial-thickness skin grafts (Figure 3). Eleven days post transplantation, the results were satisfactory. She was discharged from the hospital 44 days after admission (Figure 4).

DISCUSSION

According to the largest published retrospective population-based cohort study from Texas, USA, in a 10-year period (2001–2010) there were 4,060,201 pregnancy-associated hospitalizations, of which 148 were due to necrotizing infection. Only a minority of women (17.6%) were reported to have chronic comorbid conditions, of which diabetes mellitus was the most common one (50%). Drugs and tobacco abuse were rare, while obesity was reported in 22.3% [5]. Published data on NSTI in the general population show that 52.7–82% have at least one risk factor like diabetes mellitus or immunodeficiency of various degrees [6, 7, 8]. In a systematic review of Angoules et al. [9], diabetes mellitus was a predominant risk factor in 31%, smoking in 27%, alcoholism in 17%, cirrhosis in 8%, HIV in 6%, various stages of malignancy in 3%, corticosteroid therapy and chronic kidney insufficiency in 3% of NSTI cases. In this case, the patient's past medical history was unremarkable, suggesting that pregnancy might be as risk factor for necrotizing infection. We found only one similar case report on NSTI in pregnancy published in the English language, presenting a 15-year-old primigravid in the 29th week of pregnancy [10], since the majority of necrotizing infections related to pregnancy appears during the postpartum period (82.4%) [5]. In the presented case report by Nikolaou et al. [10], diabetes was diagnosed incidentally at the time of hospital admission. In addition to diabetes mellitus, pregnancy was suggested as a risk factor for necrotizing infection due to suppression of immune system during the second and third trimester and in postpartum period. This argument should be carefully considered since pregnancy is not a state of generalized immunosuppression, but instead, immune response is modulated in both systemic and, more effectively, local manner, which is focused on the maternal–fetus interface [11].

The course of NSTI varies, is often deceitful, and 35% of patients are initially misdiagnosed. The beginning ailment may suggest many other conditions, e.g., cellulitis, erysipelas, phlebitis, etc. [12]. A cardinal early symptom is disproportionately strong pain in comparison to clinical finding at examination. In a publication by Goh et al. [13], in nine studies, swelling was the most common presenting symptom (80.8%), followed by pain (79%) and erythema (70.7%). Initial finding in this case was not suggestive of NSTI, but rather of perianal abscess of cryptoglandular etiology, with one distinction – unusually intensive pain. In a previously mentioned case report, the diagnosis was established on the third day of hospitalization after unsuccessful treatment with incision and antibiotics, and after magnetic resonance imaging (MRI) confirmation [10]. According to data from literature, imaging techniques could be useful. Ultrasound or plain X-ray cannot reliably detect NSTI. Fascial thickening on T2-weighted MRI has a sensitivity of 90–100%, but a specificity of only 50–85% for NSTI. Computerized tomography should be considered as a diagnostic aid only when it can be obtained very quickly, having in mind that it may miss one in five cases of deep NSTI. Macroscopic findings seem to be most reliable, those such as pasty gray necrotic tissue, thin purulent fluid with a gray-brown "dishwater" appearance, a lack of resistance to digital pressure against fascial planes (the finger test), a generalized lack of bleeding, visibly thrombosed vessels, and/or muscle that does not contract to electrocautery stimulation [2]. Although diagnosis of NF is clinical, it is often delayed, because the infection begins and progresses in the deep layers of subcutaneous tissues, giving initially a false impression of a typical cellulitis [14]. Meanwhile, infection spreads qickly, with the speed of 2–3 cm per hour in the anorectal region, as seen in this case – in less than 24 hours, infection spread more than 50 cm, from perianal region to the anterior chest wall [6, 15].

The treatment of NSTI implies wide incisions and excisions of the affected region, operative debridement, tissue decompression, and the use of broad-spectrum antibiotics.

Historical data report that the exclusive use of antibiotics leads to 100% mortality, indicating the necessity of surgical intervention, which substantially decreased mortality [7]. Timely intervention is probably more important. Multiple studies confirmed that mortality is increased when surgical treatment is delayed, as well in cases in which repeated excisions are needed [16]. According to Gallup et al. [17], any patient with inordinate pain and unilateral edema in the pelvis, especially in the puerperium, should be suspected of necrotizing infection. The triad of pelvic pain, edema, and any sign of septicemia carries an extremely grave prognosis and mandates immediate surgical intervention.

In the present case, rapidly progressive infection, treatment delay, and development of septic shock unfortunately led to intrauterine death. In another reported case by Nikolaou et al. [10], necrotizing infection caused preterm delivery of viable male fetus weighing 1,470 g by normal labor despite tocolytic therapy. The baby died due to septicemia after 48 hours. Described complications of sepsis during pregnancy are increased rates of premature births, fetal infection, hypoxia and acidosis, higher fetal mortality, and increased probability for cesarean section. In the obstetric context, the assessment of fetal vitality has particular relevance, as the balance between fetal oxygen supply and consumption might be severely altered. No study has yet analyzed the best approach for fetal vitality assessment under this circumstance [18]. The best approach to ensure fetal vitality is to stabilize the mother's condition. The base treatment, which also applies to pregnant women with sepsis, is provided by the therapeutic guidelines based on the Surviving Sepsis Campaign [19]. The aim of initial hemodynamic resuscitation is to restore tissue perfusion to an adequate level and to ensure that cell metabolism and oxygen supply return to normal levels to avoid acidosis and consequent multiorgan dysfunction. In pregnancy, one further aim of initial hemodynamic resuscitation is to improve fetal vitality [18, 19].

Empirically selected antibiotics must be initiated immediately. A wide variety of pathogens has been reported to be responsible for NSTI. The recent clinical classification

distinguishes the following four types: type I (70–80%, polymicrobial/synergistic) as in this case, type II (20% of cases; usually monomicrobial), type III (Gram-negative monomicrobial, including marine-related organisms), and type IV (fungal) [16].

After massive debridement and repeated surgery like in the case presented here, the patient is left with defect. When primary closure is not possible, soft tissue reconstruction can be considered, after the stabilization of the patient. Usually, it's been performed using skin grafts and myocutaneous flaps, as in burn reconstructions. In cases with excessively large amounts of soft tissue involvement (> 25% body surface area), autograft reconstruction may be restricted by the limited donor-site availability [20].

CONCLUSION

Necrotizing soft tissue infection is a rapidly progressive, life-threatening condition that requires early aggressive treatment. Clinical findings on presentation are crucial for diagnosis. It should be suspected in pregnancy and postpartum period in cases with unusually intensive pain, local edema, and systemic signs of infection. Postponing treatment leads to septic shock with high mortality. The treatment is based on "source control" principle with aggressive surgical debridement, broad spectrum antibiotics, and resuscitation. In cases such as described above, the assessment of fetal vitality is relevant, demanding joint efforts of surgeon, obstetrician, and intensive care specialists.

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Некротизирајућа инфекција меких ткива код трудница

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САЖЕТАК

Увод Некротизирајућа инфекција меких ткива (НИМТ) јесте по живот опасно стање које карактерише опсежна некроза коже, поткожног масног ткива, фасције и мишића. Лечење је хируршко уз антибиотике широког спектра. Морталитет је висок услед касног постављања дијагнозе. НИМТ је ретка у општој популацији и још ређа код трудница. На НИМТ се мора посумњати код перианалних инфекција, нарочито у трудноћи, као имуносупресивном стању.

