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ЗА ЦЕЛОКУПНО ЛЕКАРСТВО

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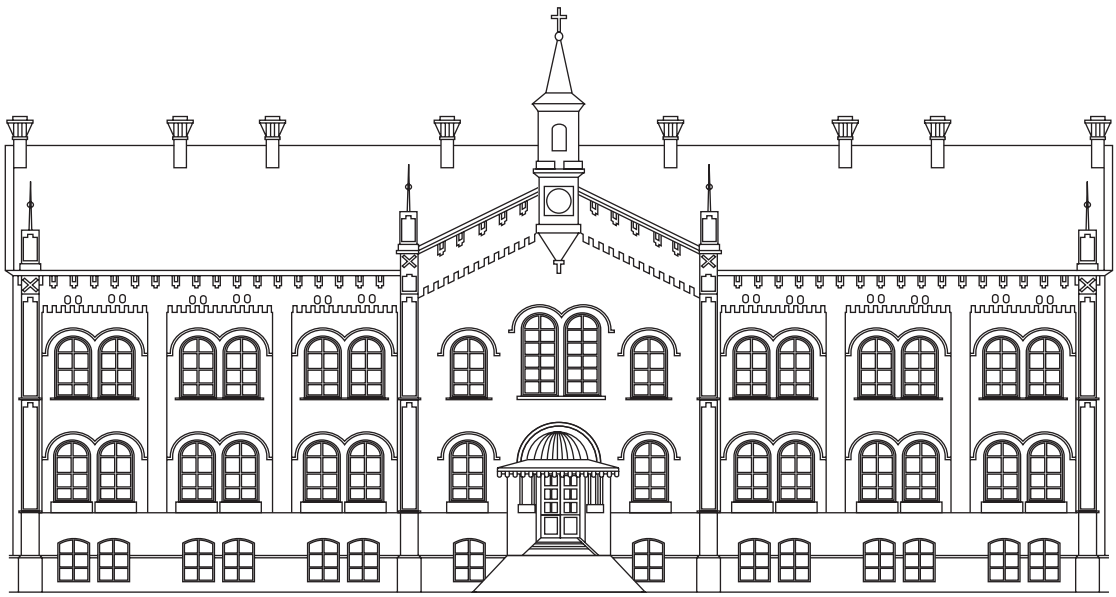

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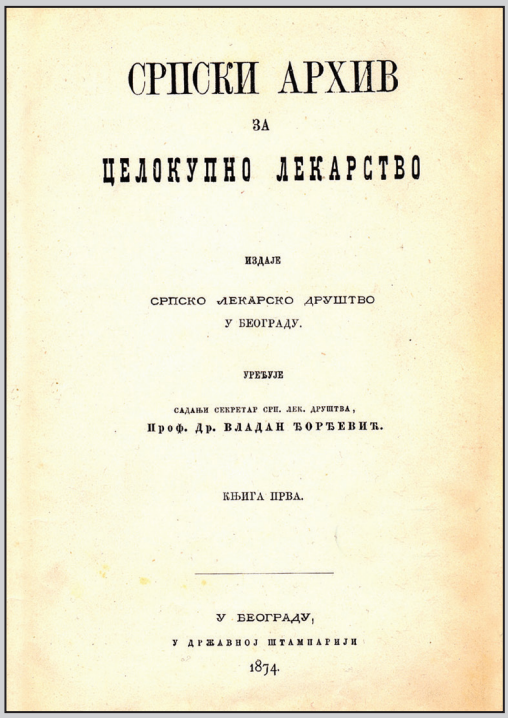

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У БЕОГРАДУ.

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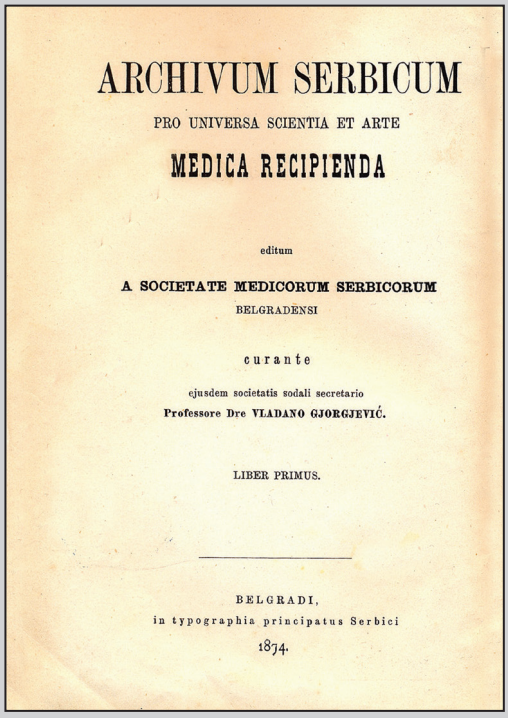
У БЕОГРАДУ,

У ДРЖАВНОЈ ШТАМПАРИЈИ

1874.

Прва страна првог броја часописа на српском језику



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BELGRADI,

in typographia principatus Serbici

1874.

The title page of the first journal volume in Latin

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Highly cited papers in dental medicine based on Essential Science Indicators

Jelena Jaćimović[1], Renata Petrović[2], Tihana Divnić-Resnik[3], Tina Pajević[4], Milica Popović[5], Dejan Stamenković[6], Đorđe Stratimirović[7]

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SUMMARY

Introduction/Objective Essential Science Indicators (ESI) Highly Cited Papers (HCPs) refer to the most influential scientific articles and breakthrough research within a research field in the past decade. This study aimed to identify the characteristics of ESI HCPs in the subject category Dentistry, Oral Surgery and Medicine, to recognize authors, institutions and countries of origin, and determine research trends that attracted the most scientific interest in dentistry.

Methods A descriptive analysis of bibliographic data, network extraction and visualization were completed. Furthermore, analyzed ESI HCPs were classified according to a field of interest, main research domain, type of study, and level of evidence.

Results The set of 185 dental HCPs was published in 42 journals from 2010 to 2020, with an average number of 211.7 citations per paper. Nearly half of HCPs were issued by the *Journal of Dental Research*, *Dental Materials*, and *Journal of Clinical Periodontology*. There were 765 authors affiliated with 351 institutions from 42 countries. The most productive institutions were the University of Hong Kong, the University of Michigan, and the University of Bern. The United States of America contributed with the highest number of publications, followed by China, and the United Kingdom. Dental materials, dental implantology, periodontology, and oral and maxillofacial surgery represented areas of significant interest within this subject category. The highest proportion of HCPs were narrative and systematic reviews, expert opinions, consensus reports, and *in vitro* or lab studies.

Conclusion Results obtained from this study can provide valuable information for researchers to better identify present and future hotspots in dental research.

Keywords: bibliometrics; social network analysis; knowledge discovery; research fronts; dentistry

INTRODUCTION

The number of citations a scientific article receives describes one aspect of its academic impact. Citing, as one of the principal forms of scientific communication, can be quantified by bibliometric methods. Citation analysis and indicators, such as the total number of citations or h-index, can be used to identify highly cited articles, authors, institutions, countries, or source journals. Additionally, popular research topics can be recognized, allowing scientists to efficiently comprehend current research trends and design future studies.

The Clarivate Analytics' InCites Essential Science Indicators (ESI) stands out as one of the datasets delivering in-depth coverage for efficient analysis and benchmarking research performance [1]. It is an integrated collection of data on the most inspiring, readable, and citable papers in the sciences, including research fronts as one of its features. Highly Cited Papers (HCPs) represent the most influential research articles in each research field annually. Since its introduction in 2002, ESI HCPs have given great importance to the science of science, significantly supporting the empirical study of scientific practice [2]. They have been widely used to evaluate research performance at different levels (researcher, research institution, university, country of origin) and from different aspects, comparing the quality of an article with the highly-rated papers published each year [3]. ESI HCPs data may be used by various stakeholders in the decision-making process related to research funding or university promotions [4].

Bibliometric performance based on citation data has been widely analyzed in various fields of dentistry. Previously published bibliometric studies have described the most cited (also labeled as highly or top-cited) articles published in the field of dentistry, oral surgery and medicine (DOSM) [5, 6] or in specific journal/s [7, 8]

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or subareas, such as prosthodontics [9], pediatric dentistry [10], endodontics [11], orthodontics [12], dental implantology [13], periodontology [14], oral pathology and medicine [15], or oral and maxillofacial surgery [16]. However, in all these studies, the term highly cited paper refers to articles whose total number of received citations, mostly from the Web of Science (WoS) Core Collection, is equal to or higher than 100, regardless of the papers' publication date. The analysis of dental ESI HCPs, related to the top 1% of papers in the medical research area, has so far not been the subject of bibliometric studies.

The main aim of this study was to identify the characteristics of ESI HCPs in the WoS subject category of DOSM, to recognize authorships, institutions, and countries of origin and determine emerging research trends in dentistry. This knowledge related to the current status within dental research areas of significant interest may provide a valuable reference to original investigations available for decision making and clinical practice support, allowing scientists to efficiently comprehend prevailing research trends and design future studies.

METHODS

Data sources

To identify breakthrough research within the field of dentistry, we searched WoS for all papers published in journals related to the subject category DOSM, using the WoS subject category field. Subsequently, ESI HCPs were selected on the retrieval result page. The identified set of 185 HCPs, obtained on July 12, 2020, was part of the second bi-monthly of 2020 (updated on July 9, 2020), which covered the period from January 1, 2010 to April 30, 2020.

Data extraction

Complete ESI HCP metadata was exported in plain text format from WoS and imported into the R environment for statistical computing and graphics [17]. In addition to bibliographic attributes stored in WoS, such as authors' names and affiliations, year of publication, document title, abstract, publication name, document type, language, citation count, cited references, authors' keywords, or Keywords Plus, Journal Impact Factor (JIF) Quartile in the category DOSM (based on the Journal Citation Reports 2019) was also recorded. Three reviewers classified selected ESI HCPs according to a field of interest, main research domain, type of study, and level of evidence (LoE). Any disagreement between the reviewers was resolved by discussion with the fourth reviewer.

Data analysis

A descriptive analysis of bibliographic data and network extraction were completed using the Bibliometrix R-package version 3.0.2 (Bibliometrix, Naples, Italy) [18]. The main results of the bibliometric analysis describe the collection in terms of the number of papers, authors, institutions, countries, sources, and keywords. Collaboration analysis was used to identify co-authorships and determine collaboration networks. Word co-occurrence analysis mapped and clustered terms extracted from keywords, titles, or abstracts. Correspondence analysis was performed to illustrate a conceptual structure of the analyzed dataset and K-means clustering to identify clusters of papers that express common concepts. Bibliometric networks were graphically presented using VOSviewer software (VOSviewer, Leiden, the Netherlands) [19] and the R packages Bibliometrix version 3.0.2 [18], as well as Wordcloud2 (R project, Vienna, Austria) version 0.2.1. Geomapping was completed using the R project Rworldmap version 1.3.6 [20].

RESULTS

The search yielded the top 185 papers related to the category of DOSM that had been identified in the ESI database. A complete list of analyzed HCPs is given in Appendix Table 1 (Available from: http://srpskiarhiv.rs/global/pdf/tables/AppendixTable1.pdf). Of the 185 papers, 168 journal articles and 17 proceedings papers published in English were selected for the analysis. The annual distribution of all HCPs published from 2010 to 2020 is presented in Table 1. The average number of years since the publication in the dataset was 5.3 years. One of the HCPs was published early online [21]. Almost half (44.9%) of HCPs (n = 185) were Open Access articles, mostly published in *Journal of Dental Research* (n = 18), *Journal of Clinical Periodontology* (n = 13), *Dental Materials* (n = 8), or *Journal of Periodontology* (n = 7). More than half of dental HCPs (56.8%) were funded studies, published mainly in *Journal of Dental Research* (n = 25), *Journal of Clinical Periodontology* (n = 21), *Journal of Periodontology* (n = 10), and *Dental Materials* (n = 9) respectively.

Table 1. Annual distribution of Highly Cited Papers

Year	N	mTCA	mTCY	Citable Years
2010	14	420.7	42.1	10
2011	16	383.8	42.6	9
2012	23	310.3	38.8	8
2013	23	237.4	33.9	7
2014	16	288.5	48.1	6
2015	24	192.2	38.4	5
2016	9	162.1	40.5	4
2017	13	104.9	35.0	3
2018	25	74.2	37.1	2
2019	11	24.3	24.3	1
2020	11	32.7		0

N – the total number of articles; mTC_A – mean total number of citations per article; mTC_Y – mean total number of citations per year

Authorship, institution and country of origin

The analysis showed that 765 authors were responsible for 1058 authorships and affiliated to 351 institutions from 42

Table 2. Study design and the distribution of Highly Cited Papers within the pyramid of evidence

Study design/LoE	0	I	II	III	IV	V	VII	**Total**
Case control study					1			1
Case series						7		7
Cohort study					4			4
Controlled clinical trial				3				3
Cross sectional study						6		6
N/A; economic evaluation	2							2
Epidemiological report					2			2
Expert opinion and panel consensus							25	25
In vitro/lab studies	15							15
Meta-analysis		1						1
Narrative literature review							70	70
RCT			3					3
N/A; short communication/ others	1							1
Systematic literature review	2	20			12	3		37
Systematic review and meta-analysis		5			3			8
Total	20	26	3	3	22	16	95	185

RCT – randomized clinical trial; LoE – level of evidence; LoE I – systematic review or meta-analysis of RCTs; LoE II – randomized controlled clinical trial; LoE III – controlled clinical trials; LoE IV – cohort study, case control study, systematic review or meta-analysis of cohort and case control; LoE V – cross–sectional study and case series, systematic review or meta-analysis of cross–sectional studies and case series; LoE VI – case report; LoE VII – narrative literature review, panel and expert opinions; LoE 0 – animal research, in vitro / lab studies; the level of evidence of systematic reviews and meta-analyses depend on the types of studies reviewed

countries. The number of authors in HCPs ranged 1–34, while the average number of authors per article was 4.14. Of the 185 HCPs in dentistry, eight (4.3%) were single-authored, 41 (22.2%) were written by two, 28 (21.5%) by three, and the remaining 41.6% by four or more authors. Ferracane [22, 23] (Oregon Hlth & Sci Univ, USA) stood out as the only author who had written two single-authored HCPs, both dealing with resin composites and being published in *Dental Materials*. Appendix Table 2 (Available from: http://srpskiarhiv.rs/global/pdf/tables/AppendixTable2.pdf) provides a list of the 30 most productive authors of dental HCPs. The ranking of authors was based on their number of total articles and adjusted frequency that reflected multiple-authored articles (for instance, if an article was published by two authors, each received half a credit). According to number of total articles, the most productive author who appeared in nine HCPs was Tonetti MS, whose fractionalized frequency was equal to 1.7, considering the number of co-authors. However, based on adjusted frequency, two other top productive authors with 2.17 fractionalized frequency were Parirokh M (Kerman Univ Med Sci, Iran) and Torabinejad M (Loma Linda Univ, USA). Authors of the analyzed HCPs were also ranked based on dominance factor, which reflected the proportion of the number of multi-authored publications of an author as the first author to the total number of multi-authored publications of an author. According to dominance factor value, Isola G (Univ Catania, Italy) and Eke PI (CDC, Ctr Dis Control & Prevent, USA) dominated their research teams as they were the first authors in all of their papers (four for Isola G and three for Eke PI). Scientific collaboration network is given in Figure 1, which discovers regular study groups, hidden scholarly groups, and pivotal authors of the HCPs in dentistry.

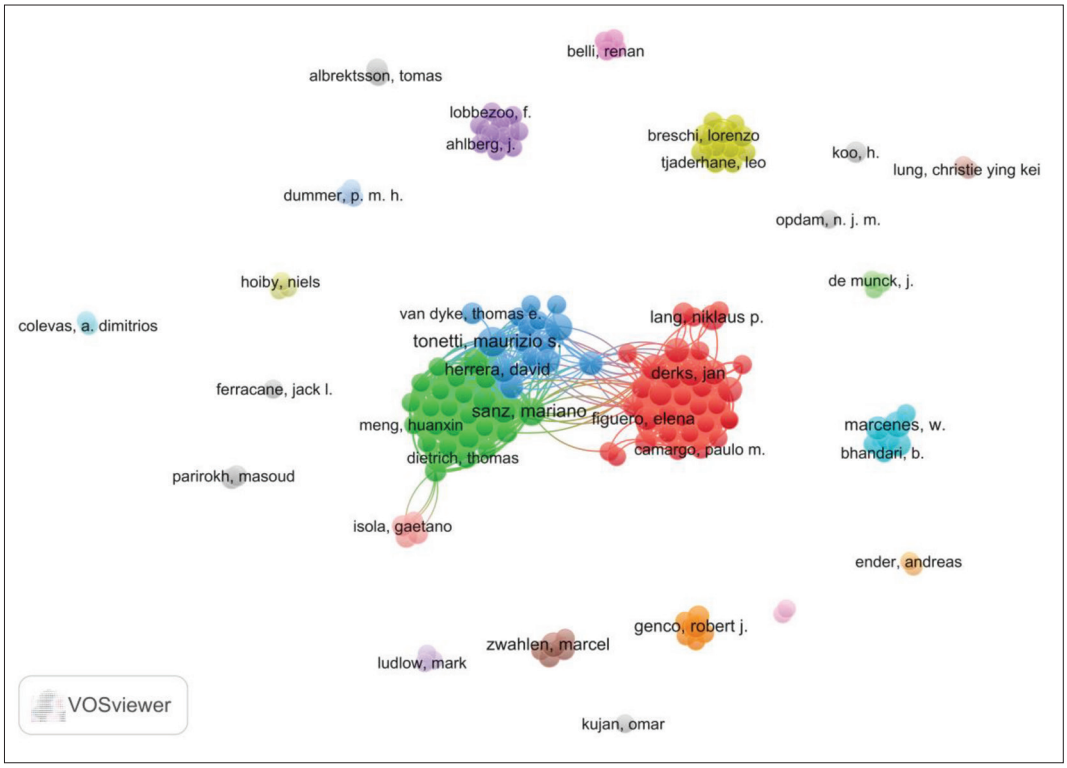

Figure 1. Author collaboration network

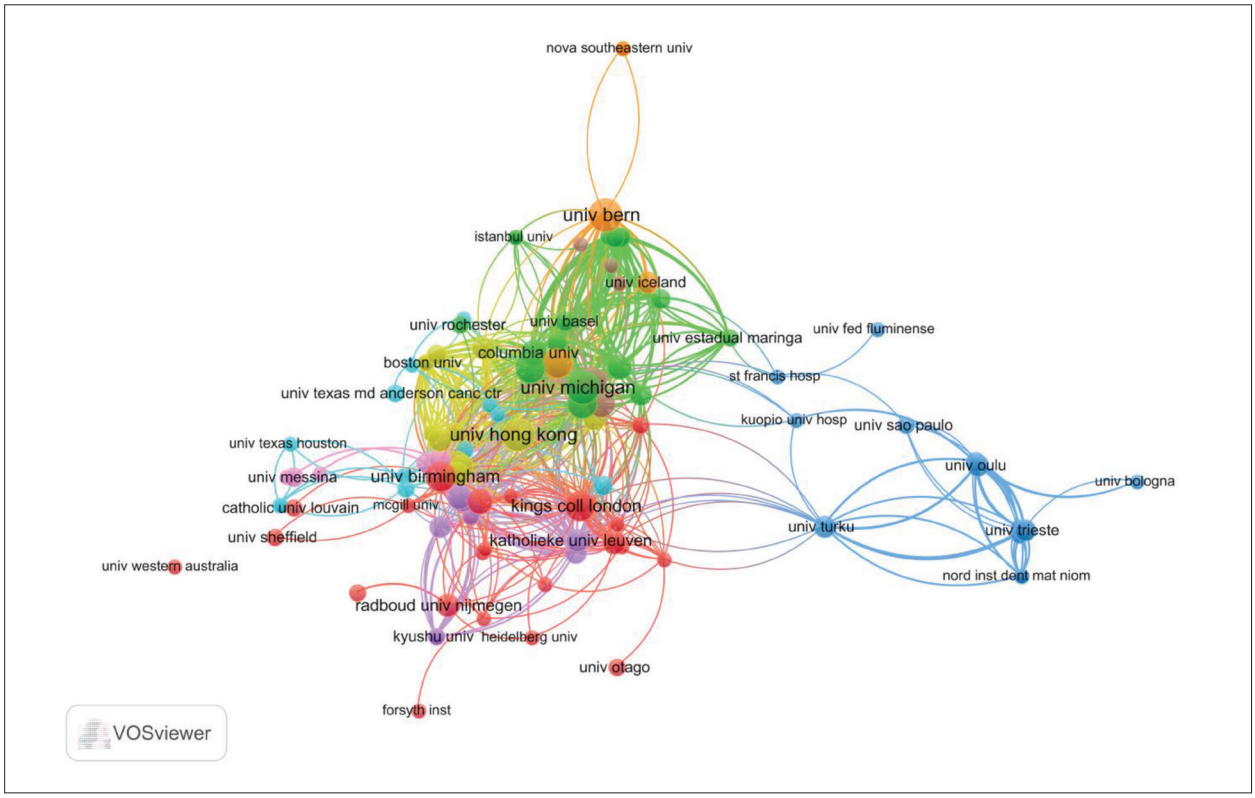

Figure 2. Institutional collaboration network

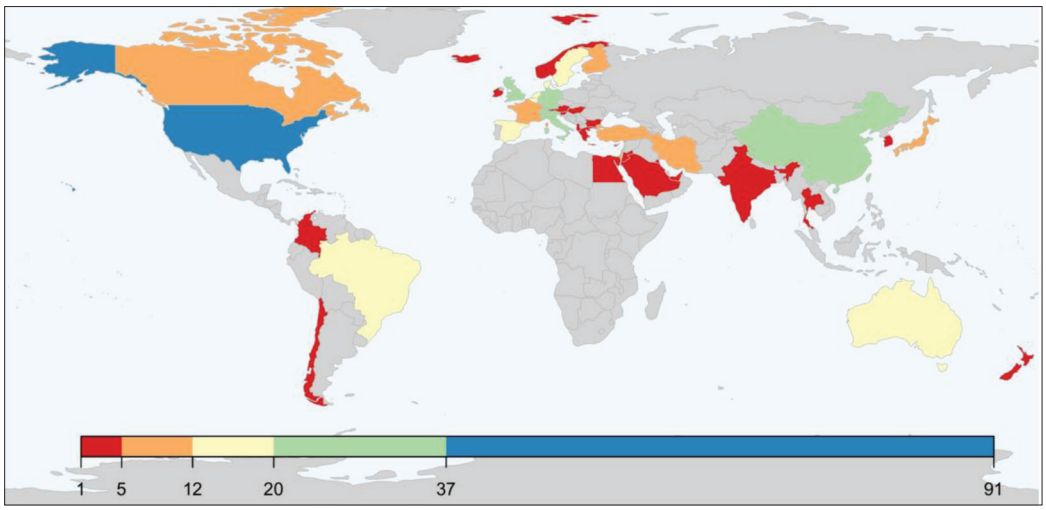

Figure 3. Participation of each country in the distribution of Highly Cited Papers

Almost a quarter of the analyzed HCPs (n = 43) were single-institution papers. Appendix Table 3 (Available from: http://srpskiarhiv.rs/global/pdf/tables/AppendixTable3.pdf) presents the institutions with at least five HCPs, ordered according to the number of total articles. Among 351 institutions, the most productive institution was the University of Hong Kong (n = 17), followed by the University of Michigan (n = 16), the University of Bern (n = 16), the Complutense University of Madrid (n = 13), and the University of Birmingham (n = 13). The collaboration network among the 70 most productive institutions is given in Figure 2, color-coded by clusters. The Complutense University of Madrid, Spain was the most collaborative institution, having the greatest number of links (n = 64).

The top 185 HCPs originated from 42 countries, including 22 European, 11 Asian, five American, two Oceanian, and two transcontinental countries (Turkey and Egypt) (Appendix Table 4 (Available from: http://srpskiarhiv.rs/global/pdf/tables/AppendixTable4.pdf)). Nearly half of the ESI HCPs (47.6%) were single-country papers, originating from 20 countries. Figure 3 shows the participation of each country in the distribution of HCPs. The USA stood out as the most productive country with an apparent advantage (n = 91), followed by China, the United Kingdom, and Germany. In addition to total HCPs Appendix Table

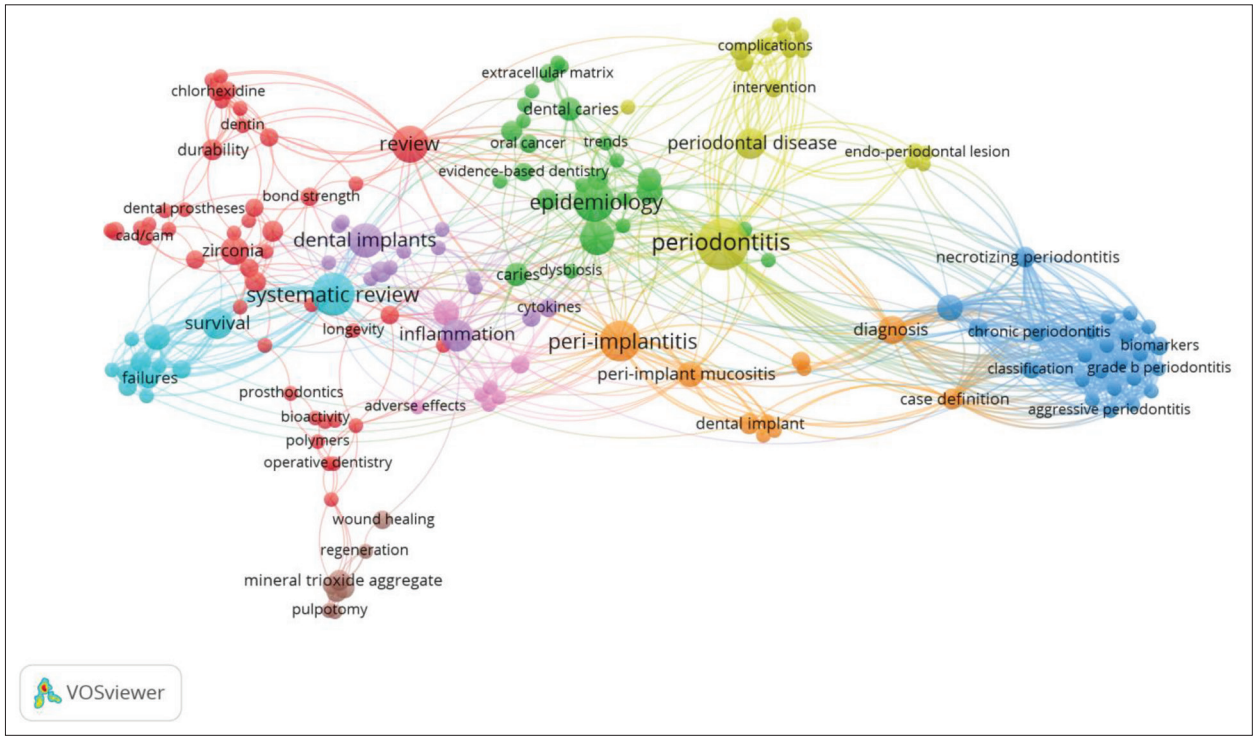

Figure 4. The most frequently used author's keywords

4 (Available from: http://srpskiarhiv.rs/global/pdf/tables/AppendixTable4.pdf) presents the number of HCPs based on the corresponding author's country of origin, as well as the number of single or multi-country papers. Among 91 HCPs from the USA, 54 were papers in which the corresponding author was from the USA. The 31 papers originated from single-country, while 60 were the result of international collaboration.

Journals

HCPs in DOSM were published in 42 journals. Appendix Table 5 (Available from: http://srpskiarhiv.rs/global/pdf/tables/AppendixTable5.pdf) shows journals' information regarding the JIF 2019 Quartile in DOSM Category, the total number of published HCPs, the total number of received citations, the average number of citations per paper, and the year when the first HCP was published in a journal. The highest number of HCPs (43.24%) was published in the *Journal of Dental Research*, followed by *Dental Materials* and *Journal of Clinical Periodontology*. HCPs published in these journals were papers with the highest total number of received citations. The highest number of average citations per paper had articles published in the *Journal of Oral & Facial Pain and Headache* (mTC_A = 820) and *Journal of Oral and Maxillofacial Surgery* (mTC_A = 771). Most of the HCPs were published in the first (42.1%) and second (31%) JIF Quartiles in DOSM.

Citations

The 185 HCPs received a total of 39,161 citations, ranging 4–1471 citations per paper. The average number of

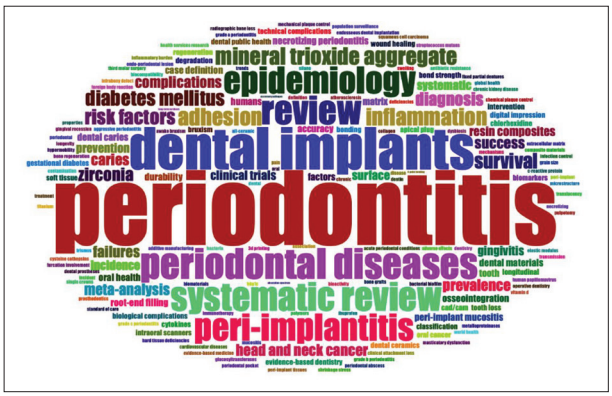

Figure 5. Word cloud of the most frequently used author's keywords

citations per paper was 211.7, while the average number of citations per year per paper was 31.34. The 20 most influential HCPs ranked by the total number of citations and the total citations per year are presented in Appendix Table 6 (Available from: http://srpskiarhiv.rs/global/pdf/tables/AppendixTable6.pdf). The most cited HCP was published in the *Journal of Dental Research* [24], with a total of 1,471 citations. However, when the date of publishing is taken into account, the HCP published in the *International Journal of Oral Sciences* [25] stood out as the most cited paper, with 135 total citations per year. Among the top 20 cited HCPs, 65% were review articles including seven narrative and six systematic literature reviews. The citation analysis revealed that the most cited papers were from the USA with a total of 15,307 citations, followed by the UK (total citations = 3621), China (total citations = 2744), and Germany (total citations = 2229).

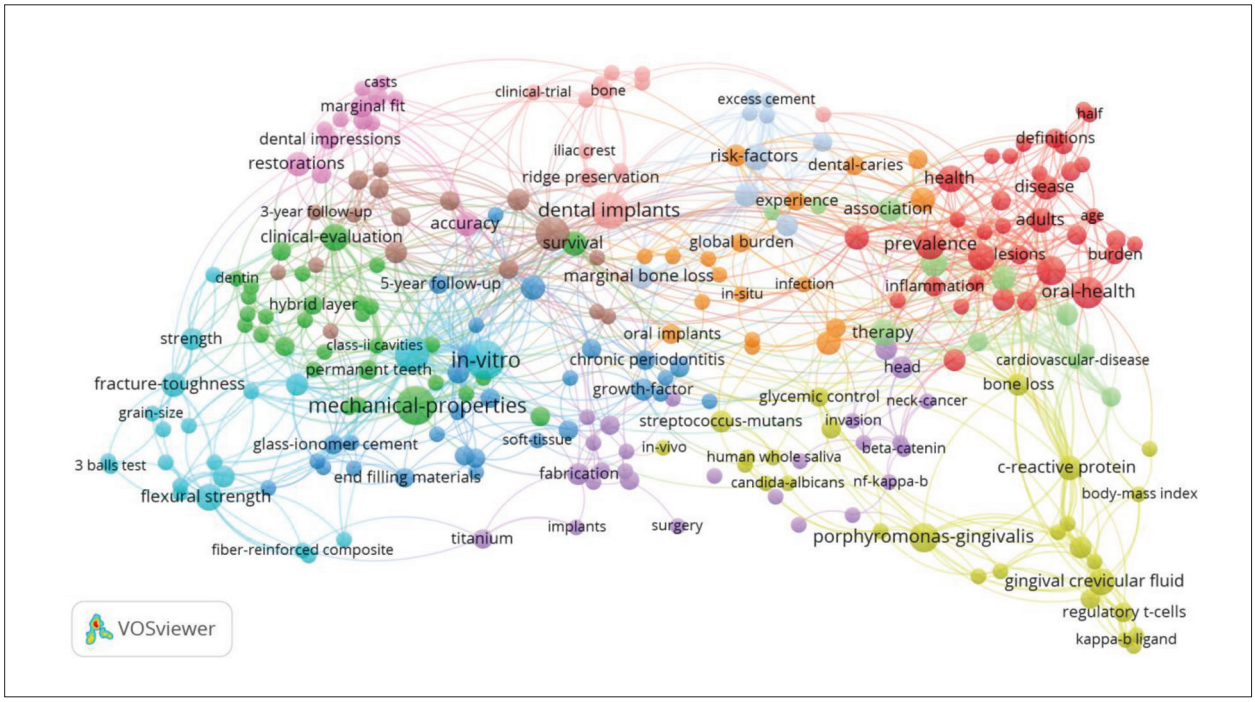

Figure 6. The most frequently used Keywords Plus
Note: Keywords Plus – keywords obtained from the titles of references that were not necessarily provided by the authors

Research topics

Hot topics of ESI HCPs in DOSM are shown in Figure 4 and through the word cloud of the most frequently used author's keywords (Figure 5). The size of the displayed terms indicated that papers related to 'periodontitis' and 'periodontal diseases', as well as 'dental implants', comprised a substantial portion of HCPs in dentistry. Other keywords such as 'review', 'systematic review', 'epidemiology', 'peri-implantitis', 'adhesion', 'inflammation', or 'mineral trioxide aggregate' were also highly prevalent. Figure 6 illustrates the co-occurrence network of used Keywords Plus.

Field of interest, study design, research question and level of evidence

Analysis of 185 HCPs based on a field of interest classification, revealed that almost 25% of papers were related to dental materials, followed by 21.62% and 16.22% related to periodontology and dental implantology respectively (Appendix Table 7 (Available from: http://srpskiarhiv.rs/global/pdf/tables/AppendixTable7.pdf)). In addition to papers focused on therapy (56.22%), the most common research issues were etiology (16.22%), pathogenesis (12.97%), diagnosis (11.89%), and epidemiology (10.81%). The highest proportion of HCPs were narrative (37.84%) and systematic (24.32%) literature reviews, followed by expert opinion and panel consensus papers (13.51%) and in vitro/lab studies (8.11%) (Table 2). The lowest portion of HCPs were case-control studies and short communications (1.08%). The highest number of HCPs were within LoE VII (n = 95), followed by LoE I (n = 26), LoE IV (n = 22), and LoE 0 (n = 20).

DISCUSSION

This bibliometric study is the first of its kind to identify and describe the main characteristics of ESI HCPs in the field of DOSM. The analysis of 185 HCPs published 2010–2020 revealed key concepts of dental research, prominent individuals, institutions, articles, journals, and countries that have contributed to the advancement of the field.

Most of the analyzed HCPs were published in 2018, which supports the fact that the time of publication of the article does not necessarily have to be a critical factor for inclusion in the ESI database [26]. Moreover, one of the most recent HCPs published in 2020 was the article with the highest number of received citations per year [25]. The paper, dealing with the potential routes of 2019-nCov infection of the oral cavity mucosa, directly reflects ESI's capacities for recognizing hot topics and future research directions.

The characteristics of the analyzed set of HCPs are mostly in line with the verified features of the ESI highly cited articles in terms of a large number of authors and significant international cooperation [4, 27]. More than half of analyzed HCPs (63.1%) were written by three or more authors, with an average number of authors per paper being 4.14, which is slightly higher compared to other studies exploring the most cited documents in dentistry [6]. The central position of the largest identified co-authorship network is occupied by the most productive author Tonetti MS, who achieved the greatest number of links (co-author relationships, n = 35). International cooperation, previously recognized as one of the main qualities of highly cited articles, appears to be less significant [28]. The present study found that only 52.43% of dental ESI HCPs are the

result of international collaboration. Additionally, most of the analyzed HCPs (73.1%) were published in the journals from the first and second JIF Quartiles in DOSM, which was in line with the results of the previously conducted bibliometric studies in dentistry [5]. The close relationship between citations and impact is confirmed by the fact that 43.24% of the HCPs are published in three journals (*Journal of Dental Research*, *Dental Materials*, and *Journal of Clinical Periodontology*), representing the core sources of ESI HCPs in DOSM.

Regarding the field of study, dental materials, dental implantology, periodontology, and oral and maxillofacial surgery represent areas of significant interest within the DOSM category. The results of this study are supported by previous research that demonstrated a greater interest in surgical rather than prosthodontic topics [29]. The notable portion of the analyzed HCPs addresses issues related to the classification, prevalence, incidence, and risk factors of periodontal and peri-implant diseases and conditions. Furthermore, a significant number of HCPs explores the association between periodontal and systemic diseases such as diabetes and cardiovascular diseases. These papers aim to draw attention to gaps in knowledge and emphasize the opportunities for oral rehabilitation, periodontal and general health improvement, highlighting the global burden of tooth loss due to untreated caries and severe periodontitis. In this context, the scientific evidence of high reputation should be instrumental in the alignment of national public interest and initiatives in strategies for prevention and oral health promotion.

In general, there was no positive relationship in dental research between citation counts and the LoE. The overall set of the analyzed HCPs was populated by a large number of lower-level evidence studies. Narrative literature reviews, expert opinions, and consensus reports with LoE VII were the most frequent types of study design (51.35%). Higher-level evidence studies, such as systematic reviews (24.32%), clinical trials, and cohort studies (3.24%), represented nearly a third of the sample. However, only half of the systematic literature reviews (55.56%) were systematic reviews of RCTs with the highest LoE I. The remaining systematic reviews, consisting of a cohort, case-control, or cross-sectional studies, were graded as studies of LoE IV or V. A notable portion of analyzed HCPs consisted of in vitro studies (8.11%), conducted mainly in the fields of dental materials, prosthodontics, and oral medicine. Only three RCTs (LoE II) and one case-control study (LoE IV) related to oral and maxillofacial surgery were included in ESI HCPs. These findings are consistent with previous data implying that the overall LoE in highly cited dental literature is low, with a high prevalence of narrative reviews [5, 30].

The limitation of the study was the inclusion of self-citations in the total number of citations, which could have inflated the citation rate. Nevertheless, the data in this study still provides significant insight into the evolving trends of dental research and prominent individuals, institutions, articles, journals, and countries that have significantly contributed to the progress of the field.

CONCLUSION

The present study outlines the main characteristics of the most recent 185 ESI HCPs in the field of DOSM published in the last decade. The majority of HCPs were narrative and systematic literature reviews in the field of dental materials, implantology, or periodontology. The highest number of frequently cited and most inspiring articles was published by the *Journal of Dental Research*, *Dental Materials* and *Journal of Clinical Periodontology*, which are the core sources of ESI HCPs representing the highest standards of research and clinical practice in dentistry. Since the citation counts are continuously evolving and the ESI HCPs are updated every two months, the results presented in this study provide a snapshot of the most prominent contemporary dental research fronts.

To provide more comprehensive insight into this research system, it would be valuable to conduct further analysis of existing co-citation and bibliographic coupling networks. Future studies comparing previous bimonthly clusters of ESI HCPs over longer periods are desirable to explain the evolution of dental research and the identification of rapidly growing or changing areas.

Conflict of interest: None declared.

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Високо цитирани радови из области стоматологије на основу сервиса Суштински научни показатељи (*Essential Science Indicators*)

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САЖЕТАК

Увод/Циљ Високо цитирани радови на основу сервиса Суштински научни показатељи (*Essential Science Indicators*) обухватају најутицајније научне чланке и револуционарна истраживања протекле деценије.

Циљ ове студије је да идентификује карактеристике високо цитираних радова на основу сервиса *Essential Science Indicators* у области стоматологије, оралне хирургије и медицине, да препозна ауторе, институције и земље порекла и утврди трендове истраживања који су привукли највише научног интереса.

Методе Спроведена је дескриптивна анализа библиографских података, екстракција мрежа и њихова визуализација. Даље, анализирани радови класификовани су према области интереса, главном домену истраживања, врсти студије и нивоу доказа.

Резултати Скуп од 185 високо цитираних радова објављен је у 42 часописа у периоду од 2010. до 2020. године, са просечним бројем цитата по раду 211,7. Готово половина ових радова објављена је у часописима *Journal of Dental Research*, *Dental Materials* и *Journal of Clinical Periodontology*. Заступљено је 765 аутора из 351 институције и 42 земље. Најпродуктивније институције биле су универзитети у Хонг Конгу, Мичигену и Берну. Највећи број публикација потиче из САД-а, Кине и Велике Британије. Стоматолошки материјали, зубна имплантологија, пародонтологија и орална и максилофацијална хирургија представљали су подручја од значајног интереса у овој категорији предмета. Највећи део високо цитираних радова били су наративни и систематски прегледи литературе, стручна мишљења, консензус извештаји и *in vitro* или лабораторијске студије.

Закључак Резултати добијени овом студијом могу пружити драгоцене информације истраживачима ради бољег препознавања садашњих и будућих актуелних тема стоматолошких истраживања.

Кључне речи: библиометрија; анализа друштвених мрежа; откривање знања; фронтови истраживања; стоматологија

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Validation of the translated and cross-culturally adapted Malocclusion Impact Questionnaire in young people seeking orthodontic treatment in Serbia

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SUMMARY
Introduction/Objective This study aimed to translate the original disease-specific Malocclusion Impact Questionnaire (MIQ) into Serbian and validate the new version in the cohort of young Serbian orthodontic patients.
Methods At the university clinic, 154 patients filled out the MIQ in Serbian before the start of the orthodontic treatment; 112 participants filled out the same questionnaire four weeks later. The Index of Orthodontic Treatment Need – Dental Health Component (IOTN-DHC) and Peer Assessment Rating (PAR) pretreatment scores were recorded. Descriptive statistic, Cronbach's α, Spearman's ρ, and exploratory factor analysis, followed by parallel analysis for factor reduction, were calculated and analyzed.
Results One hundred forty-eight patients with no missing responses (51 male, 97 female), of the average age of 13.3 ± 2 years, had MIQ total scores mean of 10.14 ± 7.451. Internal reliability (α = 0.913), external reliability (ρ = 0.906; p = 0.000), construct validity tested by MIQ total scores and two global question scores' correlations (ρ = 0.682; p = 0.000 and ρ = 0.366; p = 0.000), as well as clinical validity tested by correlations of MIQ total scores with PAR pretreatment scores (ρ = 0.181; p < 0.05), and IOTN-DHC scores (ρ = 0.192; p < 0.05) were positive and statistically significant. One item factor was extracted and it explained a large part of the cumulative variance.
Conclusion Reliability and validity of the translated and cross-culturally adapted MIQ in Serbian is satisfactory. It could be used for malocclusion-related quality of life assessments in young Serbian orthodontic patients.
Keywords: MIQ, malocclusion impact questionnaire; quality of life; malocclusion; cross-cultural adaptation; validation

INTRODUCTION

Most patients with malocclusion seek treatment to improve esthetics, psychosocial relationships, and the quality of life (QOL) [1]. However, orthodontic treatment could also improve oral functions and oral health [2, 3]. Demand for orthodontic treatment and treatment outcome satisfaction is not necessarily in correlation with the objective malocclusion records and the clinician's opinion [4]. Moreover, insight into the patients' perception of the malocclusion impact on different aspects of their life might be beneficial in the treatment decision-making process and treatment outcome considerations [5]. Standardized and validated patient questionnaires could be a suitable tool for obtaining such information [6]. Nowadays, we can see a constant increase in the use of the patient-reported outcome measures in orthodontic researches and patient-centered orthodontic care, especially in the form of generic and specific health-related QOL questionnaires [7]. Patients seeking orthodontic treatment are mostly young, healthy individuals with malocclusion that are not related to pain or tissue damage. Therefore, generic health and oral health-related QOL measures might not be sensitive enough to record the malocclusion impact on their everyday life or the QOL changes generated during and after orthodontic treatment [8]. Thus, several malocclusion-specific QOL questionnaires have been developed and validated by now. The Orthognathic QOL Questionnaire was created for young adult orthognathic patients [9]. It was originally developed in English and have been translated and culturally adapted to other languages including Serbian [10]. On the other hand, Malocclusion Impact Questionnaire (MIQ) was developed in English to assess young orthodontic patients' concerns regarding the appearance of teeth, social interactions and oral health and functions [11]. The original questionnaire was successfully validated in young persons with malocclusion in the United Kingdom (UK) and New Zealand (NZ) [12, 13]. It has since been cross-culturally adapted and translated into different languages: Chinese, Arabic, and Spanish [14, 15, 16].

This study aimed to validate the new MIQ in Serbian in the cohort of young Serbian orthodontic patients.

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METHODS

This cross-sectional study was conducted at the University Clinic from December 2018 to May 2019. The sample was selected by convenience from new consecutive patients referred to the University Clinic. The inclusion criteria were the presence of malocclusion, age 10–17 years, and signed written informed consent. Exclusion criteria were previous orthodontic treatment, the presence of craniofacial syndromes, the presence of cleft lip and palate, and the presence of learning disabilities.

All the patients and their parents/guardians were informed orally and were given a written explanation of the research aims and study design. Parents/guardians signed informed consent for participation in the study. Baseline data that included participants' demographic characteristics, sex, and age, were collected together with the questionnaire. Malocclusion status was assessed on study casts using the Peer Assessment Rating index (PAR index) and the Index Orthodontic Treatment Need – Dental Health Component (IOTN-DHC) [17, 18]. Measuring was done by one orthodontic specialist, previously trained in using PAR and IOTN indices. The MIQ was used to measure the subjective impact of the malocclusion on the participant's QOL. The MIQ consists of 17 items with three-point severity responses (0–2) and two global questions with five-point intensity responses (0–4). Total MIQ score has range 0–34, and higher scores represent the poorer QOL related to malocclusion. Two global questions are intended to help in the validation process and are presented separately [12, 13].

Translation and cross-cultural adaptation

The original version of the MIQ, MIQ handbook, preliminary permission for non-commercial use, and the Users License Agreement (signed and returned) were received from the MIQ corresponding author. A multidisciplinary committee for translation and cross-cultural validation was established to produce the most appropriate semantical and conceptual version of the MIQ in Serbian. The committee members were experts in the English–Serbian–English translation, QOL research, and orthodontics. According to the internationally accepted methodology for the translation and cultural adaptation of the health-related QOL questionnaires, the "forward–backward" translation methodology for preparing the MIQ in Serbian was used [19].

A pilot study was conducted on 10 patients in order to check understanding and interpretation of the questionnaire in Serbian. The results were discussed between the committee members regarding the patients' doubts and suggestions. This stage led to the second version of the MIQ in Serbian, which was tested on 20 new patients, who filled it out in the presence of researchers who assisted if necessary. The patients were asked additional questions regarding the simplicity, clarity, and relevance of the questions. Once more, the committee members discussed the original MIQ in English and the final version of the MIQ in Serbian, tracked all the changes made during the pretesting process, reviewed the introduction and instructions as well as scaling of the responses. The final version of the MIQ in Serbian was printed, to be used in the validation process.

Validation

During regular appointments at the University Clinic, before the start of the orthodontic treatment, 154 patients with malocclusion who fulfilled the study inclusion criteria were asked to fill out the MIQ in Serbian without any assistance from the doctor or parent/guardian. To assess the patient's acceptance of the MIQ in Serbian, the mean time required for completing the questionnaire and assistance in reading/writing were also noted. Four weeks later, 112 participants were asked to complete the same questionnaire before tooth extractions and the start of any orthodontic treatment, for retest purposes.

Statistical analysis

Descriptive statistics included sample demographics, clinical characteristics, test scores, and questionnaire completion times. Cronbach's α coefficient and item-total correlation were calculated to assess the internal reliability of the MIQ in Serbian. Spearman–Brown reliability coefficient was used to evaluate external reliability. Construct and clinical validity were explored correlating the MIQ in Serbian total scores with two global question scores and PAR index pretreatment scores and IOTN-DHC, respectively, using Spearman's correlation. Explorative factor analysis was conducted to assess whether items distribution in the MIQ in Serbian corresponded with the original MIQ items' distribution. Parallel analysis, a method for determining the number of factors to retain, was used for final factor extraction.

The study was approved by the Ethics Committee (approval No 36/19).

RESULTS

The original sample included 154 patients who met the inclusion criteria. However, six participants (3.9%) were excluded due to missing item responses. In the final sample (n = 148), 51 (34.5%) participants were male, and 97 (65.5%) were female, the average age was 13.3 ± 2 years (range 10–17 years). All the participants were able to read and answer the questions without assistance. Response rates for each item were high (96.7%) with no evidence of significant floor or ceiling effects. Thirteen participants (8.78%) had the total MIQ score of 0 and no one had the maximum (34). No one found MIQ in Serbian items ambiguous or embarrassing. The mean time to complete the MIQ in Serbian was 4.84 ± 2.355 minutes (range 2–12 minutes). Correlation analysis between age and time needed to complete the questionnaire showed significant negative correlation ($r = -0.751$; $p = 0.000$). The MIQ in Serbian total scores mean was 10.14 ± 7.451. The MIQ in Serbian descriptive statistics and frequencies

Table 1. The Malocclusion Impact Questionnaire in Serbian descriptive statistics and frequencies of single item responses and descriptive statistics of the Malocclusion Impact Questionnaire in Serbian total score

(n = 148)					Frequencies of score				
Question No	Mean	SD	Median	Mode	0 n (%)	1 n (%)	2 n (%)	3 n (%)	4 n (%)
1.	1.29	1.241	1	0	52 (35.1)	38 (25.7)	30 (20.3)	19 (12.8)	9 (6.1)
2	1.74	1.347	2	1	32 (21.6)	40 (27)	33 (22.3)	21 (14.2)	22 (14.9)
3	1.15	0.75	1	1	32 (21.6)	62 (41.9)	54 (36.5)		
4	1.11	0.757	1	1	35 (23.6)	62 (41.9)	51 (34.5)		
5	0.79	0.758	1	0	61 (41.2)	57 (38.5)	30 (20.3)		
6	0.43	0.63	0	0	95 (64.2)	42 (28.4)	11 (7.4)		
7	0.51	0.685	0	0	88 (59.5)	44 (29.7)	16 (10.8)		
8	0.43	0.64	0	0	97 (65.5)	39 (26.4)	12 (8.1)		
9	0.7	0.744	1	0	70 (47.3)	53 (35.8)	25 (16.9)		
10	0.9	0.735	1	1	48 (32.4)	67 (45.3)	33 (22.3)		
11	0.68	0.712	1	0	69 (46.6)	58 (39.2)	21 (14.2)		
12	0.9	0.771	1	1	52 (35.1)	59 (39.9)	37 (25)		
13	0.45	0.609	0	0	91 (61.5)	48 (32.4)	9 (6.1)		
14	0.55	0.722	0	0	87 (58.8)	41 (27.7)	20 (13.5)		
15	0.27	0.59	0	0	119 (80.4)	18 (12.2)	11 (7.4)		
16	0.36	0.596	0	0	103 (69.6)	36 (24.3)	9 (6.1)		
17	0.24	0.542	0	0	120 (81.1)	20 (13.5)	8 (5.4)		
18	0.47	0.664	0	0	92 (62.2)	42 (28.4)	14 (9.5)		
19	0.2	0.507	0	0	125 (84.5)	16 (10.8)	7 (4.8)		
MIQ **Total score**	**10.14**	**7.451**	**9**	**0**					

MIQ – Malocclusion Impact Questionnaire; 1 – Bother you; 2 – Affect life; 3 – Happy; 4 – Good looking; 5 – Confident; 6 – Normal; 7 – Sad; 8 – Nervous; 9 – Shy; 10 – Smiling; 11 – Laughing; 12 – Seeing photographs of yourself; 13 – Talking in public; 14 – Other people having nicer teeth than you; 15 – Being bullied; 16 – Making friends; 17 – Fitting in with friends; 18 – Covering teeth with hand when smiling; 19 – Biting some foods

of single item responses as well as descriptive statistics of MIQ in Serbian total score are shown in Table 1. The PAR index mean pretreatment score value (n = 129) was 23.5 ± 10.73 (range 1–48). All five IOTN-DHC grades were described in the sample (n = 125). Grade 4 was the most frequent one (40.8%), followed by grade 5 (29.6%), grade 3 (20%), grade 2 (8%), and grade 1 (1.6%).

Reliability

Internal reliability analysis using Cronbach's α showed excellent internal consistency of the MIQ in Serbian (α = 0.913), indicating that all scale items measured the same concept. If question number 19 would be deleted, coefficient α would be slightly higher (α = 0.920, Table 2). Item-total correlation was moderate for 15 out of 17 questions, while it was poor for questions number 19 and 14 (Table 2).

External reliability evaluated by Spearmen–Brown reliability coefficient comparing the MIQ in Serbian test and retest total scores (n = 110) was excellent (ρ = 0.906; p = 0.000, Table 3).

Table 2. Internal reliability of the Malocclusion Impact Questionnaire in Serbian items calculated with Cronbach's α coefficient and item-total correlation

Question No (n = 148)	Corrected item-total correlation	Cronbach's α if item deleted
3 Happy	0.656	0.906
4 Good looking	0.700	0.905
5 Confident	0.613	0.908
6 Normal	0.649	0.907
7 Sad	0.738	0.904
8 Nervous	0.583	0.908
9 Shy	0.665	0.906
10 Smiling	0.673	0.906
11 Laughing	0.627	0.907
12 Seeing photographs of yourself	0.675	0.906
13 Talking in public	0.624	0.907
14 Other people having nicer teeth than you	0.443	0.913
15 Being bullied	0.560	0.909
16 Making friends	0.601	0.908
17 Fitting in with friends	0.558	0.909
18 Covering teeth with hand when smiling	0.531	0.910
19 Biting some foods	0.082	**0.920**

Validity

Construct validity was assessed by correlating the first and the second global question single scores and the MIQ in Serbian total scores, which showed good correlation. Clinical validity performed by correlating the MIQ in Serbian total score with PAR index pretreatment scores and IOTN-DHC showed weak but statistically significant correlation. The results of the correlation analysis are shown in Table 3.

Exploratory factor analysis was used to explore the allocation of items in the MIQ in the Serbian version. The Kaiser–Meyer–Olkin measure (KMO = 0.891) and Bartlett's test of sphericity (χ^2 = 1270.84; p = 0.000) verified that the sample number and correlation structure were adequate for factor analysis. Three components with an eigenvalue more significant than 1 were extracted explaining the 59.18% of the cumulative variance. All items showed cross-loadings indicating that every item might correspond to all three extracted factors (Table 4). Scree plot indicated that all questions could fit one factor (Figure 1).

According to the parallel analysis, the model justifies only one factor, explaining the 41.65% of the variance. The component matrix showed that 16 items had factor loading from 0.504 to 0.783. Only item number 19 regarding

Table 3. External reliability, construct, and clinical validity of the Malocclusion Impact Questionnaire total score in Serbian

Parameter		External reliability	Construct validity		Clinical validity	
		MIQ Total score (retest) (n = 110)	1 Bothers you (n = 148)	2 Affects life (n = 148)	PAR index pretreatment score (n = 129)	IOTN-DHC (n = 125)
MIQ Total score (test)	Spearman's rho	0.906**	0.682**	0.366**	0.181*	0.192*
	Sig. (2-tailed)	0.000	0.000	0.000	0.040	0.032

MIQ – Malocclusion Impact Questionnaire; PAR – peer assessment rating; IOTN-DHC – Index Orthodontic Treatment Need – Dental Health Component;
*p < 0.05;
**p < 0.01

Table 4. Exploratory factor analyses for the Malocclusion Impact Questionnaire in Serbian items

Question No (n = 148)	Communalities		Component matrix		
	Initial	Extraction	1	2	3
3 Happy	1.000	0.756	0.701	0.513	0.046
4 Good looking	1.000	0.742	0.744	0.434	0.013
5 Confident	1.000	0.499	0.672	0.208	0.068
6 Normal	1.000	0.629	0.702	0.092	0.358
7 Sad	1.000	0.686	0.783	0.110	0.248
8 Nervous	1.000	0.472	0.644	-0.107	0.215
9 Shy	1.000	0.521	0.713	0.090	-0.062
10 Smiling	1.000	0.753	0.730	0.112	-0.455
11 Laughing	1.000	0.644	0.688	-0.055	-0.410
12 Seeing photographs of yourself	1.000	0.669	0.723	0.230	-0.307
13 Talking in public	1.000	0.537	0.676	-0.278	0.053
14 Other people having nicer teeth than you	1.000	0.282	0.504	-0.164	0.033
15 Being bullied	1.000	0.653	0.627	-0.451	0.239
16 Making friends	1.000	0.543	0.666	-0.313	0.043
17 Fitting in with friends	1.000	0.734	0.627	-0.567	0.135
18 Covering teeth with hand when smiling	1.000	0.457	0.595	-0.156	-0.279
19 Biting some foods	1.000	0.483	0.099	0.328	0.604

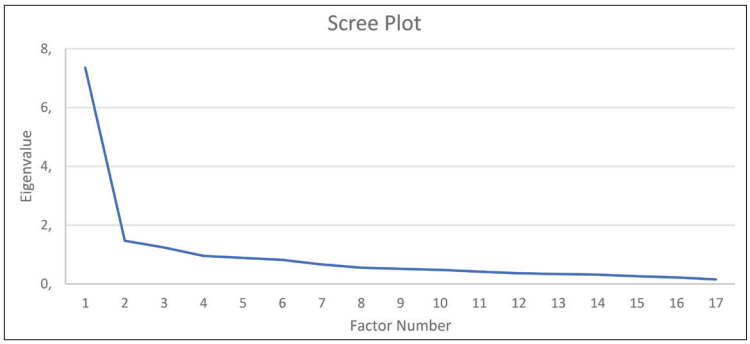

Figure 1. Scree plot for the Malocclusion Impact Questionnaire items

problems with biting certain foods explained deficient factor loading 0.099 (Table 5).

DISCUSSION

It has been shown that malocclusions have a significant impact on QOL of orthodontic patients [20]. Specific instruments for health-related QOL have consistently shown to be more sensitive than the generic tools [21]. Common use of a translated and properly validated questionnaire has the advantage of data collection from different cultures that can be compared. Original MIQ in English is a formally validated, self-administered malocclusion disease-specific questionnaire. It was developed in English to measure the malocclusion impact on young people in the UK. The English to Serbian translation and cross-cultural adaptation was done according to the internationally accepted standards [19]. Linguistic structure and the conceptual meaning were prioritized over the word-to-word translation. Appropriate MIQ version suitable for the socio-cultural environment of Serbia was attained through forward–backward translation, expert panel discussions, and pre-testing with cognitive debriefing. MIQ in Serbian was easy to use and 100% of the participants found the questionnaire comprehensible. None of the respondents needed assistance in completing the MIQ in Serbian scale and no question was found embarrassing. Most missing data were recorded as whole-page data and can be considered an accidental omission in completing the questionnaire.

To the best of our knowledge, this is the first report and the first time the MIQ was used in young people with malocclusion in Serbia. The study sample was chosen by convenience, similarly to the original MIQ validation study [12]. Even though clear scientifically sound recommendations for sample size calculation in validation studies are still lacking, the recommended minimum is 100 [22]. Six participants that had missed some of the items in the MIQ in Serbian were excluded from the validation study. The high Kaiser–Meyer–Olkin coefficient showed the sample size of 148 was suitable for performing factor analysis to explore the MIQ in Serbian dimensionality. The sample mean age was similar to the UK study [12]. Sex distribution was similar in both samples [12].

The mean time to complete the questionnaire was acceptable and indicated that the questionnaire was simple and easy to understand. The statistically significant negative correlation between age and time to complete the scale suggested that younger participants needed more time to complete the MIQ compared to the older ones. This finding was in concordance with Marek Fuchs's conclusion that children and younger adolescents rely more heavily on

Table 5. Extraction of one factor – parallel analysis for the Malocclusion Impact Questionnaire in Serbian items

Question No (n = 148)	Communalities		Component 1
	Initial	Extraction	1
3 Happy	1.000	0.492	0.701
4 Good looking	1.000	0.554	0.744
5 Confident	1.000	0.451	0.672
6 Normal	1.000	0.493	0.702
7 Sad	1.000	0.612	0.783
8 Nervous	1.000	0.414	0.644
9 Shy	1.000	0.509	0.713
10 Smiling	1.000	0.534	0.730
11 Laughing	1.000	0.473	0.688
12 Seeing photographs of yourself	1.000	0.522	0.723
13 Talking in public	1.000	0.457	0.676
14 Other people having nicer teeth than you	1.000	0.254	0.504
15 Being bullied	1.000	0.393	0.627
16 Making friends	1.000	0.443	0.666
17 Fitting in with friends	1.000	0.394	0.627
18 Covering teeth with hand when smiling	1.000	0.354	0.595
19 Biting some foods	1.000	0.010	0.099

the information provided in the questionnaire to produce an answer [23].

The MIQ in Serbian mean total score (10.14 ± 7.451) implicates that malocclusion disturbed the QOL of our participants. Malocclusion-specific QOL of our patients was better compared to the UK sample (11.6 ± 7), similar to the Arabic (10.1 ± 7), and inferior to the NZ (7.1 ± 6.6) and Chilean (8.3 ± 6) samples [12, 13, 15, 16]. Differences in the frequency of responses and the MIQ total scores in different populations can be attributed to the socio-cultural context of the respondents. Also, linguistic ambiguities and differences between the survey protocols among studies should not be completely neglected.

The MIQ in Serbian showed excellent internal consistency ($\alpha = 0.913$; $\alpha > 0.7$ is recommended), suggesting that the scale was consistent and that all items measured the same concept. Similar results were presented in the UK, NZ, Arabic, and Chilean validation studies [12, 13, 15, 16]. Question number 19 about biting certain foods showed a low item-total correlation. However, given the strong α coefficient and a minimal increase in case of question number 19 omission, removing the item was not considered. External reliability of the MIQ in Serbian was statistically significant and strong. This finding suggests that if the malocclusion does not change, MIQ in Serbian score stays reliable over time.

The validity of health-related QOL questionnaires is usually assessed according to how much the information from the questionnaire agrees with the results obtained based on other measurements. Moreover, criterion validity refers to whether a questionnaire measures what it was intended to measure, and it is known that questionnaire validity could be explored by comparison with previously existing questionnaires intended to measure the same concept. When there is no gold standard in the targeted population, assessing clinical validity could be useful. Thus, we explored the correlation among MIQ total scores and objective clinical measures. MIQ total scores and PAR index pretreatment scores, as well as IOTN-DHC, had statistically significant positive correlation. This indicates that patients with more severe malocclusion and higher treatment need reported poorer malocclusion-related QOL. The moderate to weak correlation was expected according to previous findings. The subjective health-related QOL measure do not necessarily correlate with objective findings [4, 24]. Also, malocclusion has a more of emotional and social impact compared to the oral symptoms and functional limitations [25].

The MIQ in Serbian construct validity was explored by correlating MIQ total score with global question scores, as suggested in the UK validation study [12]. Statistically significant positive correlations were found, meaning that participants who reported that teeth bothered them and affected their life also appear to have impaired malocclusion QOL. In all reported MIQ validation studies (UK, NZ, Chinese, Arabic, and Chilean) this correlation was significant and positive although the strength of correlations differed [12–16]. It could be assumed that a possible influence of cultural tradition and linguistic specificities in different populations may have a consequential impact when cross-cultural adaptations are performed.

Based on exploratory factor analysis, 17 items in the scale were divided into three potential factors explaining eigenvalues greater than one. All items corresponded to all displayed factors. This approach could overestimate the number of significant factors. Thus, the scree plot and parallel analysis as a more rigorous approach to determine the number of factors to retain was performed [26]. The scree plot (a graph of the generated eigenvalues) suggested that a model for this study data may have one factor. Moreover, only one produced eigenvalue was higher than random eigenvalues achieved in the parallel analysis. These findings suggest that one factor could be retained and that all items in the MIQ scale fit into one dimension, which is in line with the UK validation study results [12].

The MIQ in English was successfully adapted for the Serbian population in a cohort of orthodontic patients aged 10–17 years. According to the new MIQ in Serbian (MIQ-SR) scores, young people with malocclusion experienced the condition-specific QOL impairment before the orthodontic treatment.

Strength of the study

The MIQ in Serbian was filled out by patients without any parent/guardian assistance. All questionnaires were filled out at the University Clinic.

Study weakness

Although all participants were informed that their responses would not affect further orthodontic treatment, subject bias may still be present.

Further recommendation

MIQ-SR longitudinal studies are recommended to determine the responsiveness or ability to detect change over time.

CONCLUSION

Translated and culturally adapted MIQ in Serbian has satisfactory psychometric properties. It is a reliable and valid instrument for the evaluation of dental malocclusions' impact on the QOL of young people in the Serbian sociocultural environment. Clinically, this condition-specific QOL instrument might help orthodontic care professionals in patient prioritization and treatment options' selection.

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Валидација преведеног и културолошки адаптираног Упитника о утицају малоклузија на квалитет живота на узорку младих ортодонтских пацијената у Србији

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САЖЕТАК

Увод/Циљ Циљ студије је био да се валидира преведена и културолошки прилагођена оригинална верзија специфичног упитника о утицају малоклузија на квалитет живота (енгл. *Malocclusion Impact Questionnaire, MIQ*) на узорку младих ортодонтских пацијената у Србији.

Методе На Универзитетској клиници 154 пацијента су попунила *MIQ* на српском језику пре почетка ортодонтске терапије. Четири недеље касније, 112 пацијента је поново попунило идентичне упитнике. Мерени су индекс потребе за ортодонтском терапијом и индекс резултата ортодонтске терапије пре терапије. Урађени су дескриптивна статистика, тест интерне конзистентности (Кронбахова α), корелације (Спирманов ρ), експлоративна факторска анализа за екстракцију и паралелна анализа за редукцију броја фактора упитника.

Резултати Код 148 пацијената без недостајућих одговора (51 мушког и 97 женског пола) узраста 13,3 ± 2,00 година, средња вредност укупног скора упитника *MIQ* је била 10,14 ± 7,451. Интерна ($\alpha = 0{,}913$) и екстерна конзистентност ($\rho = 0{,}906$; $p = 0{,}000$) показали су високу статистичку значајност. Корелације укупног укупног скора *MIQ* са појединачним скоровима два глобална питања ($\rho = 0{,}682$; $p = 0{,}000$ и $\rho = 0{,}366$; $p = 0{,}000$, редом), скора индекса резултата ортодонтске терапије пре терапије ($\rho = 0{,}181$; $p < 0{,}05$) и скора индекса потребе за ортодонтском терапијом ($\rho = 0{,}192$; $p < 0{,}05$) биле су позитивне и статистички значајне. Након експлоративне факторске анализе и паралелне анализе издвојен је један фактор који је објаснио значајан део укупне варијансе.

Закључак *MIQ* на српском језику има задовољавајућу интерну и екстерну конзистенцију и валидност и може се примењивати приликом разматрања утицаја малоклузија на квалитет живота код младих особа са ортодонтским неправилностима у Србији.

Кључне речи: *MIQ*, упитник о утицају малоклузија; квалитет живота; међукултурално прилагођавање; валидација

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Verapamil administration alleviates microcytosis and tissue accumulation after chronic aluminum exposure in rats

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SUMMARY

Introduction/Objective Research has demonstrated the toxicant potential of aluminum, but no therapeutic options have been suggested. The aim of the study was to investigate the extent of the aluminum-induced toxicity, evaluated by hematological/biochemical disarrangements, hepcidin concentration and tissue accumulation after chronic aluminum exposure and to determine possible protection with Ca^{2+}-channel blockage, verapamil.

Methods Experimental animals (36 rats) were treated with different doses of $AlCl_3$ during eight weeks and after that their blood and tissues were analyzed.

Results The significant differences, regardless of the aluminum dose administered, were documented in all evaluated hematological ($p < 0.001$) and biochemical parameters ($p < 0.001$), as well as in aluminum tissue deposition in liver, kidneys and testicles ($p < 0.001$), respectively. After verapamil administration, a significant improvement in some hematological and biochemical parameters was demonstrated, $p < 0.001$, as well as the attenuation of aluminum deposits in liver and testes, $p < 0.001$. Evaluated parameters of inflammation and kidney deposition did not show significant change after verapamil application.

Conclusion The findings indicate that chronic $AlCl_3$ intoxication, regardless of the dose, results in the microcytic anemia associated with high hepcidin levels, numerous biochemical abnormalities and significant aluminum deposition in liver, kidney and testes and that these effects may be attenuated by verapamil administration. Overall, the results emphasize the significance of calcium homeostasis preservation in chronic aluminum exposure and propose possible therapeutic option.

Keywords: aluminum-induced toxicity; verapamil; chronic exposure; hepcidin; microcytosis

INTRODUCTION

Aluminum is one of the most widespread elements on earth; therefore, a progressive overexposure of biota to biologically active aluminum has been reported, resulting in growing evidence of aluminum-related diseases [1]. The primary and physiological source of aluminum intake is food and drinking water, where an average ingestion stands for 5–40 mg of aluminum on a daily basis, while the accepted limit for aluminum intake is recognized as being 2 mg/kg/week [1].

In recent years, there has been abundant data on aluminum systemic toxicity, in order to clarify its role in the pathophysiology of many disorders: reproductive and breast diseases [2], bone impairment [3], lung disorders [4], impacts on the immune system [5], as well as neurological diseases [6], whereas inhibition of Ca^{2+} channels may represent potential therapeutic strategy for addressing aluminum-induced toxicity.

Therefore, we postulated the following aims of the study: to investigate the extent of the obtained toxicity, evaluated by hematological and biochemical disarrangements and hepcidin plasma concentration and tissue accumulation after chronic aluminum exposure; and to determine a possible protection from aluminum-associated toxicity with Ca^{2+}-channel blockage by verapamil.

METHODS

Animals

The experiment was performed on 36 male Sprague Dawley rats (Vivarium of the Research Center for Biomedicine of the Faculty of Medicine in Niš). The rats were two months old and weighed 130–160 g. They were given *ad libitum* access to food (Zemun Veterinary Institute standard rat chow pellets) and tap water. The ambient temperature was 20 ± 2°C, the relative humidity was 55 ± 10%, with a 12-hour light–dark cycle. The care and handling of the animals were in accordance with the guidelines and recommendations specified by the Federa-

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tion of European Laboratory Animal Science Associations (FELASA). Upon the request of the Ethical Commission for Experimental Animal Welfare of the Faculty of Medicine of Niš, the Ministry of Agriculture, Forestry and Water Management of the Republic of Serbia approved the experiment, decision number 323-07-01762/2019-05/10.

Experimental design

Rats were randomly divided into six groups (n = 6). The animals were treated daily during a period of eight weeks, as follows: 10 mg/kg of body weight (b.wt) $AlCl_3$, i.p. (group E1); 5 mg/kg (b.wt) verapamil by gavage, and after 60 minutes 10 mg/kg (b.wt) $AlCl_3$ i.p. (group E2); 20 mg/kg (b.wt) $AlCl_3$ i.p. (group E3); 5 mg/kg (b.wt) verapamil by gavage, and after 60 minutes 20 mg/kg (b.wt) $AlCl_3$ i.p. (group E4); 5 mg/kg (b.wt) verapamil by gavage (group V), and saline i.p. (control group C). The material used was as described: aluminum chloride, $AlCl_3$ (Honeywell Fluka, Fisher Scientific, Hampton, NH, USA) and verapamil hydrochloride (Verapamil, Alkaloid AD Skopje, Skopje, North Macedonia).

Blood analyses and tissue sampling

At the end of the study, the rats were euthanized with an overdose of Ketonal® (Richter Pharma AG, Wels, Austria). Hematological parameters: red blood cells (RBC), white blood cells, platelets, and relative white blood cell counts were determined by electrical impedance. The colorimetric method was used to determine hemoglobin (Hg) concentration. The hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and absolute white blood cell count were calculated. All hematological parameters were determined from whole blood, with EDTA K2 anticoagulant, and were determined on the hematology counter Celltac MEK 6510K (Nihon Kohden, Tokyo, Japan).

The biochemical analyzer AU 680 (Beckman Coulter, Inc., Brea, CA, USA) with original manufacturer reagents was used for biochemical parameters which were determined from serum. The concentration of ferritin, transferrin, and unsaturated iron binding capacity (UIBC) was determined by immunoturbidimetric method, and iron concentration by colorimetric method. Total iron binding capacity (TIBC) and transferrin saturation (TSAT) were then calculated. The activity of the following enzymes was determined by enzymatic colorimetric method: aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), amylase (AMY), lactate dehydrogenase (LDH), creatine kinase (CK), and γ-glutamyl transferase (γGT).

The concentrations of hepcidin and C-reactive protein (CRP) were measured in plasma samples using commercially available ELISA kits, according to the manufacturer's instructions.

All animals were carefully examined macroscopically: body surfaces and orifices as well as all cranial, thoracic, and abdominal organs. The liver, kidney, and testes were excised, frozen, and stored at -80°C prior to measuring the aluminum tissue concentration. The samples were prepared according to the method by Banni et al. [7], slightly modified. The samples were oven-dried (60°C) to a constant weight. The dried tissues (0.3 g from each sample) were then mixed with 3 mL of pure nitric acid at 90°C for 24–48 hours. The volume was then adjusted to 25 mL with 0.5% nitric acid.

Aluminum tissue concentration measurement

All analyses were carried out on an iCAP 6000 inductively coupled plasma optical emission spectrometer (Fisher Scientific, Hampton, NH, USA) which uses the echelle optical design and a charge injection device solid-state detector. The operating conditions for the inductively coupled plasma atomic emission spectrometers were the following: flush pump rate – 100 rpm, analysis pump rate – 50 rpm, RF power – 1150 W, nebulizer gas flow rate – 0.7 L min^{-1}, coolant gas flow rate – 12 L min^{-1}, auxiliary gas flow rate – 0.5 L min^{-1}, dual (axial/radial) viewed plasma mode and sample uptake delay – 30 s. The emission wavelength, the detection and quantification limits, and the correlation coefficient of the calibration curve for the aluminum are 308.215 nm, 0.3661 μg g^{-1}, 1.2203 μg g^{-1}, and 0.99998, respectively.

A TraceCERT® (Fluka Analytical, Sigma-Aldrich Chemie GmbH, Buchs SG, Switzerland) ICP multi-element standard solution of about 40 ± 0.1 mg L^{-1} of aluminum was used as a stock solution for calibration. The plastic containers used for storing the samples were cleaned to avoid contamination of the samples with trace residues of any metals. Containers were treated with 20% nitric acid and then washed with ultra-pure water 0.05 μS cm^{-1} (MicroMed high purity water system, TKA Wasseraufbereitungssysteme GmbH, Niederelbert, Germany). The nitric acid (65%) was of analytical grade.

Statistical analysis

Data are presented as mean ± standard deviation. One-way ANOVA was performed for comparing values among different animal groups. For non-normally distributed variables Kruskal–Wallis test was used, followed by Mann–Whitney test for comparing values between two groups. The null hypothesis was tested at the 0.05 level of significance. Statistical analysis was performed in R, version 4.0.3, 2020-10-10 (The R Project for Statistical Computing, the R Foundation).

RESULTS

Our study sample included 36 rats, whereas 12 of them were treated with saline and verapamil and were considered the control group, and 24 of them were treated with $AlCl_3$ in higher and lower doses, during eight weeks. We demonstrated that chronic aluminum exposure resulted in a significant change in almost all evaluated hematological

Table 1. The comparison of chronic aluminum chloride treatment effects on hematological parameters and the effects after verapamil administration

Hematological parameters	Control group	Verapamil group	$AlCl_3$ 10 mg/kg b.wt	$AlCl_3$ 10 mg/kg b.wt/ verapamil	$AlCl_3$ 20 mg/kg b.wt	$AlCl_3$ 20 mg/kg b.wt/ verapamil	p
RBC	8.33 ± 0.52	8.56 ± 0.42	7.86 ± 0.38[a,b]	8.14 ± 0.38[a,b,c]	7.14 ± 0.38[a,b,c,d]	7.67 ± 0.52[a,b,c,d,e]	**< 0.001**
Hemoglobin	155.17 ± 1.47	152.67 ± 2.07	138.71 ± 11.95[a,b]	151.29 ± 1.38[a,b,c]	126.57 ± 4.68[a,b,c,d]	139.83 ± 2.79[a,b,c,d,e]	**< 0.001**
Hematocrit	47 ± 0.89	46.17 ± 0.75	39.57 ± 0.98[a,b]	44.14 ± 1.34[a,b,c]	33 ± 2[a,b,c,d]	37.5 ± 1.38[a,b,c,d,e]	**< 0.001**
MCV	55.17 ± 1.17	55 ± 1.26	50.57 ± 1.9[a,b]	53.71 ± 0.95[a,b,c]	46.29 ± 1.6[a,b,c,d]	49.83 ± 0.75[a,b,c,d,e]	**< 0.001**
MCH	18.5 ± 0.55	18.33 ± 0.52	17.57 ± 1.51[a,b]	18.29 ± 0.49[a,b,c]	17.57 ± 0.79[a,b,c,d]	18.5 ± 0.55[a,b,c]	0.136
MCHC	329.67 ± 4.23	330.83 ± 0.75	352.14 ± 26.64[a,b]	341.86 ± 8.76[a,b]	381.29 ± 16.89[a,b,d]	372.5 ± 10.88[a,b,c,d,e]	**< 0.001**
Hepcidin	71 ± 5.4	72 ± 10.94	185 ± 64.54[a,b]	132.14 ± 33.78[a,b]	196.43 ± 45.25[a,b,c]	160.33 ± 31.46[a,b,c]	**< 0.001**
Ferritin	190.17 ± 48.35	187.33 ± 7.26	309.29 ± 19.33[a,b]	282.71 ± 31.45[a,b]	546.14 ± 85.1[a,b,c]	456.5 ± 98.92[a,b,c,d]	**< 0.001**
Transferrin	1.47 ± 0.18	1.53 ± 0.08	1.78 ± 0.07[a,b]	1.67 ± 0.05[a,b]	1.85 ± 0.11[a,b,c,d]	1.68 ± 0.06[a,b,c,d]	**< 0.001**
UIBC	52.17 ± 7.83	48.17 ± 11.58	78 ± 7.21[a,b]	60.29 ± 9.71[a,b]	80.29 ± 8.08[a,b,c,d]	59.67 ± 7.34[a,b,c,d]	**< 0.001**
TIBC	91.5 ± 8.41	92.33 ± 10.71	105.86 ± 6.56[a,b]	88.14 ± 10.25[a,b]	101.14 ± 10.14[a,b,d]	83.17 ± 6.08[a,b,c,d]	**0.001**
Fe	39.67 ± 1.86	38.17 ± 8.84	27.86 ± 2.26[a,b]	28 ± 3.92[a,b]	20.86 ± 3.08[a,b,c,d]	21.83 ± 0.75[a,b,c,d]	**< 0.001**
TSAT	0.43 ± 0.04	0.41 ± 0.1	0.26 ± 0.03[a,b]	0.32 ± 0.05[a,b]	0.2 ± 0.02[a,b,c,d]	0.26 ± 0.02[a,b,c,d]	**< 0.001**

Values are presented as mean ± S.D; $p < 0.05$;
RBC – red blood cells; MCV – mean corpuscular volume; MCH – mean corpuscular hemoglobin; MCHC – mean corpuscular hemoglobin concentration; UIBC – unsaturated iron-binding capacity; TIBC – total iron-binding capacity; TSAT – transferrin saturation;
[a]*vs.* control group;
[b]*vs.* verapamil group;
[c]*vs.* $AlCl_3$ 10 mg/kg b.wt;
[d]*vs.* $AlCl_3$ 10 mg/kg b.wt/verapamil;
[e]*vs.* $AlCl_3$ 20 mg mg/kg b.wt

Table 2. The comparison of chronic aluminum chloride treatment effects on the platelets and white cells and the effects after verapamil administration

Parameters	Control group	Verapamil group	$AlCl_3$ 10 mg/kg b.wt	$AlCl_3$ 10 mg/kg b.wt/verapamil	$AlCl_3$ 20 mg/kg b.wt	$AlCl_3$ 20 mg/kg b.wt/verapamil	p
PLT	776.5 ± 63.46	760.17 ± 77.08	846.43 ± 90.65[a,b]	820.71 ± 79.43[a,b]	1133.17 ± 1.12[a,b,c,d]	1017.17 ± 41.1[a,b,c,d]	**< 0.001**
WBC	7.95 ± 0.83	7.67 ± 0.52	10 ± 1.83[a,b]	8.71 ± 0.49[a,b,c]	11.5 ± 0.53[a,b,c]	9.33 ± 0.82[a,b,c,d]	**< 0.001**
Ly %	53.2 ± 5.15	52.97 ± 1.72	57.16 ± 6.96[a,b]	55.83 ± 9.37[a,b]	50.1 ± 6.51[a,b,c]	40.41 ± 9.82[a,b,c,d]	**0.004**
Seg %	39.82 ± 7.13	40.725 ± 1.93	32.91 ± 6.51[a,b]	34.81 ± 10.17[a,b]	40.87 ± 7.93[a,b,c]	51.65 ± 1.43[a,b,c,d]	**0.005**
Mo %	6.98 ± 2.05	6.28 ± 0.75	9.93 ± 1.15[a,b]	9.36 ± 1.11[a,b]	9.03 ± 1.92[a,b,c]	7.85 ± 74[a,b,c,d]	**0.001**
Ly	4.2 ± 0.6	4.09 ± 0.24	5.78 ± 1.14[a,b]	4.82 ± 1.01[a,b]	5.7 ± 0.76[a,b,c]	3.8 ± 0.97[a,b,c,d]	**< 0.001**
Seg	3.07 ± 0.6	3.15 ± 0.3	3.33 ± 0.83[a,b]	2.96 ± 0.77[a,b]	4.66 ± 0.94[a,b,c]	4.87 ± 1.25[a,b,c,d]	**< 0.001**
Mo	0.54 ± 0.17	0.48 ± 0.06	1.02 ± 0.31[a,b]	0.8 ± 0.11[a,b]	1.03 ± 0.22[a,b,c]	0.74 ± 0.17[a,b,c,d]	**< 0.001**

PLT – platelets; WBC – white blood cells; Ly – lymphocytes; Seg – segmented leukocytes; Mo – monocytes;
[a]*vs.* control group;
[b]*vs.* verapamil group;
[c]*vs.* $AlCl_3$ 10 mg/kg b.wt;
[d]*vs.* $AlCl_3$ 10 mg/kg b.wt/verapamil;
[e]*vs.* $AlCl_3$ 20 mg mg/kg b.wt

parameters (Table 1). The hematological parameters that were found to be significantly decreased after administration of 10 mg of $AlCl_3$ mg/kg b.wt. are as follows: the number of RBC ($p < 0.001$), Hg ($p < 0.001$), Hct ($p < 0.001$), MCV ($p < 0.001$), MCHC ($p < 0.001$), iron plasma concentration ($p < 0.001$), and TSAT ($p < 0.001$). Statistically significant increase was observed in plasma concentrations of hepcidin ($p < 0.001$), ferritin ($p < 0.001$), and transferrin, as well in values of UIBC ($p < 0.001$) and TIBC ($p < 0.001$). The identical result was obtained after 20 mg of $AlCl_3$ mg/kg b.wt. was administered, with $p < 0.001$ for all evaluated parameters (Table 1).

After verapamil administration, a statistically significant improvement in hematological parameters was demonstrated as follows: in the number of RBC ($p < 0.001$), Hg concentration ($p < 0.001$), Hct ($p < 0.001$), MCV ($p < 0.001$) and MCHC ($p < 0.001$). Parameters such as hepcidin, ferritin, transferrin, UIBC, TIBC, iron in plasma, and TSAT did not show statistically significant change after verapamil was applicated, regardless of the $AlCl_3$ dose.

The results presented in Table 2 refer to the changes in parameters of WBC and platelets after $AlCl_3$ was administered. The lower dose of aluminum (10 mg/kg b.wt) presented with the significant increase in all evaluated parameters: the number of platelets ($p < 0.001$), WBC ($p < 0.001$), percentage of lymphocytes ($p = 0.004$), segmented leukocytes ($p = 0.005$) and monocytes ($p = 0.001$), as well as in absolute count of the same cells, number of lymphocytes ($p < 0.001$), segmented leukocytes ($p < 0.001$) and monocytes ($p < 0.001$). The same result was achieved with the higher dose of aluminum (20 mg/kg b.wt), for all analyzed parameters. However, no protective effects of verapamil application were observed, since no statistically significant differences were documented after verapamil was used, Table 2.

Table 3 presents comparison of chronic $AlCl_3$ treatment (10/20 mg/kg b.wt) on biochemical parameters and the changes after verapamil administration. In the cases of lower $AlCl_3$ dose, significant increase of evaluated parameters was observed in the following: AST ($p = 0.001$) and ALT ($p < 0.001$), and concentration of ALP ($p = 0.002$),

Table 3. The comparison of chronic aluminum chloride treatment effects on biochemical parameters and the effects after verapamil administration

Biochemical parameters	Control group	Verapamil group	$AlCl_3$ 10 mg/kg b.wt	$AlCl_3$ 10 mg/kg b.wt/verapamil	$AlCl_3$ 20 mg/kg b.wt	$AlCl_3$ 20 mg/kg b.wt/ verapamil	p
AST	245.93 ± 17.56	246.17 ± 70.89	407.11 ± 143.95[a,b]	285.21 ± 41.11[a,b]	527.39 ± 227.91[a,b,c,d]	342.6 ± 58.89[a,b,c,d]	**0.001**
ALT	75.55 ± 5.91	75.70 ± 8.32	121.97 ± 48.33[a,b]	94.2 ± 10.09[a,b]	168.2 ± 32.09[a,b,c,d]	117.85 ± 19.5[a,b,c,d]	**< 0.001**
ALP	405.65 ± 16.51	405.18 ± 11.73	421.24 ± 28.04[a,b]	385.22 ± 34.22[a,b,c]	469.87 ± 63.59[a,b,c,d,]	379 ± 42.78[a,b,c,d,e]	**0.002**
AMY	2356.12 ± 219.65	2364.1 ± 131.81	3362.58 ± 124.79[a,b]	2710.39 ± 499.39[a,b,c]	3451.43 ± 112.79[a,b,c,d]	2387.85 ± 569.49[a,b,c,d,e]	**< 0.001**
LDH	2837.30 ± 510.14	2789.9 ± 403.31	4516.35 ± 1166.46[a,b]	3058.11 ± 581.03[a,b,c]	7147.86 ± 1278.45[a,b,c,d]	4911.72 ± 2332.59[a,b,c,d,e]	**< 0.001**
CK	1302.63 ± 32.04	1299.77 ± 237.76	2719.81 ± 817.52[a,b]	2113.59 ± 687.64[a,b,c]	3446.76 ± 1492.33[a,b,c,d]	2320.35 ± 331.43[a,b,c,d,e]	**< 0.001**
γGT	0.1 ± 0.13	0.33 ± 0.2	1.51 ± 0.23[a,b]	0.91 ± 0.4[a,b,c]	2.09 ± 0.34[a,b,c,d]	0.67 ± 0.39[a,b,c,d,e]	**< 0.001**
CRP	2.1 ± 0.11	2.1 ± 0.16	15.5 ± 4.5[a,b]	14.6 ± 5.2[a,b]	22.6 ± 6.6[a,b,c,d]	20.5 ± 4.5[a,b,c,d]	**0.004**

AST – aspartate transaminase; ALT – alanine transaminase; ALP – alkaline phosphatase; AMY – amylase; CK – creatine kinase; γGT – glutamyl transferase; CRP – C-reactive protein;
[a]*vs.* control group;
[b]*vs.* verapamil group;
[c]*vs.* $AlCl_3$ 10 mg/kg b.wt;
[d]*vs.* $AlCl_3$ 10 mg/kg b.wt/verapamil;
[e]*vs.* $AlCl_3$ 20 mg mg/kg b.wt

AMY ($p < 0.001$), lactate dehydrogenase ($p < 0.001$), CK ($p < 0.001$), γGT ($p < 0.001$), and CRP ($p = 0.004$). The same, significant results were obtained in higher (20 mg/kg b.wt) $AlCl_3$ doses for all the parameters. The protective effects of verapamil administration were observed in animals treated with aluminum 10 mg/kg b.wt as a significant decrease in plasma concentrations of ALP ($p < 0.001$), AMY ($p < 0.001$), LDH ($p < 0.001$), CK ($p < 0.001$), and γGT ($p < 0.001$). The identical effects of verapamil administration were documented in animals treated with higher doses of $AlCl_3$, seen also as significantly decreased values of ALP ($p < 0.001$), AMY ($p < 0.001$), LDH ($p < 0.001$), CK ($p < 0.001$), and γGT ($p < 0.001$) (Table 3). Plasma concentration of CRP and activity of AST and ALT did not change after verapamil administration.

Figure 1 presents results after comparison of chronic $AlCl_3$ treatment in tissue aluminum deposition and the effects after verapamil administration. Significant increase in aluminum tissue accumulation was observed in tissue measurements: the liver ($p < 0.001$), kidneys ($p < 0.001$), and testes ($p < 0.001$), regardless of the aluminum dose. The protective effect of verapamil was obtained in the liver ($p < 0.001$) and testes ($p < 0.001$), irrespective of the dose. In kidneys, however, no protective effect of verapamil was documented. The results are presented in Table 4.

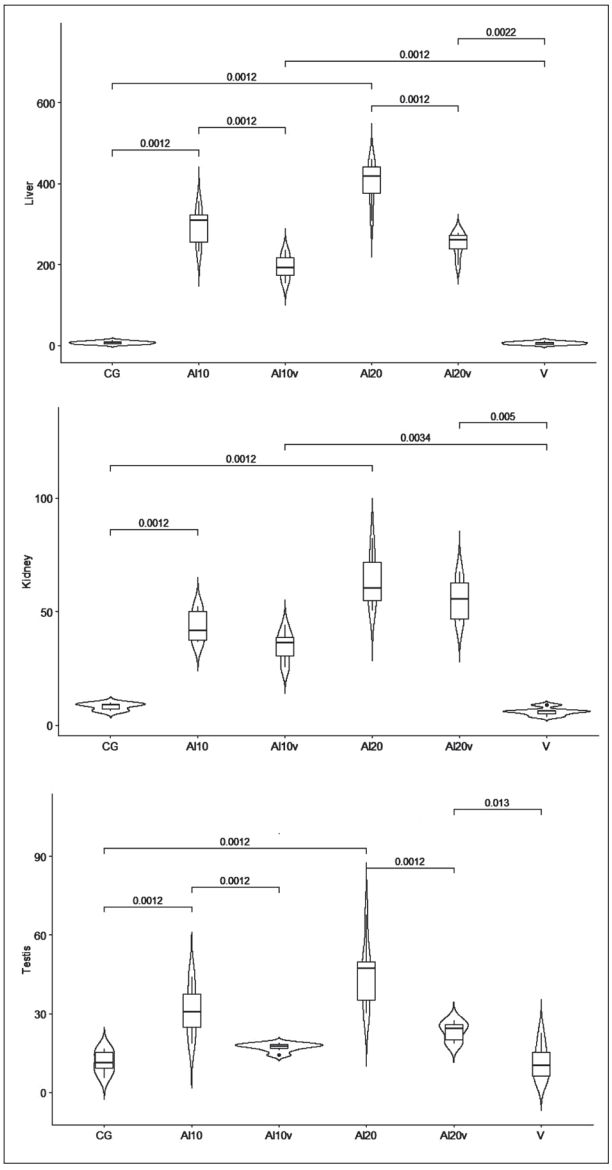

Figure 1. The comparison of chronic AlCl3 treatment of tissue aluminum deposition and the effects after verapamil administration;
CG – control group; Al 10 – AlCl3 10 mg/kg b.wt; Al 10v – AlCl3 10 mg/kg b.wt/verapamil; Al 20 – AlCl3 20 mg mg/kg b.wt; Al 20v – AlCl3 10 mg/kg b.wt/verapamil; V – verapamil group

DISCUSSION

In this study, it is documented that verapamil administration significantly mitigates aluminum-induced hematology intoxication and tissue accumulation, suggesting that it may be a protective agent against $AlCl_3$-induced toxicity. However, based on the results, the application of verapamil did not significantly decrease inflammatory parameters, implying that the inflammatory pathways in aluminum-associated toxicity are most likely independent of this mechanism of action and cannot be prevented by Ca^{2+} channel blocking.

It was evidenced that aluminum oral intake leads to its extensive accumulation in the liver, spleen, kidneys, and bones in higher rates compared to that of muscles, brain, lungs, and heart. After aluminum is deposited, its toxicity

Table 4. The comparison of chronic aluminum chloride treatment effects on tissue aluminum deposition and the effects after verapamil administration

Tissue/ groups	Control group	Verapamil group	$AlCl_3$ 10 mg/kg b.wt	$AlCl_3$ 10 mg/kg b.wt/verapamil	$AlCl_3$ 20 mg mg/kg b.wt	$AlCl_3$ 20 mg/kg b.wt/verapamil	p
Liver	7.18 ± 3.78	6.19 ± 3.70	293.24 ± 46.86[a,b]	194.44 ± 29.8[a,b,c]	403.29 ± 53.92[a,b,c,d]	251.17 ± 29.98[a,b,c,d,e]	**< 0.001**
Kidney	8.38 ± 1.56	6.05 ± 1.78	43.7 ± 9.96[a,b]	34.91 ± 6.8[a,b]	63.76 ± 11.85[a,b,c,d]	55.65 ± 9.6[a,b,c,d]	**< 0.001**
Testicle	11.63 ± 4.36	11.8 ± 6.79	31.14 ± 9.91[a,b]	17.27 ± 1.63[a,b,c]	45.01 ± 13.1[a,b,c,d]	23.35 ± 3.74[a,b,c,d,e]	**< 0.001**

[a]*vs.* control group;
[b]*vs.* verapamil group;
[c]*vs.* $AlCl_3$ 10 mg/kg b.wt;
[d]*vs.* $AlCl_3$ 10 mg/kg b.wt/verapamil;
[e]*vs.* $AlCl_3$ 20 mg mg/kg b.wt

is achieved through many diverse pathways, the following being the most studied: oxidant/antioxidant/apoptosis [8], pro-inflammatory [9], immunosuppression [10], and interference with enzymes, proteins and metabolism [11]. In addition to these findings, free intracellular Ca^{2+} variations have been proposed as an index of aluminum-associated toxic injury [12]. Indeed, reports from many experimental models implicate that chronic aluminum exposure disrupts pathways of Ca^{2+}-mediated homeostasis.

Nevertheless, Al^{3+} and Ca^{2+} share the same atomic radius; therefore, it is plausible that aluminum interacts with the binding sites of calcium, disrupting its homeostasis. In the milieu of disrupted Ca^{2+} homeostasis, mitochondria increase production of reactive oxygen species, resulting in oxidative damage, autophagy and apoptosis precipitation. Similarly, the function of calcium-ATPase enzymes may also be disrupted, resulting in cellular/organelles malfunction [13]. The inhibition of cytochrome oxidase, followed by the inhibition of respiratory chain, may lead to the depletion of ATP production via the aerobic pathway. In other words, the inhibition of cytochrome oxidase may enhance anaerobic metabolism, finally enabling the accumulation of Ca^{2+} [14]. All of this may provide a plausible pathophysiological link between Ca^{2+} disruption and oxidative damage.

Anemia was suggested as the first manifestation of aluminum toxicity, whereas much research has proved the manifestation of microcytic anemia without iron deficiency in cases of aluminum overload [15]. The results of the present study concur with these findings, since after chronic $AlCl_3$ exposure all experimental rats developed microcytic/hypochromic anemia. Importantly, a significant improvement of hematological parameters after verapamil administration was observed, regardless of the aluminum dose employed. It may, therefore, be postulated that verapamil mitigates aluminum-induced hematological toxicity.

The obtained results of a ~2.6-fold increase in hepcidin concentration suggest that the aluminum load indirectly reduces the possibilities of iron entering the erythrocyte precursors. Hepcidin concentrations are demonstrated to be significantly elevated in states of proinflammatory cytokines overproduction, leading to iron tissue sequestration, iron restriction of erythropoiesis, hypoferremia and low saturation of transferrin, which was demonstrated to be significantly decreased in this study [16, 17].

Hepcidin-related anemia is mostly presented as moderate normochromic-normocytic anemia, whereas significantly decreased values of MCV and MCHC were obtained here, so an additional pathophysiology explanation should be provided for this aluminum-related microcytosis. The result of very low iron-transferrin saturation, is most likely due to increased hepcidin production, and this implicates the likeliness of a formation of a complex of aluminum-transferrin, delivering to the erythroid precursors aluminum, instead of iron. Finally, it is plausible that it is the elevation of hepcidin concentration that initially impairs iron metabolism and that therapeutic interference with hepcidin may improve plasma concentrations of iron and iron-associated proteins.

The most important finding, that MCV and MCHC values were significantly reversed after verapamil administration, may be explained by the hypothesis that $AlCl_3$ changes erythrocyte membrane permeability for ions, interrupting the function of the cation pumps, a phenomenon that, based on presented findings, may be alleviated by calcium channels' blockage [17].

Concerning the liver, after the application of verapamil, a statistically significant decrease in liver aluminum deposition was observed, but not in the biochemical markers of liver necrosis. It has been stated that tissue exposure to aluminum leads to necrosis and biochemical abnormalities, which is in accordance with the present findings [18, 19]. The necrosis of hepatocytes is presumed by the ability of aluminum to bind to microtubule-associated phosphoprotein, located in the cytoskeleton that is an integral part of the plasma membranes of the hepatic cells, resulting in liver cell necrosis. It may be suggested that it is more likely that necrosis of hepatocytes is achieved by impaired glycolysis, ATP production, Krebs cycles and lipids' and proteins' oxidation, whereas intracellular calcium deposition represents an additional mechanism, in a way to potentiate impaired ATP production via Krebs cycle. The blockage of Ca^{2+} channels by verapamil indisputably ameliorated aluminum liver deposition, regardless of the dosage, but did not significantly reverse the concentration of liver enzymes.

The reproductive system in experimental animals is known to be significantly affected by aluminum intoxication, resulting in decreased fertility. The key factors leading to infertility are reduced sperm production and count, decreased motility and production of aberrant sperm [20]. We demonstrated increased aluminum tissue concentrations, regardless of the dose, and more importantly that this effect may be alleviated by the verapamil administration. At odds with these findings, some authors did not prove aluminum accumulation in testes, but reported the impairment of testicular function, referred to as aberrant spermatogenesis and sperm malformation [20]. The

researchers postulated that aluminum exposure may cause inhibition of acid phosphatase, succinate dehydrogenase, and LDH isoenzyme. Aluminum presumably inhibits activity of Ca^{2+}-ATPase, resulting in the dysfunction of testicular membrane activity.