Приказ болесника Приказан је случај двадесетједногодишње труднице са НИМТ која полази од перианалне регије. Примљена је у болницу у 31. недељи некомпликоване близаначке трудноће, дан након инцизије перианалног абсцеса. На пријему је стабилних виталних параметара прегледана и од гинеколога. Лабораторијски налази указивали су на присуство инфекције. Урађена је још једна хируршка интервенција и укључени су антибиотици широког спектра. Следећег јутра болесница се жалила на јак бол у трбуху. Клинички налаз је показао дискретно црвенило коже и њену повећану осетљивост, уз присуство крепитација. Индикована је хитна операција и интраоперативно је нађена масивна инфекција меких ткива која се пружа до зида грудног коша. Изведене су широке инцизије уз дебридман. Болесница је развила септични шок и после иницијалне ресусцитације гинеколог је ехосонографски утврдио интраутерину смрт оба плода и индуковао порођај. Током наредних дана опште стање болеснице се поправило. У неколико наврата рађен је агресивни дебридман у условима опште анестезије, што је довело до великог дефекта предњег трбушног зида. Двадесет три дана после иницијалне операције дефект је реконструисан коришћењем кожног графта са задовољавајућим резултатом.

Закључак Дијагноза и преживљавање НИМТ зависе од времена постављања дијагнозе и почетка третмана, јер је ток болести брз и скривен.

Кључне речи: некротизирајућа инфекција; некротизирајући фасциитис; трудноћа

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REWIEV ARTICLE / ПРЕГЛЕД ЛИТЕРАТУРЕ

Role of iodine in pathogenesis of thyroid disease – Is induction of apoptosis consequence of iodine cytotoxicity?

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SUMMARY
Iodine is one of the best-characterized environmental factors associated with autoimmune thyroid disease (ATD). Epidemiological studies have shown that ATD incidence has increased following the introduction of salt iodination in the 1920s; in addition, ATD patients can improve upon iodine restriction. In animal models such as BioBreeding/Worcester and Buffalo rats, obese chicken strain, and non-obese diabetic H-2h4 mice, excess iodine is associated with autoimmunity. Analyses of Hashimoto thyroiditis (HT) have shown enlarged number of apoptotic follicular cells, and the destruction is an effect of death receptor-mediated apoptosis. Excess of iodine induces rapid apoptosis of goitrogen Wistar pretreated rats, possibly connected with inhibition of polyamine synthesis, inhibitors of DNA fragmentation. Percentage of apoptotic cells was statistically higher in patients with HT than in those with euthyroid goiter, with significant increase of caspase 32. Genes for Bcl-2 and Bax proteins are under the transcriptional control of p53. In TAD-2 cell cultures, apoptosis is p53-independed, suggesting that DNA damage is not primarily evoked by potassium iodide (KI). High concentrations of NaI increase the proportion of apoptotic cells in FTRL5 thyroid cell line. Iodide cytotoxicity is inhibited by a TPO inhibitor and is relieved with an anti-oxidant agent. Chronic iodine excess induces apoptosis and necrosis of thyroid follicular and endothelial cells, leading to thyroglobulin accumulation in connective tissue. Iodide excess requires peroxidase enzymatic activity to induce apoptosis. Ionic iodide is not directly toxic, whereas its molecular form I_2 mediates the apoptotic effect of KI.
Keywords: iodine; apoptosis; autoimmune thyroiditis

INTRODUCTION

Iodine is a necessary component of normal thyroid hormonogenesis. It is incorporated into tyrosine moieties of thyroglobulin (Tg) as monoiodotyrosine and diiodotyrosine residues that subsequently undergo an oxidative coupling event leading to the formation of triiodothyronine (T3) and thyroxine (T4) [1]. The recommended daily allowance of iodine by the World Health Organization is 150 μg for adults (median urinary iodine concentration: 100–199 μg/l) [2, 3]. However, there is a relatively narrow interval of optimal iodine intake and both iodine deficiency and iodine excess can result in an increased prevalence of thyroid disorders [4, 5]. Environmental iodine deficiency had been a cause of iodine deficiency disorders for a long time round the world. It has been substantially reduced thanks to the implementation of programs of mandatory food iodine fortification in numerous countries. However, while this endeavor has led to virtual eradication in these regions of severe iodine deficiency, it has in parallel resulted in an increase in the prevalence of autoimmune thyroiditis (AIT). Meanwhile, it has recently been noted in various parts of the world that a decrease in iodine intake results in a lowering of the incidence of AIT [6].

Nowadays, the average dietary iodide intake can often exceed the recommended level [2]. Although it is usually considered to be safe to ingest a relatively large amount of iodine through diet, as most people are highly tolerant to iodine, the elderly population, pregnant women, fetuses, neonates, and those with pre-existing goiter or iodine deficiency are more susceptible to excess iodine-induced disorders, including autoimmune thyroid disease (ATD). Thus, iodine is indeed an environmental risk factor for the development of ATD, especially in susceptible individuals [7].

Epidemiologic studies in humans have reported an increased prevalence of thyroiditis with the administration of supplementary dietary iodine [1]. In addition, different animal models indicate that excess iodine is associated with thyroid autoimmunity. BioBreeding/Worcester (BB/W rats), an obese chicken strain, Buffalo rats, and non-obese diabetic (NOD) H-2h4 mice are all prone to develop AIT after high iodide intake [8–12]. Also, high doses of iodide have been known to cause direct thyroid cell injury on human thyroid follicles in vitro [2]. In vitro, iodide is cytotoxic, inhibits cell growth, and induces morphological changes in thyroid cells of some species [13].

Apoptosis (or programmed cell death) is an active process of cell self-destruction requiring the activation of a genetic program, leading to changes in morphology, DNA fragmentation, and protein cross-linking [13]. Physiological

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cell death is an essential mechanism which contributes to the growth and permanent maintenance of the human body [14]. The apoptotic pathways are activated by physiological stimuli such as environmental signals, cytokines, and growth factors, e.g. p53, caspases 2, 3, 8, and 9, BCL-XS and Bax [14]; they can also be induced by pathological stimuli, radiation, and anticancer drugs [13]. However, other mediators like B-cell lymphoma/leukemia-2 protein (Bcl-2), Bcl-XL, are antiapoptotic [14].

The two main pathways by which apoptosis can be initiated are (1) the mitochondrial or intrinsic apoptosis pathway, and (2) the death receptor-mediated or extrinsic apoptosis pathway.

(1) A number of internal stimuli cause an increase in mitochondrial membrane permeability. These different stressors are recognized by several intracellular proteins that send the signal to the mitochondria, ending in mitochondrial outer membrane permeabilization (MOMP). MOMP is most commonly mediated via a variety of protein–membrane and protein–protein interactions of the B-cell lymphoma-2 protein (BCL-2) family. Following apoptotic stimuli, members of BCL-2 family (BAX and BAK) activate and insert into the outer mitochondrial membrane to cause the release of cytochrome c and other mitochondrial proteins. Subsequently, in the cytosol, cytochrome c interacts with apoptosis protease-activating factor 1 (Apaf-1), and forms a complex recognized as the apoptosome. The apoptosome, a multi-protein platform comprising a seven-spoke ring-shaped complex, leads to activation of initiator caspase (usually caspase-9), which in turn activates executioner caspase-3 and initiates a caspase cascade, which eventually leads to the demolition of the cell [15]. Mitochondria-mediated apoptosis may be caspase-independent and it is mediated through apoptosis-inducing factor (AIF) 3 and endonuclease G [16].