Finally, aluminum is proven to contribute to the pathogenesis of inflammation, by up-regulating proinflammatory cytokines and giving the signal for hepcidin synthesis. Within the study, inflammation was proven by increased CRP and ferritin concentration and, indirectly, by hepcidin evaluation. Based on the resulting outcomes, neither of the biomarkers was significantly decreased after verapamil was employed. It may even be speculated that these effects remain in consequence of the calcium generated from the intracellular store, a phenomenon that occurs in the presence of metals and may not be mitigated by verapamil [13].

CONCLUSION

The findings of the present study indicate that chronic $AlCl_3$ intoxication, regardless of the dose, results in the development of microcytic anemia associated with high hepcidin levels, numerous biochemical disarrangements and significant aluminum deposition in the liver, kidney, and testes. In addition, a significant hemato-protective effect of verapamil was documented, as well as the attenuation of aluminum deposits in both the liver and the testes. Overall, the results of the research emphasize the significance of calcium homeostasis preservation in chronic aluminum exposure and propose a possible therapeutic option.

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Conflict of interest: None declared.

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Употреба верапамила ублажава микроцитозу и ткивну акумулацију после хроничне изложености алуминијуму

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САЖЕТАК

Увод/Циљ Циљ истраживања био је да се испита степен алуминијумске токсичности, процењен хематолошким и биохемијским параметрима, променом концентрације хепцидина и акумулацијом алуминијума у ткивима након хроничне изложености и да се утврди да ли постоји протективни ефекат верапамила, остварен блокадом канала Ca^{2+}.

Методе Експерименталне животиње (36 пацова) третиране су различитим дозама $AlCl_3$ током периода од осам недеља, а након тога анализирани су њихови крв и ткива.

Резултати Значајне разлике, без обзира на дозу алуминијума, постојале су у свим хематолошким ($p < 0,001$) и биохемијским параметрима ($p < 0,001$), као и у акумулацији алуминијума у јетри, бубрезима и тестисима. Након примене верапамила доказано је значајно побољшање вредности појединих хематолошких и биохемијских параметара, ($p < 0,001$), као и значајно смањење акумулације алуминијума у ткиву јетре и тестиса ($p < 0,001$). Вредности анализираних параметара инфламације и акумулације алуминијума у ткиву бубрега нису биле значајно различите након примене верапамила.

Закључак Резултати овог истраживања показују да хронична интоксикација $AlCl_3$, без обзира на примењену дозу, доводи до развоја микроцитне анемије праћене високим концентрацијама хепцидина, биохемијским поремећајима и значајним таложењем алуминијума у ткиву јетре, бубрега и тестиса, као и да се неки од ових ефеката могу ублажити давањем верапамила. Добијени резултати сугеришу значајну патогенетску везу између хомеостазе калцијума и алуминијумске токсичности, на основу чега се може говорити о потенцијалној терапијској опцији током хроничне експозиције алуминијуму.

Кључне речи: токсичност изазвана алуминијумом; верапамил; хронична изложеност; хепцидин; микроцитоза

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Clinical significance of proliferation index and E-cadherin expression in colorectal adenocarcinoma

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SUMMARY

Introduction/Objective The aim of this study is to examine the association of E-cadherin expression and high proliferation index (proIDX) with clinical and pathological indicators of colorectal cancer progression.

Methods The biopsy of 72 patients, obtained by resection of colorectal cancer, was routinely processed at the Institute of Pathology of the Clinical Centre of Montenegro, embedded in paraffin and archived. Based on the archived pathohistological reports, two study groups were formed: the first group (n = 72) consisted of operative biopsies of colorectal cancer, and the control group (n = 72) consisted of biopsies of adjacent non-tumor tissue. Routine hematoxylin-eosin and immunohistochemical avidin-biotin-peroxidase complex method with anti-Ki67 and anti-E-cadherin antibodies was applied on. After quantification of the results for statistical tests, the software package SPSS for Windows (19.0) was used.

Results In colorectal carcinoma, we observed a significant association of proIDX with pT stage, lymph and blood vessel invasion, perineural invasion, lymph node metastases and distant metastases, and Astler–Coller stage tumor disease. We also observed that the absence of E-cadherin was significantly associated with pT stage, lymph and blood vessel invasion, perineural invasion with lymph node metastases, distant metastases, with C2 and D Astler–Coller tumor stage. E-cadherin expression is associated with proIDX with a significantly high, negative correlation coefficient.

Conclusion Our results indicate that it is possible to differentiate patients into groups with a higher or lower risk of developing metastatic disease, based on the expression of Ki67 and E-cadherin.

Keywords: colorectal cancer; E-cadherin expression; proliferative index; clinical significance

INTRODUCTION

Colorectal cancer is a complex disease caused by the interaction of genetic, epigenetic and environmental factors. Colorectal carcinogenesis is a consequence of the "interplay" between environmental factors on the one side and different oncogenes, suppressor genes and their products on the other, where cell proliferation is one of the most significant biological events in that process [1].

A nuclear antigen is isolated from the proliferating cells, which was used to produce a monoclonal Ki67 antibody of IgG1 class. The antigen detected by the Ki67 antibody is present in the cell nuclei during the G_1, S, G_2, and M phases on the cell cycle. It cannot be detected in the G_0 phase [2].

Cadherin adhesion molecules also play an important role in colorectal carcinogenesis [3]. Cadherins are a family of glycoproteins that perform calcium (Ca_2+) dependent intercellular adhesion at intercellular junctions [4]. Cadherins participate in the processes of embryogenesis and they are involved in the malignant transformation of cells, where a significant reduction of their expression occurs. It has been proved that cadherins inhibit invasion and it is hypothesized that they activate the process of malignant cell dissemination [5, 6]. E-cadherin plays a key role in establishing epithelial architecture, in differentiation and in maintaining cell polarity [4]. Numerous studies have shown that E-cadherin is a suppressor of tumor invasion and metastasis [6, 7].

Considering the fact that the loss of E-cadherin molecules results in a disruption of cytoskeletal structure, which allows cell separation from tumor and increased cell migration [6], this study aims is to examine the correlation between E-cadherin expression and proliferation index (proIDX) on the one side, and clinical and pathological indicators of colorectal cancer progression on the other.

METHODS

Patients and samples

The biopsies and the operative material of 72 patients were obtained by resection of colorectal tumor at the Centre for Abdominal Surgery of the Clinical Centre of Montenegro (CCM)

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Table 1. Clinicopathological features of colorectal carcinoma studied

Clinicopathological factors	Number of cases (%)	Age (min/max/$\bar{X}$)
Males Females	42 (58.33) 30 (52.77)	34/83/66.1 ± 10.6 27/83/61.4 ± 14.2
Localization	**Number of cases (%)**	
Cecum (+ileocecum) / Ascendant colon / Transverse colon / Sigma (+descendant colon) / Rectum (+anorectum + rectosigma)	3 (4.2) / 11 (15.3) / 1 (1.4) / 9 (12.5) / 48 (66.7)	
Macroscopic appearance of the tumor	**Number of cases (%)**	
Ulceroinfiltrative/Infiltrative/Vegetatively-infiltrative/Vegetative/Ulcerative	27 (37.5) / 25 (34.7) / 16 (22.2) / 3 (4.2) / 1 (1.4)	
Histological grade	**Number of cases (%)**	
Grade I / Grade II / Grade III	4 (5.6) / 63 (87.5) / 5 (6.9)	
Invasion of lymphatic vessels	**Number of cases (%)**	
Not identified/Present	33 (45.8) / 39 (54.2)	
Invasion of blood vessels	**Number of cases (%)**	
Not identified / Present	61 (84.7) / 11 (15.3)	
Nerve invasion	**Number of cases (%)**	
Not identified/Present	59 (81.9) / 13 (18.1)	
Pathological stage of the tumor(pT)	**Number of cases (%)**	
pT_1 / pT_3 / pT_4	2 (2.8) /54 (75.0) / 4 (5.6)	
Lymph node metastases	**Number of cases (%)**	
No deposits/Deposits in 1–3 lymph nodes/Deposits in 4–6 lymph nodes/ Deposits in seven and more lymph nodes	35 (48.6) / 19 (26.4) / 8 (11.1) / 10 (13.9)	
Distant metastases	**Number of cases (%)**	
Not identified/Present	64 (88.9) / 8 (11.1)	
Astler–Coller stage of tumor	**Number of cases (%)**	
B1/B2/C1/C2/D	11 (15.3) / 21 (29.2) / 3 (4.2) / 30 (41.7) / 7 (9.7)	

between January 2010 and December 2012. At the Institute of Pathology CCM, 5–15 tissue samples were obtained from each surgery, according to the established protocol, depending on the size of the tumor, including 2–3 biopsies of the adjacent, non-tumorous colorectal tissue. After fixation the bioptic material was routinely processed, embedded in paraffin and archived. Based on the standard pathological reports from that period, an experimental group was formed that consisted of operative biopsies of colorectal adenocarcinoma (n = 72). The control group (n = 72) consisted of operative biopsies of the adjacent non-tumorous colorectal tissue (epithelial cells), from the same patients in the experimental group. The study protocol was approved by the local ethics committee.

Histopathology and immunohistochemistry

On paraffin blocks, where samples of tumor tissue and regional lymph nodes were embedded, 3–5 μm thick cuts were made. For histopathological verification of lesions, the routine hematoxylin-eosin (HE) method was applied.

Representative samples of tumor and adjacent non-tumor tissue were used for immunohistochemical analysis. Immunostaining was performed using the avidin-biotin-peroxidase complex (ABC) method (Vectastain ABC Elite-kit, Vector Laboratories, Burlingame, CA, USA), with mouse monoclonal anti-E-cadherin antibody (DAKO, Denmark, clone NCH-38, 1:50) and rabbit monoclonal anti-Ki67 antibody (Abcam, Burlingame, CA, USA, clone SP6, 1:100).

Expression of E-cadherin in carcinoma cells was measured in 10 fields of microscopic magnification 400× (mean value represents the final result for the case) and classified in the following manner: Intramembranous immunohistochemical reaction with < 10% of immunoreactive cells was considered a negative immunoreaction (−), and the presence of intramembrane expression in ≥ 10% was evaluated as a positive reaction; an immunohistochemical reaction present in < 50% of cells was classified as moderate expression (1+); a positive immunohistochemical reaction present in > 50% of cells was classified as pronounced expression (2+) of E-cadherin.

To evaluate the expression of Ki67+ cells per mm^2 by area, test system M42 according to Weibel was used. Objective micrometer calibrated the test system on a Nikon Eclipse Ni MBP 99 400 microscope at a magnification of 400, with a measurement field of 0.016 mm^2. To test the density of Ki67+ cells per mm^2, 10 "hot-spots" were counted successively. The absolute value of the density of positive cells in the "hot-spot" was determined stereometrically [8]. The means of the obtained values of the "hot-spots" represents the final number of Ki67+ cells in mm^2 per case. The median was subsequently determined and the absolute values of the density of positive cells were divided into two groups: those with the low level of expression (the value ≤ the median value) and those with the high level of expression (values>the median value). From absolute determined values of Ki67 regarding deviation from median Ki67 index (proIDX) was obtained.

Statistical analysis

The statistical software package SPSS for Windows, Version 19.0 (IBM Corp., Armonk, NY, USA) was used. To analyze the statistical significance of parametric and nonparametric

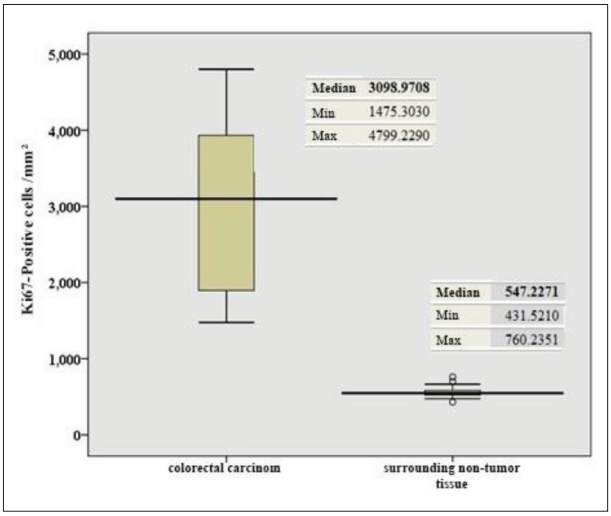

Figure 1. Distribution of Ki67–immunopositive cells in mm² of examined tissue

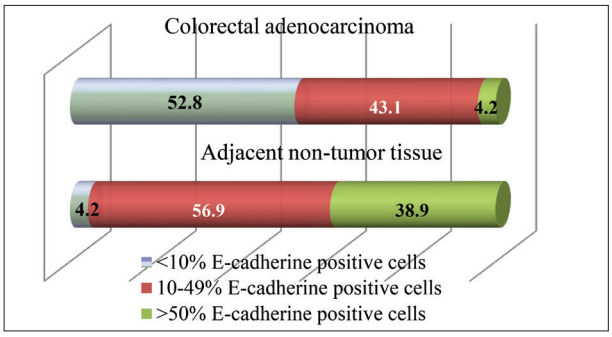

Figure 2. E-cadherin expression in colorectal cancer and adjacent non-tumor tissue

features, between and within the groups, χ^2-test, the Kruskal–Wallis test, Fisher exact test and Student's t-test were used. Afterwards, the Kolmogorov–Smirnov normality test, correlation analysis (Spearman's rank correlation coefficient).

P values less than 0.05 were considered statistically significant.

RESULTS

This study included 72 patients with colorectal adenocarcinoma (42 men, mean age 66.1 ± 10.6 years, range 34–83, and 30 women, mean age 61.4 ± 14.2 years, range 27–83). The main clinical and pathological characteristics of colorectal adenocarcinoma are shown in Table 1.

Immunohistochemical expression of Ki67 and E-cadherin in colorectal adenocarcinoma and adjacent non-tumor tissue

Immunohistochemical analysis of the Ki67 expression, gave absolute values of the number of immunopositive cells in mm² of tissue. Ki67 expression reported by the number of immunopositive cells in mm² (median = 3098.9708; min = 1475.3, max = 4799.2) in colorectal carcinoma tissue, was significantly higher compared to the control group-adjacent, non-tumor tissue (median = 547.227; min = 431.5, max = 760.2; $p < 0.001$) (Figure 1).

Immunohistochemical analysis of E-cadherin expression showed that there is a statistically significant difference in the expression of this marker between colorectal carcinoma tissue and adjacent non-tumor tissue. Namely, in colorectal adenocarcinoma, the absence of E-cadherin expression was found in a significant 52.8% of cases ($p < 0.001$). It is also observed that the expression of E-cadherin is significantly increased (38.9%, $p < 0.001$) in the adjacent non-tumor tissue compared to the colorectal carcinoma, where only 4.2% of cases expressed E-cadherin (Figure 2).

Association and correlation analysis between Ki67 expression/proliferative index (proIDX) and clinical and pathological characteristics of colorectal cancer (Table 2)

There was a significant correlation of proliferation index (proIDX) with the pT stage of the tumor because in 100% of cases there was the correlation between low pro IDX and pT1 and in 91.7% of cases between low proIDX and the pT2 stage of the tumor. This correlation is defined by a significant, positive correlation coefficient ($r = 0.352$, $p = 0.002$).

High proIDX was associated with lymphatic invasion in 82.1% of cases, while in 87.9% of cases low proIDX was associated with tumors in which lymphatic vessel invasion was not identified. Pro IDX was associated with lymph vessel invasion with a significant, positive and high correlation coefficient ($r = 0.697$, $p = 0.000$).

High proIDX was significantly associated with vascular invasion in 90.9% of cases, while in 92.3% of cases with neural invasion, there were lower but significant and positive correlation coefficients ($r = 0.347$ and $r = 0.397$, $p = 0.003$ and $p = 0.001$)

There was a significant correlation between high proIDX and lymph node metastases. High proIDX was significantly associated with metastases in 1–3 lymph nodes in 84.2%, with metastases of 4–6 lymph nodes in 100% and with metastases of seven and more lymph nodes in 100% of cases. It is also noteworthy that low proIDX was associated with the absence of lymph node metastases in 94.3% of cases. Lymph node metastases were highly, positively, and significantly correlated with proIDX ($r = 0.766$, $p = 0.000$).

High proliferation index (Figure 3) was in 100% of cases associated with distant metastases with a significant and positive correlation coefficient ($r = 0.354$, $p = 0.002$).

ProIDX was significantly associated with Astler–Coller stage of tumor disease. Low proIDX was significantly associated with stage B1 in 90.9% and stage B2 in 100% of cases. At the same time, high proIDX was significantly associated with stage C2 in 90% and with stage D in 100% of cases. This correlation was defined by a highly significant and high correlation coefficient ($r = 0.818$, $p = 0.000$).

In this study, no significant correlation was found between the proIDX/Ki67 expression and subjects' gender,

Table 2. Association of Ki67 and E-cadherin expression with clinico-pathological parameters

Type of tumor		Colorectal carcinoma				
Markers		Ki67		E-cadherin		
Tested parameters		**low**	**high**	**-**	**+**	**++**
		n (%)	**n (%)**	**n (%)**	**n (%)**	**n (%)**
Sex	Male	21 (50)	21 (50)	22 (52.4)	20 (47.6)	0 (0)
	Female	15 (50)	15 (50)	16 (53.3)	11 (36.7)	3 (10)
Significance	p	/		**0.096**		
Age of the subjects	≤ 65	14 (42.4)	19 (57.6)	18 (54.5)	14 (42.4)	1 (3)
	> 65	22 (56.4)	17 (43.6)	20 (51.3)	14 (43.6)	2 (5.1)
Significance	p	**0.237**		**0.891**		
Localization	Rectum (anorectum + rectosygma)	23 (47.9)	25 (52.1)	26 (54.2)	19 (39.6)	3 (6.2)
	Sygma (+sin. colon)	7 (77.8)	2 (22.2)	4 (44.4)	5 (55.6)	0 (0)
	Colon ascendens	5 (45.5)	6 (54.5)	5 (45.5)	6 (54.5)	0 (0)
	Transverse colon	0 (0)	1 (100)	1 (100)	0 (0)	0 (0)
	Cecum(ileocecum)	1 (33.3)	2 (66.6)	2 (66.7)	1 (33.3)	0 (0)
Significance	p	**0.369**		**0.893**		
Tumor growth pattern	Ulceroinfiltrative	12 (44.4)	15 (55.6)	16 (59.3)	10 (37)	1 (3.7)
	Infiltrative	13 (52)	12 (48)	11 (44)	13 (52)	1 (4)
	Vegetative	3 (100)	0 (0)	0 (0)	2 (66.7)	1 (33.3)
	Vegetatively-infiltrative	7 (43.8)	9 (56.2)	11 (68.8)	5 (31.2)	0 (0)
	Ulcerovegetative	1 (100)	0 (0)	0 (0)	1 (100)	0 (0)
Significance	p	**0.328**		**0.124**		
Histological grade of the tumor	Grade I	3 (75)	1 (25)	2 (50)	2 (50)	0 (0)
	Grade II	32 (50.8)	31 (49.2)	31 (49.2)	29 (46)	3 (4.8)
	Grade III	1 (20)	4 (80)	5 (100)	0 (0)	0 (0)
Significance	p	**0.245**		**0.285**		
Lymphatic invasion	Not identified	**29 (87.9)**	4 (12.1)	4 (12.1)	**26 (78.8)**	3 (9.1)
	Lymphatic invasion present	7 (17.9)	**32 (82.1)**	**34 (87.2)**	5 (12.8)	0 (0)
Significance	p	**< 0.001***		**< 0.001***		
Blood vessels invasion	Not identified	35 (57.4)	26 (42.6)	28 (45.9)	30 (49.2)	3 (4.9)
	Blood vessels invasion present	1 (9.1)	**10 (90.9)**	**10 (90.9)**	1 (9.1)	0 (0)
Significance	p	**0.003***		**0.022***		
Nerve invasion	Not identified	35 (59.3)	24 (40.7)	26 (44.1)	31 (52.5)	2 (3.4)
	Nerve invasion present	1 (7.7)	**12 (92.3)**	**12 (92.3)**	0 (0)	1 (7.7)
Significance	p	**< 0.001**		**0.020***		
Lymph node metastases	No deposit	**33 (94.3)**	2 (5.7)	2 (5.7)	**30 (85.7)**	3 (8.6)
	Deposits in 1–3 LN	3 (15.8)	**16 (84.2)**	**18 (94.7)**	1 (5.3)	0 (0)
	Deposits in 4–6 LN	0 (0)	**8 (100)**	**8 (100)**	0 (0)	0 (0)
	Deposits in > 7 LN	0 (0)	**10 (100)**	**10 (100)**	0 (0)	0 (0)
Significance	p	**< 0.001***		**< 0.001***		
Distant metastases	Not identified	36 (56.2)	28 (43.8)	30 (46.9)	31 (48.4)	3 (4.7)
	Metastases present	0 (0)	**8 (100)**	**8 (100)**	0 (0)	0 (0)
Significance	p	**0.003***		**0.018***		
Pathological stage of the tumor	pT_1	**2 (100)**	0 (0)	0 (0)	**2 (100)**	0 (0)
	pT_2	**11 (91.7)**	1 (8.3)	0 (0)	**10 (83.3)**	2 (16.7)
	pT_3	21 (38.9)	33 (61.1)	**34 (63)**	19 (35.2)	1 (1.9)
	pT_4	2 (50)	2 (50)	4 (100)	0 (0)	0 (0)
Significance	p	**0.005***		**< 0.001***		
Astler–Coller stage of tumor	B1	**10 (90.9)**	1 (9.10)	**0 (0)**	**9 (81.8)**	2 (18.2)
	B2	**21 (100)**	0 (0)	1 (4.8)	**19 (90.5)**	1 (4.8)
	C1	2 (66.7)	1 (33.3)	0 (0)	3 (100)	0 (0)
	C2	3 (10)	**27 (90)**	**30 (100)**	0 (0)	0 (0)
	D	0 (0)	**7 (100)**	7 (100)	0 (0)	0 (0)
Significance	p	**< 0.001***		**< 0.001***		

*significant difference $p < 0.05$, χ^2 test, Fisher's exact test

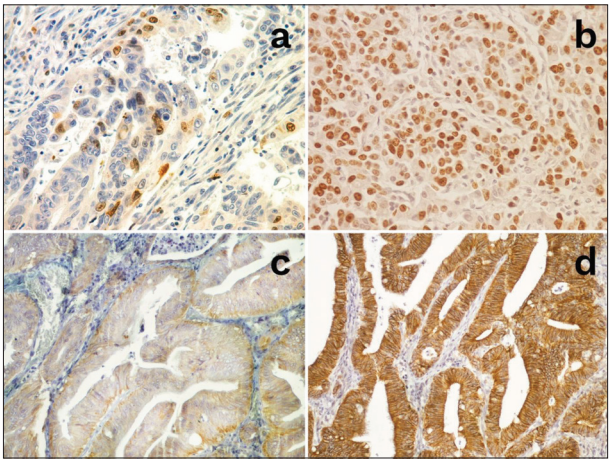

Figure 3. Immunohistochemical expression of Ki67 and E-cadherin in colorectal adenocarcinoma; a – low Ki67 expression index; b – high Ki67 expression index; c – lack of expression and expression of trace E-cadherin; d – pronounced E-catherin expression (ABC × 400)

age, localization of the tumor in the colorectum and the histological grade of the tumor ($p > 0.05$).

Association and correlation analysis between E-cadherin expression and clinical and pathological characteristics of colorectal cancer (Table 2)

A statistically significant correlation between E-cadherin expression and pT stage of the tumor was observed. In 63% of cases, there was a correlation between absent E-cadherin expression and pT3 stage of the tumor ($p = 0.039$). There was also a significant correlation between moderate E-cadherin expression and pT1 and pT2 stages ($p = 0.021$). This correlation was defined by a significant, inverse, moderate correlation coefficient ($r = -0.515$).

There was a statistically significant correlation between E-cadherin expression and lymphatic vessel invasion in colorectal cancer. Namely, the absence of E-cadherin expression was associated with lymphatic vessel invasion in 87.2% of cases. In cases where lymph vessel invasion was not identified, there was a moderate expression of E-cadherin in 78.8% of cases. E-cadherin expression was also associated with lymph vessel invasion with a high negative and significant correlation coefficient ($r = -0.726$, $p = 0.000$).

The expression of E-cadherin was associated with the invasion of blood vessels with a significant, negative and weak correlation coefficient ($r = -0.311$, $p = 0.008$), so that in 90.9% of cases, the absence of E-cadherin expression was associated with vascular invasion.

The absence of E-cadherin expression was significantly associated with nerve invasion in 92.3% of cases. Neural invasion and E-cadherin expression were associated with weak, significant negative correlation coefficient ($r = -0.293$, $p = 0.013$).

In a significant number of cases, the absence of E-cadherin expression was associated with lymph node metastases. In 94.7% of cases, there was an absence of E-cadherin expression associated with metastases in 1–3 lymph nodes, in 100% of cases with metastases in 4–6 lymph nodes, as well as in 100% of cases with metastases in seven or more lymph nodes. In lymph nodes, where no metastatic deposits were identified, moderate expression of E-cadherin was present in a significant number of cases (85.7%, $p < 0.001$). Lymph node metastases and E-cadherin expression were associated with a significant, high but negative correlation coefficient ($r = -0.729$, $p = 0.000$).

In 100% of cases, there was a significant correlation between the absence of E-cadherin expression and distant metastases.

There was a statistically significant correlation between E-cadherin expression and the Astler–Coller stage of tumor disease. Moderate expression of E-cadherin was associated with B1 and B2 Astler–Coller stage in a significantly higher number of cases (81.2% and 90.5%, $p = 0.021$). Absence of E-cadherin expression was significantly associated with C2 and D Astler–Coller stage tumors in 100% of cases ($p < 0.001$). E-cadherin expression was associated with the Astler–Colller stage of tumor disease with a very high, negative correlation coefficient ($r = -0.875$, $p = 0.000$).

In this study, there was also a highly significant correlation between E-cadherin expression and proIDX defined by a high, negative correlation coefficient ($r = -0.794$, $p = 0.000$).

No statistical significance was observed between E-cadherin expression and subjects' sex, age, tumor localization and histological grade of colorectal cancer ($p > 0.05$).

DISCUSSION

Numerous studies have observed the association of cell proliferation with the aggressive biological behavior of tumors of different localization [9–12], while the expression of Ki67 protein has been identified as a good predictor of recurrence and as an independent prognostic factor of survival rate [12, 13].

The expression of Ki67 protein during the cell cycle is strictly regulated by the balance between synthesis and degradation, which means that its half-life is short and is only 60–90 minutes long. During the cell cycle Ki67 becomes detectable in the mid-late G_1 phase when levels are low, and then its expression increases through the S and G_2 phases, and peaks in the early M phase. In the late stages of mitosis, the expression of Ki67 falls sharply and then disappears [14].

In this study, we observed that low proIDX has a good correlation coefficient significantly associated with pT_1 and pT_2 stages, while high proIDX is associated with significant and high correlation coefficients to lymph vessel invasion, blood vessel invasion, neural invasion, lymph node metastases and distant metastases. In a recent study by Tong et al. [15], a sample of 1,090 subjects with colorectal cancer observed a significant association of Ki67 expression with pT stage, histological grade, AJCC stage, and lymph node metastases.

The proliferation index calculated in our study indicates the existence of a very strong relationship between Ki67

Table 3. Correlation matrix of clinical-pathological parameters and expression of colorectal adenocarcinoma markers

		Sex	Age	Localization.	pT stage	Histol. Grade	AC Stage	Growth pattern	Lymph vessel invas.	Blood vessel invas.	Neural invasion	Lymph node metast.	Distant metastases	Ki67	E-cadherin
Sex	r	1.000	0.188	-0.058	0.102	-0.113	0.034	-0.204	-0.071	-0.202	0.043	0.024	-0.030	0.000	0.077
	p	/	0.113	0.630	0.394	0.345	0.776	0.086	0.555	0.088	0.722	0.840	0.803	1.000	0.519
Age	r	0.188	1.000	0.099	-0.141	0.012	-0.025	-0.057	-0.029	0.068	-0.046	0.021	0.072	-0.031	0.015
	p	0.113		0.408	0.238	0.917	0.833	0.635	0.807	0.569	0.700	0.860	0.546	0.798	0.904
Localization	r	0.058	0.099	1.000	0.158	0.088	0.104	-0.155	0.160	-0.037	-0.079	0.141	0.037	0.053	-0.060
	p	0.630	0.408		0.184	0.464	0.384	0.193	0.179	0.754	0.511	0.238	0.757	0.661	0.617
pT stage	r	0.102	0.141	0.158	1.000	0.154	**0.638**	-0.131	**0.429**	0.128	0.142	**0.372**	0.187	**0.352**	**-0.515**
	p	0.394	0.238	0.184		0.196	**0.000**	0.274	**0.000**	0.284	0.236	**0.001**	0.117	**0.002**	**0.000**
Histol. Grade	r	0.113	0.012	0.088	0.154	1.000	0.120	-0.104	0.115	-0.017	0.084	0.224	-0.014	0.197	-0.171
	p	0.345	0.917	0.464	0.196		0.316	0.382	0.336	0.889	0.484	0.058	0.908	0.098	0.150
AC stage	r	0.034	0.025	0.104	**0.638**	0.120	1.000	-0.082	**0.756**	**0.468**	**0.437**	**0.726**	**0.503**	**0.818**	**-0.875**
	p	0.776	0.833	0.384	**0.000**	0.316		0.494	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**
Growth pattern	r	0.204	0.057	-0.155	-0.131	-0.104	-0.082	1.000	0.071	-0.120	0.061	-0.153	-0.083	0.059	-0.013
	p	0.086	0.635	0.193	0.274	0.382	0.494		0.551	0.316	0.609	0.201	0.489	0.625	0.912
Lymph vessel invasion	r	0.071	0.029	0.160	**0.429**	0.115	**0.756**	0.071	1.000	**0.391**	**0.359**	**0.698**	**0.325**	**0.697**	**-0.726**
	p	0.555	0.807	0.179	**0.000**	0.336	**0.000**	0.551		**0.001**	**0.002**	**0.000**	**0.005**	**0.000**	**0.000**
Blood vessel invasion	r	0.202	0.068	-0.037	0.128	-0.017	**0.468**	-0.120	**0.391**	1.000	**0.403**	**0.255**	**0.710**	**0.347**	**-0.311**
	p	0.088	0.569	0.754	0.284	0.889	**0.000**	0.316	**0.001**		**0.000**	**0.030**	**0.000**	**0.003**	**0.008**
Neural invasion	r	0.043	0.046	-0.079	0.142	0.084	**0.437**	0.061	**0.359**	**0.403**	1.000	**0.279**	**0.638**	**0.397**	**-0.293**
	p	0.722	0.700	0.511	0.236	0.484	**0.000**	0.609	**0.002**	**0.000**		**0.018**	**0.000**	**0.001**	**0.013**
Lymph node metastases	r	0.024	0.021	0.141	**0.372**	0.224	**0.726**	-0.153	**0.698**	**0.255**	**0.279**	1.000	0.156	**0.766**	**-0.729**
	p	0.840	0.860	0.238	**0.001**	0.058	**0.000**	0.201	**0.000**	**0.030**	**0.018**		0.190	**0.000**	**0.000**
Distant metastases	r	0.030	0.072	0.037	0.187	-0.014	**0.503**	-0.083	**0.325**	**0.710**	**0.638**	0.156	1.000	**0.354**	**-0.315**
	p	0.803	0.546	0.757	0.117	0.908	**0.000**	0.489	**0.005**	**0.000**	**0.000**	0.190		**0.002**	**0.007**
Ki67	r	0.000	0.031	0.053	**0.352**	0.197	**0.818**	-0.059	**0.697**	**0.347**	**0.397**	**0.766**	**0.354**	1.000	**-0.794**
	p	1.000	0.798	0.661	**0.002**	0.098	**0.000**	0.625	**0.000**	**0.003**	**0.001**	**0.000**	**0.002**		**0.000**
E-cadherin	r	0.077	0.015	-0.060	**-0.515**	-0.171	**-0.875**	-0.013	**-0.726**	**-0.311**	**-0.293**	**-0.729**	**-0.315**	**-0.794**	1.000
	p	0.519	0.904	0.617	**0.000**	0.150	**0.000**	0.912	**0.000**	**0.008**	**0.013**	**0.000**	**0.007**	**0.000**	

*statistically significant difference $p < 0,05$; Spearman correlation coefficient

expression and Astler–Coller stage, supported by the high correlation coefficient that defined this relationship (Spearman rho = 0.818). High proIDX is significantly associated with C2 and D stages, which is consistent with the suggestion of Li et al. [16], that a high proliferative index may be a useful biomarker to aid in the assessment of disease outcome in patients with stage III and IV colorectal cancer.

In this study, no significant association was found between Ki67/proIDX expression with sex, age of the subjects, localization of the tumor in the colon and with the histological grade of colorectal cancer. However, there is an observation in the literature about a significant correlation the high expression of Ki67 with an older age of patients [17].

Invasion and metastasis of malignant tumors, in addition to the kinetics and proliferative capacity of tumor cells, include the interaction between tumor cells themselves and between tumor cells and their microenvironment in which changes in cell adhesion play an important role [3, 6].

The expression of E-cadherin on epithelial cell membranes maintains cell connections and suppresses cell invasion. Mutation of the *CDH1* gene which encodes E-cadherin results in the formation of a mutated, dysfunctional protein, which further results in decreased cell adhesion and uncontrolled growth of malignant cells [18]. Many studies have shown an association between reduced /absent E cadherin expression and progression of tumors [3, 6].

We identified a loss of E-cadherin expression in 52.8% of colorectal cancers, which is in complete agreement with the results of Kim et al. [6], who examined 689 colorectal cancers in 52% of cases to verify the loss of E-cadherin expression. Also, in agreement with other reports [3, 19], a significant difference in E-cadherin expression between tumor and adjacent non-tumor tissue was observed in our study.

As expected, the data on the expression of E-cadherin in relation to the clinical-pathological characteristics of colorectal cancer are heterogeneous and contradictory [5, 6]. In this study, we identified a significant association between reduced and/or absent E-cadherin expression with pT tumor stage, lymph and blood vessel invasion, perineural invasion, lymph node metastases, distant metastases, and Astler–Coller stage tumor disease. Other authors have observed that loss of E-cadherin expression is associated with lymph node metastases, but not with other parameters of colorectal cancer progression [6]. Kim et al. [6] found significantly lower E-cadherin expression in cases with infiltrative tumor growth, lymph node metastasis and distant metastasis.

Our results indicate the existence of a highly significant relationship between the loss of E-cadherin expression and proIDX/Ki67 expression defined by a high, negative correlation coefficient. Also, a significant inverse correlation between E-cadherin expression and proIDX was observed in other tumors as such esophageal squamous cell carcinoma [20].

In this study, we did not observe a significant association of E-cadherin expression with sex, age of the subjects, tumor localization in the colorectum, the mode of tumor growth or with the histological grade of the tumor. Kim et al. [6] reported a significant association between the loss of E-cadherin expression with infiltrative tumor growth, while Palaghia et al. [3], reported an association of negative expression of this marker with the age of subjects, with a higher prevalence in younger patients.

It has been observed that loss of E-cadherin expression stimulates Wnt, Rho GTPs and PI3K/AKT signaling ,which are thought to play an active role in the epithelial-mesenchymal transition (EMT) process [21, 22]. E-cadherin dysregulation promotes dysfunctions of these signaling pathways and affects the polarity, survival, invasion, and migration of cancer cells [4, 5, 21]. E-cadherin dysregulation occurs mainly due to somatic alterations of the *CDH1* gene, which is often an early stage in colorectal carcinogenesis. In addition to colorectal cancer, somatic mutations in the *CDH1* gene have also been identified in sporadic diffuse gastric cancers, lobular breast cancer, ovarian cancer, and prostate cancer [23].

CONCLUSION

High proliferative index/high levels of Ki67 expression and loss/reduced expression of E-cadherin are both mutually and strongly correlated with indicators of colorectal cancer progression. The proliferation index and expression of E-cadherin do not depend on sex, age, histological grade, localization and growth pattern of colorectal cancer.

Finally, this study supports the view that it is possible to differentiate patients into groups with a higher or lower risk of developing metastatic disease, based on the expression of these two biomarkers.

Conflict of interest: None declared.

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Клинички значај индекса пролиферације и експресије е-кадхерина у колоректалном аденокарциному

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САЖЕТАК

Увод/Циљ Циљ студије је испитивање повезаности експресије е-кадхерина и високог индекса пролиферације са клиничко-патолошким показатељима прогресије колоректалног карцинома.

Методе Биопсијски материјал 72 болесника добијен ресекцијом колоректалног карцинома је у Институту за патологију Клиничког центра Црне Горе рутински обрађиван, калупљен у парафин и архивиран. На основу архивираних патохистолошких извештаја формиране су две студијске групе: прву групу ($n = 72$) чиниле су оперативне биопсије колоректалног карцинома, а контролну групу ($n = 72$) чиниле су биопсије суседног нетуморског ткива. Примењени су рутински хематоксилин-еозин бојење и имунохистохемијска *ABC* (*avidin-biotin-peroxidase complex*) метода са анти-*Ki67* и анти-е-кадхерин антителима. Након квантификације резултата за статистичка тестирања је коришћен програмски пакет *SPSS* за *Windows* (19.0).

Резултати У ткиву колоректалног карцинома је запажена значајна повезаност високог индекса пролиферације са *pT* стадијумом, инвазијом лимфних и крвних судова, перинеуралном инвазијом, метастазама у лимфним чворовима и удаљеним метастазама и стадијумом туморске болести Астлер–Колер. Такође је запажено да је одсуство експресије е-кадхерина значајно повезано са *pT* стадијумом, инвазијом лимфних и крвних судова, перинеуралном инвазијом, са метастазама у лимфним чворовима, удаљеним метастазама, и са стадијумом тумора *C2* и *D* Астлер–Колер. Експресија е-кадхерина је значајним, високим али негативним коефицијентом корелације повезана са високим индексом пролиферације.

Закључак: Наши резултати указују да је на основу експресије *Ki67* и е-кадхерина могуће издиференцирати болеснике у групе већег, односно мањег ризика од појаве метастатске болести.

Кључне речи: колоректални карцином; експресија е-кадхерина; пролиферативни индекс; клинички значај

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

The first 10 years' experience in radical retropubic prostatectomy: complications, lower urinary tract symptoms, and quality of life – a single-center experience

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SUMMARY

Introduction/Objective This 10-year prospective study's primary objective was to evaluate the incidence of complications of radical retropubic prostatectomy (RRP). The secondary objective was to analyze how RRP affects lower urinary tract symptoms (LUTS) and quality of life (QoL) by using the International Prostate Symptom Score (IPSS).

Methods We analyzed 254 patients who underwent RRP in the period 2009–2018. All complications were graded according to the Clavien–Dindo classification. To assess urinary symptoms and the QoL, all the examinees filled out the IPSS and the International Prostate Symptom Score for QoL (IPSS QoL) questionnaires during preoperative preparation and three, six, and 12 months after surgery.

Results The incidence of complications Clavien–Dindo grade ≤ II and grade ≥ III were 26.4% and 16.5%, respectively. The mean overall IPSS for the entire group of patients after 12 months of follow-up was significantly different from the preoperative baseline value ($p < 0.001$). Patients with preoperative moderate (IPSS 8–19) and severe urinary symptoms (IPSS 20+) had a statistically significant reduction of urinary symptoms after RRP ($p < 0.001$). After 12 months, IPSS QoL was statistically significantly lower than the preoperative one ($p < 0.05$).

Conclusion For patients with clinically localized prostate cancer, RRP is a safe and effective treatment option. It is associated with a higher rate of complications from the Clavien–Dindo grade ≤ II group. RRP has clinically beneficial effects on LUTS in patients with moderate and severe urinary symptoms and the QoL related to LUTS.

Keywords: prostate cancer; radical prostatectomy; urination disorders; questionnaires

INTRODUCTION

Prostate cancer (PCa) is the most common tumor in men over 50 years of age [1]. In Western Europe and the USA, it is the second leading cause of cancer death [2, 3]. Among men in Serbia, PCa is by prevalence second only to lung cancer. The screening test with serum prostate-specific antigen (PSA) improves the diagnosis of PCa at organ-confined status [3, 4, 5]. Accordingly, this has led to an increased number of patients considered candidates for radical prostatectomy (RP). RP performed as an open, laparoscopic, or robot-assisted surgery remains the standard procedure for patients with localized PCa and life-expectancy of at least 10 years [3, 6, 7]. Despite the surgeons' growing experience, better knowledge of anatomy, and refinement of surgical techniques, most patients who undergo RP experience treatment-related side effects that can significantly impact their quality of life (QoL) [8]. This 10-year prospective study's primary objective was to evaluate the incidence of complications of radical retropubic prostatectomy (RRP). The secondary objective was to analyze how RRP affects lower urinary tract symptoms (LUTS) and the QoL by using International Prostate Symptom Score (IPSS).

METHODS

The study included 254 patients with histologically confirmed PCA in clinical T2 or lower disease stage, who underwent RRP at the Urology Clinic, Niš Clinical Center, 2009–2018. Preoperative baseline data included age, PSA, Gleason score, clinical stage, and the American Society of Anesthesiologists score. Five out of 10 urologists performed 88% of operations. The anatomical approach described by Walsh was used [9]. Pelvic lymph node (PLN) dissection was done in patients with serum PSA > 10 ng/ml and/or Gleason score ≥ 7. Preservation of the cavernous nerve was done by an individual

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surgeon's assessment based on clinical disease staging. Intraoperative and postoperative baseline data included average operative time, estimated blood loss, pelvic drainage, urinary catheterization time, duration of postoperative lymphorrhea, length of hospital stay, intraoperative and postoperative transfusion rate, operative specimen weight, pathological disease stage, presence of seminal vesicle invasion and PLN involvement. All complications were evaluated, and the definitions of complications were adapted from other RP reports and graded according to the Clavien–Dindo classification [5, 10]. The evaluation of the impact of RRP on LUTS and QoL was assessed by using IPSS and the International Prostate Symptom Score for Quality of Life (IPSS QoL). Voiding and storage symptom were analyzed independently. All the patients enrolled in the study filled out both questionnaires preoperatively and three, six, and 12 months after the surgery. Time from complete preoperative preparation to the operation itself was not longer than three months. The patients who received neoadjuvant or adjuvant therapy and had inadequate data or incomplete questionnaires were excluded from the study. Data are presented as mean value ± standard deviation or as median (25th–75th percentile). The general linear model – repeated measures was used to compare questionnaire scores measured at baseline and after three months, six months, and 12 months. Statistical procedures were performed using the R software (The R Project for Statistical Computing). The probability value $p < 0.05$ was considered statistically significant [11].

All procedures on human subjects were done in accord with the ethical standards of Helsinki Declaration and approved by the Faculty of Medicine and the University of Niš as a part of a doctoral dissertation investigations (Approval No. 04-825/12).

Table 1. Preoperative clinical and pathologic parameters

Characteristics	n	%
Age	62.7 ± 4	53–71
Clinical stage		
T1	9	3.6
T2	216	85
T3	29	11.4
Gleason score biopsy		
≤ 6	156	61.4
7	92	36.2
8	6	2.4
Baseline PSA (ng/ml)†	12.4 ± 6.1	0.6–33.2
ASA score		
0	24	9.4
1	21	8.3
2	190	74.8
3	19	7.5
Pathologic stage		
T0	2	0.8
T1	3	1.2
T2	161	63.3
T3	83	32.7
T4	5	2
Nodal status		
N0	160	63
N1–N2	12	4.7
Nx	82	32.3
Seminal vesicle invasion	55	21.7
Pathologic Gleason score		
≤ 6	125	49.2
7	101	39.8
≥ 8	28	11
Operative specimen weight (g)†	38.5 ± 13.1	15–90

ASA – American Society of Anesthesiologists; PSA – prostate-specific antigen;
†mean ± standard deviation, min-max;
data are presented as n or %;

RESULTS

Fourteen out of 268 patients who underwent RRP were excluded from the study. In 12 patients, preoperative urinary catheterization was performed because of urinary retention. Descriptive, clinical, and pathological parameters are presented in Table 1. Extended PLN dissection was performed in 37.8% and limited in 29.9% of the patients (Table 2). PLN involvement (N1–2) was present in 4.7%, seminal vesicle invasion in 21.7%. Other intraoperative and postoperative parameters in patients undergoing RRP are presented in Table 2. The average operation time was 170 minutes, and the average blood loss was 867 ml. Eighty-four patients received intraoperative or immediate postoperative blood transfusions. The average length of hospital stay was 10.5 days. The average catheterization time was 15 days. Fifteen patients were incontinent, and 218 patients did not use pads 12 months after the operation.

Postoperative complications that occurred within 60 days after the surgery were defined as early complications. Complications between 60 days and 12 months are defined as late complications. The overall complication rate (OCR) was 42.9% in 72 (28.4%) patients. Complications of RRP are presented in Table 3. Immediate surgical reintervention was done in five (2%) patients due to injuries to the rectum, ureter, urine leakage at the ureterovesical anastomosis, and bleeding. Most complications graded as Clavien–Dindo I (15.4%) and II (11.1%) did not require surgical reintervention. The most common complication in the Clavien–Dindo grade ≤ II group was urinary tract infection (UTI) (4.2%). The group of complications in Clavien-Dindo grade ≥ III comprised groups IIIa, IIIb, and IVa, and these were confirmed in 4.4%, 11.1%, and 1.2% of cases, respectively. Fifty percent of them were late postoperative complications [vesicourethral anastomosis stenoses (VUAS) and incontinence] which required surgical intervention in spinal or general endotracheal anesthesia. All the patients with VUAS had UTI. Symptomatic lymphoceles were confirmed in two (0.8%) patients and treated with percutaneous drainage in short-term intravenous anesthesia. Postoperative pulmonary embolism was encountered in two patients and myocardial infarction in one patient.

IPSS categories in the 12-months follow-up period are presented in Figure 1. Compared to the preoperative

Table 2. Intraoperative and postoperative parameters of patients who underwent radical retropubic prostatectomy

Parameters	n	%
Nerve sparing procedure		
Yes	69	27.2
No	185	72.8
Lymph node dissection	172	67.7
Extended	96	37.8
Limited	76	29.9
Not done	82	32.3
Operative time (min)†	170.8 ± 41.4	90–280
Estimated blood loss (ml)†	846.1 ± 564.9	50–2750
Blood transfusion – intraoperative (ml)	84	33.1
Length of hospital stay (d)†	10.4 ± 3.1	6–21
Catheter duration (d)†	14.6 ± 2.3	9–21
Pelvic drainage < 5 (d)	192	75.6
Prolonged pelvic drainage 5–10 d	52	20.5
Prolonged pelvic drainage >14 d	10	3.9
Positive urine culture	23	9.1
Incontinence within 6 months	66	26
Incontinence after 12 months	15	5.9
None pad per day	218	85.8
Patients with complications	72	28.4
Positive surgical margins**	44	28.7

Data are presented as n or %;
†mean ± standard deviation, min-max;
**status of surgical margins was available in 153/254 patients

Table 3. Complications of radical retropubic prostatectomy n = 72 (28.4%)

Variable	Clavien–Dindo grade	≤ II		≥ III	
		n	%	n	%
Perioperative and early postoperative within 60 days					
Rectal injury preoperatively	I	5	2		
Rectal injury postoperatively	IIIb			1	0.4
Distal ureteral injury	IIIb			2	0.8
Postoperative blood transfusion	II	8	3.2		
Postoperative bleeding	IIIb			1	0.4
Significant hematuria	II	5	2		
Obturator nerve injury	II	2	0.8		
Pulmonary embolism	IVa			2	0.8
Myocardial infarction	IVa			1	0.4
Wound infection	I	7	2.8		
Wound dehiscence	IIIa			3	1.2
Prolonged lymph secretion	I	9	3.9		
Urinary tract infection/epididymitis	II	13	4.2		
Anastomotic leakage	I	2	0.8		
Anastomotic leakage	IIIa			3	1.2
Anastomotic leakage	IIIb			1	0.4
Acute urinary retention	I	7	2.8		
Dislodgment of Foley catheter	IIIa			5	2
Suture of urinary catheter	I	5	2		
Infected lymphocele	IIIb			2	0.8
Asymptomatic lymphocele	I	4	1.6		
Late postoperative > 60 days					
Anastomotic stenosis	IIIb			17	6.7
Surgery for urinary incontinence	IIIb			4	1.6
Total number of complications	109	67	26.4	42	16.5

Data are presented as n or %

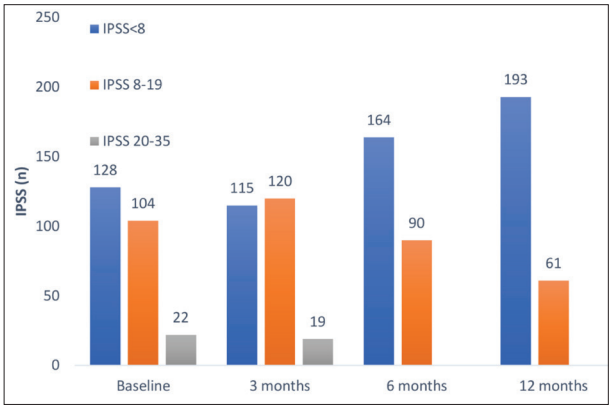

Figure 1. International Prostate Symptom Score (IPSS) categories over a 12-month follow-up period

period, after 12 months of follow-up, there were 65 patients more with mild (IPSS < 8), and 43 fewer with moderate symptoms (IPSS 8–19), while there were no patients with severe symptoms (IPSS 20+). The total IPSS score at baseline (mean ± SD) was 9.7 ± 4.32; at three months 10.69 ± 4.88; at six months 8.8 ± 3.11; and at 12 months 7.46 ± 1.85, with a statistically significant difference among the measurements ($p < 0.001$). IPSS voiding symptom score (IPSSv) at baseline (mean ± SD) was 4.57 ± 2.58; at three months it was 3.44 ± 3.21; at six months 2.68 ± 1.92; and at 12 months 1.96 ± 1.29, with a statistically significant difference among the measurements ($p < 0.001$). IPSS storage symptom score (IPSSs) at baseline (mean ± SD) was 5.13 ± 2.11; at three months 7.25 ± 2.07; at six months 6.12 ± 1.81; and at 12 months, 5.5 ± 1.48, with a statistically significant difference among the measurements ($p < 0.001$). All the mean changes in IPSSt, IPSSv, and IPSSs in the study population were significantly different among all measurement points (Table 4). In the subgroup IPSS < 8, all the mean changes in IPSSt were significantly different among all measurement points, particularly between baseline values and values after three months ($p < 0.001$). In the subgroups IPSS 8–19 and IPSS 20+, all the mean changes in IPSSt were statistically different between all the measurement points, particularly between baseline values and 12 months ($p < 0.001$). In the subgroup IPSS 20+, all the mean changes in IPSS, voiding, and storage were statistically different among all measurement points except for the mean change in storage in the first six months. All mean changes in IPSS QoL scores in the study population were statistically different among all the measurement points (Table 4). IPSS QoL scores were statistically significantly lower after 12 months compared to baseline values ($p < 0.05$). Graphical presentation of IPSS categories in the 12-month follow-up period is given in Figure 1.