(2) Apoptosis can be instigated through oligomerization of death receptors like Fas, TNFR, DR3, TRAIL-R4, and TRAIL-R5 after associating with their corresponding ligands. This oligomerization further leads to the employment of adaptor proteins and stimulation of caspase cascades. Preliminary stimulation of caspase-8 triggers apoptosis in two ways: it can directly cleave and initiate caspase-3, or it can cleave BH3 interacting domain death agonist (Bid), a proapoptotic Bcl-2 family member. This cleaved (or truncated) bid (tBid) is relocated to mitochondria, stimulating cytochrome c release, consecutively provoking caspases-9 and caspase-3, which eventually leads to DNA fragmentation and cell death [14].

In the past decade, it has become apparent that immune mediated cell death in a number of autoimmune endocrine diseases is due to the induction of apoptosis in target organ cells. This has been conclusively demonstrated for thyroid follicular cells in Hashimoto's (destructive autoimmune) thyroiditis, but the mechanisms underlying this cell death have not been made clear [17].

APOPTOSIS AND AUTOIMMUNE THYROID DISEASE

Autoimmune thyroiditis, also known as Hashimoto's thyroiditis (HT), is an organ-specific autoimmune disorder, characterized by infiltration of the thyroid gland by inflammatory cells, often followed by hypothyroidism due to destruction of the thyroid follicles and eventual fibrous replacement of the parenchymal tissue. Autoantibodies to thyroid-specific antigens also develop [18]. In AIT, lymphocytic infiltration and thyroid follicular cells apoptosis are important for the self-destructive process [19]. Thyroid gland immunohistochemical analysis in HT has shown a large number of apoptotic follicular cells, mostly in the periphery of lymphocyte infiltrates [20]; furthermore, in HT, caspase-3 and caspase-8 are upregulated and activated [21]. Thyrocyte destruction in HT might be a consequence of inadequate expression of Fas or TRAIL and reduced Bcl-2 induced by cytokines released from infiltrated lymphocytes [20]. Analysis of cytokine expression in ATD has shown, with a few exceptions, a prevalence of TH1 cytokines in HT. TH1 cells secrete IFN-γ and other cytokines that are associated with inflammation and cell-mediated immune response. IFN-γ treatment increases caspase-3 and caspase-8 expression and primes HT thyrocytes for CD95-mediated destruction [21]. In addition, some in vitro investigations have shown that low concentrations of TSH induce apoptosis and that TSH can prevent Fas-mediated apoptosis in HT. Nevertheless, some evidence suggests thyroid cell destruction in autoimmune hypothyroidism is dependent on T-cell-mediated cytotoxicity with the likely additional effect of death receptor-mediated apoptosis [20].

In addition, we performed this study in order to determine the role of apoptosis in the pathogenesis of lymphocytic thyroiditis (LT) and the existence of difference between HT and LT. We evaluated the apoptosis by in situ cell death detection TUNEL assay and the expression of Bcl-2 and Bax by immunohistochemistry in thyroid tissues from patient with HT and LT. Patients with euthyroid goiter served as a control group. We found that apoptosis of thyrocytes in HT and LT was statistically significantly higher than that in euthyroid goiter. Therefore, we concluded that apoptosis represents one of significant mechanisms in the pathogenesis of both HT and LT [22].

IODINE EXCESS AND THYROID DISEASE

Although the mechanisms are not fully elucidated, excess of iodine is a well-recognized environmental factor for ATD in autoimmune-prone individuals, particularly for AIT [7].

In animal studies it was shown that high doses of iodine induce thyrocyte injury in both the wild-type and obese strain that has a genetic background prone to spontaneous AIT. However, significant and sustained lymphocytic infiltration composed of $CD4^+$ T-cells, $CD8^+$ T-cells, B-cells, and macrophages was only observed in obese-strain chickens following iodine-induced cell injury. Pre-treatment with the antioxidant drug completely prevented both thyrocyte injury and the following lymphocytic infiltration induced by iodine. This study suggests that iodine excess can induce oxidative stress-related thyrocyte injury in individuals, although whether this cell injury leads to lymphocytic infiltration will depend on the additional effects

of genetic factors [7]. Interestingly, in a recent study, we noticed mild LT in the thyroid section from wild-type rats receiving potassium iodide (KI) [23]. This LT was characterized by diffuse mononuclear cell infiltration with lymphocytes and just a few plasma cells in the follicles and in the spaces between the follicles, with the destruction of gland acini and connective tissue proliferation.

Also, in one of our studies, we analyzed the histological changes of the thyroid gland after the administration of different doses of KI in a Wistar rat animal model. We revealed that the thyroid gland architecture was seriously damaged after the administration of KI. We compared the intensity of histological changes between rats from the Wistar strain that were treated with a low (LKI) and with a high iodine dose (HKI), while untreated non-immunized animals served as controls. The difference between them was statistically significant. Comparing controls and the group treated with LKI, a statistically highly significant difference was found, which was also the case with the group treated with HKI. However, a test revealed no statistically significant differences in animals treated with different doses of KI. The same paper proves iodine induces cell necrosis and inflammation in non-immunized animals without genetic susceptibility. Therefore, this is, in fact, a new experimental model of LT [24].

Several underlying mechanisms may explain how iodine induces AIT. Intake of large iodine quantities results in its increased incorporation into the Tg molecule. This highly iodinated Tg is characterized by alterations in its stereochemical conformation. The modifications that occur in Tg structure can change its properties, leading to loss of antigenic epitopes and to the creation of novel, iodine-containing ones. New antigenic determinants may be created by tyrosine iodination at critical points within the Tg molecule. When presented to T and/or B lymphocytes, these new determinants exhibit an increased affinity for the T-cell receptor or the MHC-presenting molecule on antigen-presenting cells (APCs). This may consequently enhance the Tg presentation by APCs and lead to specific T lymphocyte activation, thereby initiating the autoimmune process. Excessive iodination of Tg can thus heighten its immunogenic potential compared with Tg containing fewer iodine atoms. Another suggested mechanism is direct iodine toxicity to thyrocytes, possibly through induction of oxidative stress. Excessive amounts of iodine may comprise a direct threat for thyrocytes. TPO rapidly oxidizes excessive amounts of iodine in the hyperplastic thyrocytes and generates oxidative intermediates of iodine. These oxidative elements are highly reactive and able to bind to proteins, nucleic acids and membrane lipids, forming iodocompounds which damage thyroid cell and mitochondrial membrane integrity. Oxidative stress caused by the generation of free radicals can also lead to thyroid cell necrosis, while autoantigens may be released [25].

IODINE EXCESS AND THYROID CELLS APOPTOSIS

The iodide-induced cytotoxic effect on rat thyrocytes included necrotic and apoptotic features, indicating the involvement of a controlled process of cell death [13].