Table 4. IPSSt, IPSSv, IPSSs, and IPSS QoL, at baseline, three months, six months, and 12 months following surgery

Parameters	Baseline values	Three months	Six months	12 months	p
IPSSt	9.70 ± 4.32[a,b,c]	10.69 ± 4.88[b,c]	8.80 ± 3.11[c]	7.46 ± 1.85	< 0.001
IPSS < 8	6.30 ± 0.7[a,b,c]	10.02 ± 5.13[b,c]	7.62 ± 2.39[c]	7.09 ± 1.26	< 0.001
IPSS 8-19	11.64 ± 1.69[a,b,c]	10.38 ± 4.19[b,c]	9.08 ± 2.69[c]	7.38 ± 1.72	< 0.001
IPSS 20+	20.27 ± 1.2[a,b,c]	16.00 ± 2.98[b,c]	14.36 ± 2.3[c]	10.00 ± 3.06	< 0.001
IPSSv	4.57 ± 2.58[a,b,c]	3.44 ± 3.21[b,c]	2.68 ± 1.92[c]	1.96 ± 1.29	< 0.001
IPSSs	5.13 ± 2.11[a,b,c]	7.25 ± 2.07[b,c]	6.12 ± 1.81[c]	5.50 ± 1.48	< 0.001
IPSS QoL	1.95 ± 1.12[a,b,c]	2.22 ± 1.23[b,c]	1.63 ± 1.05[c]	1.42 ± 0.98	< 0.001
IPSS QoL**	1.96 ± 1.1[a,b,c]	2.20 ± 1.2[b,c]	1.61 ± 1.01[c]	1.37 ± 0.92	< 0.001
IPSS QoL*	1.94 ± 1.14[a,b,c]	2.24 ± 1.26[b,c]	1.65 ± 1.09[c]	1.47 ± 1.06	< 0.001

IPSS – International Prostate Symptom Score; data are presented as mean ± SD; $p < 0.05$; [a]*vs.* three months; [b]*vs.* six months; [c]*vs.* 12 months; *positive margins; **negative margins

DISCUSSION

Our study results suggest that RRP is a therapeutically effective method with a few severe complications, which is in line with other recently published studies [3]. Comparing different surgical approaches, De Carlo et al. [3] have reported weighted mean estimated blood loss of 935 ml, intra- and postoperative transfusion rate of 19.63%, weighted mean operative time 179.03 minutes (105–253 minutes), length of hospital stay 7.87 days (3–16 days) and catheterization time of 12.85 days (1.23–16 days) in RRP series. In our study, the mean blood loss was less (846 ml) and mean operation time was shorter (170.87 minutes), which could be explained by the well-accomplished technique of vascular control during RRP as well as an adequate operation volume of most surgeons (≥ 25 per surgeon). Comparison of operative time and transfusion rate might be misleading due to variations in reporting operative time (depending on the inclusion of set up time and PLN dissection and learning curve phase) and different clinical practices among institutions [3, 12]. Compared to minimally invasive surgical approaches, the average blood loss in RRP is three times greater, and the percentage of administered blood transfusions is five times greater [12, 13]. A great variability in length of hospital stay (6–21 days) and catheterization time (9–21 days) in our study results from hospitalization of patients from other medical centers until the time for catheter removal. Such practice is present in European counties as well, where patients remain in hospital until the urinary catheter is removed, whereas in the USA, patients are usually discharged quickly after surgery [3].

Positive surgical margins (Table 2) are an essential predictor of biochemical recurrence and additional treatment after surgery [14]. In our series of patients, it was not always available, but the overall incidence of positive surgical margins was higher (28.7%) compared to other reported series of patients (15%) [5, 14]. Relatively high incidence of pT3 patients is undoubtedly responsible for it and documented surgeon experience [14]. There was no difference in IPSS QoL during the first three months between patients with positive and negative surgical margins like in another series [15]. However, a decrease in the IPSS QoL during the follow-up could be explained by radiotherapy applied as a secondary treatment.

In our study, the prevalence of complications and their distribution by Clavien–Dindo grades are in line with results in other published studies [5, 12]. Although the OCR was relatively high (42.9%), reinterventions were done in only five (2%) patients who were operated on. Carlsson et al. [12] reported a similar OCR of 44.6%, whereas reintervention was performed in 14 (2.8%) patients. Data from a recently published review that included 29 studies reported a weighted mean OCR of 23.2% (range 6–68%) in patients who underwent RRP [3].

The incidence of Clavien–Dindo grade ≤ 2 complications was significantly higher (25.4%) compared to Clavien–Dindo grade ≥ 3 complications (16.5%) in our study. The most prevalent complications in Clavien–Dindo grade ≤ 2 group were UTI and prolonged lymph secretion. Lenart et al. [16], Cheng et al. [5], and Wang et al. [17] also reported symptomatic UTI in 8.2%, 4.2%, and 1.7%, respectively. However, there is no literature about this complication as it was not analyzed as a separate parameter. Our opinion is that there is an association of this complication with a permanent urinary catheter. UTI was postoperatively confirmed in seven out of 12 patients who had permanent catheter preoperatively. Another possible UTI mechanism is the entry of particles containing bacteria during the operation's replacement of urinary catheters. In our study, 12 of 13 patients with VUAS had UTI. However, we did not analyze this association. Prolonged lymph secretion, as a typical complication of RP, is associated with PLN dissection [7]. In published studies, the prevalence of this complication in RRP ranges 1.1–6% [5, 18]. In our study, lymphocele was confirmed in six (2.4%) out of nine (3.6%) patients with prolonged lymph secretion. Based on recent data, PLN dissection during RP is associated with a considerably increased risk for lymphocele formation compared to RP alone [19]. Our complication analysis revealed another complication that resolved spontaneously and had not been described in the literature. We defined it as urinary catheter suture and classified it among Clavien–Dindo grade I events. After emptying the balloon, a surgically placed urinary catheter could not be removed even with greater tension, but it spontaneously came off in the following 48 hours. The possible cause of this complication was that the catheter crossed the placed sutures at the anastomosis before tying, or that the catheter diameter at the site of the emptied balloon was greater than the diameter of the vesicoureteral anastomosis.

The most common complication in Clavien–Dindo grade ≥ 3 in our study was VUAS. The prevalence of this complication after RRP in the literature varies 0.5–32%, whereas we reported VUAS in 6.7%. Although we did not analyze the risk factors for VUAS, our opinion is that there is an association of this event with UTI as we confirmed symptomatic UTI in 12 (4.8%) out of 17 (6.7%) patients [7, 12, 17]. Whether UTI is the consequence or one of the causes of VUAS remains the topic for future researches.

Twelve months following RRP, there were 14.2% incontinent patients (one or more pads). The prevalence of this complication in the literature ranges 6.3–29.3% [3]. Although it is a significant complication, we reported only 1.6% of men who underwent surgical management of this complication. A similar result (2.2%) was reported by Carlsson et al. [12] as well.

The effect of RRP on LUTS in our study was reported for the entire group of patients. Only men with symptoms of IPSS ≥ 8 can significantly benefit because of symptom improvement. Slova and Lepor [20] analyzed the results of IPSSt, IPSSv, and IPSSs in 453 patients divided into two groups (IPSS < 8 and IPSS ≥ 8) 12 and 48 months after RRP. They did not report a statistically significant difference in mean IPSSt score after 12 months, but they reported a statistically significant reduction of mean IPSSv score. In contrast to the results of Slova and Lepor [20], our results demonstrated a statistically significant reduction in mean overall IPSSt after 12 months, which could be explained by greater baseline mean IPSSt (9.7 *vs.* 6.9), as well as by greater baseline number of patients with IPSS ≥ 8 in our study.

They also reported a significant difference in mean IPSSt and IPSSv for the entire group between baseline values and values after 48 months. The improvement of mean IPSSt between the baseline and after 48 months was attributed primarily to the reduction of mean IPSSv in the first 12 months and thr reduction of mean IPSSs in the period 12–48 months after the operation [20]. Our study results showed a statistically significant reduction of mean IPSSv and IPSSt six and 12 months after RRP compared to the baseline values, suggesting the role of IPSSv in the improvement of mean IPSSt. On the other hand, we could not estimate whether the results of IPSSs had a positive or negative impact on IPSSt after 12 months. The mean values of IPSSs at baseline and after 12 months were almost equal (5.1 *vs.* 5.5), and similar results for the same time interval were reported by Slova and Lepor [20] (4.2 *vs.* 4.5).

After 12 months, significant LUTS improvement was observed in subgroups IPSS 8–19 and IPSS 20+, since there was a statistically significant reduction by 4.3 and 10.3 symptom units compared to baseline values. Other studies have also reported LUTS improvement after RRP in men with IPSS8 ≥ 8 [20, 21]. Bayoud et al. [22] analyzed RP's impact on LUTS in 804 patients and reported a significant increase of mean IPSSt after one and three months (11.1 ± 7.1, 7.6 ± 6.1) compared to the baseline value (5.5 ± 6.6). There was no statistically significant difference between mean baseline value and after six, 12, and 24 months. Our study baseline means IPSSt was 9.7 ± 4.3, and after three months, IPSSt increased 10.6 ± 4.8 with a tendency of statistically significant decrease after six and 12 months (7.4 ± 1.8). This difference in decreasing tendency of mean IPSSt can be explained by very high baseline IPSSt compared to the observed study and by greater number of patients with IPSS ≥ 8 in our study (49.6% *vs.* 34.5%). The authors also demonstrated a downward trend in the number of patients in IPSS > 8 subgroup as follows: 42.4%, 32.9%, 21.7%, and 17% at three, six, 12, and 24 months, respectively [22], which was also confirmed in our study: 54.8%, 35.4% and 24.1% at three, six, and 12 months, respectively.

Numerous studies have demonstrated a beneficial role of RRP on LUTS. Papadopoulos et al. [23] reported a significant improvement of LUTS in 240 patients undergoing RRP. They studied IPSS and maximum flow rate (Q_{max}) before and after RRP. The percentage of patients with Q_{max} ≤10 ml/s was 41.3% before RRP, and during the follow-up of 12 months, Q_{max} increased from a median of 12 ml/s initially to 21 ml/s. In patients with Q_{max} of ≥ 10 ml/s there was no significant difference. They also reported significant IPSSt reduction in the group of patients with moderate and severe symptoms [23].

Analyzing LUTS in our study, we noticed a very slow decrease of mean IPSSt values in the IPSS20+ group during 12 months. This could be explained by the presence of postoperative incontinence in 36 (14.2%) patients postoperatively, which we did not analyze. Several authors have studied the negative impact of RRP on LUTS. Nocturia, frequent urination and incontinence occurring *de novo* in 2–77% of patients after RP with up to 50% recovery are dysfunctional disorders that can be associated with detrusor muscle overactivity, bladder compliance disorder, and detrusor contractility impairment [21, 24, 25]. LUTS could be explained as a consequence of pelvic plexus injury due to surgical dissection. Nerve injury of neurovascular bundles is responsible for postoperative continence impairment [25, 26]. The bladder filling and voiding phase can thus be disturbed since damaged bladder function is usually associated with sphincter weakness [25].

Our study demonstrated a significant improvement of QoL IPPS after RRP at the end of follow-up compared to the preoperative period (1.9 ± 1.1 *vs.* 1.4 ± 0.9). Therefore, it was a positive impact of RRP on QoL. Changes of mean values of IPPS QoL directly correlated with improvement and deterioration trends of LUTS and IPSSt following RRP. A positive impact of RRP on LUTS and QoL was reported as well by Mastubara et al. [24], using IPSS and QoL IPSS in 117 patients three, six, and 12 months after RRP. They reported a significant reduction of IPSS and QoL IPSS especially in patients with IPSS ≥ 8. Similar results were reported in the study focusing on LUTS and IPPS LUTS and IPPS QoL improvement in patients after robot-assisted RP [27]. Frequent urination and nocturia significantly impacted QoL IPSS in patients who did not have this symptom before RRP [21, 24, 27]. Moreover, similar results were presented for QoL IPSS regardless of the surgical approach [22, 27, 28].

Our study has several limitations. The assessment of impact of RRP on LUTS is subjective. It is based on the estimation of urinary symptoms by using IPSS questionnaire.

The study that analyzed flow rate and IPSS showed statistically significant improvement in Q_{max} (21 ml/min) and decrease in IPSS score 12 months following RRP only in patients with preoperative Q_{max} ≤ 10 ml/s and IPSS ≥ 8 [23]. We did not perform urodynamic studies, but our results showed a statistically significant reduction of IPSS score 12 months after RRP in groups of patients with

preoperative IPSS ≥ 8. Furthermore, we should be cautious regarding the uniformity of preoperative prostate biopsy and histopathology findings obtained not from one center but from some general hospitals, which presumably involve individual approaches despite a generally adopted doctrine. A single urologist did not perform the operations, and differences related to surgical skills and experience may lead to confusion when analyzing complications.

CONCLUSION

For patients with clinically localized PCa, RRP is safe and effective treatment option. It is associated with a higher rate of complications from the Clavien–Dindo grade ≤ II group, which does not require invasive surgical management. RRP has clinically beneficial effects on LUTS in patients with moderate and severe urinary symptoms and QoL related to LUTS.

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Conflict of interests: None declared.

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Прво десетогодишње искуство у радикалној ретропубичној простатектомији: компликације, уринарни симптоми и квалитет живота – искуство једног центра

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САЖЕТАК

Увод/Циљ Примарни циљ ове десетогодишње проспективне студије био је да се прикаже инциденца компликација радикалне ретропубичне простатектомије (РРП). Секундарни циљ је био да се прикаже утицај РРП на симптоме доњег уринарног тракта и квалитет живота на основу интернационалног скора симптома простате (*IPSS*).

Методе Анализирана су 254 болесника којима је урађена РРП у периоду између 2009. и 2018. године. Све верификоване компликације градиране су према класификацији Клавијен–Диндо. Процена уринарних симптома и квалитета живота свих испитаника је урађена на основу упитника *IPSS* и интернационалног скора симптома простате за квалитет живота (*IPSS QoL*) у току преоперативне припреме и 3, 6 и 12 месеци после операције.

Резултати Компликације градуса Клавијен–Диндо ≤ II забележене су у 26,4%, а Клавијен–Диндо ≥ III у 16,5% случајева. Средња вредност укупног *IPSS* целе групе испитаника након 12 месеци праћења била је статистички значајно мања ($p < 0,001$) у односу на преоперативну базичну вредност. Статистички значајно смањење уринарних симптома након операције ($p < 0,001$) забележено је код болесника са преоперативно умереним (*IPSS* 8–19) и јако израженим уринарним симптомима (*IPSS* 20+). У односу на преоперативну, средња вредност *IPSS QoL* након 12 месеци је била статистички значајно мања ($p < 0,05$), а квалитет живота бољи.

Закључак За болеснике са клинички локализованим карциномом простате, РРП је ефикасна опција лечења и повезана је са вишом стопом компликација из групе Клавијен–Диндо ≤ II. Код болесника са преоперативно умереним или јако израженим уринарним симптомима, РРП значајно доприноси њиховом смањењу и побољшава квалитет живота као последицу уринарних симптома.

Кључне речи: карцином простате; радикална простатектомија; уринарне тегобе; упитници

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

The clinical outcomes after surgical treatment of mass lesions causing sciatica – a single-center retrospective study

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SUMMARY
Introduction/Objective Sciatica is a disabling pathology with variable etiologies. The most common pathologies arise from discogenic or non-discogenic causes. Mass lesions are a rare cause of extraspinal sciatica, which have been commonly overlooked, leading to unnecessary spinal surgeries, delay in diagnosis or inadequate treatment. There is no standard surgical approach and functional outcomes after surgical treatment of these lesions are not well-known.
The aim of this study is to evaluate clinical outcomes after surgical treatment of mass lesions causing sciatica in different locations.
Methods Data were obtained by a retrospective review from 2015 to 2020. The mean duration of symptoms at the time of surgery was 10.3 months (3–48 months). The mean age of patients at the time of surgery was 43.8 years (14–73 years). The mean follow-up was 19.5 months (4–50 months). In total, 14 cases had an extrapelvic localization distal to sciatic notch. The other three cases had lesions in the intrapelvic area, including left sciatic notch (1), right acetabulum (1), sacroiliac and lumbosacral region (1). None of the patients had palpable masses. Transgluteal, infragluteal, lateral, and posteromedial approach were used depending on location and size of the lesion.
Results At the final follow-up, all patients recovered with pain relief. The median musculoskeletal tumor society score was 90% (70–100). There was no recurrence at the latest follow-up.
Conclusion Our study demonstrated that early detection by neurological examination and radiological work-up can avoid unnecessary surgeries, enable early surgical treatment of tumoral mass with satisfactory clinical outcomes. The surgical approach should be individualized according to location and size of the lesion.
Keywords: mass lesions; sciatic nerve; non-discogenic sciatica; transgluteal approach; infragluteal approach

INTRODUCTION

Sciatica is a frequently encountered complaint and described as the pain along the course of the sciatic nerve [1, 2]. It is characterized by pain radiating downward from the lumbar region to the posterior thigh. Lumbar disc herniation, spinal stenosis, and pyriformis syndrome are among the most common causes; however less common extraspinal pathologies are of infective, inflammatory, tumoral and vascular origin which include soft tissue and bone tumors, hematomas, presacral abscesses, aneurysms, sacroiliitis, and gynecological conditions such as endometriosis and tubal-ovarian abscesses [3, 4, 5].

The wide variety of extraspinal causes of sciatic nerve entrapment can be overlooked since the size of the tumor had to become enlarged enough to violate the greater sciatic foramen. Also, the increased sensitivity of magnetic resonance imaging (MRI) leads to misdiagnosis of discogenic sciatica [5]. Hence, differential diagnosis could be compelling and should be meticulously made. Nevertheless, an incidental finding on pelvic or femur X-ray can reveal the leading cause of non-discogenic sciatica. MRI is the best modality to delineate pelvic and gluteal lesions. Physical examination and detailed patient history with the awareness of the possible mass lesions aids in early diagnosis and surgical treatment. Understanding the etiology of intra- and extrapelvic causes requires a comprehensive approach for diagnosis and management.

The aim of this study is to evaluate clinical outcomes after surgical treatment of mass lesions causing sciatica in different locations.

METHODS

Informed consent was obtained from all patients for being included in the study. The study

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Table 1. Details about extrapelvic lesions

Case	Age (years)	Sex	Primary diagnosis	Previous misdiagnosis	Symptoms	Symptoms' duration (months)	Lesion size on MRI (cm)	Lesion location	Surgical approach	Follow-up (months)	MSTS score (%)
1	20	Female	Hemangioma	None	Pain	3	6 × 5 × 5	Right ischium	Transgluteal	19	100
2	28	Male	Hereditary multiple osteochondromas	None	Pain, antalgic gait	12	8 × 6 × 6	Right femoral neck	Transgluteal	21	80
3	49	Male	Low grade fibrosarcoma	Spinal stenosis	Pain	6	8 × 7 × 3.5	Right gluteal area	Infragluteal	48	85
4	64	Female	Solitary plasmacytoma	Lumbar radiculopathy	Pain, paresthesia	12	5 × 6 × 5	Left gluteal area	Infragluteal	50	90
5	28	Male	Schwannoma	None	Pain	5	5 × 2 × 5	Right hip pain	Transgluteal	10	90
6	37	Male	Schwannoma	None	Sciatica	4	3 × 1 × 2	Left gluteal area	Lateral	10	90
7	42	Female	Soft tissue chondroma	None	Hip pain	48	8 × 6 × 5	Left posteromedial femur	Posteromedial	17	100
8	40	Female	Osteochondromatous lipoma	None	Hip pain	6	6 × 5 × 3	Right proximal femur	Infragluteal	25	100
9	65	Female	Lipoma	None	Sciatica	6	4 × 3 × 5	Right proximal femur	Transgluteal	6	80
10	36	Female	Tenosynovial giant cell tumor	None	Hip pain, sciatica	18	10 × 5 × 20	Left posterior hip	Infragluteal	6	80
11	41	Female	Atypical lipomatous tumor	None	Thigh pain	4	5 × 4 × 5	Left thigh	Posteromedial	6	80
12	64	Female	Soft tissue metastasis of squamous cell carcinoma	None	Gluteal pain	2	3 × 2 × 3	Left gluteal area	Infragluteal	4	70
13	65	Female	Atypical lipomatous tumor	None	Hip pain paresthesia	3	12 × 20 × 9	Left gluteal area	Transgluteal	6	80
14	34	Male	Osteochondroma	None	Pain, paresthesia	24	4 × 3 × 4	Right posterior femoral neck	Transgluteal	6	100

MRI – magnetic resonance imaging; MSTS – the musculoskeletal tumor society

Table 2. Details about intrapelvic lesions

Case	Age (years)	Sex	Primary diagnosis	Previous misdiagnosis	Symptoms	Duration of symptoms (months)	Lesion size on MRI (cm)	Lesion location	Surgical approach	Follow-up (months)	MSTS score (%)
15	35	Male	Cyst hydatic	Lumbar disc herniation	Pain, paresthesia	12	7.5 × 6 × 8.5	1. Presacral area 2. Right ischium	Two stages: 1.Transabdominal 2. Transgluteal	30	100
16	14	Female	Non-ossifying fibroma	None	Pain, paresthesia	18	3 × 2 × 3	Right femoral neck	Transgluteal	6	100
17	73	Male	Low grade fibrosarcoma	Spinal stenosis	Pain	6	8 × 7 × 3.5	Right gluteal area	Infragluteal	48	70

MRI – magnetic resonance imaging; MSTS – the musculoskeletal tumor society

protocol was approved by the local ethics committee (No: 2020/0516 Date: 55 12.08.2020). Data were obtained by a retrospective chart review from 2015–2020. A retrospective review was made of 17 patients who were treated surgically for mass lesions with sciatica. All 17 cases, six females and eleven males were aged between 14 and 73 years old.

In extrapelvic lesions, surgical procedures were performed by using transgluteal (n = 5), infragluteal (n = 5), lateral (n = 2), and posteromedial (n = 2) approach, depending on the location and size of the mass lesion. Intrapelvic lesions were managed using different approaches: One patient with cyst hydatic at the left sciatic notch underwent a two-stage transabdominal approach followed by transgluteal incision. One patient with non-ossifying fibroma underwent curettage and grafting using the posterior sacral approach. The last patient underwent periacetabular resection and reconstruction with a saddle prosthesis.

Statistical analysis

Descriptive statistics was done was done by using the IBM SPSS Statistics for Windows, Version 20.0. (IBM Corp., Armonk, NY, USA). The median values were given with ranges, minimum, and maximum. The mean values were given with standard deviation.

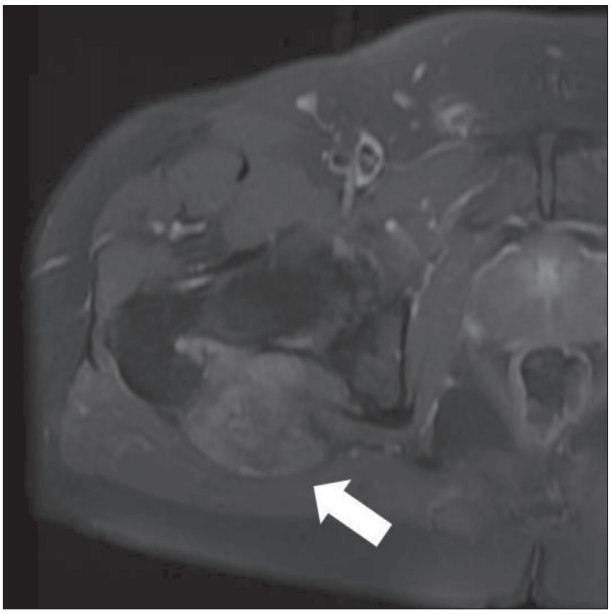

Figure 1. Case 3: a 49-year-old male with low-grade fibromyxoid sarcoma; pelvic magnetic resonance imaging demonstrated a sharp and lobulated contoured 8 × 7 × 3.5 cm lesion extending between the right gluteal muscle fibers close to the trochanter major with heterogeneous enhancement; the sciatic nerve is encroached by the lesion (white arrow)

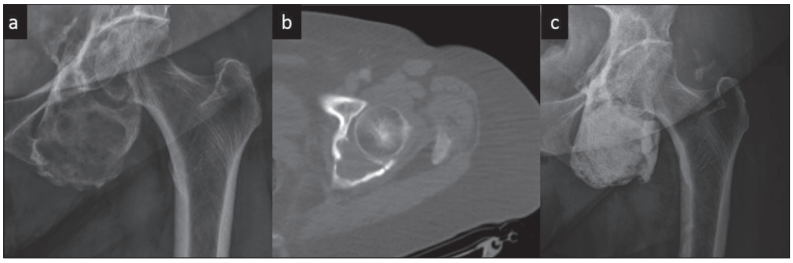

Figure 2. Case 4: a 64-year-old female with solitary plasmacytoma; a – preoperative X-ray; b – computed tomography view demonstrates an expansive lytic lesion extending from the posteroinferior part of the left acetabulum to inferior ramus pubis; c – five-year follow-up radiograph after curative resection and bioceramic antibacterial grafting shows graft consolidation

RESULTS

Demographic data

Details regarding extrapelvic and intrapelvic lesion are summarized in Table 1 and Table 2. The mean age was 43.8 years (range: 14–73 years). The mean duration of symptoms was 10.3 months (range: 3–48 months). The mean follow-up was 19.5 months (range: 4–50 months). The median Musculoskeletal Tumor Society score was 90% (range: 70–100). 14 lesions had an extrapelvic localization distal to sciatic notch. The other three lesions were in the intrapelvic area, including left sciatic notch, right acetabulum, sacroiliac and lumbosacral region. None of the patients had palpable mass.

Clinical findings

None of the patients had a well-delineated palpable mass. Remarkably, all patients experienced low back pain or buttock pain. Pain was not responding to analgesics in all patients. In extrapelvic localizations, there was positive Tinel's sign at gluteal region over the course of sciatic nerve and tenderness after deep gluteal palpation. There was no weakness, gait dysfunction, motor and sensorial deficit. The localization of all lesions with specific etiology was demonstrated by MRI. Therefore, no preoperative electromyography was performed.

Pathologic diagnosis

The diagnosis of the lesions includes osteochondrolipoma of soft tissue, soft tissue chondroma (n = 1), sciatic nerve hemangioma (n = 1), intramuscular lipoma (n = 1), atypical lipoma (n = 2), schwannoma of the sciatic nerve at the level of ramus pubis inferior (n = 1) and sciatic notch (n = 1), low-grade fibrosarcoma (n = 1) (Figure 1), solitary plasmacytoma of ischium (n = 1) (Figure 2), tenosynovial giant cell tumor (n = 1) (Figure 3), osteochondroma of the femoral neck (n = 2) (Figure 4), cyst hydatic (n = 1) (Figure 5), metastatic acetabular lesion of lung carcinoma (n = 1) (Figure 6), soft tissue metastasis of squamous cell carcinoma (n = 1), and non-ossifying fibroma of the sacrum (n = 1).

Surgical approach

Transgluteal, infragluteal, lateral, and posteromedial approach were used depending on location and size of the

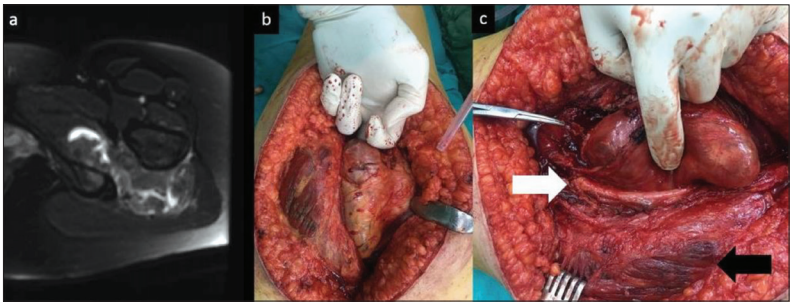

Figure 3. Case 10: a 36-year-old female with tenosynovial giant cell tumor; a – magnetic resonance imaging demonstrated a 10 × 5 × 20 cm nondestructive lesion; b – wide excision was performed using infragluteal approach; c – intraoperative view shows the close proximity of tumor to sciatic nerve (white arrow: sciatic nerve black arrow: gluteus maximus)

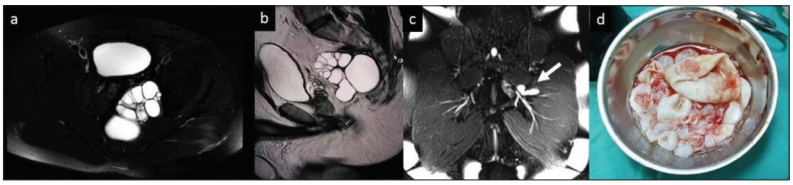

Figure 5. Case 15: a 35-year-old male with hydatid cyst; a and b – preoperative magnetic resonance imaging before the first surgery, which shows multiloculated septated cystic lesion at the presacral area; c – the patient presented to our clinic one year postoperatively; magnetic resonance imaging demonstrated a 43 × 14 mm lesion inferior to left pyriformis muscle between gluteus medius and maximus (white arrow); d – intraoperative view of daughter cysts

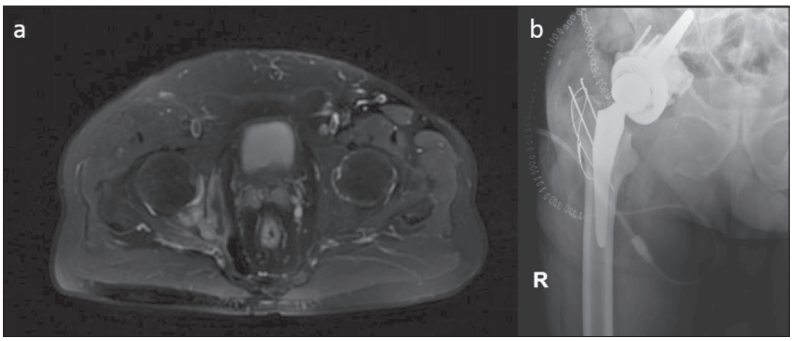

Figure 6. Case 17: a 73-year-old male with a metastatic lesion at right posterior acetabulum due to lung metastasis; a – magnetic resonance imaging; b – X-ray view after periacetabular resection and endoprosthetic reconstruction

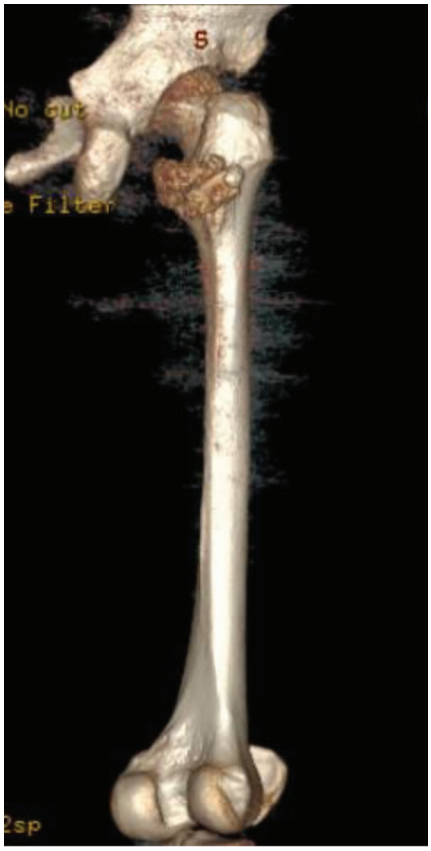

Figure 4. Case 14: a 34-year-old male with osteochondroma; preoperative three-dimensional computed tomography view showing mass lesion at posterior femoral neck

mass lesion. In proximal sciatic nerve lesions at the level or below the sciatic notch, an infragluteal or a transgluteal approach was used (Table 1). If there is suspicion about malign lesion, infragluteal approach was done in lesions to obtain wide exposure with safe surgical margins and avoid intracompartmantal contamination. In this approach, the gluteus maximus muscle is detached from iliotibial bant and reflected medially. The lesion is dissected from the sciatic nerve with wide excision. In possible benign lesions, the transgluteal approach was preferred. In this approach, gluteus maximus was splitted to enhance access to the sciatic nerve. In intrapelvic lesions, one patient with cyst hydatic underwent classical transabdominal at first stage and transgluteal approach at second stage (Table 2). For intrapelvic lesions anterior to sacrum transabdominal either using intraperitoneal or retroperitoneal approach may be used. Among intrapelvic lesions, one patient with nonossifying fibroma at right femoral neck underwent transgluteal approach. One patient with fibrosarcoma at right gluteal area underwent infragluteal approach. One patient with cyst hydatic underwent transabdominal approach. One year later, the same patient underwent transgluteal approach due to residual lesion. In all cases, no intraoperative neuromonitoring is needed as sciatic nerve was protected.

Follow-up

In benign lesions, the patients were followed every six months for the first two years, and annually after that. In malign lesions, the patients were followed every three months for the first two years, and annually after that.

DISCUSSION

To our knowledge, the current study is the third-largest series after Sim et al. [6] (38 cases) and Bickels et al. [4] (32 cases), which report space-occupying mass lesions with sciatic pain.

Bickels et al. [4] presented 32 cases with various etiologies of benign and malign lesions. The average of symptoms was 11.9 months (range: 1–59 months) at the time of diagnosis, which is similar to our study. The predominance

of malign lesions in the same series underlines the importance of detailed physical examination and patient history. Sim et al. [6] reported on 38 patients, 37 of which (14 benign, 23 malign) presented with sciatic pain. He emphasized that tumoral lesions and lumbar disc hernia can have similar presentations with low-back pain and sciatica. The duration of symptoms varied 2–58 months again similar to our findings. Different from these series, we encountered rare pathologies with non-palpable masses such as sacral melanocytic schwannoma, low-grade fibromyxoid sarcoma, solitary plasmacytoma, soft tissue metastasis of squamous cell carcinoma, non-ossifying fibroma, osteochondrolipoma and chondroma and cyst hydatic; however, the surgical strategy favoring complete removal is valid and paramount irrespective of diagnosis.

Other reports were limited to few case series and presentations [1, 2, 7–10]. Guedes et al. [2] reported on six patients with non-discogenic sciatica due to extrauterine endometriosis (one case) and tumoral lesions (five cases) three of whom (metastatic rectal adenocarcinoma, low-grade sarcoma, high-grade sarcoma) had malign lesions. He obtained clinical improvement after wide resection. All lesions were deeply located and unpalpable similar to our cases. Kulcu et al. [1] presented 11 patients with non-discogenic sciatica which includes two mass lesions, including schwannomatosis (case 2) and angiosarcoma (case 8). Matsumoto et al. [9] treated eight patients with sciatic notch dumbbell malign tumors who suffered from sciatica without back pain. Other types of lesions which are more frequently identified lesions in previous studies include pelvic heterotopic ossification, granulocytic sarcomas, osteochondromas, and ganglion cysts [7, 8, 10]. In line with these studies, we also demonstrated that sciatica can be present in extraspinal mass lesions.

Oncologic principles must be applied for all mass lesions compressing sciatic nerve since these lesions can have a malign component, which leads to unplanned resections, as evident in the existing literature. Diagnostic workup should start with detailed history taking and physical examination. The previous diagnosis of cancer and surgical history should be asked. Pain characteristics like constant or intermittent, related to activity or progressive should be noted.

Palpation of the sciatic notch and piriformis muscle eliciting pain should prompt us for possible mass lesion compressing the sciatic nerve. However mass lesions may be non-palpable due to obesity. X-rays and imaging modalities including ultrasonography, computed tomography, and MRI should be ordered when deemed necessary.

The surgical approach must be individualized according to the location and size of the lesion [11, 12]. The aim is to obtain enhance exposure. Various approaches depending on the location of the mass lesion and experience of the surgeons may be performed, providing safe surgical margins can be accomplished after resection. For proximal sciatic nerve lesions at the level of sciatic notch either an infragluteal or transgluteal approach may be utilized. During infragluteal approach, gluteus maximus muscle is detached from iliotibial bant and reflected medially; however, transgluteal approach provides access to the sciatic nerve by splitting the gluteus maximus muscle. For intrapelvic lesions anterior to sacrum transabdominal either using intraperitoneal or retroperitoneal approach may be used. In our study, we preferred different approaches. Predominantly, if the lesion is suspected to be malign, we prefer infragluteal approach rather than transgluteal approach to achieve wide surgical margins and avoid intercompartmental contamination.

To note, the size of lesion varies until the patient becomes symptomatic. In intrapelvic lesions, we observed more larger lesions compared to extrapelvic lesions. This should alert clinicians in intrapelvic lesions with a possible malign diagnosis.

Regarding neuromonitoring, there is no standard use in extraspinal bone and soft tissue tumors. Although it is commonly preferred in spinal surgery, there is no need in our cases as sciatic nerve is identified and preserved during tumor excision. Also, one recent study regarding the use of neuromonitoring in spinal cord tumors concluded that neuromonitoring do not take the role of replace clinical judgment and other perioperative information [13].

Study limitation

The small sample size, retrospective design and heterogeneity of pathologic diagnosis are major limitations of this study. Due to unequal numbers of intrapelvic (14 cases) and extrapelvic lesions (three cases), no statistics was applied. There is no preoperative and postoperative electrodiagnostic values to evaluate the effect of various surgical approaches on clinical improvement. However, all patients obtained dramatic clinical improvement. This study with these limitations will underline the need for further studies regarding the decision for surgical approach in various localizations.

CONCLUSION

Diagnostic algorithm should include detailed physical examination and radiologic imaging including pelvic and thigh area to detect mass lesions as extraspinal causes of sciatica. Patients who suffered from failed back surgery syndrome, and having persistent and progressive clinical symptoms despite physical or medical therapy should be investigated for a possible mass lesion which may be compressing the sciatic nerve. This will further avoid unnecessary and unsuccessful spinal surgeries.

Conflict of interest: None declared.

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Клинички резултати након хируршког лечења масовних лезија које узрокују ишијас – ретроспективна студија једног центра

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САЖЕТАК

Увод/Циљ Ишијас је онеспособљавајућа патологија са променљивом етиологијом. Најчешће патологије настају због дискогених или недискогених узрока. Масовне лезије су редак и често занемарен узрок екстраспиналне болести ишијаса, што доводи до непотребне операције кичме, одложеног дијагностиковања или неадекватног лечења. Не постоји стандардни хируршки приступ, а функционални исходи након хируршког лечења ових лезија нису довољно познати.

Циљ ове студије је процена клиничких резултата након хируршког лечења масовних лезија које изазивају ишијас у различитим локализацијама.

Методе Подаци су добијени ретроспективним прегледом радова између 2015. и 2020. године. Просечно трајање симптома током операције било је 10,3 месеца (3–48 месеци). Просечна старост болесника у време операције била је 43,8 (14–73 године). Просечно праћење је било 19,5 месеци (4–50 месеци). Четрнаест случајева има екстрапелвичну локализацију дистално од ишијасног зареза. У остала три случаја лезије су присутне у интрапелвичном подручју, и то леви ишијасни зарез (1), десни ацетабулум (1), сакроилијакални и лумбосакрални (1). Ниједан од болесника није имао опипљиву масу. Трансглутеални, инфраглутеални, бочни и постеромедијални приступи праћени су у складу са локацијом и величином лезије.

Резултати На последњој контроли примећено је ублажавање болова код свих болесника. Средњи резултат Друштва за мишићно-коштане туморе био је 90% (70–100). На последњој контроли није било рецидива.

Закључак Наше истраживање је показало да рана дијагноза неуролошким прегледом и радиолошком студијом може спречити непотребне операције и омогућити рано хируршко лечење туморске масе са задовољавајућим клиничким резултатима. Хируршки приступ треба индивидуализовати у складу са локацијом и димензијама лезије.

Кључне речи: масовне лезије; ишијасни нерв; недискогени ишијас; трансглутеални приступ; инфраглутеални приступ

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Comparative gait analysis of patients with different design of total knee arthroplasty

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SUMMARY

Introduction/Objective The essence of the treatment of degenerative knee joint diseases is pain relief, restoring motion range and stability of knee joints.

Methods In this study, 35 patients participated after having surgery of the knee joint. The patients had a posterior-stabilized (PS) endoprosthesis in one joint, and a posterior cruciate ligament retaining (CR) endoprosthesis in the other. Kinematic data was collected using a 3D optical system for tracking fluorescent markers in time. Based on these data, the following parameters were determined: degree of flexion, mediolateral (ML) translation, lateral gap, medial gap, and the angle of change between the transtibial and transfemoral axes.

Results The results show a more pronounced flexion degree with the PS prosthesis compared to the CR prosthesis. Also, the results show negligible values of the ML translation, lateral gap, and medial gap in both types of prostheses. Using the non-parameter Wilcoxon test, a substantial difference in the angle change between the transtibial and transfemoral axes was confirmed, that is, in the flexion angles on the CR and PS prostheses.

Conclusion This study shows that there is no great difference in the use of the PS or CR designs of endoprostheses. Better behavior and range of motion in the knee joint were established with the implantation of the PS endoprosthesis. This conclusion is confirmed by the substantial difference in the degree of flexion of the knee joint and in the position of the transversal axes of the tibia and femur.

Keywords: gait analysis; gait kinematics; gonarthrosis; PS endoprosthesis; CR endoprosthesis

INTRODUCTION

The basic role of knee arthroplasty is pain relief, restoration of knee joint motion range and stability [1]. Therapy success of implanted endoprostheses is most commonly defined by clinical and radiographic methods and tests based on the subjective feeling of patients about their pain and everyday functioning, such as the Knee Society Score (KSS), Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) and EQ-5D [2–5]. Functional comparisons of different implanted endoprostheses are difficult because of many subjective factors related to patients and their various postoperative expectations [6].

Therefore, examiners had a demanding task related to the application of the innovative objective methods for determining the level of physical activity, that is, the evaluation of operation success. The aim of this study is a simultaneous examination of motion analysis in both knees where due to gonarthrosis, a posterior-stabilized, that is, a cruciate-substituting endoprosthesis was implanted in one knee, and a cruciate-retaining endoprosthesis was implanted in the other.

METHODS

Patients

The examination was conducted at the Clinical Center of Kragujevac (Serbia). The selection of patients was done based on the following criteria:

– the patient suffers from gonarthrosis that was diagnosed based on anamnesis, clinical examination, and the analysis of radiographic records with the application of Kellgren–Lawrence (KL) classification [7, 8];

– based on the KL classification, gonarthrosis belongs to the third or fourth stage of the disease;

– all affected knees had a varus deformity with deviation of the axis of 5–15°;

– all the knee joints that were analyzed preoperatively had a flexion deformity of less than 10°;

– the PS endoprosthesis was implanted in one knee joint, and the CR endoprosthesis was implanted in the other;

– the patient does not suffer from neurological, rheumatological, or similar diseases, that is, diseases that may affect the disruption of walking pattern.

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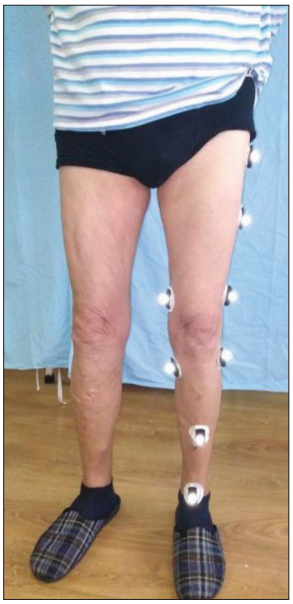

Figure 1. Clinical anatomical position of markers on a patient

Table 1. Values of the observed parameter values

Parameter	Posterior cruciate ligament		Cruciate ligament retaining	
	Stance	Swing	Stance	Swing
Flexion angle, °	13.98 ± 1.66	18.52 ± 7.67	11.35 ± 0.97	18.85 ± 7.21
Medio-lateral translation, mm	-0.19 ± 0.18	0.12 ± 0.31	-0.01 ± 0.18	-0.02 ± 0.34
Lateral gap, mm	-0.19 ± 0.33	0.03 ± 0.12	-0.11 ± 0.35	0.02 ± 0.12
Medial gap, mm	0.01 ± 0.04	0.01 ± 0.03	0.01 ± 0.02	-0.01 ± 0.03
Change between transfemoral and transtibial axes, °	0.06 ± 0.04	0.03 ± 0.01	0.11 ± 0.02	0.08 ± 0.01

In the examination, there were 35 patients who suffer from gonarthrosis (mean value of years 68.79 ± 5.98, mean value of weight 81.5±16.18 kg, and mean value of height 167.86 ± 8.51 cm). The patients were familiarized with the procedure of examination to which they voluntarily agreed. Gait analysis was done six months after the second arthroplasty. All the patients signed an agreement for participating in the study.

This study was done in accord with standards of the institutional Committee on Ethics.

Implant system

In the study, two endoprosthesis designs were used. The PS endoprosthesis was implanted in one knee (NexGen Complete Knee Solution Legacy Posterior Stabilized Knee, Zimmer, Warsaw, IN, USA), and the CR endoprosthesis was implanted in the other (DePuy Synthes, SIGMA, Primary Knee System, Cruciate Retaining design, DePuy Orthopaedics, Inc, Warsaw, IN, USA).

Clinical evaluation

All knee arthroplasties were done with a standard medial parapatellar incision. After the performed operation, verticalization and early rehabilitation were performed at the clinic. All the performed operations were done by the same group of surgeons.

Instrumentation and protocol

Kinematic data was collected using a 3D OptiTrack system (Natural Point, Inc., Oregon, www.naturalpoint.com). This system consists of 6 infrared cameras (V100:R2) all with a resolution of 640 × 480 pixels and a frame rate of 100 fps.

Using the afore-mentioned system, the tracking of positions of the 8 fluorescent markers (10 mm diameter) in space was done. The markers were placed on anatomic positions of the lower extremities to allow for repeatability of the examination, in the area of the great trochanter, on the medial and lateral femoral epicondyle, medial and lateral tibial epicondyle, on the center of the ankle joint and on the diaphysis of the femur and tibia (Figure 1) [9, 10].

Visualization was done using ARENA Software (Natural Point, Inc., Oregon, www.naturalpoint.com).

During the tracking protocol, patients moved without shoes at their own speed along a straight line (length 3 m) towards the cameras. The tracking was repeated at least twice.

Kinematic data

The obtained data from the ARENA Software was extracted to a standard VICON .c3d recording format. Furthermore, data processing was done using the MATLAB program (The MathWorks, Inc, USA, www.mathworks.com), that is, an evaluation of the following parameters was made: flexion angle, ML translation, medial gap, lateral gap, and the change between the transfemoral and transtibial axes. Depending on the implant, the processed data was divided into the PS or CR group. The kinematic analysis was based on the principles of a three-dimensional body.

The mean value and standard deviation calculated for every observed parameter, while a comparison was made using the non-parameter Wilcoxon test.

RESULTS

The results for the examined prostheses (PS and CR) were shown using the mean values of change in the observed parameters for the stance phase and the swing phase (flexion angle, ML translation, medial gap, lateral gap, change between transfemoral and transtibial axes) listed in Table 1 and using movement curves shown in Figure 2. The values of the observed parameters were approximately equal.

The flexion angle (Figure 2a, b) shows that a patient's leg is slightly bent in the stance phase, and that it stays in that position until the swing phase starts. Also, Figure 3b shows that the flexion angle in the stance phase is more expressed in the PS endoprosthesis design (Table 1).

There is relatively little ML translation (Figure 2c, d, Table 1) in both endoprosthesis designs. However, in the first 30% of the gait cycle for both types of prostheses, it can be noticed that there is a slight medial motion and that with the beginning of the swing phase, the lateral motion starts, and at the end of this phase the medial motion starts again.

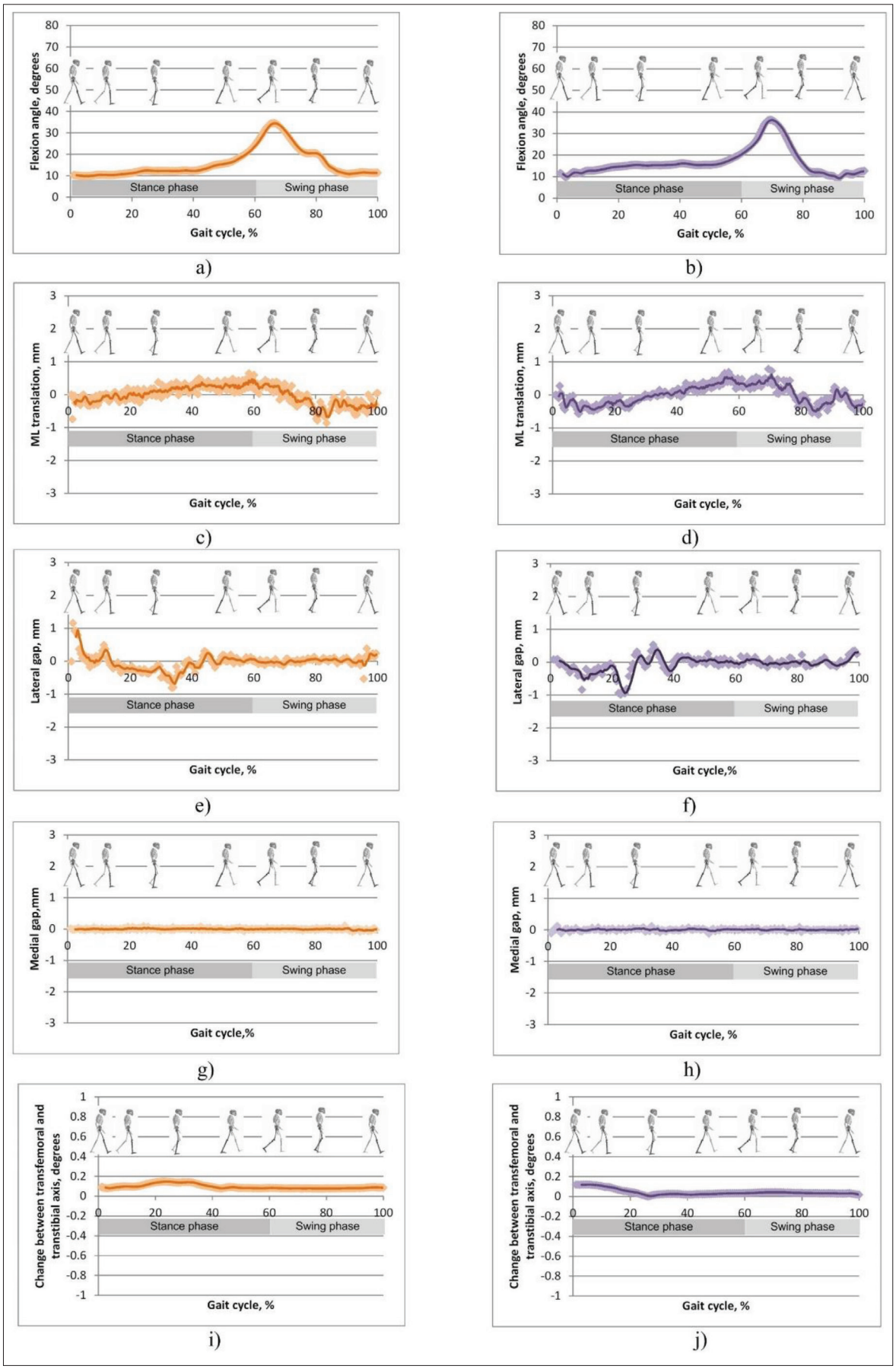

Figure 2. Graphic representation of the parameters: a) degree of flexion – CR, b) degree of flexion – PS, c) ML translation – CR, d) ML translation – PS, e) lateral gap – CR, f) lateral gap – PS, g) medial gap – CR, h) medial gap – PS, i) angle change between the transtibial and transfemoral axes – CR, and j) angle change between the transtibial and transfemoral axes – PS

The lateral gap (Figure 2 e, f, Table 1) is between -1 and 1 mm. These movements in the knee joint occur in the stance phase and are completely eliminated when the swing phase starts. With the CR endoprosthesis design, a gap increase is noticeable in the first 10% of the gait cycle, while in the PS design, the gap increase occurs in the first 30%. The medial gap (Figure 2 g, h, Table 1) is almost constant. Its changes are very little, and their values represent hundredths of a millimeter.

The angle that the transtibial and transfemoral axes form (Figure 2 i, j, Table 1) remains constant during the entire gait cycle in both endoprosthesis designs.

The non-parameter Wilcoxon test (Table 2) determines that there is no statistically substantial difference in the ML translation, and lateral and medial gap in the PS and CR prostheses. However, a substantial difference is determined in the angle change between the transtibial and transfemoral axes, that is, the flexion angles in the CR and PS prostheses. This change is not accidental – it occurs under the influence of systematic or experimental factors with a statistical significance of $p = 0.01$, a possible error of $p < 0.01$ and certainty of $p > 99\%$.

Table 2. Wilcoxon test

Compared groups	Value
Flexion angle degree cruciate ligament retaining *vs*. flexion angle degree posterior-stabilized	sig. = 0.00**
Medio-lateral translation cruciate ligament retaining *vs*. medio-lateral translation posterior cruciate ligament	sig. = 0.837
Lateral gap cruciate ligament retaining *vs*. lateral gap posterior-stabilized	sig. = 0.945
Medial gap cruciate ligament retaining *vs*. medial gap posterior-stabilized	sig. = 0.623
Change between transfemoral and transtibial axes cruciate ligament retaining *vs*. change between transfemoral and transtibial axes posterior-stabilized	sig. = 0.00**

DISCUSSION

The analysis of the success of knee arthroplasty using various endoprosthesis designs has been a topic of many examinations that are usually based on the use of subjective tests and the matching of patients with different types of endoprostheses [2, 11–16]. In our examination, an objective index of the gait pattern was used after knee arthroplasty in the same patient with, in one knee joint, an implanted endoprosthesis with sacrifice of the posterior cruciate ligament, and in the other, an endoprosthesis with a preserved posterior cruciate ligament.

Over the past years, the design and technology of implanted endoprostheses has been significantly improved, and many producers have placed various implant designs on the market. The choice of implants, in the majority of cases, depends on a surgeon`s personal experience. Apart from the surgeon`s experience, the implantation of the CR or PS endoprosthesis designs depends on the pathoanatomic change of the knee joint and on ligament stability [11, 17, 16].

Currently, there are disagreements regarding the sacrifice or retention of the posterior cruciate ligament in total knee prosthesis implantation. Marczak et al. [12] suggest that the proprioception property of patients shows better results with the PS endoprosthesis compared to the CR implant. Additionally, there are similar claims by Vanluawe et al. [13], who suggest that the implantation of the PS endoprosthesis design shows slight flexion instability, slight clinical, radiologic laxity, greater freedom of movement as well as slight complications after the implantation compared to the CR implant. A complete knee arthroplasty with retention of the posterior cruciate ligament (CR) has its own advantages compared to implantation of the total knee endoprosthesis with sacrifice of the posterior cruciate ligament (PS). The advantages are, firstly, it is based on a natural rollback of the femoral condyle during extension in the knee, as well as low osteotomy of the distal femur [11]. The disadvantage of implantation of such an endoprosthesis design is poor balance of soft tissue, which can lead to loosening of the implant. There are certain indications when it is necessary to place an implant with posterior stabilization that replaces the posterior cruciate ligament (PCL) function, such as a lack or insufficiency of the PCL, contraction of the posterior capsule that demands release, as well as noticeable flexion contractures of the knee.

Considering the divided opinions among examiners and clinicians, and in order to obtain objective results about the behavior of the knee joint, after implantation of the CR and PS endoprostheses, kinematic data were collected using the 3D OptiTrack system. A similar methodology of 3D gait analysis was used by Bytyqi et al. [14], and Prodanović et al. [15], who analyzed deformity of the gait pattern of the knee joint with degenerative change using the aforementioned methodology. The methodology has proved to be an excellent mode for diagnosing lesions of the anterior and posterior cruciate ligament, shown by Matić et al. [18], and La Prade et al. [19] in their examinations.

The method of establishing contact between implant elements has a direct influence on the functionality and durability of implants. As already mentioned, there are disputes about the placement of PS and CR implants. However, various studies have shown that there is no substantial difference in their use [20, 21]. Koga [22] belongs to the group of examiners who think that better reduction in knee joint rotation is achieved through the use of the CR implant type because of the increased tension in the PCL. Global analyses on the range of knee joint flexion have shown that rejection of the PCL increases the flexion angle by 2% [20]. Our results in Table 1 show that an increase in the degree of flexion occurs in the stance phase, while in the swing phase, the degree of flexion remains equal with the CR and PS prostheses. Similar results were obtained by Murakami et al. [23], who analyzed the walking pattern after bilateral arthroplasty of the knee joint using a treadmill with radiographic supervision and a flat panel detector. Victor et al. [24] and Broberg et al. [25], examined the comparison of motion range in both endoprosthesis designs and showed that there is a statistically greater range

of motion after knee endoprosthesis implantation with sacrifice of the posterior cruciate ligament.

With the use of the computational model and simulation of real conditions, potential conclusions of implant application can be drawn. With this model, Smith et al. [26] showed potential behavior of the PS endoprosthesis. Based on their results, it was shown that the use of this type of implant does not influence contact stresses, however it does affect ML distribution of the stress. An examination of CR prosthesis behavior in vivo was conducted by Li et al. [27]. Their results showed that, compared to the osteoarthritis (OA) joint, there is an increase in ML translation. With the increase of this translation, a change in the knee mechanics occurs, e.g., there is a change in the distribution of force and stress in the knee. Stress distribution is closely related to the realized contact [22], which our results showed. The ML translation is more noticeable with the use of the PS prosthesis rather than the CR implant. As the movement is more frequent in the gait cycle, contact is achieved on a greater surface, which influences the increased stress distribution in the ML direction.

The results show that there is a greater range of motion with the use of the PS implant. Similar results were obtained by Jiang et al. [28]. They think that these results can be connected to the removal of the PCL and a better balancing of the soft tissue.

In the gait analysis, we performed an examination of the medial and lateral gap. An increased gap occurs on the lateral side with the use of both types of endoprostheses, while the medial gap is non-existent. These gaps are noticeable in the stance phase (extensive and lateral gap). Another study proved that PCL resection does not influence the gap size. They analyzed the influence of PCL resection after knee arthroplasty [29].

In total arthroplasty of the knee joint, it is recommended that the transtibial and transfemoral axes be parallel [30], shown by the results in Figure 4e. There is a slight deviation for both endoprosthesis designs in the first 40% of the gait cycle due to the lateral gap occurring at the same moment.

This study has several shortcomings that must be considered. The main weakness of our research is that surgeries were done in two separate procedures, therefore the period from operation to movement recording is not identical for both knees. Secondly, the use of optical systems for gait analysis is not such a precise procedure as dynamic radiographic examination, however, compared to the afore-mentioned procedure, it is superior due to the possibility of 3D analysis and the ultimate safety of the patient (i.e., through lack of X-rays).

CONCLUSION

In this study, we examined the kinematic behavior of the knee joint with the application of objective methods after knee joint arthroplasty when the same patient was implanted using a PS endoprosthesis design on the one knee, and a CR on the other. The purpose of this surgical intervention is to restore the original knee joint kinematics and eliminate patients' discomfort. Even though there is no substantial difference between these two designs, better behavior and range of motion in the knee joint were achieved with the implantation of the PS endoprosthesis. This was confirmed by the substantial difference (shown using the Wilcoxon test) in the degree of flexion in the knee joint, and in the position of the transversal axes of the tibia and femur.

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Conflict of interest: None declared.

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Упоредна анализа хода пацијената са уграђеним тоталним ендопротезама зглоба колена различитог дизајна

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САЖЕТАК

Увод/Циљ Суштина лечења дегенеративних обољења зглоба колена је ублажавање болова, обнављање опсега покрета и стабилности зглоба колена.

Методе У овој студији је учествовало 35 пацијената после операције зглоба колена. Пацијенти су имали уграђену ендопротезу зглоба колена са задњом стабилизацијом на једном колену и ендопротезу са очуваним задњим укрштеним лигаментом на другом. Кинематски подаци су прикупљени коришћењем 3Д оптичког система којим се прате флуоресцентни маркери током времена. На основу ових података одређени су следећи параметри: степен флексије, медијално-латерална транслација, латерални међупростор, медијални међупростор и промена угла између транстибијалне и трансфеморалне осе.

Резултати Резултатима је показан већи степен флексије код ендопротезе са задњом стабилизацијом у односу на ендопротезу са очуваним задњим укрштеним лигаментом. Такође, резултати показују занемарљиве вредности медијално-латералне транслације, латералног међупростора и медијалног међупростора код оба типа ендопротеза. Коришћењем непараметријског теста *Wilcoxon*, потврђена је суштинска разлика у промени угла између транстибијалне и трансфеморалне осе, односно у степену флексије протеза са очуваним задњим укрштеним лигаментом и са задњом стабилизацијом.

Закључак Студијом је показано да не постоји велика разлика у коришћењу ендопротезе са задњом стабилизацијом или са задњим укрштеним лигаментом. Боље понашање и опсег покрета у зглобу колена се постиже уградњом ендопротезе са задњом стабилизацијом. Овај закључак је потврђен суштинском разликом у степену флексије зглоба колена и позицијама трансверзалних оса фемура и тибије.

Кључне речи: анализа хода; кинематика хода; гонартроза; ендопротеза са задњом стабилизацијом; ендопротеза са очуваним задњим укрштеним лигаментом

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Correlation between myocardial perfusion imaging findings and future cardiac events in patients with type 2 diabetes mellitus

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SUMMARY

Introduction/Objective Myocardial perfusion imaging (MPI) is clinically useful for the evaluation of coronary artery disease in patients with diabetes mellitus. However, the prevalence of ischemia and its ability to predict future cardiac events is less clear. The aim was to determine the incidence of cardiac events in diabetic patients and the relationship between them and MPI findings.

Methods Two cohorts of patients, 98 diabetics and 100 non-diabetics, with medium- to high-risk of coronary artery disease without previous coronary revascularization, were studied prospectively. All of them were outpatients who underwent ^{99m}Tc-sestamibi MPI with dipyridamole. The data about cardiac events were collected during a follow-up period of two years.

Results Cardiac events occurred in 17.3% diabetics and in 8% non-diabetics ($p = 0.048$). Diabetics had shorter estimated event-free time of 24.7 months (95% CI 23.2–26.2) versus non-diabetics' estimated event-free time of 28.5 months (95% CI 27.4–29.5) ($p = 0.046$). The independent predictors of cardiac events were male sex ($p = 0.010$), previous myocardial infarction ($p < 0.001$), presence of the symptoms of angina ($p = 0.014$), and all variables derived from MPI findings. After adjustment for variables derived from MPI findings, the significant predictors in diabetics were the size of stress perfusion defect ($p = 0.022$), summed stress score ($p = 0.011$), and summed difference score ($p = 0.044$).

Conclusion In diabetic patients, the cumulative rate of cardiac events was higher and the event-free survival was worse. MPI could help in prediction of cardiac events in diabetics and the most important predictors were size of stress perfusion defect, summed stress score, and summed difference score.

Keywords: myocardial perfusion imaging; diabetes mellitus; coronary artery disease; cardiac events

INTRODUCTION

Coronary artery disease (CAD) has now become a common cause of mortality and morbidity worldwide [1]. Furthermore, caring for patients with known or suspected CAD poses tremendous economic pressure on healthcare resources, not only due to costs related to testing and treatment, but also those associated with loss of productivity in afflicted individuals [1]. The worldwide prevalence of diabetes mellitus (DM) is increasing, concurrently with obesity and other comorbid conditions [2]. Despite significant advances in medical and invasive therapy, CAD is the leading cause of morbidity and mortality in patients with DM [3]. The diagnosis of CAD is complicated by the often atypical presentation of patients with DM attributable to concomitant autonomic neuropathy and other disorders. It is important to identify CAD early in these patients to optimize medical therapy and lifestyle modifications, and especially important to identify and aggressively treat those at the highest risk of events. The value of single-photon emission computed tomography (SPECT) myocardial perfusion imaging (MPI) in the evaluation of diabetic patients has been widely investigated [4, 5]. MPI is clinically useful for the evaluation of CAD in patients with DM. In diabetic patients with suspected or known CAD, a strong evidence base has been accumulated that MPI provides diagnostic and incremental prognostic information [4, 6, 7, 8]. The prognostic impact of ischemia together with other clinical and stress variables has been reported previously [4]. However, the prevalence of ischemia and its ability to predict those who experience future cardiac events is less clear in patients with DM with or without symptoms referred for MPI.

The aim of the study was to determine the incidence of cardiac events in diabetic patients and relationship between them and MPI findings.

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METHODS

Patient selection

The study population consisted of two cohorts of patients with medium- to high-risk of CAD without previous coronary revascularization. In the study group, there were 98 patients with type 2 DM and there were 100 patients without DM in the control group. All of them were outpatients who underwent ^{99m}Tc-sestamibi SPECT MPI with pharmacologic stress using dipyridamole. The test was requested for assessment of myocardial ischemia in all the patients. The patients in the study group had previously diagnosed DM and were treated with insulin or oral hypoglycemic agents. Selection of patients was performed so that the groups were matched with no significant differences between them regarding classical risk factors of CAD [age, sex, body mass index, smoking, arterial hypertension, previous myocardial infarction (MI), and symptoms of angina]. The study was conducted prospectively under the rules of the Declaration of Helsinki. The informed consent was obtained from all subjects before testing. The local medical ethics committee approved the study protocol. Before the test, a structured interview was performed and a clinical history was obtained, including assessment of cardiac risk factors. Furthermore, the measurements of patient height and weight were performed. Hypertension was defined as blood pressure above 140/90 mmHg or need for antihypertensive medication. Dyslipidemia was defined as the need for lipid-lowering medication. Subjects were considered symptomatic if they were experiencing chest pain or shortness of breath thought to be of possible cardiac origin.

Stress protocol and SPECT MPI

Stress testing and stress/rest gated SPECT MPI was performed as per guidelines of the European Association of Nuclear Medicine (EANM) [9]. The patients underwent intravenous vasodilator stress using dipyridamole (0.56 mg/kg over four minutes). At four minutes after completion of the dipyridamole infusion, a bolus of 550 MBq ^{99m}Tc-sestamibi (technetium-99m methoxy-isobutyl-isonitrile) was intravenously injected. In the event of chest pain, significant ST depression, or other symptoms, a dose of 125 mg of aminophylline was administered intravenously two minutes after injection of the radiotracer. SPECT MPI was performed using the two-day protocol. Each participant had gated stress using eight frames per R-R cycle and non-gated rest SPECT MPI. For resting studies, 550 MBq of the same tracer was injected at least 24 hours after the stress test. Image acquisition was performed with a commercially available SPECT camera system (Optima™ NM/CT 640, GE Healthcare, Chicago, IL, USA). Radiopharmaceutical dosing, SPECT acquisition, and image processing were performed within previously mentioned guidelines established by the EANM [9]. All images were obtained 60 minutes after radiotracer injection using rotating dual-head gamma camera equipped with low-energy, high-resolution, parallel hole collimator with 30% (± 15%) symmetric energy window centered at 140 keV. Sixty-four projections (40 seconds per projection), with a 64 × 64 matrix were obtained over a 180° orbit. No attenuation or scatter correction was used.

Image interpretation

Relative perfusion distribution was analyzed semiquantitatively using standardized segmentation of 17 myocardial segments. Each segment was scored by the consensus of two experienced observers using a five-point scoring system (0 = normal; 1 = equivocal; 2 = moderate; 3 = severe reduction; and 4 = absence of tracer uptake in a segment). The summed stress score (SSS) was obtained by adding the scores of the 17 segments of the stress images. The summed rest score (SRS) was obtained by similarly adding the scores of the 17 segments of the rest images. The sum of the differences between each of the 17 segments on the stress and rest images was defined as the summed difference score (SDS), a variable representing the amount of ischemia present. A scan was considered normal if the SSS was 3 or lower, mildly abnormal if the SSS was 4–8, moderately abnormal if the SSS was 9–13, and severely abnormal if the SSS was more than 13, as previously reported [10, 11]. The SDS < 2 was considered to be no ischemia, 2–4 mild ischemia, 5–8 moderate ischemia, and > 8 severe ischemia [10, 11]. An automated software program the Emory Cardiac Toolbox™ (ECTb™, Emory University School of Medicine, Atlanta, GA, USA) was used to calculate left ventricular ejection fraction (LVEF) and the variables incorporating both the extent and severity of perfusion defects.

Patient follow-up

Collection of follow-up data was obtained by reviewing hospital records, by contacting the patient's general practitioner, and/or by contacting the patient by phone during the period of approximately two years. The date of the last review or consultation was used to determine the follow-up time. End points were developments of the following cardiac events: cardiac mortality, nonfatal MI, or coronary revascularization by percutaneous coronary intervention or coronary artery bypass grafting. Cardiac mortality was defined as a death caused by acute MI, significant cardiac arrhythmias, or refractory congestive heart failure. Sudden death occurring without another explanation was included as cardiac mortality. Nonfatal MI and coronary revascularization were confirmed by reviewing hospital records. Patients with other-cause mortality were excluded from the study.

Statistical analysis

Baseline characteristics of patients were compared by Student's t or Mann–Whitney U tests for continuous variables and χ^2 or Fisher's exact tests for categorical variables where appropriate. Univariate Cox proportional hazard regression model was used to identify independent predictors of cardiac events. The risk of a variable was expressed

as a hazard ratio with corresponding 95% confidence interval (CI). Univariate Cox regression model was used to investigate the association between cardiac events and DM, after adjustment for variables derived from MPI findings LVEF, end diastolic volume, end systolic volume (ESV), systolic volume, presence of stress defect, presence of ischemia, SSS, SRS, and SDS. Survival curves as a function of time (months) were generated with the Kaplan–Meier method. A p-value < 0.05 was considered statistically significant. Statistical analyses were performed using the statistical software platform IBM SPSS Statistics, Version 22.0 (IBM Corp., Armonk, NY, USA).

RESULTS

Study population and MPI findings

The demographics, clinical characteristics and MPI results among diabetics and non-diabetics are shown in Tables 1 and 2, respectively. There were no significant differences of the prevalence of classical risk factors between the groups except of dyslipidemia and family history of diabetes, which were higher among the diabetics (p = 0.004 and p < 0.001). Perfusion and non-perfusion variables were obtained from MPI for all the patients. Diabetics had lower LVEF (p = 0.018), higher ESV (p = 0.039), and higher proportion of them were with abnormal ESV (p = 0.049). There were no significant differences of perfusion variables between the groups.

Table 1. Baseline characteristics of the cohorts

Baseline characteristics	DM (n = 98)	non-DM (n = 100)	p
Male, n (%)	55 (56.1)	45 (45)	0.118
Age, years	66.8 ± 7.2	66.9 ± 7.7	0.952
Body mass index, kg/m^2	30.2 ± 4.8	29.2 ± 5	0.137
Hypertension, n	96	97	NS
Previous MI, n (%)	18 (18.4)	18 (18)	0.947
Smokers (anytime), n (%)	50 (51)	51 (51)	0.998
Smokers (current), n (%)	11 (11.2)	15 (15)	0.432
Dyslipidemia, n (%)	72 (73.5)	54 (54)	**0.004**
Family history of DM, n (%)	61 (62.2)	28 (28)	**< 0.001**
Family history of CAD, n (%)	73 (74.5)	76 (76)	0.806
Symptoms of angina, n (%)	61 (62.2)	64 (64)	0.798

DM – diabetes mellitus; MI – myocardial infarction; CAD – coronary artery disease

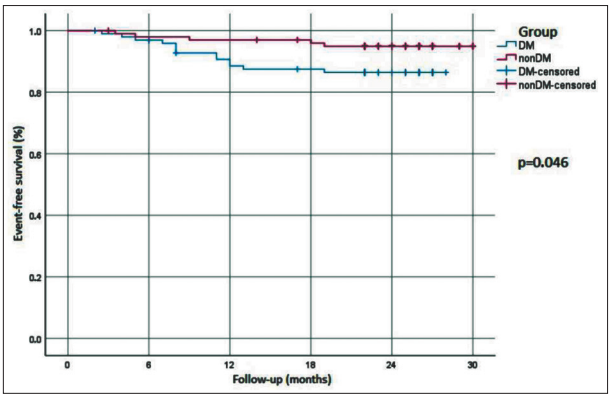

Figure 1. Kaplan–Meier event-free survival curves

Table 2. Myocardial perfusion imaging findings

Characteristics		DM (n = 98)	non-DM (n = 100)	p
LVEF US, %		54.7 ± 9.9	57.8 ± 6.9	**0.013**
LVEF SPECT, %		64 ± 14.3	68.6 ± 12.3	**0.018**
LVEF SPECT ≥ 50%, n (%)		84 (85.7)	92 (92)	0.159
EDV, ml		104 (43–318)	97 (41–214)	0.133
ESV, ml		34 (4–245)	29.5 (6–140)	**0.039**
SV, ml		67.5 ± 16.4	67.5 ± 16.8	0.983
Abnormal ESV, n (%)		27 (27.6)	16 (16)	**0.049**
SSS		0 (0–19)	0 (0–23)	0.093
SRS		0 (0–18)	0 (0–20)	0.606
SDS		0 (0–15)	0 (0–18)	0.094
Abnormal stress MPI (SSS ≥ 4), n (%)		33 (33.7)	24 (24)	0.133
Severity of stress defect, n (%)	SSS < 4 – no defect	65 (66.3)	76 (76)	0.095
	SSS 4–8 – mild	13 (13.3)	13 (13)	
	SSS 9–13 – moderate	12 (12.2)	7 (7)	
	SSS >13 – severe	8 (8.2)	4 (4)	
Ischemia (SDS ≥ 2), n (%)		18 (18.4)	12 (12)	0.212
Severity of ischemia, n (%)	SDS < 2 – no ischemia	80 (81.6)	88 (88)	0.145
	SDS 2–4 – mild	0 (0)	6 (6)	
	SDS 5–8 – moderate	6 (6.1)	2 (2)	
	SDS > 8 – severe	12 (12.2)	4 (4)	

DM – diabetes mellitus; LVEF – left ventricular ejection fraction; US – ultrasound; SPECT – single photon emission computed tomography; EDV – end diastolic volume; ESV – end systolic volume; SV – systolic volume; SSS – summed stress score; SRS – summed rest score; SDS – summed difference score; MPI – myocardial perfusion imaging

Follow-up, outcomes, and survival analysis

Median of follow-up period did not differ significantly between the two groups (26 *vs.* 24 months; p = 0.184). During this period of time, cardiac events occurred in 17.3% of the diabetics and in 8% of the nondiabetics (p = 0.048) (Table 3).

Event-free survival curves were constructed using the Kaplan–Meier method to account for censored survival times (Figure 1). The diabetics had shorter estimated event-free time (24.7 months; 95% CI 23.2–26.2) compared to the non-diabetics (28.5 months; 95% CI 27.4–29.5) (p = 0.046).

Predictors of cardiac events

The results of the univariate Cox proportional hazards analysis predicting cardiac events are given in Table 4. The independent predictors were male sex (p = 0.010), previous MI (p < 0.001), presence of the symptoms of angina (p = 0.014) and all variables derived from MPI findings. DM was not significant, but borderline predictor of cardiac events in

Table 3. Follow-up period and outcomes

Characteristics		DM (n = 98)		non-DM (n = 100)		p
Follow-up, months		26 (2–28)		24 (3–30)		0.184
Cardiac event n (%)	Cardiac death	2 (2)	17 (17.3)	1 (1)	8 (8)	**0.048**
	Non-fatal MI	2 (2)		2 (2)		
	Revascularization	13 (13.3)		5 (5)		

DM – diabetes mellitus; MI – myocardial infarction

Table 4. Predictors of cardiac events in the univariate Cox analysis

Variable	HR (95% CI)	p
DM	2.3 (0.99–5.3)	0.053
Sex (f/m)	0.3 (0.1–0.8)	**0.010**
Body mass index	1 (0.9–1.1)	0.867
Hypertension	0.6 (0.1–4.2)	0.574
Previous myocardial infarction	5.9 (2.7–12.9)	**< 0.001**
Smokers (anytime)	2.7 (1.1–6.4)	**0.027**
Smokers (current), n (%)	0.9 (0.3–3)	0.877
Dyslipidemia	1.3 (0.5–2.9)	0.596
Family history of DM	1.7 (0.7–3.6)	0.213
Family history of CAD	1 (0.4–2.6)	0.938
Symptoms of angina	4.5 (1.4–15.1)	**0.014**
LVEF SPECT, %	0.9 (0.9–1)	**< 0.001**
Presence of normal LVEF SPECT	0.2 (0.1–0.4)	**< 0.001**
EDV, mL	1.01 (1.01–1.02)	**< 0.001**
ESV, mL	1.01 (1.01–1.02)	**< 0.001**
Presence of normal ESV	0.2 (0.1–0.4)	**< 0.001**
SV, mL	1.02 (1.00–1.05)	**0.025**
Presence of stress perfusion defect	119.1 (4–3574.9)	**0.006**
Size of stress perfusion defect, %	1.1 (1.1–1.2)	**< 0.001**
Presence of ischemia	82.4 (19.3–351.1)	**< 0.001**
Size of ischemia, %	1.2 (1.1–1.2)	**< 0.001**
SSS	1.2 (1.2–1.3)	**< 0.001**
SRS	1.1 (1.1–1.2)	**< 0.001**
SDS	1.3 (1.2–1.4)	**< 0.001**
Abnormal stress MPI (SSS≥4)	498.2 (3.6–69325.6)	**0.014**
Severity of stress defect	5.2 (3.5–7.8)	**< 0.001**
Ischemia (SDS ≥ 2)	112.5 (26.3–481)	**< 0.001**
Severity of ischemia	4.6 (3.2–6.6)	**< 0.001**

HR – hazard ratio; CI – confidence interval; DM – diabetes mellitus; CAD – coronary artery disease; LVEF – left ventricular ejection fraction; SPECT – single photon emission computed tomography; EDV – end diastolic volume; ESV – end systolic volume; SV – systolic volume; SSS – summed stress score; SRS – summed rest score; SDS – summed difference score; MPI – myocardial perfusion imaging

univariate Cox proportional hazards analysis. The association between cardiac events and DM was determined using univariate Cox regression model after adjustment for variables derived from MPI findings (Table 5). The significant predictors were size of stress perfusion defect (p = 0.022), SSS (p = 0.011) and SDS (p = 0.044).

DISCUSSION

In our study, the groups were matched and there were no significant differences between them regarding classical risk factors of CAD (age, sex, body mass index, smoking, arterial hypertension, previous MI, and symptoms of angina) except of prevalence of dyslipidemia and family history of diabetes, which were higher among the diabetics (p = 0.004 and p < 0.001). These were expected since diabetics have higher prevalence of dyslipidemia and 2–4 times higher prevalence of family history of diabetes than non-diabetics [12, 13].

We found that diabetics had lower LVEF (p = 0.018), in accordance with some other studies. Ehl et al. [14] showed that diabetics had a lower LVEF determined by MPI than non-diabetics (p = 0.001) and this difference could be demonstrated regardless of CAD extent (no significant differences of SSS, SRS, and SDS) and might in part explain their generally worse cardiac survival compared with non-diabetics. Chareonthaitawee et al. [15] found that one of six asymptomatic diabetic patients without known CAD referred for MPI had reduced LVEF. The annual mortality rates of the groups with and without reduced LVEF were 7% and 4%, respectively.

In recent years, a large body of literature has established the prognostic significance of MPI in the general population [7, 16, 17]. It was shown that patients with normal stress MPI studies had remarkably low cardiac event rates (< 1% per year) and the event rate was proportional to the extent of stress-induced hypoperfusion. In patients with a normal MPI SPECT, there was an annual death rate of 0.3% compared with 2.9% in patients with severely abnormal scans [10]. The nonfatal MI rate in another study also increased in relation to the SSS [3].

Diabetic patients have multitude of characteristic features including higher prevalence of multi-vessel and small vessel CAD, frequent silent myocardial ischemia and infarction with higher cardiac event rates, and the presence of autonomic dysfunction. This together with the prevalence of diabetic cardiomyopathy contributes to a higher cardiovascular mortality [18, 19]. Furthermore, diabetic patients have higher prevalence of cardiovascular co-morbidities as compared to patients without diabetes [20, 21]. Two-thirds of diabetic patients will die of heart or vascular disease, and patients with CAD and DM have worse outcomes and a much higher cardiac event rate than their nondiabetic counterparts [22, 23].

Our study demonstrates that the two-year cumulative rate of cardiac events was higher (17.3% *vs*. 8%) and the event-free survival was worse in diabetics (24.7 *vs*. 28.5 months) than that seen in patients without DM. We found that the independent predictors of cardiac events were male sex, previous MI, presence of the symptoms of angina and all variables derived from MPI findings, but in diabetics the most important predictors were the size of stress perfusion defect, SSS, and SDS. There are numerous similar evidences in previous work of many authors. Kang et al. [24] showed that diabetics had a higher event rate than nondiabetics with the same SSS. Giri et al. [4] showed in a multicenter trial that diabetics with ischemic defects had increased cardiac events than nondiabetics with the same level of ischemia. Despite this, an abnormal scan was an independent predictor of cardiac death and MI in both diabetic and nondiabetic groups.

Table 5. Association between cardiac events and diabetes mellitus using univariate Cox regression model after adjustment for variables derived from myocardial perfusion imaging findings

Adjusting variable	HR (95% CI)	p
LVEF SPECT, %	1.8 (0.8–4.2)	0.180
Presence of normal LVEF SPECT	2.1 (0.9–4.8)	0.094
EDV, mL	1.9 (0.8–4.5)	0.148
ESV, mL	1.9 (0.8–4.4)	0.153
Presence of normal ESV	1.8 (0.8–4.3)	0.167
SV, mL	2.3 (1–5.3)	0.054
Presence of stress perfusion defect	1.9 (0.8–4.5)	0.129
Size of stress perfusion defect, %	2.8 (1.2–6.6)	**0.022**
Presence of ischemia	1.6 (0.7–3.8)	0.263
Size of ischemia, %	2.2 (0.9–5.3)	0.069
SSS	3.2 (1.3–7.9)	**0.011**
SRS	2.3 (1–5.3)	0.052
SDS	2.4 (1–5.8)	**0.044**
Abnormal stress MPI (SSS ≥ 4)	1.8 (0.8–4.2)	0.161
Severity of stress defect	1.7 (0.7–4)	0.223
Ischemia (SDS ≥ 2)	2.2 (0.9–5.1)	0.079
Severity of ischemia	0.9 (0.4–2.2)	0.802

HR – hazard ratio; CI – confidence interval; LVEF – left ventricular ejection fraction; SPECT – single photon emission computed tomography; EDV – end diastolic volume; ESV – end systolic volume; SV – systolic volume; SSS – summed stress score; SRS – summed rest score; SDS – summed difference score; MPI – myocardial perfusion imaging

In the previous analyses of perfusion imaging, the cardiac event rates in diabetic patients were significantly higher compared with nondiabetic patients, and the event rates in diabetic patients were related to the presence or absence of perfusion abnormalities. Kang et al. [24] showed a higher cardiac event rate in diabetic patients than in nondiabetic patients, and the severity and size of the perfusion abnormalities as evaluated by the SSS were significantly related to the probability of a cardiac event. Giri et al. [4] demonstrated the incremental value of perfusion imaging in predicting cardiac events, and De Lorenzo et al. [6] showed that the risk is related to the number of territories involved, and the extent and severity of the stress defects in both men and women. Similarly, Berman et al. [25] further demonstrated that the SSS predicted outcome in both diabetic men and women. Outcome was significantly higher in diabetic patients than in nondiabetics, and the severity of the defect predicted the event rates. Cardiac events are, however, significantly higher in diabetic patients with an abnormal scan, resulting in a three- to eight-fold increased risk compared with diabetic patients with a normal scan, and the severity of the perfusion abnormality in the diabetic population is proportionately related to outcome[26]. These findings are consistent with the assumption that diabetes contributes to an accelerated path of CAD complications. Diabetic patients are predisposed to a more aggressive form of vascular disease with diffuse coronary atherosclerosis and significantly higher incidence of cardiac events [4].

CONCLUSION

Our study adds to the body of evidence that the MPI continues to have an important diagnostic and prognostic value in evaluation of CAD, particularly in diabetics. In diabetic patients, the cumulative rate of cardiac events was higher and the event-free survival was worse than in patients without DM. We found that MPI could help in predicting cardiac events in diabetics and the most important predictors were the size of stress perfusion defect, SSS, and SDS.

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Корелација између налаза перфузионе сцинтиграфије миокарда и будућих срчаних догађаја код оболелих од дијабетеса типа 2

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САЖЕТАК

Увод/Циљ Перфузиона сцинтиграфија миокарда (ПСМ) корисна је у евалуацији коронарне артеријске болести код оболелих од дијабетеса типа 2 (ДТ2). Ипак, преваленца исхемије код њих и могућност предвиђања будућих срчаних догађаја су нејасни.

Циљ је био одредити инциденцу срчаних догађаја код оболелих од ДТ2 и везу између њих и налаза ПСМ.

Методе Проспективно су испитиване две групе болесника са средњим до високим ризиком за коронарну артеријску болест, 98 са ДТ2 и 100 без, који нису имали ранију коронарну реваскуларизацију. Свима је урађена *^{99m}Tc-sestamibi* ПСМ са дипиридамолом. Подаци о срчаним догађајима су сакупљени током двогодишњег праћења.

Резултати Срчани догађаји су настали код 17,3% испитаника са ДТ2 и 8% испитаника без ДТ2 ($p = 0,048$). Испитаници са ДТ2 су имали краће време преживљавања без срчаног догађаја – 24,7 месеци (95% *CI* 23,2–26,2) према 28,5 месеци (95% *CI* 27,4–29,5) код оних без ДТ2 ($p = 0,046$). Независни предиктори настанка срчаних догађаја су били мушки пол ($p = 0,010$), ранији инфаркт миокарда ($p < 0,001$), присуство ангинозних тегоба ($p = 0,014$) и све варијабле добијене из налаза ПСМ. Код испитаника са ДТ2, након корекције и прилагођавања са варијаблама добијеним из налаза ПСМ, значајни предиктори су били величина испада перфузије у оптерећењу ($p = 0,022$), укупан збир бодова у оптерећењу (*summed stress score – SSS*) ($p = 0,011$) и укупна разлика бодова између *SSS* и укупног збира бодова у мировању (*summer diference score – SDS*) ($p = 0,044$).

Закључак Код оболелих од ДТ2, кумулативна стопа срчаних догађаја је била виша, а време преживљавања до настанка срчаног догађаја краће. ПСМ може помоћи у предвиђању будућих срчаних догађаја код оболелих од ДТ2, а најважнији предиктори су били величина испада перфузије у оптерећењу, *SSS* и *SDS*.

Кључне речи: перфузиона сцинтиграфија миокарда; дијабетес; коронарна артеријска болест; срчани догађаји

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Lifetime and periodic prevalence and characteristics of violence against women committed by their alcohol-dependent partners – a cross-sectional study

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SUMMARY

Introduction/Objective We aimed to investigate the lifetime and periodic prevalence (during a year) and characteristics of violence against women and health status of women whose partners have been treated for alcohol dependence.

Methods A cross-sectional study was conducted among women whose male partners were alcohol-dependent and admitted to hospital for the inpatient treatment. Exposure to physical and sexual violence was measured by the Conflict Tactics Scale (CTS-2). Mental health status was measured by Beck Depression Inventory (BDI-II), Beck Anxiety Inventory (BAI), suicidal risk (using Mini International Neuropsychiatric Interview or MINI scale), and alcohol consumption (Alcohol Use Disorders Identification Test). The data were analyzed by descriptive and inferential statistical methods. We also constructed two logistic regression models to study associations between violence and socioeconomic status, and violence and health-related variables.

Results The lifetime prevalence of physical violence committed by alcohol-dependent partners against women was 65.4%, while the periodic prevalence (during 12 months prior to the study) was 46.2% for physical, 20.2% for sexual, and 18% for both types of violence. No women were in risk of harmful alcohol consumption. Violence was more frequent against women not living in urban areas [odds ratio (OR) 2.53, 95% confidence interval (CI) 1.08–5.94, in the univariate model], and among women with moderate/severe depression (OR 12.34, 95% CI 2.26–67.33, in the multivariate model).

Conclusion Alcohol-dependent men are very often violent toward their spouses, and inpatient treatment presents an opportunity to work with them on raising awareness on the unacceptability of violence against women.

Keywords: alcohol dependence; alcohol use disorders (AUD); violence; women; health; perpetuators

INTRODUCTION

Violence against women (later in the text: violence) is a worldwide phenomenon that is rooted in gender inequality. Most often, the perpetrator of violence is the woman's intimate partner (a spouse), either current or a former one. According to the World Health Organization, prevalence of physical and/or sexual violence among all ever-partnered women worldwide was 26% and 10%, respectively, during the past 12 months [1].

In Serbia, the most recent available data showed that 17% of women experienced physical violence and 5% experienced sexual violence during their lifetime [2]. The frequency is even higher among women whose husbands/partners have alcohol dependence [3, 4]. Intimate partner violence can manifest itself not just as a physical or sexual violence, but also psychological violence, which is very prevalent and even more difficult to bear, although it is challenging for validation and intercultural interpretation [5]. However, in real life, violence often simultaneously appears in many forms, which has cumulative negative consequences on women's health [6]. All forms of violence are associated with poor mental health of women, especially with the occurrence of depression [6].

Alcohol consumption, especially heavy drinking, facilitates expression of violence [7]. According to the social-ecological model, violence is a result of the interaction of four groups of factors that appear at the individual's, partner's, community's, and society's level [8, 9]. Men's excessive alcohol consumption is one of the risk factors for violence that belong to the individual partner's level and is associated with reduced reasoning and disinhibited behavior. Although not all men perpetuate violence under the influence of alcohol, it is well known that alcohol affects cognitive functions, alters perception, reduces inhibitory mechanisms, makes it difficult to constructively solve problems, and facilitates the manifestation of aggression [3, 7].

In this paper, we investigated the frequency and characteristics of intimate partner violence against women whose partners have been

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Table 1. Incidence of physical and sexual intimate partner violence against women during the previous 12 months, among women who experienced intimate partner violence during lifetime

Physical violence	No n (%)	Yes n (%)	Frequency		
During the last 12 months, has your current husband/partner ever…			Once n (%)	A few times n (%)	Many times n (%)
Slapped you or thrown something at you that could hurt you?	25 (24)	39 (37.5)	14 (35.9)	24 (61.5)	1 (2.6)
Pushed or shoved you or pulled your hair?	17 (16.3)	39 (37.5)	15 (38.5)	22 (56.4)	2 (5.1)
Hit you with his fist or with something else that could hurt you?	10 (9.6)	10 (9.6)	5 (55.6)	4 (44.4)	-
Kicked, dragged or beaten you up?	7 (6.7)	5 (4.8)	5 (100)	-	-
Choked or burnt you on purpose?	6 (5.8)	-	-	-	-
Threatened to use or actually used a gun, knife or other weapon against you?	9 (8.7)	10 (9.6)	4 (36.4)	6 (54.5)	1 (9.1)
Answered "Yes" to any of the above questions	-	**48 (46.2)**	-	-	-
Total number of physical violence acts	-	103	43 (41.7)	56 (51.4)	4 (3.8)
Sexual violence	No n (%)	Yes n (%)	Frequency		
			Once n (%)	A few times n (%)	Many times n (%)
Were you ever physically forced to have sexual intercourse when you did not want to?	5 (4.8)	9 (8.7)	2 (22.2)	7 (77.8)	-
Did you ever have sexual intercourse you did not want because you were afraid of what he might do?	10 (9.6)	20 (19.2)	8 (40)	12 (60)	-
Did he ever force you to do something sexual that you found degrading or humiliating?	2 (1.9)	4 (3.8)	3 (75)	1 (25)	-
Answered "Yes" to any of the above questions	-	**21 (20.2)**	-	-	-

treated for alcohol dependence. Additionally, we examined the associations between violence against women and socio-demographic characteristics, and women's health status. We used the same sample to study mental health as a main outcome variable, the results of which have been published in a previous paper, while in this paper we focused on factors associated with the experience with violence [10].

METHODS

The research was designed as a cross-sectional study, using self-administered questionnaire among women older than 18 years whose partners (spouses) were under an inpatient treatment for alcohol dependence, with an established diagnosis of alcohol dependence F10.2, according to the International Classification of Diseases, 10th revision. The research was conducted at the Special Hospital for Addiction Diseases in Belgrade, and ethics committee of this hospital approved conducting of this study.

Recruitment of participants and inclusion criteria

Women older than 18 years who appeared at the Department for consultations related to treatment of their spouses were approached and asked to fill out the questionnaire. The inclusion criteria were that they have been married or living in cohabitation for at least a year with their male partners who were alcohol dependent and currently admitted to hospital for inpatient treatment. Prior to the start of the survey, the respondents were informed that their participation in the survey was anonymous and on a voluntary basis, and women gave their informed consent. The data were collected between January and June of 2018.

Main dependent variables

The main dependent variable in the study was exposure to physical and sexual violence that women experienced during the last 12 months by their partners who had been at that time under the inpatient treatment for alcohol dependence. The occurrence of physical and/or sexual male partner violence was measured by the Conflict Tactics Scale 2 (CTS 2) [11]. This scale included six behaviorally specific questions regarding exposure to physical violence, and three behaviorally specific questions related to the exposure to sexual violence (Table 1).

Independent variables

Independent variables were socio-demographic characteristics of both partners (age, level of education, employment status); characteristics of marital union (number of years spent in marital/extramarital union, number of children), and health characteristics of both partners (existence of somatic and psychiatric diseases, on a dichotomous scale yes/no). In addition to it, we measured mental health outcomes among women, such as depression, anxiety, suicidality, and the occurrence of alcohol dependence.

Depression was measured using the Beck Depression Inventory (BDI-II) scale, which is standardized in Serbia as well [12]. The scale has 21 questions about how the respondent might feel, with four options of answers, starting from 0 (does not agree at all) to 3 (agrees very much). The total score on the scale was obtained by simply adding all the answers obtained from the first to the 21st question. No depression is indicated by the score of up to 9 points; mild depression is indicated by 10–19 points; moderate depression by 20–29 points, and severe depression by 30–63 points [12]. In the later analyses, moderate and severe depression were summarized in one category.

Anxiety was measured using Beck Anxiety Inventory (BAI), which is standardized in Serbia as well [13, 14]. This instrument comprises 21 questions related to the symptoms of general anxiety. Respondents answered each question by estimating the intensity of symptoms, on a Likert scale from 0 (not present) to 3 (very much present). The sum of all responses (maximum of 63) represents the intensity of the symptoms of general anxiety. The overall score was graded into four categories: no anxiety (0–9), mild and mild to moderate (10–19), moderate (20–29), and severe anxiety (30–64). In later analyses, moderate and severe anxiety were summarized in one category.

Suicidality among women was measured using the Mini International Neuropsychiatric Interview (MINI), which has six questions, with binary options of response (yes/no) [13]. Positive response on the first, the second, and the third statement indicated high level of suicidality risk.

Women's alcohol drinking was measured using the Alcohol Use Disorders Identification Test (AUDIT) [15]. AUDIT is a screening test designed for the early identification of risky and harmful drinking as well as alcohol dependence in the adult population, developed and recommended by the World Health Organization. Developed and evaluated over a period of two decades, it has been found to provide accurate risk measurement by both sex and age in different cultures. It consists of 10 questions related to recent alcohol consumption, the existence of symptoms of alcohol dependence, and problems related to alcohol consumption. It complies with the International Classification of Diseases 10th revision definitions of alcohol dependence and harmful alcohol use. It can be used through an oral interview or as a written questionnaire. Answers are scored in the range of 0–4. The values of all responses are summed up and grouped into the five levels: no alcohol consumption at all; zone I (1–7 points); zone II (8–15 points); zone III (16–19 points), and zone IV (20–40 points) [15]. No alcohol consumption risk comprises two categories: no alcohol consumption at all and zone I (1–7 points) [15].

Statistical analysis

The statistical analyses were done using the methods of descriptive and analytical statistics. Frequency of exposure to physical and sexual violence against women was expressed in absolute numbers and percentages. We used the χ^2 test to identify the differences in occurrence of physical and/or sexual violence among women and wide range of socioeconomic and health variables. We run two logistic regression models with the exposure to violence as an outcome variable: one model with all SES variables, and the other one with all health-related variables. For both models we ran both the univariate and the multivariate model, and the results were expressed as odds ratios (OR) with 95% confidence intervals (CI).

Probability of results was set up at 0.05 and 0.01 level (significant and highly significant results). The analyses were done by using the statistical software package IBM SPSS Statistics, Version 26.0 (IBM Corp., Armonk, NY, USA).

RESULTS

A total of 104 women whose husbands/partners have been under treatment for alcohol dependence completed the questionnaire. The age of the respondents was in the range 26–66 years, (mean age 48.19 years, SD 9.17). The age of their current partners who were being treated for alcohol dependence were in the range 33–67 years (mean age 50.09, SD 10.94). The structure of the sample in relation to age, education, and employment status of spouses is shown in Table 2.