An in vitro study by Vitale et al. [13] of immortalized thyroid cell line (TAD-2) treated with KI demonstrates that human thyroid follicular cells react to an excess of iodide activating a cell suicide program. Similar sensitivity to KI excess was shown by thyroid primary cultures, whereas cells of non-thyroid origin were resistant, indicating that iodide cytotoxicity is tissue specific [13]. In line with these results, Smerdely et al. [26] demonstrated that high concentrations of NaI increase the proportion of cells undergoing apoptosis in FTRL5 thyroid cell line. Golstein and Dumont [27] confirmed that iodide induces apoptosis in the FTRL-5 cell line, but they also noticed necrosis. In the same article, iodide cytotoxicity was inhibited by a thyroid peroxidase inhibitor and was relieved with an anti-oxidant agent, indicating involvement of reactive oxygen species (ROS) in iodine-induced thyroid cell apoptosis. In contrast, dog thyrocytes in primary culture were not sensitive to iodide [27].

However, Kostić et al. [28] failed to demonstrate KI-induced apoptosis in primary human thyroid cells, and Pitsiavas et al. [29] did not demonstrate apoptosis on electron microscopy nether in Wistar rats' nor in BB/W rats' thyroid gland treated with iodide water. Nevertheless, one recent study sustained findings made by Vitale et al. [30] demonstrating KI-induced thyroid cell apoptosis in human thyroid follicular cells in vitro (Nthy-ori 3-1 cells). Furthermore, Gao et al. [31] demonstrated that excess iodine intake induces thyroid cell apoptosis in Wistar rat animal model, and one in vivo study on healthy Wistar rats showed that long term excessive iodine exposure promoted apoptosis of thyrocytes through the ROS pathway. This effect was reversible with iodine restriction. Interestingly, this treatment had no influence on either serum levels of TSH and FT-4 or the expression of Bcl-2 and Bax [32]. Genes for Bcl-2 and Bax proteins are known to be under the transcriptional control of p53. According to the results by Vitale et al [13], apoptosis in TAD-2 cell cultures is also p53-independent, suggesting that DNA damage is not a primary event evoked by KI. In the same study they show that this type of apoptosis is a process independent of protein synthesis. One of the Bcl-2 family members, Bad, does not require neosynthesis to regulate apoptosis, because its activity is regulated at the posttranscriptional level. Therefore, they propose that factors altered by KI excess might trigger apoptosis at a posttranscriptional level.

Basalaeva and al. [33] have demonstrated a significant increase of caspase-32 concentration in the thyroid gland from inbred female rats of a local laboratory strain, after single iodide dose of 8 μg/100 g. This data suggest iodide is inducing caspase dependent apoptosis in thyroid.

Iodide excess requires peroxidase enzymatic activity to induce apoptosis. Ionic iodide is not directly toxic for the follicular cell, whereas its molecular form I_2, produced by TPO oxidation, mediates the apoptotic effect of KI excess [13, 34]. It is demonstrated that molecular iodine excess induces apoptosis in thyrocytes through formation of free oxygen radicals that induce mitochondrial damage and cytochrome c release [35].

Iodine is taken up by the thyrocytes, organified, and inserted into Tg molecules through the enzymatic action

of thyroperoxidase. In doing so, generation of ROS occurs, such as superoxide anion and hydrogen peroxide (H_2O_2), which works as a donor of oxidative equivalents for thyroperoxidase [36]. Low H_2O_2 concentrations induce apoptosis in various cell types, including pig thyrocytes [37], which once more indicate that iodine-induced oxidative stress might be involved in thyroid apoptosis.

I_2 is a highly reactive molecule, able to react with proteins, lipids, and nucleic acids to form iodocompounds. Different types of iodolipids are produced when iodide binds to membrane lipids, and this could determine the loss of cell and mitochondrial membrane integrity with the generation of ROS and peroxidation of lipids [13]. One of them, delta-iodolactone (i.e., 5-iodo-delta lactone) of arachidonic acid (IL-d), was demonstrated by electron microscopy to induce apoptosis in porcine thyroid follicles ex vivo in a three-dimensional tissue culture. Interestingly, the induction of apoptosis was lowered by pre-incubating human thyroid follicles with low concentrations of selenium, which induced glutathione peroxidase activity. This is one more piece of evidence that the induction of apoptosis is mediated by free oxygen radicals in mitochondria [34]. Furthermore, IL-d has the goiter inhibitory activity due to the inhibition of cell proliferation and the transient stimulation of apoptosis. Interestingly, apoptosis in this case does not involve oxidative stress [38].

Another important iodolipid is 2-iodohexadecanal (2-IHDA), a compound proposed to be responsible for the Wolff–Chaikoff effect [39]. An increase in Bax/Bcl-2 ratio, in the percentage of apoptotic cells and caspase-3, activity was observed on FRTL-5 thyroid cell line treated with 2-IHDA. Activation of the caspase-3 pathway is a hallmark of apoptosis [40].

It was shown that excess iodine could induce apoptosis in the thyroid gland of goitrogen Wistar pretreated rats. This effect is very rapid and possibly connected with inhibition of polyamine synthesis, which are potent inhibitors of oligonucleosomal DNA fragmentation [41]. In line with this results, Boechat et al. [42] found higher levels of FasL expression, in NOD mice with methimazole-induced goiter after the administration of KI in animals sacrificed four days after the administration [42].

Some authors consider that follicular cell injury, apoptosis, and necrosis precede lymphocytic infiltration in the thyroid gland and they are considered the initial events in, and prerequisites for, the development of iodine-induced AIT [7].

We have previously shown that percentage of cells undergoing apoptosis was statistically higher in patients with HT than in those with euthyroid goiter [43]. In addition, we have shown enhanced expression of Bax pro-apoptotic proteins in the Wistar rat experimental model of thyroiditis induced by administration of different doses of KI, which can be regarded as a model of HT. These findings indicate the roll of apoptosis in the pathogenesis of LT in Wistar rats [44].

Recent studies in endogenous settings have demonstrated key roles for CIDEC (also known as fat-specific protein 27, or Fsp27) in energy metabolism. CIDEC was reported to induce apoptosis via the mitochondrial pathway through the cleavage of caspases-3, -7, and -9, and release of cytochrome c from mitochondria [41]. Swist et al. [45] demonstrated that high levels of iodine increased mRNA and protein levels of CIDEC in thyroiditis-prone BBdp rats. The apoptotic mechanism of Fsp27, which involves caspase-9 and mitochondrial cytochrome c, requires 174-192 amino acids of its CIDEC domain. Ectopic expression of Fsp27 induces enlarged lipid droplets in multiple human cell lines, which is indicative that its mechanism involves ubiquitously present, rather than adipocyte-specific, cellular machinery, and promotion of lipid droplet formation in HeLa cells via culture in exogenous oleic acid offsets Fsp27-mediated apoptosis. [46]. Although there is also evidence that CIDEC-induced apoptosis is dependent on activation of caspase-8, but independent on Fas-associated protein with death domain (FADD) [47]. Nevertheless, iodine doesn't have this effect in thyroiditis-resistant BBc rats [45]. These results suggest that iodine induces apoptosis in thyroiditis-prone animals.