Table 2. Sociodemographic and health characteristics of women, their partners/spouses, and characteristics of the marital union

Parameters	Women (respondents) N = 104 (100%) n (%)	Partners/ spouses N = 104 (100%) n (%)
Age		
Under or 39 years old	17 (16.5)	14 (14)
40–49 years old	38 (36.9)	35 (35)
50–59 years old	33 (32)	37 (38)
60 and over	15 (14.6)	14 (13)
Education		
Primary school	6 (5.8)	9 (8.7)
High school (9–12years)	56 (53.9)	64 (61.6)
Higher education (12 years and more)	42 (40.4)	31 (29.8)
Working status		
Employed	67 (64.4)	69 (66.3)
Unemployed	26 (25)	26 (25)
Retired	11 (10.6)	9 (8.7)
Presence of physical disease		
Yes	11 (10.6)	19 (18.3)
No	93 (89.4)	85 (81.7)
Presence of psychiatric disease		
Yes	1 (1)	2 (1.9)
No	103 (99)	102 (98.1)
Characteristics of marital union and number of partners treatments for alcohol dependence	N = 104 (100%) n (%)	
Duration of marital union		
10 years or less	21 (20.4)	
11–20 years	37 (35.9)	
21–30 years	26 (25.2)	
31 years and over	19 (18.4)	
Number of children		
0	12 (11.7)	
1	31 (30.1)	
2	54 (52.4)	
3 and more	6 (11.7)	
Place of living		
City	71 (68.3)	
Countryside	11 (10.6)	
Suburb	22 (21.2)	
Differences in the level of education between spouses		
The same level of education	65 (63.7)	
The man is more educated	13 (12.7)	
The woman is more educated	24 (23.5)	
Number of treatments for alcohol addiction		
First treatment	72 (69.9)	
Second treatment	25 (24.3)	
Third treatment or more	6 (5.8)	

Lifetime and periodic prevalence of physical and/or sexual violence

The prevalence of either physical or sexual intimate partner violence during the lifetime of women whose partners are under treatment for alcohol addiction was 69.2% (Figure 1).

When looking at the last 12 months, almost half of the women (48.1%) confirmed either physical or sexual violence (Figure 2).

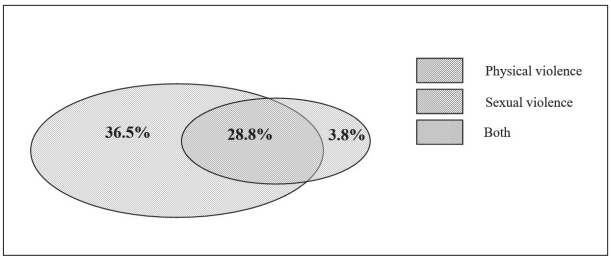

Figure 1. Exposure to violence against women during their lifetime

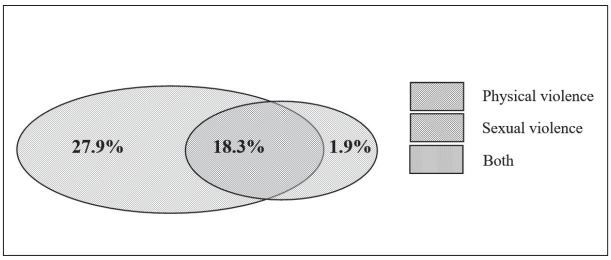

Figure 2. Exposure to violence against women during the previous 12 months

The most frequent acts of violence during the last 12 months were slapping or throwing something that could hurt women, and pushing or shoving or pulling women's hair, which were experienced by 37.5% each (Table 1).

Characteristics of women's mental health

No depression was found in 29.8% women, while mild and moderate/severe depression were found in two of three women (35.5% and 34.6%, respectively) (Table 3). No anxiety was found in 44.7% of women, and every fourth women had moderate/severe anxiety (25.2%). More than one in four women had some level of suicidal risk (27.2%). No women had the risk of harmful alcohol consumption (Table 3).

The results showed that there is statistically significant difference in the exposure of women to violence during the past 12 months in relation to the age of the husband ($p = 0.029$) and rural place of residence ($p = 0.030$) (Table 4). Living in rural areas was associated with an increased risk for experiencing violence (OR 2.53, 95% CI 1.08–5.94), which was even higher in a multivariate logistic regression model, although statistical significance was lost (OR 3.4, 95% CI 0.81–14.2).

A statistically significant difference was observed in the exposure of women to violence and the presence of depression ($p < 0.001$), anxiety ($p < 0.001$), and suicidality ($p < 0.001$) (Table 5). These health conditions were associated with several times higher odds for violence (starting from OR 3.92, 95% CI 1.48–10.35 for mild anxiety, to OR 27.96, 95% CI 7.35–106.33 for moderate/severe depression). In a multivariate logistic regression model, the direction of these associations remained, although weakened and without statistical significance, while moderate/severe depression remained to be strongly associated with violence (OR 12.34, 95% CI 2.26–67.33).

Table 3. The women's mental health status

Parameters	N = 104 (100%) n (%)
Women's depression	
Normal (0–9 points)	31 (29.8)
Mild depression (10–19 points)	37 (35.5)
Moderately depressed state (20–29 points)	26 (25)
Severe depression (> 30 points)	10 (9.6)
Women's anxiety	
Normal (0–9 points)	46 (44.7)
Mildly anxiety (10–19 points)	31 (30.1)
Moderately anxiety (20–29 points)	16 (15.5)
Severe anxiety (> 30 points)	10 (9.7)
Women's suicidal risk	
No risk	73 (72.3)
Low risk	25 (24.8)
Moderate risk	1 (1)
High risk	2 (2)
Risk of harmful alcohol consumption	
Never consume alcohol	67 (64.4)
Zone I (1–7 points) low level of alcohol related problems	37 (35.6)
Zone II (8–15 points) medium level of alcohol-related problems (hazardous drinking)	0
Zone III (16–19 points) high level of alcohol-related problems	0
Zone IV (20–40 points) high risk for alcohol dependence	0

DISCUSSION

In this paper we investigated the lifetime and periodic prevalence and factors associated with intimate partner violence against women whose spouses had been under treatment for alcohol dependence. We found that almost half of the women experienced violence in the previous year (46.2%). Other studies that also addressed alcohol-dependent men and their abusive behavior revealed that even in pregnancy women are not protected from violence: 27% of women who just delivered experienced violence [16]; Bhatta et al. [17] found that pregnant women whose partners are alcohol-dependent are exposed to violence twice as often than women whose partners are not alcohol-dependent. These findings suggest that alcohol dependent partners appeared to present an extremely high risk for perpetuation of violence, and a "red flag" that calls for an action to enhance women's protection and safety, by providing immediate protection and long-term support. However, these figures might be just the tip of the iceberg,

Table 4. Distribution of socio-demographic variables among women who were exposed to violence during the previous 12 months, along with univariate and multivariate logistic regression model

Parameters	Total n (%)[1]	Yes n (%)[2]	Univariate OR (95% CI)	Adjusted OR (95% CI)
Women's age				
Under or 39 years old	17 (16.3)	9 (52.9)	1.00	1.00
40–49 years old	38 (36.5)	12 (31.6)	0.41 (0.13–1.32)	1.20 (0.11–12.78)
50–59 years old	33 (32.7)	20 (60.6)	1.37 (0.42–4.45)	4.22 (0.15–117.44)
60 years or over	15 (14.5)	9 (60)	1.33 (0.33–5.43)	16.86 (0.29–971.20)
Women's education				
Primary school	6 (5.8)	5 (83.3)	1.00	1.00
High school (9–12 years)	56 (53.8)	28 (50)	0.20 (0.02–1.82)	0.33 (0.02–5.7)
Higher education (12 years and more)	42 (40.4)	17 (40.5)	0.14 (0.01–1.27)	0.40 (0.02–8.58)
Women's working status				
Employed	67 (64.4)	33 (49.3)	1.00	1.00
Unemployed	26 (25)	10 (38.5)	0.64 (0.26–1.62)	0.24 (0.05–1.05)
Retired	11 (10.6)	7 (63.6)	1.80 (0.48–6.74)	2.63 (0.20–35.4)
Partner's age*				
Up to 39 years old	14 (14)	8 (57.1)	1.00	1.00
40–49 years old	35 (35)	10 (28.6)	0.30 (0.08–1.09)	0.07 (0.01–0.98)*
50–59 years old	38 (38)	23 (60.5)	1.15 (0.33–3.98)	0.16 (0.01–4.42)
60 years or over	13 (13)	8 (61.5)	1.20 (0.26–5.59)	0.08 (0.00–4.78)
Partner's education				
Primary school	9 (8.6)	5 (55.6)	1.00	1.00
High school (9–12 years)	64 (61.6)	30 (46.9)	0.71 (0.17–2.87)	0.52 (0.07–3.51)
Higher education (12 years and more)	31 (29.8)	15 (48.4)	0.75 (0.17–3.33)	0.41 (0.05–3.49)
Partner's working status				
Employed	69 (66.4)	34 (49.3)	1.00	1.00
Unemployed	26 (25)	12 (46.2)	0.88 (0.36–2.18)	0.69 (0.16–3.02)
Retired	9 (8.6)	4 (44.4)	0.82 (0.20–3.33)	0.03 (0.00–1.24)
Duration of marital union				
Up to 10 years	21 (20.4)	6 (28.6)	1.00	1.00
11–20 years	37 (35.9)	17 (45.9)	2.12 (0.67–6.68)	6.46 (0.89–46.95)
21–30 years	26 (25.2)	16 (61.5)	4.00 (1.17–13.73)	4.03 (0.38–42.68)
31 years and more	19 (18.4)	11 (57.9)	3.44 (0.92–12.79)	4.39 (0.17–110.42)
Number of children				
0	12 (11.6)	5 (41.7)	1.00	1.00
1	31 (30.1)	12 (38.7)	0.88 (0.23–3.43)	0.41 (0.04–4.2)
2	54 (52.5)	28 (51.9)	1.50 (0.42–5.35)	0.79 (0.08–8.02)
3 and more	6 (5.8)	4 (66.7)	2.80 (0.36–21.73)	1.9 (0.07–49.02)
Place of living*				
City	71 (68.3)	29 (40.8)	1.00	1.00
Else	33 (31.7)	21 (63.6)	2.53* (1.08–5.94)	3.4 (0.81–14.2)
Differences in the level of education among spouses[3]				
The same level of education	65 (63.7)	32 (49.2)	1.00	-
The man is more educated	13 (12.7)	8 (61.5)	1.64 (0.49–5.58)	-
The woman is more educated	24 (23.6)	9 (37.5)	0.62 (0.24–1.61)	-

*$p < 0.05$;
[1] % of total in that variable;
[2] % of total in that category;
[3] the variable was not included in the multivariate model due to the collinearity level of education variables at individual level

as during the research, many women might still hesitate to confirm that partners perpetuated violence against them, or even they are not aware that such partner behavior constitutes violent acts that are forbidden and unjustifiable.

In regard to the partner's age, we found that older men are more violent, while some other authors identified that violence occurred more often among young married couples and young parents [3, 18]. Although in our study we did not inquire about the timing of the first violent acts that happened during the marital life, other researches indicated that this happens early, at the beginning of the union [3, 17, 18].

Our results corroborate findings related to the associations between women's mental health, especially depression, and exposure to partner violence, which actually might present a *circulus vitiosus*, which is very difficult to escape without comprehensive treatment and support [10, 19, 20]. As identified in a previous paper by Dostanić et al.

Table 5. Distribution of health-related variables among women who are exposed to violence, along with univariate and multivariate logistic regression model

Exposure to violence during the previous 12 months				
Parameters	Total n (%)[1]	Yes n (%)[2]	OR (95% CI)	Adjusted OR (95% CI)
Presence of physical disease in women				
No	93 (89.42)	43 (46.2)	1.00	1.00
Yes	11 (10.58)	7 (63.6)	2.03 (0.56–7.42)	1.51 (0.26–8.79)
Presence of psychiatric disease in women				
No	103 (99.03)	49 (47.6)	1.00	-
Yes	1 (0.97)	1 (100)	-	-
Presence of physical disease in partner				
No	85 (81.73)	41 (48.2)	1.00	1.00
Yes	19 (18.27)	9 (47.4)	0.97 (0.36–2.61)	1.00 (0.24–4.24)
Presence of psychiatric disease in partner				
No	102 (98.08)	49 (48)	1.00	1.00
Yes	2 (1.92)	1 (50)	1.08 (0.07–17.77)	-
The number of treatments for alcohol dependence				
The first treatment	72 (69.23)	32 (44.4)	1.00	1.00
Second treatment or more	31 (30.77)	18 (58.1)	1.73 (0.74–4.05)	1.07 (0.33–3.43)
Women's depression**				
Normal	31 (29.81)	4 (12.9)	1.00	1.00
Mild	37 (35.58)	17 (45.9)	5.74 (1.67–19.69)**	2.58 (0.62–10.74)
Moderate/ Severe	36 (34.61)	29 (80.6)	27.96 (7.35–106.33)**	12.34 (2.26–67.33)**
Women's anxiety**				
Normal	46 (44.66)	12 (26.1)	1.00	1.00
Mild	31 (30.09)	18 (58.1)	3.92 (1.48–10.35)*	1.74 (0.49–6.22)
Moderate/ Severe	26 (25.24)	19 (73.1)	7.69 (2.59–22.83)**	1.44 (0.33–6.33)
Women's suicidal risk**				
No	73 (72.28)	24 (32.9)	1.00	1.00
Yes	28 (27.72)	23 (82.1)	9.39 (3.18–27.75)**	3.33 (0.89–12.48)

*p < 0.05
**p < 0.01
[1] % of total in that variable
[2] % of total in that category

[10], occurrence of depression in women whose partners have alcohol dependence is associated with older partners, and if they spent more than 20 years together, which was also found in other studies [21, 22]. The link between violence and older age of husbands with alcohol dependence can be explained by the fact that these men probably hold traditional values that justify gender-based violence, along with cognitive deficits they developed over time as a consequence of the continuous and excessive use of alcohol.

We found that women who live in rural areas in Serbia have experienced violence more often. It can be explained by the fact that they are much more vulnerable due to the adverse socio-economic conditions and lower social status they have [23]. Therefore, particular attention has to be given to recognition and timely protection and support of this population group.

CONCLUSION

Alcohol-dependent men perpetuate violence toward their female spouses very often. Inpatient treatment presents a window of opportunity to work with them, and professionals who are focused on the treatment should extend their professional competencies in a way to acquire knowledge and skills that are relevant for the identification and work with violent alcohol-dependent patients [24]. This would be particularly important when dealing with younger alcohol-dependent men, whose brain structure and cognitive functions are not yet largely compromised, and who therefore might possess the largest potential for change. They have to be aware that violence is completely unacceptable and to learn how to control their impulses for violent behavior.

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Целоживотна и периодична преваленција и карактеристике насиља према женама од стране њихових партнера који су зависни од алкохола – студија пресека

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САЖЕТАК

Увод/Циљ Циљ овог истраживања је утврђивање целоживотне и периодичне (12 месеци) преваленције и карактеристика партнерског насиља према женама чији се мужеви болнички лече од алкохолне зависности.

Методе Студија пресека је спроведена међу женама чији се партнери хоспитално лече због алкохолне зависности. Изложеност физичком и сексуалном насиљу током живота и претходних 12 месеци мерена је Скалом за оцењивање тактика у конфликтима, а ментално здравље жена је процењивано Бековом скалом за депресију, Бековим упитником за анксиозност, Мини интернационалним неуропсихијатријским интервјуом (МИНИ) за процену суицидалности и тестом за идентификацију алкохолом узрокованих поремећаја (АУДИТ). Изложеност насиљу и његова повезаност са социодемографским факторима и здравственим стањем анализирана је методама дескриптивне и инференцијалне статистичке анализе, као и помоћу два модела логистичке регресионе анализе.

Резултати Целоживотна преваленција физичког насиља међу женама чији се мужеви лече од алкохолизма била је 65,4%, док је изложеност насиљу током 12 месеци који су претходили истраживању (периодична преваленција) износила 46,2% за физичко, 20,2% за сексуално и 18% за оба типа насиља. Међу женама није регистровано ризично конзумирање алкохола. Насиље је било чешће међу женама које нису живеле у градовима (*OR* 2,53, 95% *CI* 1,08–5,94, у униваријантном моделу) и међу женама са умереном/тешком депресијом (*OR* 12,34, 95% *CI* 2,26–67,33, у мултиваријантном моделу).

Закључак Мушкарци зависни од алкохола су веома често насилни према својим супругама, и болничко лечење представља могућност да се са њима ради на подизању свести о недопустивости насиља према женама.

Кључне речи: алкохолизам; алкохолна зависност; насиље; жене; здравље; насилник

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

The role of interleukin-4 and interleukin-5 Th2 cytokines in assessing severity and prognosis of acute pancreatitis

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SUMMARY

Introduction/Objective Acute pancreatitis (AP) is a relatively common disease which in most patients has favorable course. However, in approximately 20% patients, the course of the disease is more severe with high mortality (40–50%). The evaluation of disease severity is now primarily based on protocols that includes clinical, laboratory, and radiographic diagnostic procedures, APACHE II score, Ranson score, CT index, and CT necrosis score. Key cells in the immunopathogenesis of AP are T-lymphocytes, and recent studies indicate the role of Th2 and their effector cytokines: interleukin (IL) -4 and interleukin (IL) -5. The purpose of our study was to determine the potential clinical value of IL-4 and IL-5 as biochemical markers for predicting development of severe, necrotizing form of acute pancreatitis with systemic complication such as systemic inflammatory response syndrome (SIRS).

Methods This prospective study included 240 patients hospitalized at The Clinic for Emergency Surgery of Clinical Center of Serbia as AP. Levels of IL-4 and IL-5 in serum were detected using commercial Bender Med Systems (BMS716FF) kits.

Results IL-4 and IL-5 were statistically significant increased on the second day of hospitalization with maximum values on the third day. In patients with severe AP complicated with necrosis and/or sepsis values were rising all through the seventh day.

Conclusion Levels of IL-4 and IL-5 in peripheral blood correlate with SIRS, Ranson score and clinical outcome in AP patients, therefore these cytokines are potential early biomarkers of disease progression and related complications.

Keywords: acute pancreatitis; interleukin-4; interleukin-5; biomarker

INTRODUCTION

Acute pancreatitis (AP) is a common inflammatory condition with a highly variable clinical course that can cause severe local and extrapancreatic organ dysfunction and failure. The most common causes are gallstones and alcohol, accounting for up to 80% of cases [1]. The diagnosis of AP requires the patient to present with abdominal pain consistent with AP and the elevation of serum amylase or lipase (> 3 times upper limit of normal). Although advancement in diagnosis and treatment of AP has been made, this disease is still serious and potentially lethal [2]. The clinical course of AP can show minimal organ dysfunction with recovery in several days, but patients may experience a severe attack involving organ failure and severe complications as well as a high mortality rate. Severe acute pancreatitis (SAP) is defined as an episode of pancreatitis with persistent organ failure [3]. SAP is associated with high morbidity and mortality due to the development of pancreatic and extra-pancreatic necrosis, their subsequent infection and multisystem organ failure (MOF). In the case of SAP with sterile necrosis mortality rate can be as low as 12%, but in those with multiple organ failure mortality rate could be as high as 85% [4]. Thus, early assessment of the severity and initial aggressive fluid resuscitation decreases morbidity and mortality [5]. Patients with SAP benefit considerably from early management in an intensive care unit [5, 6]. Therefore, esearches focus on possibilities of an early assessment of the severity of an attack. Development of an Early severe acute pancreatitis (ESAP), in first week, accounts for 37.5–44% mortality among patients with AP [7]. During the second week AP progresses to Infected pancreatic necrosis (IPN), which results in Systemic inflammatory response syndrome(SIRS), Multiple organ dysfunction syndrome MODS, sepsis and a second wave of mortality [8]. Nevertheless, not all patients with acute necrotizing pancreatitis (ANP) develop IPN, suggesting that certain susceptibility factors make the patients with ANP prone to IPN. In AP innate immunity recognize damage-associated molecular patterns released from necrotizing pancreatic cells and stimulate adaptive immune response [9]. Cytokines play an important role in etiopathogenesis of AP by causing an inflammatory response that leads to tissue damage and organ dysfunction or failure in patients with SAP. The inflammatory response trigger both

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recruitment and activation of inflammatory cells that can progress to pancreatic necrosis [10]. Local recruitment and activation of inflammatory cells in acute pancreatitis induce the production of pro-inflammatory (IL-1β, IL-6, and IL-8as well as tumor necrosis factor-alpha [11]) and anti-inflammatory cytokines (IL-4, IL-10, IL-13, and TGFβ) [9]. Previous studies have shown increased production of IFN-γ, typical Th1 cytokine in patients with AP [12]. On the other hand, role of Th2 cells and their cytokines (IL-4 and IL-5) in development of acute and chronic pancreatitis is still controversial. In Th2-mediated response IL-4 stimulates B-cells to produce IgE and induce alternative activation of macrophages resulting in M2 phenotype, while IL-5 is important in eosinophil recruitment and activation [13].

Therefore, the purpose of our study was to determine the potential clinical value of IL-4 and IL-5 as biochemical marker for predicting development of severe, necrotizing form of acute pancreatitis with systemic complications (SIRS, ARDS, MODS, POF).

METHODS

Patients

A total of 240 patients at the Clinic for emergency surgery, Emergency center, Clinical center of Serbia, aged 18–75 years, with no history of malignancy and/or systemic diseases, were included in our study. Study was conducted multidisciplinary. Informed consent was obtained from all cases before enrollment into the study. The protocol of this study was approved by the Ethics Committee of the Clinical Center of Serbia. The diagnosis of AP was established based on the following 3 criteria: 1) abdominal pain or signs of AP; 2) serum amylase and/or lipase 5 times the upper limit of normal, and 3) characteristic findings for AP on computed tomography scan. We monitored 75 parameters divided in 7 groups: demographic, clinical, radiographic, operations, laboratory, systemic complications and scores (SIRS, APACHE II, Ranson, CT index, necrosis score) [14, 15].

Radiographic method: native RTG, abdominal US, CT and/or MR.

Three group of laboratory analysis were followed:

1. standard and specific biochemical analysis: D-dimer, CRP, INR and plasminogen;
2. microbiological findings for diagnosis of infection in necrotic pancreatitis;
3. serum levels of IL-4 and IL-5.

Monitoring levels of cytokines and laboratory findings

Daily samples of blood were routinely tested for standard, specific biochemical analysis and for determining the levels of serum cytokines from day 1 to day 7. Blood samples were centrifuged with the speed of 3000 rpm for 10 minutes and prior to the analysis the serum was kept at -20^0 C. The cutoff of 6 pg/ml was taken as a minimal concentration of IL-4 and IL-5.

Cytokines were quantified according to the manufacturer's instructions using Bender Med Systems GmbH (BMS716FF). The samples were run on Beckman Coulter system. CRP was quantified according to the manufacturer's instructions with commercial Olympus test using Olympus analyzer AU400. Standard laboratory methods were used for hemostatic, blood and enzyme activity.

Statistical analysis

Continuous variables are reported as mean ± standard deviation (SD), and categorical variables are expressed as N (%) of study participants. The student t-test was used to compare continuous variables between the two groups, and ANOVA for comparing continuous variables between three or more groups. The Pearson's correlation analysis was used for determining the correlation between the biochemical parameters and scores. ROC (receiver operative characteristics) curves were used to assess the predictive power of v methods were used. The most predictive parameters were approximately under 1 of the ROC curve. On the other hand, 0.5 parameters proved to be with no significance. All p values were two-sided, and $p \leq 0.05$ was considered to be statistically significant. All statistical analyses were conducted using SPSS for Windows, Version 11.1 (SPSS Inc., Chicago, IL, USA).

The study was done in compliance with the institutional standards on ethics.

RESULTS

There was a total of 240 patients with AP that fulfilled inclusion criteria, 144 subjects (53.3%) were male and 96 (41.7%) were female. Forty-five percent had SIRS score 3 and 4 at the time of admission and 95% had SIRS score two or more. Ranson score was 3–6 in 65% of patients and 16% of patients had maximal values (6 and 7) of Ranson score.

Overall, 78 (32.5%) patients died during hospitalization.

We quantified the circulating cytokines IL-4 and IL-5 on the first day (day of admission into hospital) and after 24, 48, 72, and 168 hours. We evaluated these cytokines in

Table 1. Measurement time IL-4 and IL-5

Measurement time (h)	Mean (pg/ml)	Standard deviation	95% confidence interval	
			Lower limit	Upper limit
IL-4				
0	1920.67	3323.52	1423.37	2417.97
24	2305.84	6261.21	1441.49	3170.19
48	7184.94	15536.24	5007.54	9362.33
72	3040.39	5627.20	2239.36	3841.43
168	2175.86	4640.94	1504.51	2847.21
IL-5				
0	325.50	631.18	231.05	419.94
24	262.11	538.30	187.80	336.42
48	584.32	1509.28	369.47	799.17
72	319.77	443.65	256.61	382.92
168	236.52	405.06	176.94	296.09

Table 2. Intragroup and intergroup dependence (concentration of IL-4 and IL-5)

Source of variation	Variance	F	p
IL-4			
Between groups	941061024.49	13.240	0.000
Within groups	71077579.37		
IL-5			
Between groups	3675826.141	5.469	0.000
Within groups	672141.520		

Table 3. Tukey HSD (parameter interdependence-dependent variable: IL-4 and IL-5)

(I) Measurement time (h)	(J) Measurement time (h)	Mean difference (I-J) (h)	Standard error	p	95% confidence interval	
					Lower limit	Upper limit
IL-4						
0	24	-385.17	870.00	0.992	-2762.92	1992.58
	48	-5264.26 (*)	876.05	0.000	-7658.54	-2869.98
	72	-1119.72	882.43	0.710	-3531.43	1291.99
	168	-255.18	889.17	0.999	-2685.32	2174.94
24	0	385.17	870.00	0.992	-1992.58	2762.92
	48	-4879.09 (*)	841.06	0.000	-7177.76	-2580.42
	72	-734.54	847.71	0.909	-3051.37	1582.27
	168	129.98	854.72	1.000	-2206.00	2465.97
48	0	5264.26(*)	876.05	0.000	2869.98	7658.54
	24	4879.09(*)	841.06	0.000	2580.42	7177.76
	72	4144.54(*)	853.91	0.000	1810.76	6478.32
	168	5009.07(*)	860.88	0.000	2656.26	7361.88
72	0	1119.72	882.43	0.710	-1291.99	3531.43
	24	734.54	847.71	0.909	-1582.27	3051.37
	48	-4144.54(*)	853.91	0.000	-6478.32	-1810.76
	168	864.53	867.37	0.857	-1506.01	3235.08
168	0	255.18	889.17	0.999	-2174.94	2685.32
	24	-129.98	854.72	1.000	-2465.97	2206.00
	48	-5009.07(*)	860.88	0.000	-7361.88	-2656.26
	72	-864.53	867.37	0.857	-3235.08	1506.01
IL-5						
0	24	63.38	84.60	0.945	-167.84	294.61
	48	-258.82 (*)	85.81	0.022	-493.35	-24.29
	72	5.72	85.81	1.000	-228.80	240.25
	168	88.98	87.16	0.846	-149.23	327.19
24	0	-63.38	84.60	0.945	-294.61	167.84
	48	-322.20 (*)	82.43	0.001	-547.51	-96.90
	72	-57.65	82.43	0.957	-282.95	167.64
	168	25.59	83.83	0.998	-203.54	254.73
48	0	258.82 (*)	85.81	0.022	24.29	493.35
	24	322.20 (*)	82.43	0.001	96.90	547.51
	72	264.55 (*)	83.67	0.014	35.86	493.24
	168	347.80 (*)	85.05	0.000	115.33	580.27
72	0	-5.72	85.81	1.000	-240.25	228.80
	24	57.65	82.43	0.957	-167.64	282.95
	48	-264.55 (*)	83.67	0.014	-493.24	-35.86
	168	83.25	85.05	0.865	-149.21	315.72
168	0	-88.98	87.16	0.846	-327.19	149.23
	24	-25.59	83.83	0.998	-254.73	203.54
	48	-347.80 (*)	85.05	0.000	-580.27	-115.33
	72	-83.25	85.05	0.865	-315.72	149.21

*The mean difference is statistically significant, $p < 0.05$.

patients with SAP and early mortality. There was statistically significant increase in the concentration of circulating IL-4 and IL-5 on the second day of hospitalization with maximum values on the third day (Table 1). In patients with SAP complicated with necrosis and/or sepsis values were rising all through the seventh day (Tables 1, 2, and 3).

There was highly statistically significant correlation between concentrations of IL-4, IL-5 and SIRS (Tables 4 and 5). Concentrations of these cytokines in serum of our patients did not corelate with CRP values and Apache II score (Tables 4 and 5). Levels of IL-5 were significantly higher in group of patients with negative outcome (Tables 5 and 6). Interleukin-4 was also higher in these patients, but correlation with outcome have not reached statistical significance (Tables 4 and 6). Correlation between Ranson score and measured levels of IL-5 was satistically highly significant (Table 5) in contrast to IL-4 (Table 4). Amylase levels, important for diagnosis of AP, were lower in patients with negtive outcome, and there is negative correlation with concetrations of both cytokines that we analyzed in this study (Tables 4, 5, and 6).

DISSCUSION

The aim of this study was to analyze serum levels of Th2 cytokines in AP patients and potential correlation with the severity of disease. Initial clinical response to pancreatitis is SIRS which can develop in sepsis with MODS. SIRS usually happen in the first week and its progression is a critical step in the prognosis of AP [16]. Most of our patients had SIRS score two or more (95%) and 45% had SIRS score 3 and 4 at the time of admission. This indicates that on admission most of our patient had complicated AP with highly developed inflammatory process, and almost 50% had developed septic syndrome. After initial injury of acinar cells progression of AP could be divided in three stages: local inflammation, generalized inflammatory response and final stage of sepsis with MODS. In our study group most of our patients were in stage of generalized inflammatory response. Ranson score was in 65% of patients 3–6, and 16% of patients had maximal values (6 and 7) of Ranson score. These values can explain, and certainly highly correlate with, the high SIRS scores on admission. Overall, in this study 78 (32.5%) patients died during hospitalization, while in literature mortality rates as high as 30% [17]. The main cause of early death in AP patients is ARDS, 60% of our

Table 4. Correlations of IL-4 concentration and protocol parameters

Parameters		Sex	SIRS	Outcome	Years	CRP	Amylase	Apache II	Ranson	IL-4
Sex	r	1	-0.139*	-0.194**	0.185**	-0.306**	-0.130	-0.062	-0.104	-0.083
	p		0.043	0.004	0.006	0.000	0.059	0.367	0.140	0.295
SIRS	r	-0.139*	1	0.677**	0.232**	0.274**	0.028	0.268**	0.331**	0.201**
	p	0.043		0.000	0.000	0.000	0.683	0.000	0.000	0.009
Outcome	r	-0.194**	0.677**	1	0.569**	0.502**	-0.110	0.481**	0.520**	0.099
	p	0.004	0.000		0.000	0.000	0.102	0.000	0.000	0.186
Years	r	0.185**	0.232**	0.569**	1	0.414**	0.146*	0.373**	0.334**	0.168*
	p	0.006	0.000	0.000		0.000	0.032	0.000	0.000	0.027
CRP	r	-0.306**	0.274**	0.502**	0.414**	1	-0.181**	0.274**	0.277**	-0.023
	p	0.000	0.000	0.000	0.000		0.000	0.000	0.000	0.469
Amylase	r	-0.130	0.028	-0.110	0.146*	-0.181**	1	0.066*	-0.202**	-0.079*
	p	0.059	0.683	0.102	0.032	0.000		0.041	0.003	0.025
Apache II	r	-0.062	0.268**	0.481**	0.373**	0.274**	0.066*	1	0.327**	0.013
	p	0.367	0.000	0.000	0.000	0.000	0.041		0.000	0.686
Ranson	r	-0.104	0.331**	0.520**	0.334**	0.277**	-0.202**	0.327**	1	0.069
	p	0.140	0.000	0.000	0.000	0.000	0.003	0.000		0.390
IL-4	r	-0.083	0.201**	0.099	0.168*	-0.023	-0.079*	0.013	0.069	1
	p	0.295	0.009	0.186	0.027	0.469	0.025	0.686	0.390	

*The correlation is statistically significant, $p < 0.05$;
**the correlation is statistically significant, $p < 0.01$;
r – Pearson correlation coefficient

Table 5. Correlations of IL-5 concentration and protocol parameters

Parameters		Sex	SIRS	Outcome	Years	CRP	Amylase	Apache II	Ranson	IL-5
Sex	r	1	-0.139*	-0.194**	0.185**	-0.306**	-0.130	-0.062	-0.104	-0.087
	p		0.043	0.004	0.006	0.000	0.059	0.367	0.140	0.269
SIRS	r	-0.139*	1	0.677**	0.232**	0.274**	0.028	0.268**	0.331**	0.332**
	p	0.043		0.000	0.000	0.000	0.683	0.000	0.000	0.000
Outcome	r	-0.194**	0.677**	1	0.569**	0.502**	-0.110	0.481**	0.520**	0.254**
	p	0.004	0.000		0.000	0.000	0.102	0.000	0.000	0.001
Years	r	0.185**	0.232**	0.569**	1	0.414**	0.146*	0.373**	0.334**	0.082
	p	0.006	0.000	0.000		0.000	0.032	0.000	0.000	0.284
CRP	r	-0.306**	0.274**	0.502**	0.414**	1	-0.181**	0.274**	0.277**	0.001
	p	0.000	0.000	0.000	0.000		0.000	0.000	0.000	0.966
Amylase	r	-0.130	0.028	-0.110	0.146*	-0.181**	1	0.066*	-0.202**	-0.059
	p	0.059	0.683	0.102	0.032	0.000		0.041	0.003	0.094
Apache II	r	-0.062	0.268**	0.481**	0.373**	0.274**	0.066*	1	0.327**	-0.007
	p	0.367	0.000	0.000	0.000	0.000	0.041		0.000	0.828
Ranson	r	-0.104	0.331**	0.520**	0.334**	0.277**	-0.202**	0.327**	1	0.280**
	p	0.140	0.000	0.000	0.000	0.000	0.003	0.000		0.000
IL-5	r	-0.087	0.332**	0.254**	0.082	0.001	-0.059	-0.007	0.280**	1
	p	0.269	0.000	0.001	0.284	0.966	0.094	0.828	0.000	

*The correlation is statistically significant, $p < 0.05$;
**the correlation is statistically significant, $p < 0.01$;
r – Pearson correlation coefficient

Table 6. Values of IL-4, IL-5, and other parameters relating to outcome

Parameters	Outcome	Mean	Standard deviation
Years	alive	46.38	12.08
	dead	62.30	8.02
CRP	alive	104.60	113.98
	dead	251.28	128.51
Amylase	alive	1383.62	1257.38
	dead	1094.20	867.69
Apache II	alive	12.44	3.75
	dead	17.84	6.04
IL-5	alive	214.27	452.21
	dead	557.57	860.65
IL-4	alive	1653.14	3550.45
	dead	2360.03	2504.26

Table 7. Results of comparison of two patient groups in respect of outcome (Student's t-test)

Parameters	t	p
Years	-10.529	0.000
CRP	-8.953	0.000
Apache II	-8.475	0.000
IL-4	-1.327	0.186
IL-5	-3.498	0.001

Grouping variable: OUTCOME

patients died in the first six days of hospitalization mostly because of pulmonary complication [18].

Wide spectrum of inflammatory mediators are included in inflammatory process which consequently modulates migration of leukocytes, increases vascular permeability, damages local tissue and can cause generalized inflammation with damages to the kidneys, lungs and other organs [19]. T-helper cells are grouped with varieties of cytokines they produce. Th1 cells are involved in defense mechanisms against intracellular pathogens and produce TNF-α and interferon-γ [9]. Various factors can influence the polarization of Th cells, including the cytokine profile of the environment in which Th cells undergo the process of transdifferentiation. Th1 and Th2 cytokine products reciprocally reduce each other's activity [9].

In our study, according to the similarities between these two interleukins, their behavior in severe forms of acute pancreatitis is practically identical. The values of their concentrations increase significantly on the second day of the disease, reaching the highest values on the third day, and in severe forms of infected necrosis and sepsis, these values increase until the seventh day, when the last measurements were made (Tables 1, 2, and 3). Table 6 shows the values of IL-4 and IL-5 in correlation to the outcome. In our study the survivors, both IL-4 and IL-5, had lower values than non-survivors but only IL-5 was statistically significant (Table 7). Rogriguez et al. [20] reported lower levels of IL-4 but significantly higher IL-5 in serum of patients with severe AP and non-survivors. Similarly, our results showed that IL-5 highly significantly correlates with the outcome of acute pancreatitis (Table 5). Intracellular labeling of IL-4 and IL-10 showed that Th2 and regulatory T cells were induced in a mouse model of severe acute pancreatitis. These findings suggest that anti- inflammatory response develops not only after but also during tissue damage in AP. Sendler et al. [21] also found higher IL-4 analyzing human samples, but levels of this cytokine were not in correlation with disease severity. Alternative macrophage activation induced by IL-4 and IL-13 is responsible not only for anti-inflammatory response in AP but also for fibrosis in chronic pancreatitis which can be reduced by blocking these cytokines [22].

IL-4 and IL-5 correlate with SIRS (Tables 4 and 5), as reported in the literature [23], although more attention is paid to the values of these interleukins in some other conditions (asthma) [24, 25].

CONCLUSION

Values of IL-4 and IL-5 can potentially be used as an early marker of severity of AP, early marker of sepsis and outcome because of a significant statistical correlation with outcome, SIRS and Ranson score.

Conflict of interest: None declared.

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Улога *Th2* цитокинâ интерлеукина-4 и интерлеукина-5 у процени тежине и прогнозе акутног панкреатитиса

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САЖЕТАК

Увод/Циљ Акутни панкреатитис (АП) релативно је често обољење које у већини случајева има бениган ток. Међутим, код око 20% болесника ток болести је много тежи, а смртност и даље врло висока (40–50%). Процена тежине болести сада се превасходно заснива на протоколима који укључују клиничке, лабораторијске и радиографске дијагностичке поступке, скор *APACHE* II, Рансонов скор, индекс компјутеризоване томографије и скор некрозе мерен компјутеризованом томографијом. У имунопатогенези АП кључне ћелије су Т-лимфоцити, а новија истраживања указују на улогу *Th*2 и њихових ефекторских цитокина: интерлеукина (ИЛ)-4 и ИЛ-5. Циљ ове студије био је да се испита клинички значај серумских нивоа ИЛ-4 и ИЛ-5 као потенцијалних биомаркера тешке форме АП са системским компликацијама, као што је синдром системског инфламаторног одговора.

Методе Ова проспективна студија укључила је 240 болесника са АП хоспитализованих на Клиници за ургентну хирургију Универзитетског клиничког центра Србије. Серумски нивои ИЛ-4 и ИЛ-5 свакодневно су детектовани употребом комерцијално доступних китова *Bender Med Systems* (*BMS716FF*).

Резултати Нивои ИЛ-4 и ИЛ-5 у серуму били су статистички значајно повишени другог дана, док су максималне вредности достигли трећег дана хоспитализације. Код оболелих од тешке форме АП са системским компликацијама (синдром системског инфламаторног одговора) нивои поменутих цитокина били су повишени до седмог дана.

Закључак Вредности концентрација ИЛ-4 и ИЛ-5 показују високо значајну корелацију са синдромом системског инфламаторног одговора и Рансоновим скором и исходом, те наша студија показује да ови цитокини могу бити рани биомаркер тежине АП, појаве системских компликација и исхода болести.

Кључне речи: акутни панкреатитис; интерлеукин-4; интерлеукин-5; биомаркер

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CASE REPORT / ПРИКАЗ БОЛЕСНИКА

A multisystem inflammatory syndrome in children associated with COVID-19 in an 11-year-old girl

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SUMMARY

Introduction Multisystem inflammatory syndrome in children (MIS-C) is a post-viral, life-threatening, inflammatory state with multisystem involvement that typically manifests 3–4 weeks after SARS-CoV-2 infection. In this article, we present the first case of MIS-C at the Institute for Child and Youth Health Care of Vojvodina at the beginning of the COVID-19 pandemic.

Case outline A previously healthy 11-year-old girl got sick two days before admission to the hospital with a fever, headache, vomiting, abdominal pain, and fatigue. She was tested positive for COVID-19 by a nasopharyngeal PCR swab with positive IgM and IgG antibodies. In the further course, the illness presented with prolonged fever, laboratory evidence of inflammation, multiorgan involvement such as respiratory, gastrointestinal, cardiovascular, and dermatologic. Based on CDC and WHO criteria, the diagnosis of MIS-C was made and IVIG and methylprednisolone were introduced with favorable clinical course.

Conclusion Every prolonged and unusual febrile state, especially if it is accompanied by gastrointestinal symptoms, in a school-age child, should be investigated in the direction of recent COVID-19 infection or exposure. In a case of a positive COVID-19 history or history of exposure, the MIS-C diagnosis should be considered.

Keywords: SARS-CoV-2; child; inflammation; immunoglobulins; glucocorticoids

INTRODUCTION

Initially, the SARS-CoV-2 infection was not considered to be a severe infection in children. However, the first cases of multisystem inflammatory syndrome in children (MIS-C) showed the potential of this virus to produce a life-threatening state with multisystem involvement [1, 2]. Still, the vast majority of children with COVID-19 present with mild symptoms and have excellent outcomes. MIS-C remains a rare complication of SARS-CoV-2 infection in the pediatric population [2, 3]. The mortality rate of MIS-C is 1.7–1.8% [4, 5].

MIS-C has been defined by the Center for Disease Control and Prevention (CDC) and the World Health Organization (WHO). Both definitions are similar and include the same main criteria: age under 21 years (CDC) or 19 years (WHO), fever for more than one day (CDC) or three days (WHO), clinical signs of multisystem involvement, laboratory evidence of inflammation, no alternative diagnosis and evidence of SARS-CoV-2 infection or exposure [6, 7]. MIS-C is a post-viral inflammatory state that typically manifests 3–4 weeks after SARS-CoV-2 infection and may rapidly progress to multiorgan failure [1, 4, 8]. Rarely, MIS-C occurs during the acute phase of SARS-CoV-2 infection [2]. Clinical presentation of MIS-C may have some overlapping features with Kawasaki disease (KD) and toxic shock syndrome but is presently understood to be a separate phenomenon [1]. Unlike traditional KD, affected patients with MIS-C often require intensive care and additional targeted therapy against the inflammatory response [8].

In this article, we present the first case of MIS-C in the Institute for Child and Youth Health Care of Vojvodina at the beginning of the COVID-19 pandemic.

CASE REPORT

A previously healthy 11-year-old girl, apart from congenital hypothyroidism (well controlled with oral levothyroxine replacement therapy), with the 75% of body weight and height, got sick two days before admission to the hospital with a fever, headache, vomiting, abdominal pain, and fatigue. Upon admission, the patient tested positive for COVID-19 by a nasopharyngeal PCR swab. IgM and IgG antibodies to SARS-CoV-2 were positive. No other family members were tested and all remained asymptomatic. On addmition laboratory tests revealed an elevated C-reactive protein (CRP) of 11.6 mg/dL with a leukocyte count of 5.8×10^9/L, neutrophilia (84.7%), and lymphopenia (3%). An abdominal ultrasound was performed and was inconclusive for appendicitis; enlarged mesenteric lymph nodes were found. Oral intake was stopped, parenteral hydration started and the patient was observed for the acute surgical abdominal disease. On the second day of hospitalization,

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the patient remained febrile with the appearance of tachypnea, dry cough, and episodes of oxygen desaturations. The patient developed red and cracked lips, macular rash on limbs with petechiae on the feet, and swelling of the ankles. The acute phase reactants continued to increase (CRP 21.5 mg/dL, procalcitonin 1.6 ng/mL, ERS 62 mm/hr, fibrinogen 4,57 g/l, IL6 1077 pg/ml, ferritin 385 ug/L. D-dimer values were elevated (over 2500 ng/mL) with prolonged PT and PTT. Troponin I (95.5 ng/mL) was also elevated. There was hypoalbuminemia (34 g/L), slightly elevated uric acid values (362 umol/L), elevated aspartate aminotransferase (AST) values (0.480 ukat/L). T3 and TSH were decreased, T4 was in a normal range. Other parameters of renal, hepatic, and pancreatic function were normal, as well as LDH and lipid profile. Serum immunoglobulin levels and serum components of complement C3 and C4 were in a normal range. Antinuclear antibodies were positive. Anti-neutrophil cytoplasmic antibodies, anticardiolipin antibodies, beta-2 glycoprotein antibodies, anti-double stranded DNA antibodies, and anti-cyclic citrullinated peptides were negative. Blood, urine, and stool cultures were negative. A chest radiograph was performed to verify bilateral interstitial pneumonia (Figure 1). Oxygen therapy and antibiotic therapy (ceftriaxone, metronidazole, azithromycin) were introduced. Since the patient had pneumonia and confirmed SARS-CoV-2 infection, according to the RECOVERY collaborative group recommendations, parenteral corticosteroid therapy (dexamethasone 0.15 mg/kg, 6 mg) was started. Vitamin C and vitamin D were also introduced. Initially, nadroparin was given, followed by acetylsalicylic acid.

The patient was treated by a multidisciplinary team including a pediatric pulmonologist, immunologist, hematologist, intensive care pediatrician, and surgeon.

On the fourth day of hospitalization, fever persisted, as well as abdominal pain with the further increase of the acute phase reactants (CRP 22.8 mg/dL, procalcitonin 2.5 ng/ml). Additional blood cultures were negative. Echocardiography verified pericardial effusion of 6mm with no changes in coronary arteries and normal ejection fraction. The lung ultrasound revealed bilateral pleural effusions up to 19 mm. The abdominal ultrasound showed the non-compressible appendix surrounded by reactively altered adipose tissue. The pediatric surgeon recommended appendectomy which was done and catarrhal appendicitis was found.

Antibiotic therapy was changed, meropenem and linezolid were introduced. Since the patient was febrile with laboratory evidence of inflammation, multiorgan involvement such as respiratory, gastrointestinal, cardiovascular, and dermatologic and proven SARS-CoV-2 infection, the multidisciplinary team considered MIS-C. The intravenous immunoglobulins (IVIG) at a dose of 2 g/kg (1 g/kg for two days) were introduced on the fourth day of hospitalization, and methylprednisolone at a dose of 2 mg/kg/day was introduced on the fifth hospital day. The day after the introduction of IVIG, IL-6 levels halved (561 pg/ml) with a constant decrease in the further course. The level of CRP started decreasing two days after the introduction of IVIG and methylprednisolon. Levels of procalcitonin continued

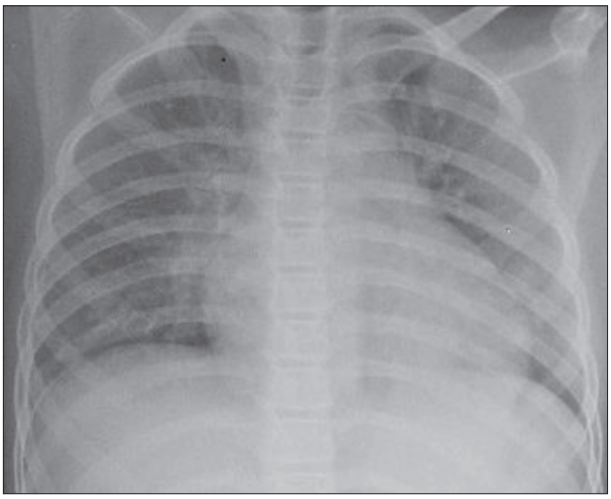

Figure 1. The chest X-ray of the 11-year-old girl with multisystem inflammatory syndrome associated with COVID-19 bilateral ground glass opacities

to rise until the fifth hospital day (maximum 25.9 ng/ml) and then decreased slowly. Ferritin levels increased to the maximum level of 468 μg/l on the fifth hospital day and then started to decrease slowly. The further clinical course was complicated with the appearance of acute renal injury (urea 12.9 mmol/L, creatinine 129.1 μmol/L, uric acid 417 μmol/L), hypotension, and signs of shock. Acute renal failure was treated conservatively. Due to hypotension, a vasopressor (norepinephrine) was introduced. There was a good clinical response to the applied therapy. The patient became afebrile from the fifth day of hospitalization with a gradual normalization of the acute phase reactants as well as renal function parameter levels. There was also a gradual cardiovascular and respiratory recovery with normalization of the chest radiograph and lung ultrasound. From the 12th day of hospitalization, no oxygen therapy was needed. The further clinical course was complicated with acute pancreatitis (amylase 4.36 ukat/L, lipase 4.22 ukat/L), which was treated conservatively. After 23 days the patient was discharged from the hospital in a good general condition. During a regular check-up, she complained of occasional chest pain and increased hair loss that resolved after a couple of months. Control chest X-ray, laboratory, and echocardiography findings were normal.

We confirm that we have read the journal's position on issues involving ethical publication and affirm that this work is consistent with those guidelines.

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Written consent to publish all shown material was obtained from the patient.

DISCUSSION

MIS-C associated with SARS-CoV-2 is thought to occur due to a dysregulated innate immune response and a

subsequent cytokine storm and endothelial damage that affects numerous organ systems [8, 9]. Still, much remains unknown regarding epidemiology, pathogenesis, clinical spectrum, and long-term outcomes of MIS-C [9].

We present the first case of MIS-C in our Institute in the first months of the COVID-19 pandemic.

It is known that children can develop MIS-C 3–4 weeks after a symptomatic or asymptomatic COVID-19 infection, often causing the patients with MIS-C to have positive antibodies to SARS-CoV-2 and negative PCR. This suggests that MIS-C is not mediated by direct viral invasion, but coincides with the development of the acquired immune response to SARS-COV-2. In a minority of cases, MIS-C occurs during the acute phase of SARS-CoV-2 infection. This underlines the necessity of meticulous follow-up of children with COVID-19 infection even when they don't have serious symptoms [2, 4, 9]. Our patient at the time of MIS-C presentation had both positive PCR and IgM and IgG antibodies. As the SARS-COV-2 infection in children is usually asymptomatic with no multiorgan involvement, and since the PCR test may still be positive several days after infection, a multiorgan presentation in our patient is considered to be MIS-C.

Until today, risk factors for developing MIS-C are not completely defined. The literature data show that the mean age at presentation is around nine years with no specific difference between genders [4, 5, 10]. Our patient is a 11-year-old girl, slightly older than the published mean age. Some studies in the USA showed that there was a higher rate of MIS-C in the African American/Afro-Caribbean population, suggesting genetic susceptibility. Obesity in children is considered to be a risk factor due to an accumulation of inflammatory cells in adipose tissue, impaired respiratory function, and more SARS-CoV-2 binding receptors in adipose cells [4]. Our patient had body weight on the 75%, slightly above the average, but still was not obese.

Diagnosing MIS-C can be challenging, especially when it develops after the episode of asymptomatic or unrecognized COVID-19 infection. MIS-C can start with nonspecific symptoms and over time can develop all necessary criteria defined by CDC and WHO [4, 6, 7]. The obligatory criterion is prolonged fever, as it was in the case of our patient. Other usual symptoms are gastrointestinal, which occur in around 70% of patients with MIS-C [4]. Vomiting and abdominal pain can be so intense to mimic acute appendicitis [4, 5, 8, 10]. The same clinical picture was present in our patient, which was firstly observed in the light of acute abdominal disease and undergone an appendectomy. In the three weeks clinical course she also developed pancreatitis, as previously was described in the literature as one of the spectra of gastrointestinal pathology in MIS-C [11]. Other clinical signs and symptoms of MIS-C are: respiratory, cardiovascular, renal, hematological, neurological, and dermatological [6, 7]. Our patient had bilateral pleuropneumonia, hypotension, shock, pericarditis, acute kidney injury, coagulopathy, and skin rash. All these clinical signs appeared in the latter course, after the initial gastrointestinal presentation.

Elevated markers of inflammation are among the criteria of the case definition of MIS-C. They include elevated CRP, ERS, fibrinogen, procalcitonin, D-dimer, ferritin, LDH, IL-6 levels, neutrophilia, lymphocytopenia, and hypoalbuminemia [6, 7]. All these criteria were fulfilled in the case of our patient, except elevated LDH.

One of the criteria for making the diagnosis of MIS-C is the exclusion of other obvious microbial causes or inflammation. Multiple bloods, urine, and stool cultures in our patient were negative. Also, immunological analyses were done and all results were normal, except positive antinuclear antibodies.

One of the problems with making the diagnosis of MIS-C is that it resembles KD. Still, there are few important differences. Firstly, KD occurs in young children under five years of age, whereas the mean age in MIS-C is nine years. Secondly, there is an increased incidence of MIS-C in patients of African, Afro-Caribbean, and Hispanic descent, but a lower incidence in those of East Asian descent, in contrast to KD [4]. Thirdly, the inflammatory storm observed in MIS-C is much more intense with a greater elevation of inflammatory markers. At presentation, patients with MIS-C tend to have higher CRP levels, lower platelet counts, and lower absolute lymphocyte counts than patients with KD. Fourthly, the clinical presentation of KD and MIS-C is slightly different. Distinguishing clinical characteristics found in MIS-C are vomiting, diarrhea, abdominal pain, shock, and cardiac dysfunction, while they could be present, but are not usual in KD. MIS-C and KD may share overlapping clinical features, including fever, conjunctival injection, oropharyngeal findings (red and/or cracked lips, strawberry tongue), rash, swollen and/or erythematous hands, and feet, and cervical lymphadenopathy [3, 4]. According to the published data conjunctivitis and rash are present in around 50% of children with MIS-C [4]. Epidemiologic studies of MIS-C suggest that younger children are more likely to present with KD-like features while older children are more likely to develop myocarditis and shock [3, 4]. Our patient, although an older child, had the clinical picture that mimics KD with prolonged fever, oropharyngeal changes, rash, and swelling of the ankles. Still, she did not have the enlargement of cervical lymph nodes and bilateral bulbar conjunctival injections and no other criteria for KD. On the other hand, predominant symptoms were gastrointestinal with the further involvement of other organ systems; laboratory findings were consistent with MIS-C and SARS-CoV-2 infection was confirmed. These are reasons why our patient was diagnosed and treated as MIS-C.

The overlapping characteristics between these syndromes might suggest that they may have similar pathophysiology and as a consequence similar therapeutical approach. Most children with MIS-C recover with standard KD therapies, IVIG, and glucocorticoids and only a small number of patients require immunomodulating agents, such as IL-1 or IL-6 antagonists. The majority of the children with MIS-C need some degree of intensive care, most often respiratory support and inotropes [3, 4, 9]. There are no widely accepted guidelines concerning the

therapy of MIS-C, but several organizations have published their guidelines [3, 12–17]. The combination of IVIG and methylprednisolone has been proven to be associated with a more favorable fever course in patients with MIS-C than IVIG alone [18]. Our patient had a good clinical response to IVIG and glucocorticoid therapy (initially dexamethasone, then methylprednisolone). Vitamin C, vitamin D, and initially nadroparin followed with acetylsalicylic acid, were also introduced. During the first 12 days of hospitalization oxygen therapy was required as well as the inotropes in the further course.

It is currently unknown whether MIS-C has potential long-term sequelae [4, 9]. It is known that the inflammatory disorders triggered by SARS-CoV-2 can result in coronary aneurysms, so cardiac assessment and follow-up are essential in all cases [9]. It is unknown if the incidence of coronary artery aneurysms (CAA) is different in MIS-C compared to KD. However, MIS-C patients without KD features can develop CAA [3]. A follow-up of our patient revealed the occasional chest pain and increased hair loss in the weeks following discharge from the hospital. Echocardiography and 24-hour ECG monitoring were without pathological changes and all laboratory analyses were within referral ranges, including control antinuclear antibodies which were negative. These symptoms disappeared after a few months.

This case report highlights the necessity of close monitoring of children during and after COVID-19. Every prolonged and unusual febrile state, especially if it is accompanied by gastrointestinal symptoms, in a school-age child should be investigated in the direction of recent COVID-19 infection or exposure. In a case of a positive COVID-19 history or history of exposure, the MIS-C diagnosis should be considered. Otherwise, the child should be observed for any other cause of the febrile state, including other infections or KD. Overlaps in the clinical picture of MIS-C, KD, and acute appendicitis should be kept in mind in order to make the right diagnosis and introduce the appropriate treatment.

Conflict of interest: None declared.

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Мултисистемски инфламаторни синдром код деце повезан са болешћу *COVID*-19 код девојчице узраста 11 година

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САЖЕТАК

Увод Мултисистемски инфламаторни синдром код деце животно је угрожавајуће инфламаторно стање са мултисистемском презентацијом које се типично јавља 3-4 недеље после инфекције *SARS-CoV*-2. У овом раду приказујемо први случај мултисистемског инфламаторног синдрома код деце на Институту за здравствену заштиту деце и омладине Војводине на почетку пандемије болести *COVID*-19.

Приказ болесника Претходно здрава девојчица узраста 11 година разболела се појавом фебрилности, малаксалости, главобоље, повраћања и болова у трбуху. *PCR* тест на *SARS-CoV*-2 из назофарингеалног бриса је био позитиван, као и ИгМ и ИгГ антитела на *SARS-CoV*-2. У даљем току фебрилност се одржавала уз лабораторијске показатеље инфламације и мултиорганску презентацију укључујући респираторни, гастроинтестинални, кардиоваскуларни систем и кожне промене. На основу критеријума Центра за контролу и превенцију болести и Светске здравствене организације постављена је дијагноза мултисистемског инфламаторног синдрома код деце и започета је терапија интравенским имуноглобулинима и метилпреднизолоном са добрим одговором на примењену терапију и повољним клиничким током.

Закључак Свако пролонгирано или неуобичајено фебрилно стање код школског детета, нарочито ако је праћено гастроинтестиналним симптомима, треба да буде испитано у правцу претходне инфекције *COVID*-19 или изложености овом вирусу. Уколико постоје анамнестички подаци о инфекцији *COVID*-19 или њеној изложености, потребно је размотрити мултисистемски инфламаторни синдром код деце.

Кључне речи: *SARS-CoV*-2; дете; инфламација; имуноглобулини; кортикостероиди

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CASE REPORT / ПРИКАЗ БОЛЕСНИКА

Surgical extraction of the impacted mandibular third molar – vestibular bone window technique

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SUMMARY

Introduction Surgical extraction of impacted mandibular third molar can lead to the periodontal defect on distal aspect of the mandibular second molar causing pocket formation, tooth sensitivity, food retention, postoperative infections. Different surgical techniques have been proposed to reduce periodontal complications.

Case outline We presented five cases treated with Vestibular Bone Window Technique. Considering data from the literature review, benefits and limitations of the technique are discussed and compared to the Standard Surgical Technique.

Conclusion Vestibular Bone Window Technique might be recommended surgical procedure for the extraction of impacted mandibular third molar when bucco-distal bone wall is present preoperatively. Taking into account only five cases, future work should consider a randomized clinical trial with the larger stratified samples.

Keywords: mandibular third molar; periodontal defect; vestibular bone window technique

INTRODUCTION

Surgical extraction of impacted mandibular third molar (M3M) is one of the most frequent procedures in oral surgery. It is followed by pain, swelling, trismus, or alveolitis that impairs the patient`s overall quality of life [1, 2]. If the impaction is deep, periodontal defect on distal aspect of the mandibular second molar (M2M) can occur postoperatively resulting in tooth sensitivity/mobility, food retention, fetor, or postoperative infections. Different treatment strategies, such as vestibular bone window technique (VBWT) have been proposed to reduce secondary periodontal complications [3].

CASE REPORT

Five patients (20–29 years old) were treated at the Clinic for Oral Surgery, School of Dental Medicine in Belgrade, where surgical extraction of impacted M3M was performed using VBWT. The procedure was carried out under local anesthesia and full-thickness envelope flap (Figure 1). Prior to surgery, the depth of periodontal pocket on the distal side of M2M was measured. Comparing to the standard surgical technique (SST) in which osteotomy is performed on the crestal part of the alveolar ridge (distally from M2M), in VBWT osteotomy is performed only as a small window on vestibular cortex (3–5 mm below the highest point of bone). The procedure was done using the round bur and a handpiece with copious saline irrigation. The bone tunnel was prepared obliquely and downwards, till contact with the impacted tooth. If M3M was fully impacted, additional osteotomy above the crown was performed, on the bucco-distal side of the crown. Depending on the position, tooth was divided using a tungsten fissure bur. Throughout the bony window, a straight elevator was introduced pushing first the crown, and the roots upwards and dislodging them outside the alveolus (Figure 2). After the inspection and curettage of granulation tissue, the wound was rinsed with saline and single sutures were used. The postoperative period was uneventful in all five cases. Sutures were removed on the seventh day and additional periodontal probe measurement was conducted one month after the surgery (Figure 3).

All the subjects from the study had bucco-distal bony bridge present before the surgery. Using the VBWT, we managed to preserve it and prevent soft tissue from collapsing. The results of this approach were confirmed on periodontal measurements, one month after the surgery. The patients did not declare any complaints postoperatively (short-term nor long-term). Postoperative pain, swelling, and trismus were recorded and estimated as acceptable (similar to the ones in SST) but were not evaluated additionally.

We confirm that we have read the journal's position on issues involving ethical publication and affirm that this work is consistent with those guidelines.

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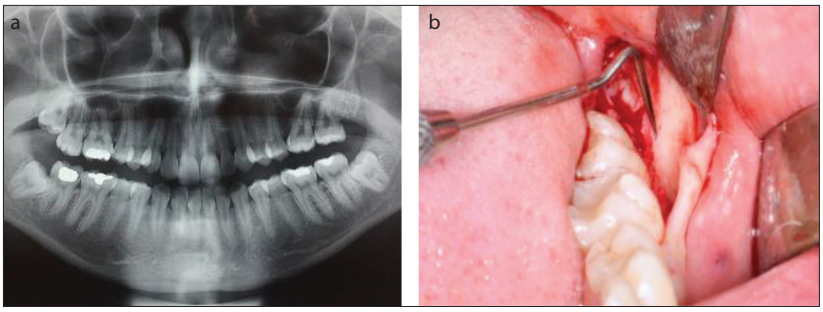

Figure 1. Preoperative X-ray and intraoperative insight: a – mandibular lower left third molar with Pell–Gregory II-b type of the impaction; b – bucco-distal bone is present preoperatively

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Written consent to publish all shown material was obtained from the patient.

DISCUSSION

Our findings indicate that VBWT preserves periodontal tissues on the distal aspect of M2M. The creation of a bony window on the vestibular aspect of the impacted third molar maintains bucco-distal bony bridge. That bone structure is essential because it supports soft tissues, preventing them from collapsing (Figure 4). Furthermore, in all of the five cases, periodontal pockets depth on the distal side of M2M were the same as before surgery. There were no changes in soft tissue architecture on one-month follow-up, and post-operative discomfort abstained.

These findings correlate to the research of Peñarrocha et al. [4]. The authors were the first to describe VBWT, stating that it minimizes the time required for ostectomy, postoperative pain, swelling, trismus, and periodontal risks for the second molar. However, they conducted the pilot study without the randomization on just four patients, so it is a little bit presumptuous to conclude all of that. Without the control group, there are doubts on the conclusions on postoperative pain, swelling, and trismus since they are not related to the ostectomy site. Then again, alteration of a bone window to the vestibular cortex and preservation of bucco-distal bony bridge inexplicably promote periodontal healing on distal aspects of M2M, maintaining the soft tissue structures, thus resulting in less postoperative complications.

Periodontal status of M2M after wisdom tooth surgical extraction is not only affected by ostectomy location but with flap design, alveolar preservation, or suturing techniques. When standard ostectomy is performed (bucco-distal from the second molar), the periodontal status of M2M is unpredictable. It depends on the patient's age, third molar impaction type and depth, and pre-surgical periodontal defects [5]. However, there are no clear scientific data to explain the outcome of third molar surgical extraction on M2M periodontal status [6, 7, 8]. It is also unclear how the flap design [9, 10, 11] or suturing technique [12] will alter the results.

Although we managed to maintain bucco-distal bone bridge in all of the five cases and retain periodontal health

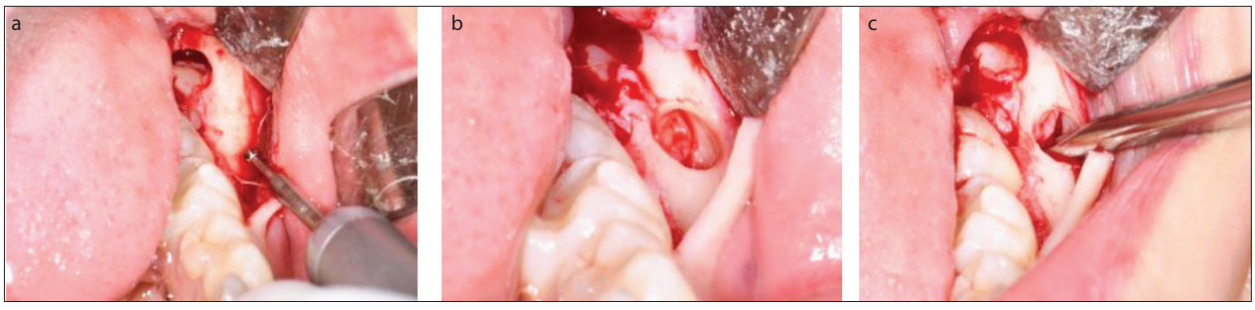

Figure 2. Surgical procedure for vestibular bone window technique: a and b – bone tunnel preparation; c – tooth extraction through bony window

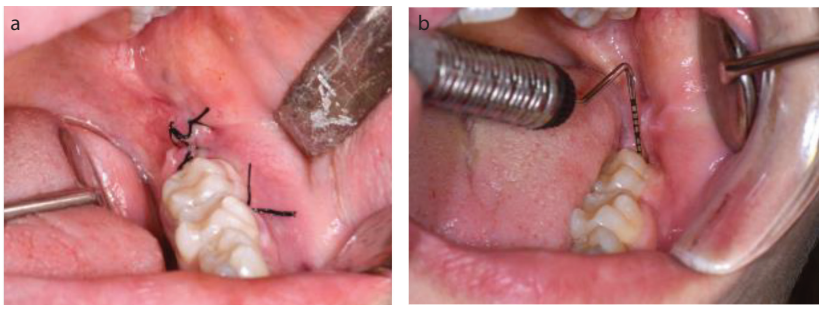

Figure 3. Postoperative insight and periodontal measurement: a – the wounds are sutured with single sutures; b – postoperative periodontal measurement on follow-up was conducted

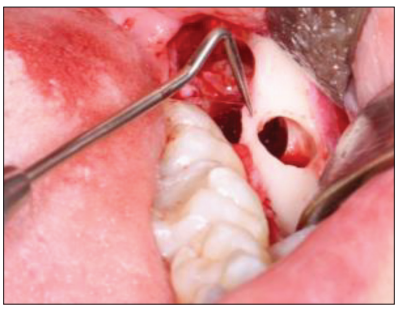

Figure 4. Bucco-distal bony bridge: using vestibular bone window technique, preservation of bucco-distal bony bridge was maintained

of M2M, this investigation was conducted only on the subjects that had this part of the bone present preoperatively. We assumed there was no point in testing VBWT on semi-impacted wisdom teeth that are already missing this part of the bone. However, SST in that clinical condition requires additional osteotomy that will generate larger post-extraction defect on alveolar bone distal to the second molar. In those assets, it is reasonable to anticipate the loss of periodontal support and periodontal pocket formation. We believe that VBWT could benefit periodontal health of the second molar in those impaction types where M3M undermines the second molar. Future work should consider a randomized clinical trial with the stratified samples that will compare M2M periodontal health (pre and postoperatively) between VBWT and SST groups in different preoperative clinical conditions.

Additionally, we observed that VBWT also differs from SST by its sensitivity since it requires additional surgical routine and skills. Bone osteotomy is performed on the vestibular cortex, mesial from the roots of the M3M. It is very important not to damage the distal root of M2M when creating this window. If the risk is too high or the surgeon is unable to extract the tooth this way, we suggest alteration of the protocol to SST.

Taken together the experience from case reports and literature review, VBWT might be a recommended surgical procedure for the extraction of impacted M3M when bucco-distal bone is present preoperatively and SST generates the risk for development of periodontal complications.

Conflict of interest: None declared.

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Хируршка екстракција импактираног доњег умњака – техника вестибуларног коштаног прозора

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САЖЕТАК

Увод Хируршко вађење импактираног доњег умњака може проузроковати појаву пародонталног дефекта дистално од другог молара. Он доводи до настанка пародонталног џепа, осетљивости зуба, западања хране, постоперативне инфекције. Предложене су различите хируршке технике како би се спречио настанак ових компликација.

Приказ болесника У раду је приказано пет пацијената којима су хируршки извађени импактирани доњи умњаци техником вестибуларног коштаног прозора. Разматрајући податке из литературе, описали смо предности и мане ове технике поредећи је са стандардном хируршком техником.

Закључак Техника вестибуларног коштаног прозора може се препоручити за хируршку екстракцију импактираног доњег умњака онда када је букодистална кост присутна преоперативно. С обзиром на мали узорак од свега пет пацијената, препорука је да се будуће истраживање реализује као рандомизована клиничка студија на стратификованом узорку.

Кључне речи: импактирани доњи умњак; пародонтални дефект; техника вестибуларног коштаног прозора

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CASE REPORT / ПРИКАЗ БОЛЕСНИКА

"TAP" technique on bifurcation lesion of the Y graft in a patient with NSTEMI complicated with cardiogenic shock

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SUMMARY

Introduction Bifurcation lesions on venous Y grafts are rare. We present a case of a woman who developed non-ST segment elevation myocardial infarction complicated by cardiogenic shock due to a bifurcation lesion on the venous Y graft for left anterior descending artery (LAD) and ramus circumflex artery (RCX).

Case outline A 72-year-old woman was admitted to the coronary care unit as an emergency in September 2017 due to acute heart failure followed by the development of cardiogenic shock. Urgent coronarography revealed severe atherosclerotic disease of native coronary arteries with significant bifurcation lesion on venous Y graft for LAD and RCX (medina classification of 1,1,1) with thrombolysis in myocardial infarction (TIMI) grade 2 flow. According to the general condition of patient, a life-saving *ad hoc* percutaneous coronary intervention (PCI) was performed. Two stents were implanted in the Y graft with T and protrusion (TAP) technique achieving optimal result followed with patient stabilization. On one-year follow-up, the patient was without symptoms of angina, and computed tomography coronarography revealed patent both stents in the Y graft. To the best of our knowledge, this is the first described TAP technique used on the Y graft.

Conclusion The PCI on a vein graft is not uncommon either in elective cases or in cases with acute coronary syndrome due to the poorer persistence and more frequent progression of atherosclerotic disease in the venous grafts. The use of bifurcation techniques for the treatment of lesions on a vein graft and especially on the Y graft is rare, but it can be used the same way it is used in native vessels.

Keywords: PCI; Y graft; TAP technique

INTRODUCTION

In routine practice, percutaneous coronary intervention (PCI) of bifurcation lesions in native coronary vessels is found in about 15–20% of cases [1]. However, the incidence of bifurcation lesions on venous Y grafts is rarely found. The use of Y grafts in surgical myocardial revascularization is not so common. They are mainly used when the vein grafts (VG) are of lower quality or when it is desirable to reduce manipulation on the aorta altered by atheromas [2]. PCI on venous grafts is known to be more associated with short-term as well as long-term adverse events when compared to native blood vessels [3]. Especially challenging are bifurcation lesions on the Y graft that lead to the acute coronary event. Practically, such lesions are in some cases equivalent to lesions on the left main coronary artery. In consequence, a larger area of the myocardium is affected by ischemia, which is more often followed by serious complications such as the development of cardiogenic shock, malignant rhythm disorders, or fatal outcome.

Herein, we present a case of a woman who developed non-ST segment elevation myocardial infarction (NSTEMI) complicated by cardiogenic shock due to a bifurcation lesion (medina classification 1,1,1) on the venous Y graft for left anterior descending artery (LAD) and ramus circumflex artery (RCX).

CASE REPORT

A 72-year-old woman was admitted to the coronary care unit (CCU) as an emergency in September 2017 due to the acute heart failure followed by the development of cardiogenic shock. Dyspnea discomforts occurred suddenly around one hour before arriving in the emergency room.

It was a patient who underwent coronary artery bypass grafting (CABG) in 2008 [LAD – left internal mammary artery (LIMA), VG at right internal mammary artery (RIMA) and obtuse marginal (OM) artery]. Due to unstable angina pectoris, she underwent recoronarography in March 2017 when severe multivessel coronary artery disease of the native blood vessels was registered as well as LIMA occlusion, and VG subocclusion on RIMA, while the only graft without significant lesions was on RCX–OM (Figure 1).

In March 2017, emergency surgical myocardial revascularization with three venous grafts [LAD, RIMA, right coronary artery – posterior

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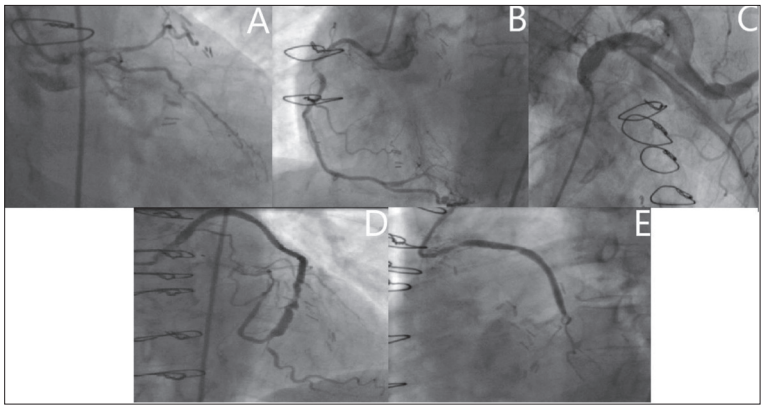

Figure 1. A) Acute coronary syndrome system – the left main 90%, subocclusion of the proximal left anterior descending artery and occlusion of distal segment of the ramus circumflex artery (RCX) [right anterior oblique (RAO) 1, caudal (CAU) 36); B) right coronary artery – proximal segment significantly changed [left anterior oblique (LAO) 42, CAU 3]; C) left internal mammary artery – occluded (RAO 46, CAU 3); D) vein graft on RCX–OM (obtuse marginal artery) – without significant lesions (RAO 3, cranial 24); E) vein graft on the right internal mammary artery – subocclusion in the proximal segment (LAO 43, CAU 2)

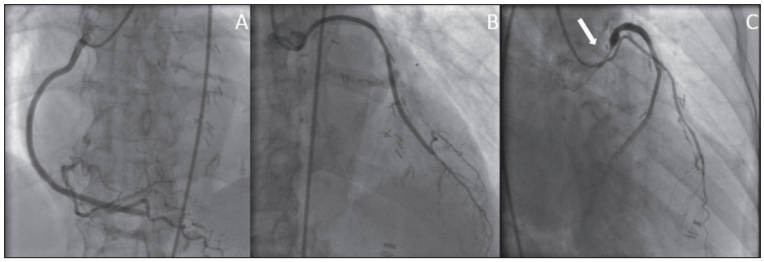

Figure 2. A) Vein graft (VG) for right internal mammary artery – without significant lesions [right anterior oblique (RAO) 9, caudal (CAU) 19]; B) VG for right internal mammary artery – without significant lesions (RAO 2, cranial 24); C) Y graft for left anterior descending artery and ramus circumflex artery – bifurcation lesion of medina 1,1,1 (right anterior oblique 37, CAU 5) – arrow is pointing to the lesion

descending artery (RCA–PD)] was performed. Since the aorta was altered by atheromas and venous grafts were of poor quality, Y graft technique was done and the venous graft on the LAD was sutured to the proximal segment of the venous graft for OM from 2008.

Immediately after the admission to the CCU in September 2017, the intensive treatment began. The patient was sedated, intubated, and supported by invasive mechanical ventilation. Medication circulatory support was also introduced (noradrenaline 0.5 μg/kg/minute and dobutamine 10 μg/kg/minute) in order to achieve hemodynamic stabilization. Non-invasive diagnostics were performed. Emergency echocardiography examination registered a fall in ejection fraction (EF) from the previous 60% to 48% and new abnormalities of segmental kinetics in the form of inferolateral akinesia and hypokinesia partly medially and apically anterolaterally. There was also a significant increase in myocardial necrosis markers.

After the performed emergency diagnostics, it was concluded that it was NSTEMI, which was complicated by cardiogenic shock. Therefore, following the recommendations, urgent coronarography was indicated and performed.

Urgent coronarography finding showed the significantly altered acute coronary syndrome (ACS) system, significant lesion on the left main (LM) artery, proximal subocclusion of LAD, the occlusion of RCX in the proximal segment, as well as the occlusion of native RCA, while it showed the VG for RCA and RIMA patency. It was also registered that the venous graft for LAD was sutured to the graft for RCX, whose patency had been shown by the previous coronarography. The bifurcation lesion of medina classification 1,1,1 developed with thrombolysis in myocardial infarction (TIMI) grade 2 flow at the insertion of the venous graft for LAD to the venous graft for RCX (Figure 2).

According to the general condition of the patient who was in a state of cardiogenic shock, intubated, and mechanically ventilated as well as on medication circulatory support, a life-saving PCI was performed.

Since it was the bifurcation lesion that, in this case, was the equivalent of the LM, it was decided to go up front with the two-stent technique. Two guidewires were placed (Runthrough, Terumo, Tokyo, Japan) and after the predilatation of both grafts, the T and protrusion (TAP) technique was performed with the implantation of two stents. Firstly, drug-eluting stent (DES) (Coroflex ISAR NEO, B. Braun Melsungen AG, Melsungen, Germany) 3.5 × 16 mm with inflation on 18 atmospheres (atm) was implanted in VG for RCX. Afterwards, proximal optimization technique (POT) was performed with non-complaint (NC) balloon 4.5 × 12 mm (Quantum Apex, Boston Scientific, Marlborough, MA, USA) with inflation on 20 atm. After successful rewiring through distal strut of the implanted stent towards the VG for LAD, predilatation was performed with low profile balloon (Sprinter Legend, Medtronic, Dublin, Ireland) to open the struts. According to TAP technique propositions, NC balloon 3.5 × 15 mm in size (Quantum Apex, Boston Scientific) was positioned towards the VG for RCX and, with a small protrusion in previously implanted stent, DES 2.25 × 13 mm (Orsiro, Biotronik, Berlin, Germany) was implanted in VG for LAD with inflation on 8 atm. Afterwards, post-dilatation of the ostial part of the stent was performed with an inflation on 16 atm. Next, kissing was performed with an NC balloon in VG for RCX and balloon from the stent in VG for LAD. At the end, final POT was performed with an NC balloon 4.5 × 12 mm in size (Quantum Apex, Boston Scientific) inflated on 20 atm (Figure 3).

During the first 24 hours after the procedure, satisfactory clinical stabilization was achieved, medication circulatory support was stopped, and the patient was extubated. On the 10th hospital day, she was discharged for outpatient treatment.

On the one-year follow-up, the patient had no anginal discomforts, echocardiography registered EF of 55%, with

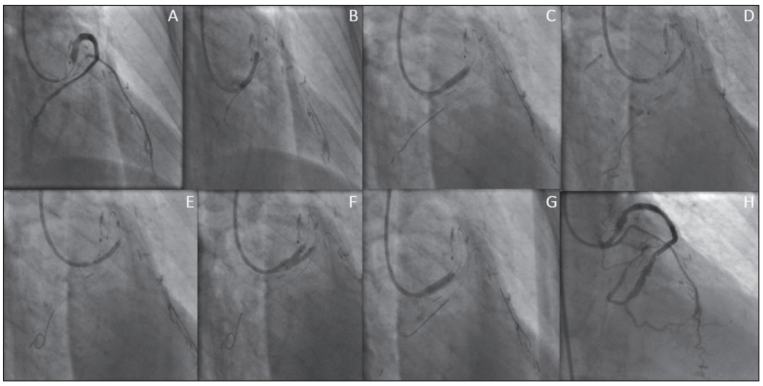

Figure 3. A) Stent positioning in vein graft (VG) for ramus circumflex artery (RCX); B) stent implantation in VG for RCX; C) proximal optimization technique; D) stent implantation in VG for left anterior descending artery (LAD); E) post-dilatation of the ostial part of the stent implanted in VG for LAD; F) kissing; G) final proximal optimization technique; H) final result

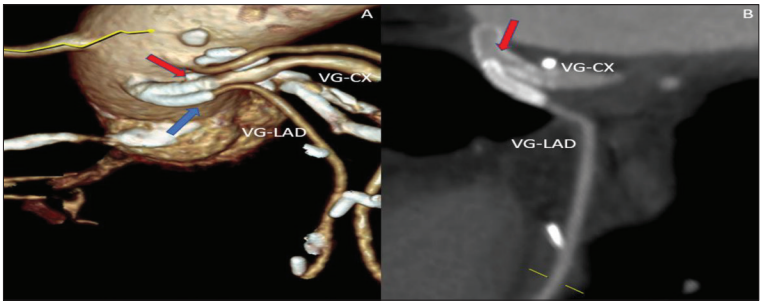

Figure 4. A) CT coronary angiography – 3D reconstruction of the aorta and implanted stents in the Y graft; the red arrow points towards the stent implanted in the vein graft (VG) for ramus circumflex artery (RCX), while the blue arrow points towards the stent implanted in the VG for left anterior descending artery (LAD); B) both patent stents and VGs; the red arrow points towards the area of neo carina

discrete segmental kinetics abnormalities, while CT coronarography registered the potency of stents implanted using the TAP technique into grafts for LAD and RCX (Figure 4).

This case report was approved by the institutional ethics committee, and written consent was obtained from the patient for the publication of the case report and any accompanying images.

DISCUSSION

Treating true bifurcation lesions with percutaneous coronary intervention is a major challenge, primarily because of the selection of an appropriate technique to be followed with fewer potential adverse events. The current European Society of Cardiology guidelines for myocardial revascularization, as well as the 15th consensus from the European Bifurcation Club (EBC), recommend the stent implantation into the main vessel, followed by provisional balloon angioplasty of the side vessel with or without the stent implantation. However, the implantation of two stents is required in 5–20% of cases [4, 5]. No randomized studies addressing the treatment of bifurcation lesions in ACS are available at present. Data are available mainly from registers, the largest of which is the Korean Coronary Bifurcation Stenting (COBIS) registry.

Analyzing data from the COBIS registry, Song et al. [6] found that the outcome of bifurcation PCI in patients with NSTE-ACS and patients with stable angina differed and that patients with NSTE-ACS were associated with the poorer clinical outcome than those with stable angina.

Kim et al. [7] published the first study based on data from the COBIS II registry that compared the long-term clinical outcome (three years) of PCI bifurcation lesions in patients with and without the acute coronary syndrome. It showed that adverse cardiac events were significantly more common with the two-stent technique than the provisional technique in patients with ACS, as opposed to the patients without ACS. Although it should be taken into consideration that this was not a randomized study and that lesions treated with the implantation of two stents had a higher prevalence of more complex lesions, true bifurcations, left main stem (LMS) lesions, as well as multivessel disease.

While the data for selecting one of the techniques for PCI revascularization of bifurcation lesions of native coronary vessels in patients with NSTE-ACS are available, the data for revascularization of patients with Y graft bifurcation lesions can be found sporadically in the literature, primarily through case reports. It is well known that the long-term patency of arterial grafts is significantly better than that of venous grafts. Accordingly, the progression of coronary artery disease in surgically revascularized patients imposes an increasing need for subsequent PCI revascularization of lesions on any of the venous grafts or native blood vessels. According to the study conducted by Redfors et al. [8], there is a significantly higher risk of ischemic adverse events after PCI on VG than in native blood vessels. In some case reports of PCI on the Y graft, various bifurcation techniques have been successfully used, such as DK crush, classic crush, and even the implantation of a dedicated triton bifurcation stent [9, 10, 11].

As there is still no clear consensus on the choice of a technique for revascularization of patients with bifurcation lesions on the Y graft, we were guided by the consensus of the EBC on the revascularization of the LM with a complex bifurcation lesion, and accordingly the decision was made to first stent the vessel with a more pronounced disease, since both branches of the Y graft are of equal importance [2, 5, 12].

According to the available published data in the medical literature, our case is the first to use the TAP technique for the treatment of Y grafts. The decision to use the two-stent technique was made since it was a bifurcation lesion (medina 1,1,1) on the Y graft for LAD and RCX, which was equivalent to a lesion on the LMS.

Given the larger caliber as well as the angle, VG for RCX was taken as the main branch, while VG for LAD was taken as a side branch. In this case, the TAP technique had an advantage over the other two stent techniques due to its lower complexity, which allowed faster revascularization of a highly demanding patient in cardiogenic shock.

Clinical stabilization was soon achieved after the revascularization. During the follow-up, the patient had a satisfactory performance status, without angina, and CT graftography confirmed the patency of the stents in the Y graft.

In our center, more than 1000 primary PCIs are performed per year because of the STEMI, and only 15–20 procedures are performed in VG. According to our data, around 15% are bifurcation lesions which are, in the majority of cases, treated by provisional strategy. Up front two stent techniques are used only when both of the vessels are diseased and of great importance.

In conclusion, although associated with poorer outcomes when compared to the PCI of native vessels, the PCI on VG is not uncommon either in elective cases or in cases with ACS due to the poorer persistence and more frequent progression of atherosclerotic disease in the venous grafts. The use of bifurcation techniques for the treatment of lesions on VG and especially on the Y graft is rare, but it can be used the same way it is used in native vessels.

Conflict of interest: None declared.

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Техника *TAP* на бифуркационој лезији графта *Y* код болесника са *NSTEMI* компликованим кардиогеним шоком

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САЖЕТАК

Увод Бифуркационе лезије на венском *Y* графту се ретко налазе. Приказали смо случај болеснице која је развила *NSTEMI* компликован кардиогеним шоком због бифуркационе лезије на *Y* графту за *LAD* и *RCX*.

Приказ болесника Болесница старости 72 године примљена је као хитан случај у коронарну јединцу због акутне срчане инсуфицијенције праћене развојем кардиогеног шока. Ургентном коронарографијом је регистрована тешка вишесудовна коронарна болест нативних суда као и сигнификантна бифуркациона лезија на венском *Y* графту за *LAD* и *RCX* (медина класификације 1,1,1) са тромболизом код инфаркта миокарда (*TIMI*) протока 2. Сходно општем статусу болеснице урађена је спасавајућа *ad hoc* перкутана коронарна интервенција. Два стента су имплантирана техником *TAP* постижући оптималан резултат, након чега се убрзо постигла стабилизација општег стања. На годишњој контроли болесница је била без ангинозних тегоба, а *CT* коронарографијом регистрована су проходна оба стента имплантирана у *Y* графт. Према нашим сазнањима, ово је први описани случај технике *TAP* коришћене на *Y* графту.

Закључак Перкутана коронарна интервенција на венском графту није ретка ни код елективних случајева, ни код акутног коронарног синдрома, због мање постојаности као и чешће прогресије атеросклеротске болести на венском графту. Примена бифуркационих техника за третман лезија на венским графтовима а посебно *Y* графту је ретка, али се може користити по истом принципу као и на нативним судовима.

Кључне речи: перкутана коронарна интервенција; графт *Y*; техника *TAP*

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CASE REPORT / ПРИКАЗ БОЛЕСНИКА

Penetrating and blunt injuries of the heart – an abdominal surgeon's personal experience in Serbia

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SUMMARY
Introduction In the world with constantly increasing incidence of violence and trauma on one side, and more and more specialized surgeons on the other, the question about the role of abdominal surgeons in cardiac trauma emerges. The objective of this article is to show personal experience of an abdominal surgeon in managing heart trauma.
Outlines of cases We present two penetrating injuries and one blunt trauma of the heart successfully managed by an abdominal surgeon.
Conclusion Abdominal surgeons should feel comfortable with the decision to operate on greatly physiologically deranged patients with penetrating chest trauma, and not to delay the operation with conservative measures or with time-consuming transport to remote specialized facilities, since that could lead to greater death percentage of these patients.
Keywords: heart trauma; penetrating wound; abdominal surgeon

INTRODUCTION

Traumatic injuries are the leading cause of death among people younger than 45 years [1, 2]. Right after injuries of the brain and spinal cord, cardiac injuries are the second most common cause of lethal outcome of trauma victims [2].

Heart can be injured in two possible manners, by non-penetrating/blunt trauma (blunt cardiac injury) and penetrating trauma [3, 4].

Trauma caused from blunt forces can have a wide clinical presentation, from practically silent presentation or transient arrhythmias to fatal cardiac rupture [5, 6], with the latter as the most common cause of death in 64% of blunt cardiac injury cases, followed by tears at the venous–atrial confluence (33%) and dissection of coronary arteries (2%) [7]. The most common site of myocardial rupture is the wall of the right ventricle, usually immediately fatal. Those who survive long enough to reach the hospital can present with cardiac tamponade or pseudoaneurysm [8, 9].

One of the most lethal medical emergencies is certainly penetrating injury of the heart [10]. Estimations are that almost 94% of the injured die before reaching the hospital [10]; reports about in-hospital death rate vary 8.5–50% or even more [10, 11, 12]. The range of mortality in these reports can be explained by patient inclusion criteria, variety of conjoined injuries, and the mechanism of the occurrence. As in blunt cardiac injuries, the right ventricle is the most common site of injury in penetrating injuries, but with similar percentage of injury for both ventricles [2, 10]. Cardiac tamponade is the common manifestation in initial survivors, who are usually young males, with stab/gunshot wound to the chest [3].

Objective of this article is to show personal experience of an abdominal surgeon in managing heart trauma.

Surgery of the wounded heart – the beginnings

The first steps in cardiac surgery were made in trauma, as lifesaving procedures [13, 14]. The first attempt to suture a human heart was performed by a Norwegian doctor named Axel Hermansen Cappelen in 1895. The patient was a young male with a stab wound to the left ventricle. After initial improvement, the patient died on the third postoperative day [15]. The first successful suture of the heart is considered to be the one performed by Ludwig Rehn on September 9, 1896 in Frankfurt am Mein. He operated on a 22-year-old male with a stab wound to the right ventricle. As a peculiarity, the unfortunate patient got stabbed two days earlier, and Rehn operated after returning from a journey. After the procedure, the patient completely recovered [16]. The first successful suture of the left ventricle described in the literature was done in Rome, Italy, by Antonio Parrozzani on April 18, 1897. The patient was also a young male with a stab wound to the left side of the chest. The man recovered completely

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after a 75-minute-long operation [17]. The first large series of successfully managed heart wounds was published by Dwight Harken, an American war surgeon [14, 15].

First steps in Serbia

The first successful operation of a wounded heart in Serbia was performed in 1928 by Jovan Mijušković, in the city of Valjevo [14, 18]. The patient was a 15-year-old male with a gunshot wound to the right ventricle. The operation was performed in local anesthesia, and lasted only 35 minutes as the author described it. The postoperative period was long and with numerous complications, but eventually the boy was discharged in good condition [18]. This operation was the very first step in surgery of the heart, decades before cardiac surgery even emerged as a separate field, in former Kingdom of Yugoslavia, now Serbia. Jovan Mijušković (1886–1944) studied medicine in Vienna, and conducted his surgery residency in Vienna and Belgrade. He was chief of surgery in the cities of Ćuprija and Valjevo. In 1936 he was appointed as a professor for the subject History of Medicine, at the Faculty of Medicine, University of Belgrade. During his career, he published numerous papers in domestic and foreign scientific Journals [14]. He was briefly the minister of health during World War II. In 1942 he founded Department of Surgery at the Belgrade City Hospital, currently Nikola Spasić Surgical Clinic, Zvezdara University Clinical Center in Belgrade [14]. One of the cases was managed at this institution, at which some of the authors of this paper practice surgery.

The role of general surgeons in heart trauma, in the era of modern cardiac surgery

Several authors published results of specialized centers in managing heart wounds, combining interventional, radiologic, and also surgical approach [19–22]. It is obvious that diagnosis made swiftly and very early, with an organized and available heart team (interventional cardiologists, radiologists, and cardiac surgeons), which are available in specialized centers, provide favorable results [4, 22]. In some parts of the world, where trauma and cardiothoracic surgeons are not easily available, penetrating thoracic trauma is becoming more frequent, as well as the need for thoracotomy [10, 23]. It is also worth mentioning that general surgeons and general surgery residents are becoming more specialized by the day, and less "adventurous" when it comes to operating beyond their area of specialization [24]. Fear of being sued in case of failure, is the most frequent reason for referring chest trauma to more specialized centers and staff [24]. In recent years, some authors published results with an intent to encourage general surgeons to engage in cardiac trauma and emergency thoracotomy in general [24, 25]. Doll et al. [24] stated that in all cardiac wounds they applied a simple direct suture of the heart wall, highlighting that there was no need for an expertise of a cardiac surgeon nor extracorporeal circulation. They speculated that patients with complicated injuries which would require bypass of the heart would most likely die before reaching the hospital. It is their strong opinion that, apart from mediastinal vessels and esophageal injuries, a great majority of penetrating thoracic trauma can be managed with simple operative techniques. They concluded that general surgeons should feel comfortable with the decision to operate on greatly physiologically deranged patients with penetrating chest trauma, and not to delay the operation with conservative measures or with time-consuming transport to remote specialized facilities, since that could lead to greater death percentage of these patients.

REPORTS OF CASES

Case 1

A 23-year-old patient was immediately admitted to the Clinic of Emergency surgery, University Clinical Center of Serbia in Belgrade for suicide attempt, stabbed in the epigastrium. He was hemodynamically unstable with clinical signs of hemorrhagic shock. An emergency operation was performed. Abdominal cavity was opened by medial laparotomy, penetrating injuries of the left liver lobe, the diaphragm, and the diaphragmatic side of the left heart ventricle were verified intraoperatively. Furthermore, left ventricle penetrating hole was sewn by transdiaphragmatic approach with several interrupted stiches preserving left coronary artery (Figures 1 and 2). Then, the diaphragm was reconstructed and the left lobe of the liver was sutured.

The postoperative period was uneventful and the psychiatrist has been consulted. The hospital discharge took place on the 10th postoperative day. The patient was in good condition.

Case 2

A 75-year-old man with blunt thorax injuries was hospitalized at the Clinic of Emergency Surgery, University Clinical Center of Serbia in Belgrade, due to a fall from a height of approximately 2.5 m. In this case, there were fractures of ribs V–VIII and consequential hemopneumothorax. Firstly, the thoracic tube was placed through the second intercostal space rightward on the midclavicular line. Initially, air and blood appeared in the tube, after which the lung re-expansion and blood control was achieved.

The next day, the massive hemothorax was treated and the patient underwent emergency surgery.

Right posterolateral thoracotomy was performed through the fifth intercostal space. Intraoperatively, hemothorax and laceration of the right atrium auricle as a consequence of previous adhesions following acceleration-deceleration injuries was verified. A vascular clamp was placed under laceration and auricle sutured continuously (Figures 3 and 4).

The postoperative period was uneventful. The patient was discharged on the 12th postoperative day in relatively good condition.