Cultured thyrocytes, from NOD.H2h4 mice prone to develop AIT after high iodide intake, exposed to low NaI concentrations in vitro, are more susceptible to apoptosis compared to thyrocytes from CBA/J mice, which are resistant to iodide-accelerated spontaneous AIT. Explanation possibly lies in a fact that NaI intake upregulates the expression of 22 genes involved in ROS metabolism and/or antioxidant function in CBA/J thyrocytes, whereas only two of these genes were upregulated in NOD.H2h4 thyrocytes. The results demonstrate that an impaired control of oxidative stress mechanisms is associated with the observed high susceptibility of NOD.H2h4 thyrocytes to NaI-mediated apoptosis [48]. Iodine induced apoptosis in AIT might be through mechanisms that involve the activation of the BH3-interacting domain death agonist (BID) proapoptotic protein. BID is a proapoptotic Bcl-2 family member that functions as a bridge molecule between two classic apoptotic pathways, cell death receptors and mitochondrial elements, to augment apoptotic signaling. It was demonstrated that the increasing BID expression specifically in the thyroid gland in CBA/J (H-2 k) mice does not cause AIT. However, same strains of mice with thyroid-specific BID over-expression that were given iodine water are at high risk of developing AIT [49].

A number of apoptosis signaling pathways, including Fas ligand and tumor necrosis factor (TNF)-related apoptosis, inducing ligand (TRAIL), are thought to be implicated in destructive thyroiditis [6]. Excessive iodine could induce TRAIL and DR5 abnormal expression in the thyroid gland. Furthermore, one study suggests that TRAIL band with DR5 promotes follicular cells apoptosis, thus mediating thyroid destruction in experimental AIT in NOD mice [19].

Amiodarone, a potent antiarrhythmic drug containing two iodine atoms per molecule, may induce either hypo- or hyperthyroidism [13]. Rats receiving amiodarone expressed hypothyroidism with specific ultra-structural features of necrosis and apoptosis of the thyroid gland. Amiodarone induces thyroiditis that might be a form of endoplasmatic reticulum storage disease. This could be explained by excess iodide, from amiodarone or its metabolites, resulting in heavily iodinated proteins such as thyroglobulin and other polypeptides, which cannot be processed, folded, or trans-

ported to appropriate sites. Disruption in protein production may prevent synthesis of apoptosis inhibitors such as Bcl-2, or it may result in loss of essential proteins involved in cellular homeostasis, leading to cellular death [29].

Some studies indicate that amiodarone and its metabolite DEA (desethylamiodarone) induce apoptosis in thyroid and non-thyroid cells through an iodine-independent mechanism. Apoptosis induced by amiodarone and its main metabolite DEA is not mediated by modulation of p53, Bcl-2, Bcl-XL, or Bax protein expression and does not involve the generation of free radicals, whereas it induces the release of mitochondrial cytochrome c into the cytosol [50]. Since there is evidence that iodine induces apoptosis in thyrocytes, it remains to be resolved if apoptosis in amiodarone-induced hypothyroidism is iodine-induced or a result of direct drug cytotoxicity [13, 28].

Also, iodine can induce apoptosis in some non-thyroid tissues. Iodine excess increases the apoptosis rate in rat aorta endothelial cells that were cultured with iodide ion for 48 hours. Iodine also reduces the activity of superoxide dismutase, glutathione peroxidase, and concentrations of glutathione, suggesting that excessive exposure to iodine increases oxidative stress [51]. Furthermore, it has been shown experimentally that IL-d is able to trigger apoptosis in various cancer cell lines, including thyroid cancer and breast cancer [39]. Epidemiological studies have shown that sufficient iodine supply can prevent the development of thyroid cancer. Iodine can induce mitochondrial-mediated apoptosis in three different types of thyroid cancer cells. Thus, iodine-induced apoptosis may be the key mechanism that contributes to the prevention of thyroid cancer [52]. In addition, iodine induces apoptosis in cultured human breast cancer cell lines, namely MCF-7, MDA-MB-453, ZR-75–1, and T-47D. In MCF-7 cells, iodine treatment activates a caspase-independent and mitochondria-mediated apoptotic pathway. An interesting fact is that iodine treatment leads to a decrease in cellular ROS in MCF-7 after 24 hours [16].

CONCLUSION

An interesting question whether iodide itself displays cytotoxic effects on thyroid cells in the human thyroid gland and on experimental models, or its cytotoxicity represents an apoptotic phenomenon, still remains to be completely elucidated.

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Улога јода у патогенези болести штитасте жлезде: Да ли је апоптоза у штитастој жлезди узрокована цитотоксичношћу јода?

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САЖЕТАК

Јод је један од најпознатијих егзогених етиолошких фактора повезан са аутоимуним тироидитисом (АТ). Епидемиолошке студије су показале да је инциденција АТ порасла након увођења јодирања соли 1920. године. Такође, стање особа које болују од АТ се може поправити смањењем уноса јода. На животињским моделима као што су *biobreeding/worcester* и *buffalo* пацови, пилићи гојазног соја, као и мишеви *NOD.H-2h4*, показана је повезаност вишка јода са аутоимуношћу. У Хашимотовом тироидитису (ХТ) повећан је број апоптотичних фоликуларних ћелија у жлезди. Вишак јода узрокује брзу апоптозу у струми *wistar* пацова, што је можда подстакнуто кочењем синтезе полиамина, инхибитора ДНК фрагментације. Проценат ћелија у апоптози је статистички значајно већи у ХТ него у еутироидној струми. Једнократно давање јода довело је до повећања каспазе 32 у штитастој жлезди. Гени *Bcl2* и *Bax* су под транскрипцијском контролом *p53*. У *TAD* 2 ћелијским културама тиреоцита апоптоза је *p53* независна, из чега произилази да ДНК оштећење није примарно узроковано калијум-јодидом (KJ). Високе концентрације натријум-јодида (*NaJ*) повећавају проценат ћелија у апоптози у *FTRL5* ћелијској линији тиреоцита. Цитотоксичност јода је спречена инхибитором *TPO*, а слаби применом антиоксидансног агенса. Хронични вишак јода узрокује апоптозу и некрозу фоликуларних и ендотелних ћелија, те се онда у везивном ткиву нагомилава тиреоглобулин. Вишак јода подстиче активност пероксидазе у индукцији апоптоза. Јон јод није директно токсичан, док је молекулски облик J_2 медијатор апоптотског ефекта KJ.

Кључне речи: јод; апоптоза; аутоимуни тиреоидитис

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HISTORY OF MEDICINE / ИСТОРИЈА МЕДИЦИНЕ

Dragoljub (Bata) Adamov (1927–1996) – The first pacemaker implantation in Serbia

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SUMMARY
It has been over half a century since the implementation of pacemaker therapy in our country and the region. The first successful implantation of a pacemaker in former Yugoslavia and in Serbia took place on September 16, 1965 in "Dr. Dragiša Mišović" Clinical Hospital Centre, and this operation, with a team of doctors of the institution, was performed by surgeon Dragoljub (Bata) Adamov (1927–1996). The first permanent pacemaker implantation was with epicardial leads with thoracotomy approach. The patient was operated on under general anesthesia, administered by anesthesiologist Predrag Lalević (1927–), and Dr. Adamov was assisted by Dr. Miša Albrecht (1933–) and Dr. Milan Dragović (1933–2009).
Although pacemaker therapy has since been widely proven and confirmed, it is necessary to remember the pioneers who introduced this kind of therapy to the region, as they deserve a distinguished place in the history of medicine in Serbia.
Keywords: pacemaker; first implantation; history of medicine

INTRODUCTION

It has been over half a century since the implementation of pacemaker therapy in our country and the region. Today, in this area, the National Reference Center, "Prof. Dr. Milan Bane Đorđević" Pacemaker Center of the Clinical Center of Serbia epitomizes the highest level of application of this type of therapy. Pacemaker Center of the Clinical Center of Serbia bears the name of Professor Milan (Bane) Đorđević (1933–1993), the founder of this center, who established the standards for the use of pacemaker therapy and contributed immensely in its development and implementation [1].

However, it should be emphasized that the first successful implantation of a pacemaker in former Yugoslavia, and in Serbia, took place on September 16, 1965 at "Dr. Dragiša Mišović" Clinical Hospital Centre, and that operation, with a team of doctors of the institution, was performed by surgeon Dragoljub (Bata) Adamov (1927–1996) (Figure 1).

Figure 1. Dr. Dragoljub (Bata) Adamov

At the time, in Novi Sad, Serbia, Dr. Ivan Fajgelj (1919–2002) tried to save a patient with a complete atrioventricular block by connecting an electrode attached to the heart muscle with a low-voltage power source, a triggering switch being an ordinary musical metronome. Unfortunately, there are no precise data about this endeavor, which would be welcome, if for no other reason, as an illustration of doctoral inventiveness in solving the problem of life-threatening bradycardia.

Dragoljub Adamov was born in Kikinda, where he finished elementary school and began his high school education. When he was 14, he was arrested together with his father, Rada, by the collaborationist government police and brought to Topovske Šupe (Cannon Sheds in Serbian) in Kragujevac on October 21, 1941. The total number of hostages that were arrested amounted to around 6,000. The shooting of hostages began on October 21 at seven o'clock in the morning. Initially, German soldiers executed 2,301 persons, as a reprisal for the 10 dead and 26 wounded German soldiers. Immediately afterwards, they executed another 500, since in the struggle that arose while people were being rounded up and taken away to be shot, another five German soldiers got killed. Two hundred and fifty hostages were kept in reserve for ex-

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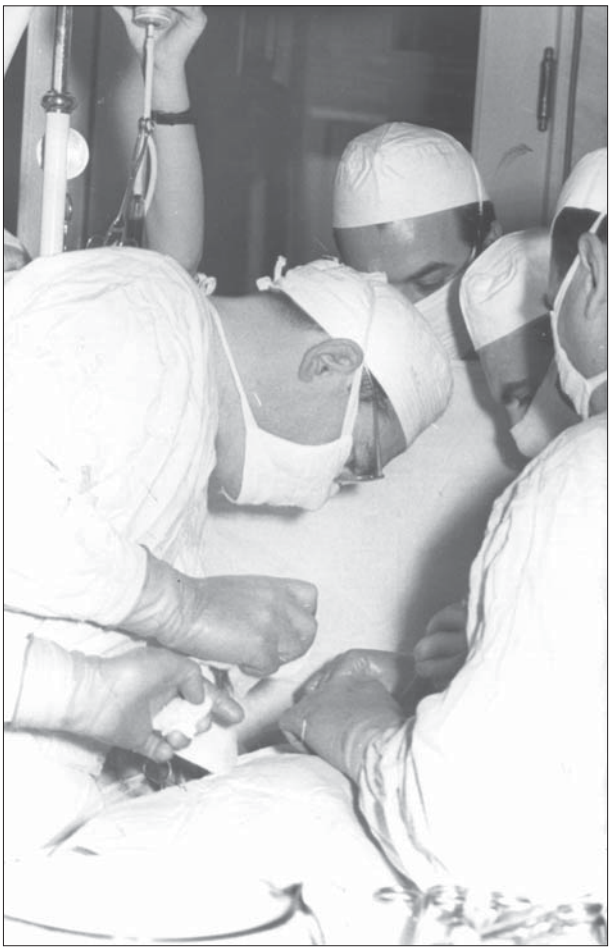

Figure 2. Dr. Adamov in the operating room during surgery

ecution, in case of riots in the city [2]. Among people who got released were Rada Adamov and his son Dragoljub, by pure chance. It should be noted that Dragoljub's parents, Nadežda and Rada, were teachers of history and geography, respectively, at the Kragujevac High School at the time.

After the war, Dragoljub Adamov began his medical studies, which ended in 1952 at the School of Medicine of Belgrade University. As a student he was interested in endoscopic procedures and began to work on them at the Military Medical Academy. He specialized surgery in Belgrade, after which he started working at the Bežanijska Kosa Hospital, predominantly as a thoracic surgeon.

Then he went on to the United States for a one-year training with Dr. Paul W. Sanger (1907–1968) in Charlotte, North Carolina, at the hospital which now bears the name of Dr. Sanger (Sanger Heart & Vascular Institute). After his training with Dr. Sanger, with whom he remained close friends, he continued his professional training at the Karolinska Hospital in Stockholm, Sweden, where he worked with Dr. Viking Bjork (1918–2009); his training was continued with Dr. Libor Hejhal (1924–1979) in Prague, then Czechoslovakia, where he studied in the field of vascular surgery [3]. During his training at the Karolinska Hospital, he had the opportunity to get acquainted with the concept of implantation of a whole pacemaker in the human body. In 1958, at this hospital, Dr. Åke Senning (1915–2000) successfully implanted the first pacemaker to patient Arne H. W. Larsson (1915–2001). The pacemaker was designed by engineer Rune Elmquist (1906–1996) [4].

After returning from professional surgical training, Dr. Adamov went to "Dr. Dragiša Mišović" Hospital in Belgrade. This hospital was founded in 1952 and had a department of general surgery, but very quickly specific groups of pathological conditions began to dictate further development of vascular, pulmonary, and cardiac surgery (application of hypothermia was made possible for the first time). It was the dominant motive for young surgeons to continue improving in exactly these areas [5]. The first complex cardiovascular surgery at this hospital was performed by Ivo Popovic Đani (1915–1986), and Dragoljub (Bata) Adamov [5].

In his later career, Dr. Adamov made great efforts to define and establish the Department of Thoracic and Vascular Surgery, as the first independent Center for Vascular Surgery in our country, which was realized in 1970. During the first several years, this department was headed by Dr. Adamov, with his primacy in cardiac pacing, but he focused his interest on the problems of vascular surgery, and made great efforts in allocating resources for founding a special hospital for vascular surgery and training of its staff. The 1970s began and ended with the construction of a new hospital building in Dedinje, a part of Belgrade, which became operational in October 1977 under the name of Department of Cardiovascular Surgery, what is today Dedinje Institute for Cardiovascular Diseases. Its first director was Dr. Adamov. The Department of Vascular Surgery was led by chief physician Miodrag Jevremović, and the Service of Cardiac Surgery by Professor Mihajlo Vučinić (1932–2004) [5].

In 1986, Dr. Adamov's illness forced him into early retirement, and he died in 1996.

FIRST PACEMAKER IMPLANTATION

In 1965, Dr. Adamov implanted the first permanent pacemaker with epicardial leads with thoracotomy approach (Figure 2).

This first implantation occurred on September 16, 1965 (hospital protocol number 1281, operations' protocol sequence number 1002) (Figure 3). The patient was operated on under general anesthesia, which was administered by anesthesiologist Predrag Lalević (1927–), and Dr. Adamov was assisted by Dr. Miša Albrecht (1933–) and Dr. Milan Dragović (1933–2009). Scrub nurse was Nada. Patient K.D., born in 1904, was operated on due to complete heart block.

The protocol is a description of the entire procedure, so it contains the following (the copy written into the protocol) (Figure 4):

Implantation of a pacemaker

Type: Permanent subcutaneous subepicardial automatical pacemaker-battery, made by Electrodyne[1] company, produced in June 1965. Impulse rhythm of 70 bpm.

[1] Pacemaker company Elctrodyne from the US was producing pacemakers at the time; subsequently it was sold to Becton Dickinson Company, which stopped pacemaker production in 1971.

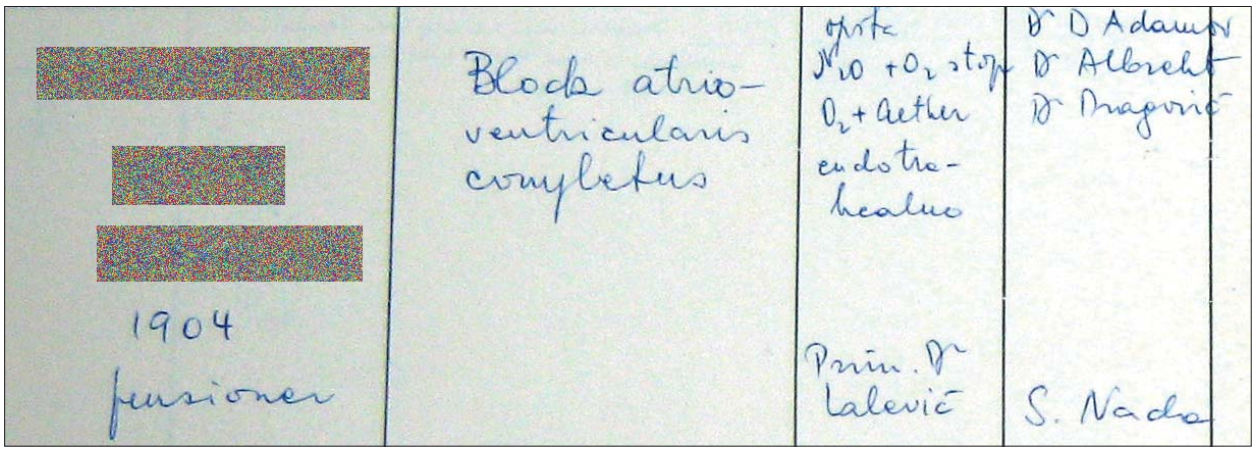

Figure 3. Part of the hospital protocol with the above diagnosis of the patient and the team that took part in the first pacemaker implantation

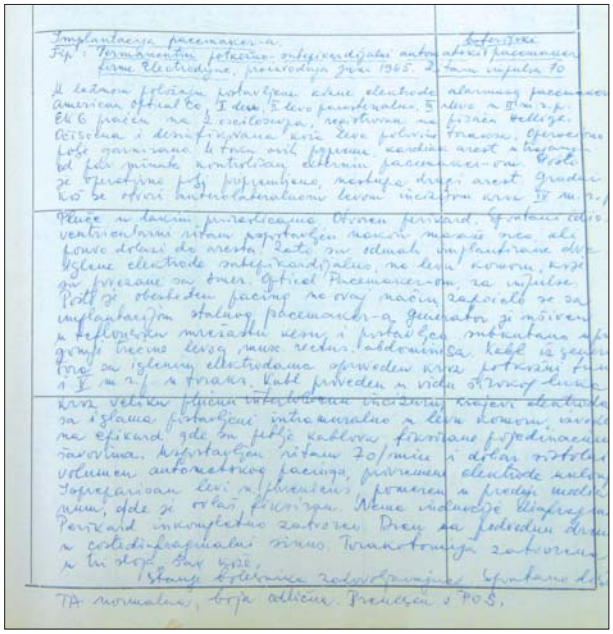

Figure 4. Page of the original protocol with the description of the entire procedure of the first pacemaker implantation

In the dorsal position, a set of skin electrodes of an alarm pacemaker by American Optical Co.[2] was set – I to the right, II left parasternal, III left in the second i.c.s. (intercostal space – author's note).

ECG monitored on two oscilloscopes, registered on a Hellige printer.

Skin of the left half of the thorax cleaned and disinfected. Operative area draped.

During preparations, cardiac arrest for a period of a few minutes, controlled by an external pacemaker. After the operative field is prepared, the second arrest ensues. The chest opened by anterolateral incision in the fourth i.c.s. Lungs in light adhesions. Opening the pericardium. Idioventricular rhythm spontaneously established after cardiac massage, but arrest ensues again. Thus, two needle electrodes immediately placed subepicardially, the left ventricle connected with Amer. Optical external pacemaker. Having secured pacing in this way, the implantation of a permanent pacemaker started. The generator sewn into a Teflon mesh bag and placed subcutaneously in the upper third of the left musculus rectus abdominals. The cable from the generator with a needle electrode administered through the subcutaneous tunnel and in the intercostal space in the thorax. The cable implemented in the form of a wide arc through the large pulmonary interlobar posterior, the ends of the electrodes with needles placed intramurally into the left ventricle, sewn onto the epicardium, where cable loops are held in place by sutures. Automatic pacing rhythm of 70 bpm established, good systolic volume, temporary electrodes removed. Preparation of the left n. phrenicus, it is moved to the front mediastinum, where lightly fixed. No induction of phrenic nerve stimulation and diaphragm stimulation. Pericardium incompletely closed. Underwater drainage in the costo-diaphragmal sinus. Thoracotomy closed in three layers, the skin sutured.

Condition of patient satisfactory. Spontaneous breathing, BP normal, excellent color, transferred to the ICU.

The operation was successful, and the patient survived for the next several years without significant complications and disorders. However, according to Professor Lalević, the whole story has a tragic outcome – the patient committed suicide, afraid that because of the pacemaker he would not be able to die [10]. Documents confirming this outcome do not exist, but this is what Dr. Adamov said in a conversation with Professor Lalević about the sad fate of the first patient with an implanted pacemaker in the history of Serbian medicine.

CONCLUSION

Dr. Adamov has left a large and significant trace in surgery in general, and will be remembered as a pioneer and the first doctor who implanted a permanent pacemaker, not

[2] American Optical Company (AO) from Southbridge (Massachusetts, USA), was founded in the 19th century; in the 1960s it began to produce equipment related to cardiology, which is linked to the arrival of engineer Berković (Barouh Vojtec Berkovits, 1926–2012). He constructed defibrillators and first pacemakers "on demand," which was not enough for commercial success in the beginning.

only in Serbia, but also in this part of Europe. Thus began the era of application of pacing in our country.

Although pacemaker therapy has since been widely proven and confirmed, it is necessary to remember the pioneers who introduced this kind of therapy to the region, as they deserve a distinguished place in the history of medicine in Serbia. The desire and intention of the author of this review was to remind all of us of it.

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Special thanks are also owed to Professor Aleksandar Nešković and to the family of Mr. Josip Klinger from Zurich, especially to his daughter Vesna, for locating and establishing contact with the Adamov family, Mr. Rade, Dr. Bata Adamov's son, and Rade's wife Bogdanka, to whom I owe special thanks for understand my desire for all of us to participate in recalling the indelible mark made by Dr. Dragoljub (Bata) Adamov in the application of this type of therapy.

Information on Dr. Ivan Fajgelj's attempt to create a pacemaker in Novi Sad using a metronome is derived from personal contact with Dr. Josip Lavac, whose father proposed a technical solution to this attempt to save a patient's life.

I express gratitude to Professor Predrag Lalević, who was a witness to the first pacemaker implantation and who has shared his memories with us.

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Драгољуб Бата Адамов (1927–1996) – прва уградња пејсмејкера у Србији

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САЖЕТАК

Протекло је преко пола века од почетака примене пејсмејкер терапије у нашој земљи. Прва, успешна уградња пејсмејкера у бившој Југославији, па тиме и у Србији, учињена је 16. септембра 1965. у болници „Др Драгиша Мишовић" у Београду, а операцију је, заједно са тимом лекара ове болнице, извршио хирург, примаријус др Драгољуб Бата Адамов (1927–1996). Прва уградња пејсмејкера је учињена приступом торакотомијом са применом епикардијалних електрода. Пацијент је оперисан у условима опште анестезије, коју је водио анестезиолог др Предраг Лалевић (1927–), а асистирали су др Миша Албрехт (1933–) и др Милан Драговић (1933–2009).

Примена пејсмејкер терапије касније је широко примењивана, али неопходно је подсетити на пионире примене ове врсте терапије у овом региону, јер су они својим местом у историји српске медицине такву пажњу засигурно и заслужили.

Кључне речи: пејсмејкер; прва уградња; историја медицине

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ERRATUM

Doppler changes as the earliest parameter in fetal surveillance to detect fetal compromise in intrauterine growth-restricted fetuses: Erratum

In the article that appeared on pages 69–73 of the January–February 2016 issue of the *Serbian Archives of Medicine* ("Srpski arhiv za celokupno lekarstvo"; Srp Arh Celok Lek. 2016 Jan-Feb; 144(1-2):69–73; doi: 10.2298/SARH1602069B), there is an inaccuracy regarding the stated authors of the article (Saloni Bansal, Deepika Deka, Vatsla Dhadwal, Rajiv Mahendru); the authors should be in fact listed in the following manner: **Saloni Bansal**, **Dipika Deka**, **Vatsla Dadhwal**, **Neeta Singh**, **Smriti Hari.** The authors, as well as the publisher, regret this error.

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Промене на доплеру као најранији показатељ у праћењу фетуса за установљавање угрожености плодова с интраутерусним застојем у расту: *Erratum*

У чланку који је објављен на странама 69–73 у јануарско–фебруарској свесци из 2016. године часописа „Српски архив за целокупно лекарство" (Srp Arh Celok Lek. 2016 Jan-Feb; 144(1-2):69–73; doi: 10.2298/SARH1602069B) постоји нетачност у вези са ауторима рада (Салони Бансал, Дипика Дека, Ватсла Дхадвал, Рацив Махендру); исправан списак аутора рада гласи: **Салони Бансал**, **Дипика Дека, Ватсла Дадхвал**, **Нита Синг, Смрити Хари**. Аутори, као и и издавач, искрено жале због грешке.

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 CA 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.

АДРЕСА:
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Уредништво часописа „Српски архив за целокупно лекарство“
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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.

ETHICAL APPROVAL. Manuscripts with human medical research should contain a statement that the subjects' written consent was obtained, according to the Declaration of Helsinki, the study has been approved by competent ethics committee, and conforms to the legal standards. Experimental studies with human material and animal studies should contain statement of the institutional ethics committee and meet legal standards.

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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.

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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.

Summaries and articles written in Serbian by authors from Serbia need to be written in the Serbian Cyrillic alphabet.

STRUCTURE OF THE MANUSCRIPT. All section headings should be in capital letters using boldface. Original articles, meta-analyses and preliminary and short communications should have the following section headings: Introduction (objective is to be stated in the final paragraph of the Introduction), Methods, Results, Discussion, Conclusion, References. A review article includes: Introduction, corresponding section headings, Conclusion, References. The firstly named author of a meta-analysis or a review article should cite at least five auto-citations (as the author or co-author of the paper) of papers published in peer-reviewed journals. Co-authors, if any, should cite at least one auto-citation of papers also published in peer-reviewed journals. A case report should consist of: Introduction (objective is to be stated in the final paragraph of the Introduction), Case Report, Discussion, References. No names of patients, initials or numbers of medical records, particularly in illustrations, should be mentioned. Case reports cannot have more than five authors. Letters to the editor need to refer to papers published in the *Serbian Archives of Medicine* within previous six months; their form is to be comment, critique, or stating own experiences. Publication of articles unrelated to previously published papers will be permitted only when the journal's Editorial Office finds it beneficial.

All enclosures (tables, graphs, photographs, etc.) should be placed at the end of the manuscript, while in the body of the text a particular enclosure should only be mentioned and its preferred place indicated. The final arrangement (position) of the enclosures will depend on page layout.

ABBREVIATIONS. To be used only if appropriate, for very long names of chemical compounds, or as well-known abbreviations (standard abbreviations such as DNA, AIDS, HIV, ATP, etc.). Full meaning of each abbreviation should be indicated when it is first mentioned in the text unless it is a standard unit of measure. No abbreviations are allowed in the title. Abbreviations in the summary should be avoided, but if they have to be used, each of them should be explained when first mentioned in the text of the paper.

DECIMAL NUMBERS. In papers written in English, including text of the manuscript and all enclosures, a decimal point should be used in decimal numbers (e.g. 12.5 ± 3.8), while in Serbian papers a decimal comma should be used (e.g. 12,5 ± 3,8). Wherever applicable, a number should be rounded up to one decimal place.

UNITS OF MEASURE. Length, height, weight and volume should be expressed in metric units (meter – m, kilogram – kg, gram – g, liter – l) or subunits. Temperature should be in Celsius degrees (°C), quantity of substance in moles (mol), and blood pressure in millimeters of mercury column (mm Hg). All results of hematological, clinical and biochemical measurements should be expressed in the metric system according to the International System of Units (SI units).

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.

Video-articles are to last 5–7 minutes and need to be submitted in the *flv* video format. The first shot of the video must contain the following: title of the journal in the heading (*Serbian Archives of Medicine*), title of the work, last names and initials of first and middle names of the paper's authors (not those of the creators of the video), year of creation. The second shot must show summary of the paper, up to 350 words long. The final shot of the video may list technical staff (director, cameraman, lighting, sound, photography, etc.). Video-articles need to be submitted along with a separate summary (up to 350 words), a single still/photograph as an illustration of the video, and a statement signed by the technical staff renouncing copyrights in favor of the paper's authors.

To check the required number of words in the manuscript, please use the menu *Tools–Word Count*, or *File–Properties–Statistics*.

ARTICLE ENCLOSURES are tables, figures (photographs, schemes, sketches, graphs) and video-enclosures.

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