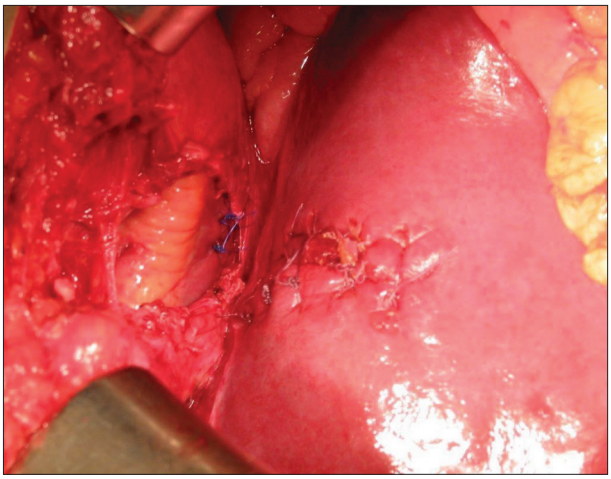

Figure 1. Case 1, transabdominal view of the sutured heart, sutured liver, and the rupture point of the diaphragm

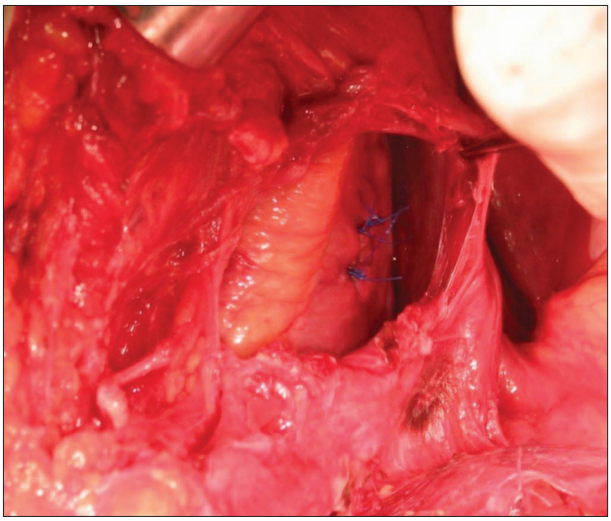

Figure 2. Case 1, view of the sutured heart as seen transabdominally through the ruptured diaphragm

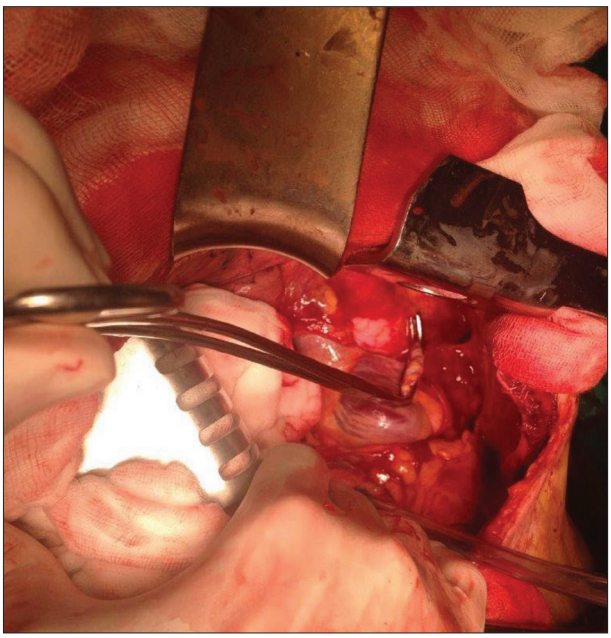

Figure 3. Case 2, clamped auricle of the right atrium of the heart

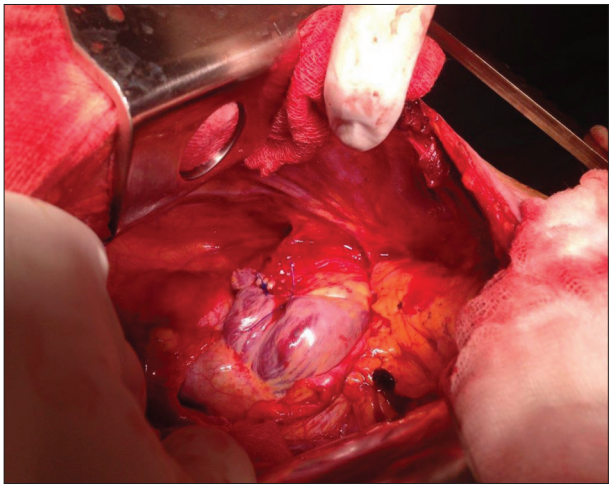

Figure 4. Case 2, sutured auricle of the right atrium of the heart

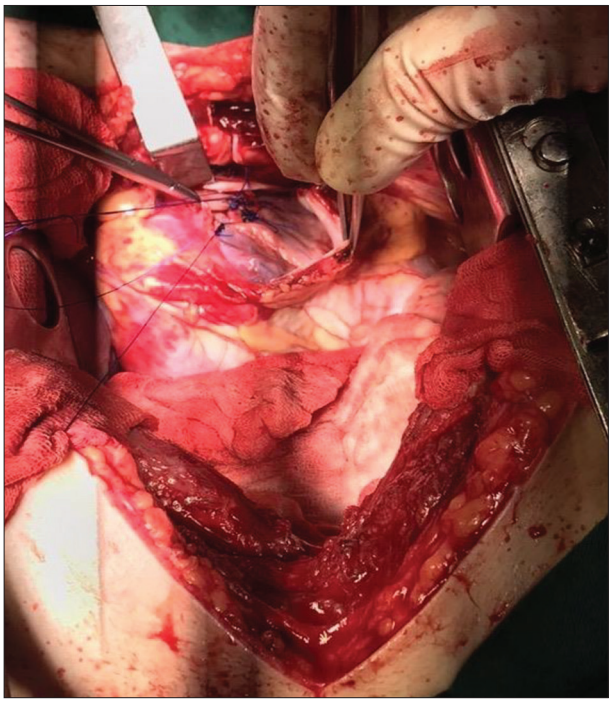

Figure 5. Case 3, interrupted sutures on the myocardium of the left ventricle

Case 3

A young 17-year-old patient was found lying down on the street, in close proximity to the Nikola Spasić Surgical Clinic, with a penetrating wound through the left side of the chest, close to the sternum. According to an eyewitness, no more than 10 minutes had passed since the stabbing, by an unknown perpetrator. He was immediately admitted to an intensive care unit, hemodynamically unstable and somnolent, with hemoglobin values of 10.7 g/dl and hematocrit 31.5%. Inotropes were administered along with massive fluid resuscitation, two units of fresh frozen plasma and two units of erythrocytes. A stab wound through the chest wall at the intersection of the fifth intercostal space and left midclavicular line was identified with moderate

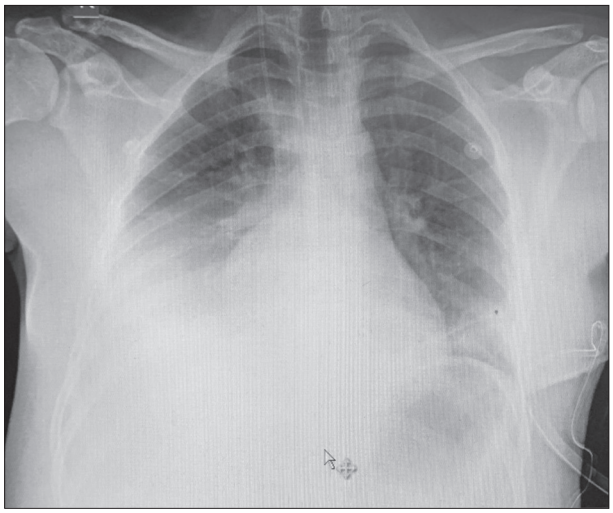

Figure 6. Case 3, chest X-ray before pleural punction on the right side due to pleural effusion, third postoperative day

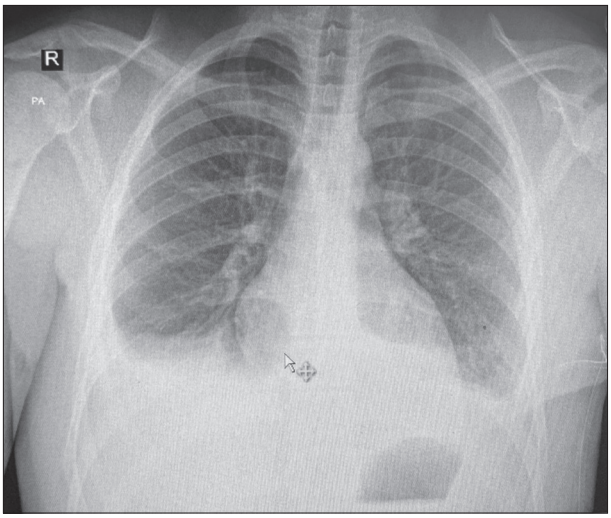

Figure 7. Case 3, chest X-ray after pleural punction on the right side due to pleural effusion, third postoperative day

active bleeding. Immediately after the admission to the Intensive Care Unit, chest X-ray revealed a massive hemothorax, and a chest tube was inserted through the wound. After a short period of observation, the tube evacuated a total amount of 1500 ml of blood, and a sudden drop in hemoglobin values to 4.9 g/dl and in hematocrit to 14.5% occurred. He was promptly transferred to the operating room, with continuous norepinephrine and dopamine i.v. drip; two additional units of fresh frozen plasma and three units of blood were administered, as well as one unit of cryoprecipitate. Left hemithorax was opened via anterolateral thoracotomy through the fifth intercostal space. A stab wound through the full thickness of the left ventricle wall was identified, with the breach of endocardium and synchronous, pulsatile bleeding. The myocardial wound was approximately 4 cm long, with much wider pericardial laceration. Also, left internal thoracic artery was incompletely transected, with a lesion of the sternum at the same level and hemothorax and hemopericardium present. Other intrathoracic and mediastinal organs were intact. The myocardium was closed with prolene 2.0 and 3.0 interrupted sutures; pericardium was sutured in the same fashion, with narrow drain inserted in the pericardial space (Figure 5). The internal thoracic artery was suture ligated, and after thorough rinsing of the hemothorax and chest tube placements, the chest wall was closed. Postoperatively, regular transthoracic echocardiographs were performed on five occasions, with no rhythm or hemodynamic irregularities. On the second postoperative day he was extubated. Due to renal impairment caused by massive blood loss, a nephrologist was called into consult twice, but significant therapeutic changes and dialysis were not necessary. On the third postoperative day, due to right pleural effusion, a pleural punction and fluid evacuation was performed (Figures 6 and 7). All drains and tubes were gradually pulled out. The patient received 11 units of blood, 10 units of fresh frozen plasma, one unit of thrombocytes, and six units of cryoprecipitate. The patient spent seven days in the Intensive Care Unit and four more days in the Semi-Intensive Care Ward, and was finally discharged on the 12th postoperative day in good condition.

Written consent for publication of this article has been obtained by the patients or the patients' family members.

DISCUSSION

The incidence of cardiac injuries has risen in the past few decades severalfold. In civilian settings, most penetrating injuries are stab wounds, with an increasing percentage of fire arm lesions [24, 26]. Already recognized as one of the most lethal medical emergencies, penetrating heart injuries have an in-hospital death rate, as said earlier in this paper, from 8.5% to 50% or even more [10–12]. We had the fortune to see all three of our patients successfully discharged alive.

"The quadrangle of death" is the most frequent localization of cardiac injuries on the chest wall. It goes above the diaphragm, under the clavicles and medial to the nipples. Some previous authors reported that one chamber injury accounts for 70%, and multiple chamber injury accounts for 30% of all injuries [27]. This complies with our small series, in which all three patients had one chamber injury, two left ventricle injuries, and one right atrium auricle injury. According to previous findings, in penetrating injuries, both ventricles are affected with similar frequency, but with the right ventricle as more common site of entry, since it forms most of the anterior surface of the heart. In only three cases (66.66%), injuries were of the left ventricle [2].

At least one of the three symptoms of Beck's trinity (hypotension, elevated central venous pressure, dull heart murmurs) is present in penetrating heart injuries [4]. One must have in mind that tension pneumothorax, also a chest injury, can mimic all of these symptoms, but with auscultatory silence on the affected side. Full preoperative diagnostics can only be conducted in hemodynamically stable patients [4]. On the other hand, when we face massive bleeding, cardiac tamponade, parasternal

wounds, unstable patients in general, immediate thoracotomy, without detailed diagnostics, is mandatory [4, 24]. Echocardiography, if allowed by hemodynamic stability, is very useful in detecting trauma of the heart [28]. One of very informative but time-consuming diagnostic procedures, heart catheterization, is by some authors not necessary in the initial phase of diagnostics, but is very important in solving complex injuries, and practically mandatory in the post-surgical phase, in order to exclude secondary complications, which can be expected in up to 10% of cases [4, 22].

Swift and precise diagnosis, reanimation, expeditious transport and timely surgical intervention are of utmost importance in managing cardiac injuries. Equipped centers and specialized cardiac teams are certainly ideal for injured patients. However, even in the era of more and more specialized branches in medicine and surgery, the role of abdominal surgeons in cardiac trauma is still important. Apart from mediastinal vessels and esophageal injuries, a great majority of penetrating thoracic trauma can be managed with simple operative techniques. Abdominal surgeons should feel comfortable with the decision to operate on greatly physiologically deranged patients with penetrating chest trauma, and not to delay the operation with conservative measures or with time-consuming transport to remote specialized facilities, since that could lead to greater death percentage of these patients.

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Пенетрантне и тупе повреде срца – лична искуства абдоминалног хирурга у Србији

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САЖЕТАК

Увод У свету у ком се стално повећава инциденца насиља и повреда, а хирурзи се едукују за све уже хириршке гране, поставља се питање о улози абдоминалних хирурга у кардијалној трауми.

Циљ овог рада је да прикаже лична искуства абдоминалног хирурга у решавању повреда срца.

Прикази болесникâ Приказана су два случаја пенетрантне повреде срца и једна тупа повреда срца, које је успешно излечио абдоминални хирург.

Закључак Требало би да се абдоминални хирурзи слободно упусте у операцију хемодинамски нестабилних болесника са пенетрантним повредама грудног коша. Не треба да одлажу операцију спровођењем конзервативних мера или транспортовањем у удаљене специјализоване центре, јер то може довести до веће смртности ових болесника.

Кључне речи: повреде срца; пенетрантне повреде; абдоминални хирург

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CASE REPORT / ПРИКАЗ БОЛЕСНИКА

Spleen-preserving surgical treatment of splenic artery aneurysm secondary to chronic pancreatitis and primary biliary cholangitis

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SUMMARY

Introduction Splenic artery aneurysm (SAA) represents the third cause of abdominal aneurysms, just after abdominal aorta and iliac arteries aneurysms, with overall prevalence of 1%. Pancreatitis has been linked with pseudoaneurysm formation of SA due to destruction of arterial wall by pancreatic enzymes, however true SAA associated with pancreatitis has not been described yet. We are presenting the first case of true SAA in a patient with chronic pancreatitis and primary biliary cholangitis successfully treated by surgical excision, direct arterial reconstruction and spleen preservation.

Case outline A 74-year-old male patient was admitted for multidetector computed tomography angiography due to suspected SAA and renal artery aneurysm (RAA). He was previously treated for chronic pancreatitis and primary biliary cholangitis. Upon admission, computed tomography arteriography showed SAA 32 mm in diameter and RAA 12 mm with SAA being in direct contact with superior margin of the pancreas. Surgical treatment of SAA was indicated while RAA was treated conservatively. Intraoperatively, SAA adherent to the superior margin of pancreas was noted, followed by complete exclusion of the aneurysm and end-to-end splenic artery anastomosis. Histopathology showed atherosclerotic degeneration of arterial wall with all three layers presenting as true aneurysm. Two years after the surgery, control computed tomography angiography showed regular postoperative findings without further progression of RAA.

Conclusion This is the first case to describe a true SAA aneurysm originated on the field of previous episodes of chronic pancreatitis and primary biliary cholangitis. Surgical treatment including aneurysm resection and direct arterial reconstruction with spleen preservation showed satisfactory results.

Keywords: splenic artery aneurysm; chronic pancreatitis; primary biliary cholangitis; spleen-preservation surgery

INTRODUCTION

Splenic artery aneurysm (SAA) represents the third cause of abdominal aneurysms, just after abdominal aorta and aneurysms of iliac arteries [1, 2]. SAA is defined as dilatation of splenic artery > 1 cm in diameter while in patients with SA > 2 cm surgical or endovascular treatment should be indicated. [3] The majority of patients with SAA are asymptomatic, while unspecific symptoms such as epigastric abdominal or the left upper quadrant pain, nausea, melena and anemia, vomiting or anorexia hematemesis could be seen as well [4, 5].

The exact cause of SAA is not entirely known, however atherosclerosis, trauma, portal hypertension, medial degeneration or dysplasia, pregnancy, and the female gender are known risk factors [3, 4]. Spontaneous rupture could be seen in 2–10% of the patients associated with high mortality up to 40%, especially in patients with pseudoaneurysm (PSA) or during pregnancy [6, 7, 8]. PSAs of SA are rare, more prone to rupture than true aneurysms and mostly associated with pancreatitis, trauma, iatrogenic cause or rarely peptic ulcer disease [9]. Pancreatitis has been linked with PSA formation of SA due to destruction of arterial wall by pancreatic enzymes causing inflammation, fragmentation of elastic tissue and consequent PSA formation, however true SAA associated with pancreatitis has not been described yet [10, 11]. We are presenting the first case of true SAA in a patient with chronic pancreatitis and primary biliary cholangitis successfully treated by surgical excision and arterial reconstruction.

CASE REPORT

A 74-year-old male patient was admitted to our Institution for multidetector computed tomography (MDCT) angiography. Prior to admission, abdominal computed tomography and ultrasonography showed SAA 31 mm in diameter and left renal artery aneurysm (RAA) 12 mm. The patient denied any abdominal or back pain. His past medical history included hypertension and hyperlipidemia, two years earlier radiofrequency ablation was performed for persistent atrial fibrillation. He was also previously treated for chronic pancreatitis and

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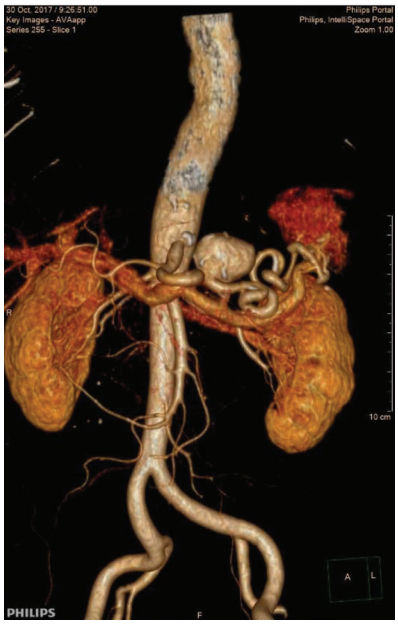

Figure 1. Multidetector computed tomography angiography; splenic artery aneurysm – 32 mm

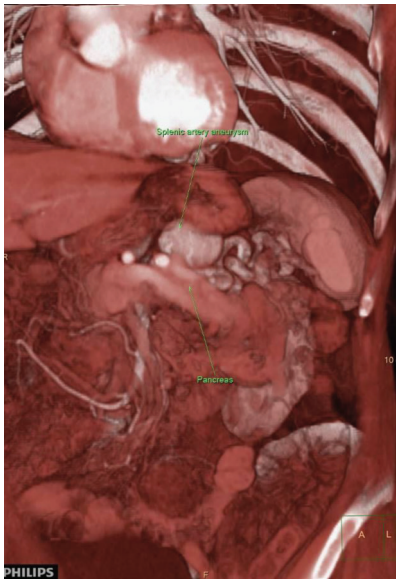

Figure 2. Multidetector computed tomography angiography; splenic artery aneurysm adherent to superior margin of the pancreas

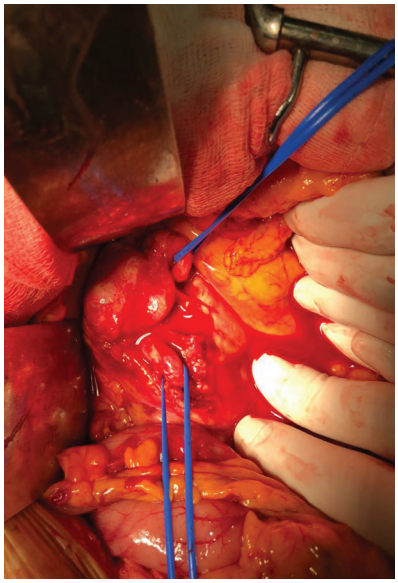

Figure 3. Intraoperative findings showing splenic artery aneurysm

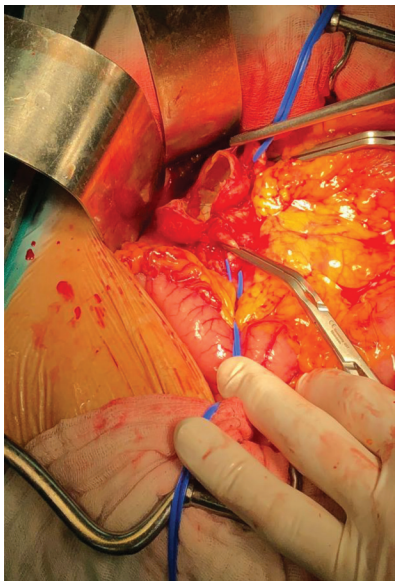

Figure 4. Intraoperative findings; resection of splenic artery aneurysm – thin arterial wall without thrombotic mass

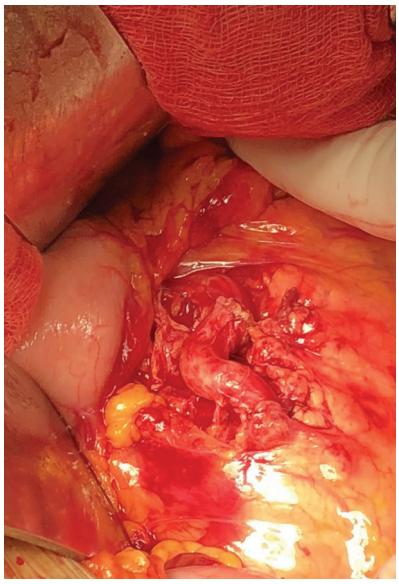

Figure 5. Intraoperative findings; successful splenic artery reconstruction by end-to-end anastomosis

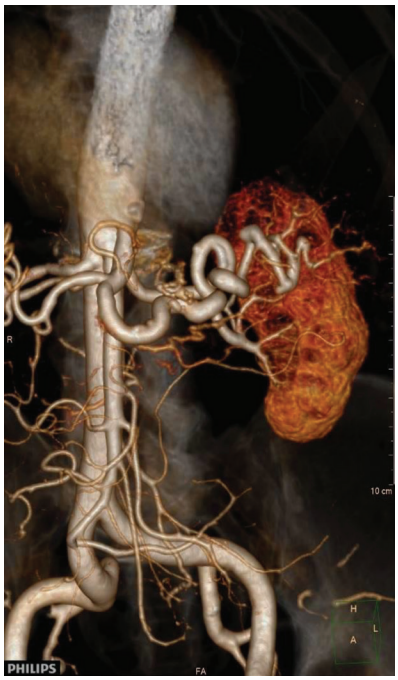

Figure 6. Multidetector computed tomography angiography; splenic artery after the reconstruction

primary biliary cholangitis. Echocardiography showed regular findings with ejection fraction > 60% and aortic and mitral valve mild insufficiency. All laboratory values were within referent values including pancreatic enzymes and parameters of kidney function.

Clinical examination showed regular pulses on lower extremities, ankle-brachial index was 1.0 on both legs. MDCT arteriography (Philips®, 128 slice device, Amsterdam, Netherlands) showed SAA 32 mm in diameter (Figures 1 and 2) as well as left RAA 12 mm. Direct contact between superior margin of the pancreas and the aneurysmal sac was clearly visible (Figure 2).

Surgical treatment of SAA was indicated while RAA was treated conservatively. Prior to surgery, 24-hour Holter monitoring was performed that revealed insignificant number of supraventricular extrasystoles and occasional first-grade atrioventricular block.

Following medial laparotomy, SAA was approached through lesser sac (Figure 3).

Intraoperatively, close contact of SAA sac with the superior margin of pancreas was noted to which it was partially adherent. Complete exclusion of SAA was performed followed by end-to-end splenic artery anastomosis (Figures 4 and 5).

Surgical procedure went uneventfully, still the first postoperative day, due to low hemoglobin, abdominal pain and suspected intrabdominal hematoma the patient was returned to the operating room. Hematoma was evacuated and the bleeding was taken care of from the superior margin of the pancreas. In the further postoperative course elevated values of alpha amylase (1200 U/L) and total bilirubin (120 mmol/L) were verified. Six days after the procedure there was significant decrease of alpha-amylase (350 U/L) values while total bilirubin was normal as well as all other laboratory values. Histopathology showed atherosclerotic degeneration of arterial wall with all three layers presenting as true aneurysm. The patient was discharged on the 13th postoperative day. After six months follow up, the patient was doing well, all laboratory finding were within the referent values. Two years after the surgery control MDCT angiography showed regular findings after SA reconstruction (Figure 6) without further progression of RAA.

Ethics Committee of the Dedinje Cardiovascular Institute approved this case – number 127/20. Informed consent has been obtained from the patient for publication of the case report and accompanying images.

DISCUSSION

SAA accounts nearly 60% of all visceral artery aneurysms with overall prevalence of 1% [3]. SAA are up to four times more common in females while overall incidence increases to 10% in patients over the age of 60, and in patients with portal hypertension [12, 13]. Spontaneous rupture is associated with high mortality, up to 40% for true aneurysms, and even 90% for PSAs [8, 9, 10]. In patients with intraperitoneal SAA rupture, acute abdomen and hypovolemic shock, splenectomy and SA ligation are mostly performed [14, 15]. Spontaneous rupture is more frequent in true aneurysms > 2 cm making this diameter cut-off point for surgical or endovascular treatment.

Pancreatitis, on the other hand, has been associated with SA PSA development with high risk of rupture up to 40% and mortality up to 90% [10, 16]. It has been proposed that in the case of pancreatitis, pancreatic enzymes are responsible for destruction of arterial wall architecture and disintegration of elastic tissues, leading to formation of PSA [17]. Apart from direct damage of vascular structures by severe inflammation, a longstanding pseudocyst may also induce a PSA formation, by compression, ischemia or vascular erosion from enzymes within the pseudocyst [18].

In the presented case, true aneurysm of SA was verified, that occurred due to repeated episodes of chronic pancreatitis as evident contact was seen between the pancreas and aneurysmal sac, both, on computed tomography imaging and intraoperatively, with SAA being adherent to superior margin of pancreas. However, histopathology findings showed all three layers of arterial wall without any thrombotic mass, which speaks in favor of aneurysm rather than PSA of the SA. Local secretion of pancreatic enzymes appears to have led to a weakening of the artery wall, loss of elastic tissue and aneurysmal degeneration. Increased incidence of SAA has been also reported in cirrhosis [19] and the patient in the presented case was previously treated for primary biliary cholangitis (cirrhosis).

As for the treatment options, surgical, endovascular or conservative approach could be applied in the management of patients with SAA, although endovascular treatment might be followed by serious complications [20–23]. Open surgery remains the gold standard for SAA treatment, although endovascular treatment with coil embolization or covered stenting and laparoscopic surgery are increasingly used for SAAs treatment in recent years [2]. In case of ruptured SAA, SA ligation and splenectomy could be justified, however, due to the important immune function of the spleen, spleen-preserving surgery is recommended whenever possible. In case of SAA that is sufficiently far away from the hilum, as seen in the presented case, aneurysm resection and direct arterial reconstruction with the spleen preservation should be a priority.

This is the first case to describe a true SAA aneurysm originated on the field of previous episodes of chronic pancreatitis and primary biliary cholangitis. Surgical treatment including aneurysm resection and direct arterial reconstruction with spleen preservation showed satisfactory results.

Conflict of interest: None declared.

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Хируршки третман анеуризме лијеналне артерије као последица хроничног панкреатитиса и примарног билијарног холангитиса уз очување слезине

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САЖЕТАК

Увод Анеуризма лијеналне артерије (АЛА) представља трећи узрок свих анеуризми абдомена, након анеуризми трбушне аорте и илијачних артерија, са укупном преваленцијом од 1%. Познато је да је панкреатитис повезан са стварањем псеудоанеуризме ЛА, као последица деструкције артеријског зида панкреасним ензимима, али повезаност панкреатитиса и праве АЛА још увек није описана. Приказујемо први случај праве АЛА код болесника са хроничним панкреатитисом и примарним билијарним холангитисом, успешно решен хируршком екцизијом, директном реконструкцијом артерије и очувањем слезине.

Приказ болесника Седамдесетчетворогодишњи болесник мушког пола примљен је ради мултидетекторске компјутеризоване томографске ангиографије, а због сумње на АЛА и анеуризму бубрежне артерије (АБА). Претходно је лечен од хроничног панкреатитиса и примарног билијарног холангитиса. По пријему, компјутеризована томографска артериографија је показала пречник АЛА 32 *mm* и АБА 12 *mm*, при чему је АЛА у директном контакту са горњом ивицом панкреаса. Индиковано је хируршко лечење АЛА, док је АБА лечена конзервативно. Интраоперативно је верификована АЛА адхерентна за горњу ивицу панкреаса, након чега је учињена комплетна ексклузија анеуризме и термино-терминална анастомоза ЛА. Хистопалогија је показала атеросклеротску дегенерацију артеријског зида са сва три слоја, што говори у прилог праве анеуризме. Две године након операције, контролна компјутеризована томографска ангиографија је показала уредан постоперативни налаз, без даље прогресије АБА.

Закључак Ово је први случај који описује праву АЛА, насталу на пољу претходних епизода хроничног панкреатитиса и примарног билијарног холангитиса. Хируршко лечење, укључујући ресекцију анеуризме и директну реконструкцију артерије уз очување слезине, показало је задовољавајуће резултате.

Кључне речи: анеуризма лијеналне артерије; хронични панкреатитис; примарни билијарни холангитис; хирургија очувања слезине

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CASE REPORT / ПРИКАЗ БОЛЕСНИКА

Arthroscopically assisted resection of overlooked fracture of posterior talar process

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SUMMARY

Introduction The fractures of the posterior process of the talus are relatively rare injuries of the ankle. They most frequently occur via the mechanism of the forced hyper plantar flexion and inversion. Sometimes they are not initially diagnosed, since over 40% of cases of the fractures of the posterior process of the talus are not seen in the initial radiography. The objective of this work is the review of the case study of the arthroscopically treated unhealed fracture of the posterior process of the talus.

Case outline In our case report we present a 30-year-old male, professional soccer player, with a three-month-long history of chronic pain in the region of the left ankle and heel and the fracture of the posterior process of the talus.

Conclusion The work shows all the advantages of minimally invasive surgery – arthroscopic excision of the fragment, quick recovery and returning to physical activities.

Keywords: the fracture of the posterior process of the talus; radiography of the ankle; arthroscopic excision

INTRODUCTION

Talus has a complex anatomy since it is involved in the formation of the tibiofibular, the talocalcaneal, and the talonavicular joint. About 60% of the talus surface is covered with joint cartilage. The talus consists of a body, neck, and head. Talus tissue is poorly vascularized, which often leads to avascular posttraumatic necrosis of the talus. The posterior process of the talus is composed of the lateral tubercule, the medial tubercule, and the groove for the *flexor hallucis longus* (FHL) tendon. The lateral tubercule, also known as the Stieda process, is more posterior than the medial tubercule. There are attachments for the posterior talocalcaneal and posterior talofibular ligaments on the lateral tubercule. The medial tubercule is usually smaller but varies more in size. It includes attachments for the posterior third of the deltoid ligament superior and the medial bundle of the talocalcaneal ligament inferior. The joint surface which articulates with calcaneus is located under both tubercules [1, 2].

Mechanism of injury

There are two most frequent mechanisms of injury to the posterior process of the talus. The first one is the forced hyper plantar flexion and inversion, which causes the direct pressure of the lateral tubercule between the posterior edge of the tibia and the posterior facet of the calcaneus [2, 3]. The second mechanism of injury is forced dorsiflexion and inversion, which leads to the avulsion fracture of the lateral tubercule (posterior talofibular ligament) [3]. Cedell [4] described the avulsion fracture of the posteromedial tubercule during forced pronation and dorsiflexion of the foot. The rarer mechanism is the direct trauma of the posteromedial facet by impingement of the sustentaculum during supination and forced dorsiflexion with injuries caused by high intensity forces [5].

Diagnosis and clinical features

The diagnosis is made via clinical features and imaging procedures. Swelling and pain in the posterior side of the ankle are usually present, and a positive talar impingement test, with the pain increasing during the active movements of the thumb toe flexion and extension. In a clinical examination, palpation is extremely significant in the posterior talar process as well as apprehension test with great toe flexion (FHL) in order to differentiate it from an ankle sprain [3].

The standard radiography of the ankle is a routine procedure (anteroposterior, mortise and lateral view) [6]. Sometimes Ebraheim X-ray is necessary when standard radiography is insufficient. They are angled beams with external rotation of 45°and 70° [7]. In more than 40% of cases, the fracture of the posterior process of talus is not registered in initial radiography. In such cases, when difficulties are persistent, it is necessary to do computed tomography (CT) and magnetic resonance imaging (MRI). CT and MRI are not needed both, but on CT we could see position of fractured fragments and the relationship between them,

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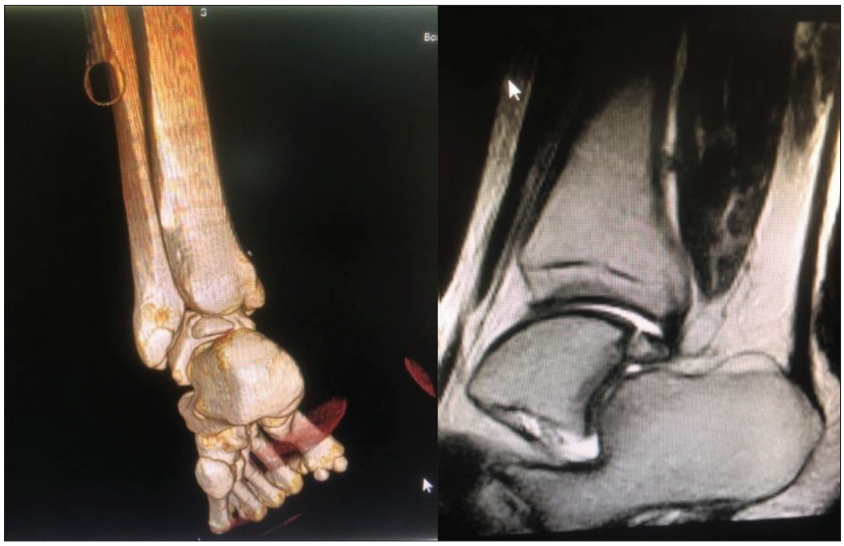

Figure 1. Computed tomography and magnetic resonance imaging showed posterior talar process fracture

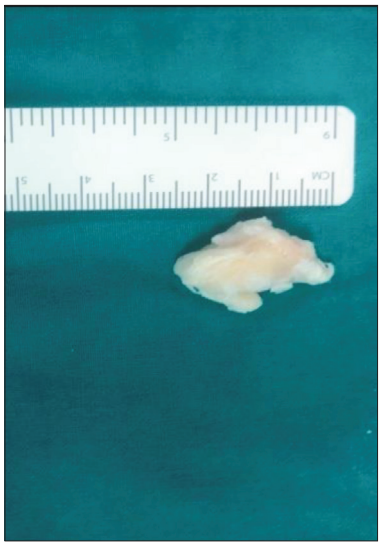

Figure 3. The free body was removed through the posteromedial portal

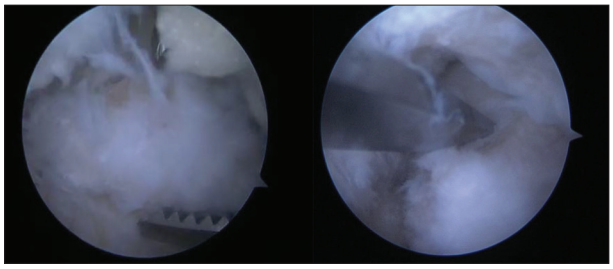

Figure 2. Arthroscopic excision of the posterior process of the talus

and on MRI we could see if there is damage to the soft tissue, especially the FHL tendon [8].

Fractures of the posterior process of the talus

Fractures of posterolateral process (Shepherd fracture)

Fractures of the posterolateral process can be mistaken for an *os trigonum*, which develops as a consequence of secondary ossification when the fusion with the talus body fails. The differentiation between the fracture of the posterolateral process of the talus and *os trigonum* can be performed via CT and nuclear magnetic resonance diagnostics. The clinical picture of the Shepherd fracture can be very similar to an ankle sprain. It differs in the pain during movements in the subtalar joint and the passive movement of the FHL tendon.

Fractures of the posteromedial process of the talus (Cedell fracture)

Cedell [4] first described the fracture of the medial part of the posterior process of the talus. It can also be mistaken for an ankle sprain if the posteromedial pain is ignored.

Fractures of the posterior process of the talus

The fractures of the entire posterior process of the talus are very rare. There are only several described cases [9, 10].

The aim of this work is the case study of the unhealed fracture of the posterior process of the talus with a professional footballer. The applied method of treatment – arthroscopic excision of the bone fragment of the posterior process of the talus – was chosen as a recommended surgical treatment in the modern orthopedic surgery.

CASE REPORT

A 30-year-old male, professional footballer with a three-month-long chronic pain in the region of the left ankle and heel was examined in our hospital for the first time in August 2018 when he was sent to additional CT and MRI diagnostics (Figure 1). The examination revealed the presence of posteromedial pain on palpation and a positive FHL test. Prior to this, he had been treated with physical procedures in another hospital, after the injury by a distortion mechanism in April of the same year. CT and MRI diagnostics revealed a nonunion fracture of the posterior process of the talus and an indication for surgical treatment was set.

The surgery was performed in the conditions of block anesthesia and tourniquet control (280 mmHG) with the patient in prone position. After the surgical field cleaning and preparation, posteromedial and posterolateral portals were created at the level of the lateral malleolus top, medially and laterally in regard to the Achilles tendon. The arthroscope, 4 mm in size, was inserted through the posterolateral portal, and shaver was inserted through the posteromedial portal. After debridement had been performed, a free body by fibrous tissue, i.e., the broken fragment of the posterior process of the talus fixed, was discovered (Figure 2). The free body was then removed through the posteromedial portal (Figure 3). After irrigation, both

portals were closed. Antibiotic and thromboprophylaxis therapy were conducted with the patient according to the protocol (cefazolin 1 g in bolus administered intravenously and nadroparin 0.4 ml subcutaneously 1 × 1 until the 21st postoperative day).

The patient was suggested physical therapy after the discharge, which he conducted completely.

Two weeks after the surgery, at the control examination, the patient was free of any difficulties and with the full range of motion of the left ankle, the FHL test was negative, without any pain posteromedially.

Written consent for publication of this this case report and any accompanying images has been obtained by the patient's family member.

DISCUSSION

The fracture of the posterior process of the talus is an infrequent injury difficult to notice by radiography and is frequently overlooked. The treatment of the fracture of the posterior process of the talus are the following: restoring the talus anatomy which prevents a later nonunion of bones or posterior impingement syndrome. Non-displaced fractures of the posterior process of the talus are treated by immobilization in a period of six to eight weeks. With displaced fractures of the posterior process of the talus, surgical treatment is recommended depending on the fragment size (osteosynthesis or excision) [11, 12]. Surgical treatment can be performed by open reduction and internal fixation or the excision of the bone fragment of the posterior process of the talus. Posterolateral or posteromedial approaches can be used for this surgery depending on the localization of the bone fragment. In both of these approaches, surgical trauma is significant due to the deep position of the posterior process in the posterior aspect of the ankle. The complications of an open surgery are a non-healing wound, considering the region, and a greater possibility of infection [13, 14].

Many patients who are treated non-surgically need surgical treatment later because of the fracture nonunion or tarsal tunnel syndrome. Consequently, Swords et al. [15] recommend acute excision of the posterior process of the talus. Due to significant surgical trauma in open surgery, these arthroscopic surgical techniques are being increasingly applied.

Arthroscopic surgery is a minimally invasive surgery with two small portals posterolaterally and posteromedially in regard to the Achilles tendon at the level of the posterior process of the talus [16]. In an arthroscopic procedure, the complications may be damage to neurovascular structures in the posteromedial compartment, the branch of the posterior tibial artery, the lateral and medial plantar nerve, and an injury to the FHL tendon [17]. In our case, an excision of the bone fragment was performed arthroscopically.

American Orthopaedic Foot and Ankle Society (AOFAS) rating score is a clinical score that evaluates the function of the ankle and foot before and after treatment, where the maximal score of 100 corresponds to the normal function of the ankle. In our case, after the arthroscopic removal of the posterior process of the talus, AOFAS score was 96.3, and before the surgery it was 60.5.

The use of the arthroscopic method of treatment of this injury is on the increase nowadays because of all of the advantages of minimally invasive surgery [18, 19, 20]. In their study, Zwiers et al. [21] followed 410 patients treated by open or endoscopic treatment methods. They proved that the arthroscopic treatment of the fracture of the posterior process of the talus enables significantly faster recovery and returning to sports activities (15.9 weeks with open surgery and 7.2 weeks with arthroscopic surgery). They also proved that major and minor complications are less frequent (15.9% with open surgery *vs.* 7.2% with arthroscopic surgery) and that the AOFAS score is higher in patients treated with arthroscopic method compared to patients treated with open surgery. Fractures of the posterior process of the talus are injuries which appear with younger physically active persons. These fractures can sometimes be overlooked due to poor radiography signs. This fracture can be diagnosed late, and can give nonunion, pseudoarthrosis, and a consequential posterior impingement syndrome. CT of the ankle must be performed with all suspicious cases. If the fracture is diagnosed late, or the bone fragment is too small, the arthroscopic excision of the bone fragment of the posterior process of the talus is recommended. Osteosynthesis can be performed in acute trauma and if the fragment is large enough. In such cases of late diagnosed fractures, we suggest arthroscopic excision of the fragment as a preferred method and not osteosynthesis.

Conflict of interest: None declared.

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Артроскопски асистирана ресекција превиђеног прелома задњег наставка талуса

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САЖЕТАК

Увод Преломи задњег наставка талуса су релативно ретке повреде скочног зглоба. Настају најчешће механизмом форсиране плантарне флексије и инверзије. Понекад се иницијално не дијагностикују, јер се преко 40% случајева прелома задњег наставка талуса не види на иницијалним радиографијама.

Циљ овог рада је приказ случаја артроскопски леченог несраслог прелома задњег наставка талуса.

Приказ болесника У нашем приказу болесника приказали смо прелом задњег наставка талуса код тридесетогодишњег мушкарца, професионалог фудбалера, са хроничним болом који траје три месеца у пределу левог скочног зглоба и пете.

Закључак Рад показује предности минимално инвазивне хирургије – артроскопске ексцизије фрагмента, бржи опоравак и враћање физичким активностима.

Кључне речи: прелом задњег наставка талуса; радиографија скочног зглоба; артроскопска ексцизија

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CASE REPORT / ПРИКАЗ БОЛЕСНИКА

Breast implant rupture 37 years after breast augmentation

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SUMMARY

Introduction Silicone implants have been used ever since the second half of the 20th century. Over that period, several generations of implants have been developed that differed in thickness of the shell and viscosity of the silicone gel. Development of these generations of implants was accompanied with different complication rates. The first-generation implants had the lowest tendency to rupture, but were more prone to capsular contracture and calcification formation.

Case outline An 81-year-old female patient had her silicone implants placed in 1983. After a chest injury in 2015, on the lateral aspect of the left breast a tumefaction becomes palpable and she complains of pain. She denied any subjective problems before the injury. After pertinent diagnostic procedures and clinical examination, silicone implant rupture was suspected. Surgical findings confirmed ruptures of both implants so that they were extracted, capsulectomy was performed and the surrounding tissue imbibed with silicone removed. Samples were sent for histopathological examination.

Conclusion Implant rupture is one of late complications of breast augmentation. The incidence of ruptures has changed with development of newer generations of silicone implants. We believe that our patient had the first-generation silicone implants, knowing the time of their placement to the occurrence of symptoms and macroscopic appearance of the shell after extraction. The fact is that these implants have proved to be very durable, but regardless of the lack of symptoms, current guidelines recommend regular screening for rupture, while possible preventive extraction, particularly in case of so old implants should be considered.

Keywords: implant rupture; silicone implants; breast augmentation

INTRODUCTION

Augmentation mammoplasty is a surgical procedure where the use of silicone implants or transfer of fatty tissue result in breast enlargement, regaining of the volume or achieving the desired shape [1]. Augmentation mammoplasty is one of the most commonly performed procedures in esthetic surgery worldwide. Since 2006 it has been the most commonly performed esthetic operation in the US. In 2019 only in the US 2.3 million esthetic operations were performed, excluding minimally invasive procedures. Out of these, 193,073 were augmentation mammoplasties, accounting for 8% of the total number [2].

Silicone implants have been used for over half a century. Generations of implants have been developed that differed in thickness of the shell and composition of the filling [3]. Complications after breast enlargement can be classified into early and late. Early complications include infection, asymmetry, hematoma, seroma, pain, altered sensations. Late complications include change of implant position, implant rupture, contracture and other [4, 5]. Implant rupture most commonly results from the implant age, trauma or can occur due to iatrogenic damage [6]. Silicone implant rupture could potentially require surgical treatment with extraction of the ruptured implant. Depending on whether it is an asymptomatic or symptomatic rupture, treatment options should be discussed with the patient while presenting the potential benefits, risks, and costs of implant removal. Patients with asymptomatic rupture should be presented with a choice between continued periodic imaging or surgical treatment [3], while those with symptomatic rupture should be advised to undergo surgical treatment in order to eliminate subjective symptoms or additional clinical problems [3]. Treatment of other complications that can potentially develop as a result of rupture and imbibition of the surrounding tissue with silicone gel could also be required. The purpose of this report is to describe a potential longevity of older breast implant generations and absence of symptomatic rupture in the presented case for more than 37 years, with highlighting screening, diagnostic and treatment options.

CASE REPORT

An 81-year-old female patient was admitted to the Clinic for Burns, Plastic and Reconstructive Surgery of the University Clinical Center of Serbia in August 2020 complaining of pain and

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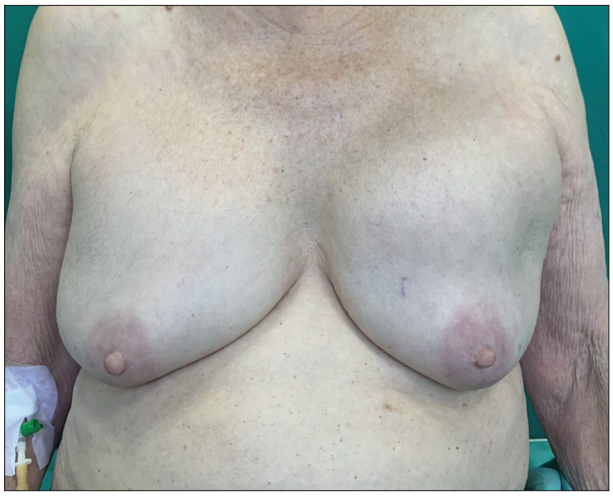

Figure 1. Clinical examination revealed breast asymmetry

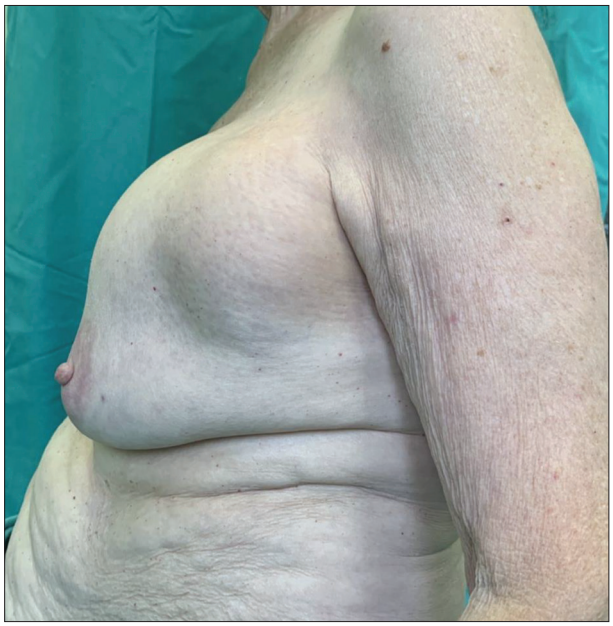

Figure 2. In the upper left quadrant, there was a tumefaction of about 5 × 5 cm, insensitive to palpation, partially fixated, of hard consistency, without signs of inflammation present

presence of tumefaction in the area of her left breast. Her medical history revealed that she had breast implants placed in 1983 for augmentation purposes. She said that she had fell five years previously and injured her chest on the left. Ever since, she could feel a tumefaction of about 1 × 1 cm that had gradually grew. Clinical examination revealed breast asymmetry (Figure 1). In the upper left quadrant there was a tumefaction of about 5 × 5 cm, insensitive to palpation, partially fixated, of hard consistency, without signs of inflammation present (Figure 2). Mammography suggested signs of herniation of the implant towards the axillary extension, i.e., differential diagnosis suggested a rupture. The right implant also had uneven edges. Ultrasound scan revealed blurred lines of the capsule in the external quadrant of the left breast above which there was a hyperechogenic area that was suggestive of imbibition of the surrounding tissue due to extravasation of the implant filling. In the upper external quadrant of the left breast, there was a non-homogenous

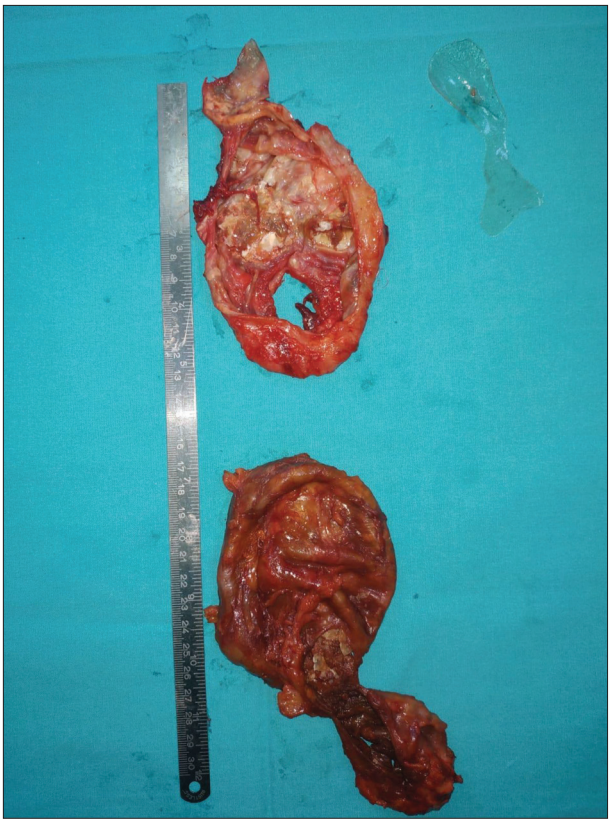

Figure 3. Both implants, both connective tissue capsules and silicone imbibed surrounding tissue were removed

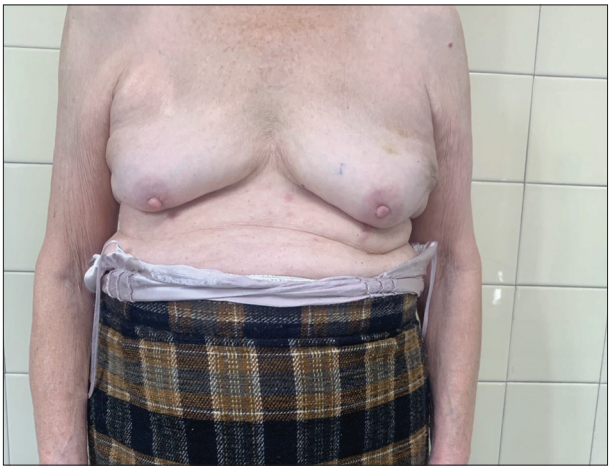

Figure 4. Postoperative follow-up

area with mildly affected tissue architecture, 26 × 14 mm, along the implant itself. Towards the axillary extension of the left breast an oval discrete structure, about 68 × 46 mm, suggestive of herniated part of the implant is seen.

On the basis of mammography, echotomography and clinical examination, surgical treatment was indicated. Both implants, both connective tissue capsules and silicone imbibed surrounding tissue were removed (Figure 3). The tissue was sent for histopathological examination. The results verified the presence of hyalinized capsule with calcifications and multinuclear giant cells filled with polarized foreign matter (silicone). On follow-up, the patient was overall satisfied with the outcome (Figure 4).

We confirm that we have read the journal's position on issues involving ethical publication and affirm that this work is consistent with those guidelines.

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Written consent to publish all shown material was obtained from the patient.

DISCUSSION

A rupture can be intracapsular or extracapsular. Normal body reaction to the presence of an implant as a foreign body is to produce a fibrous tissue capsule in order to limit it. Intracapsular rupture refers to spilling of the content within the fibrous capsule. With leaking of the content beyond the fibrous capsule limits, it becomes an extracapsular rupture. An extracapsular rupture enables further spreading of the content and imbibing of the surrounding tissues. Possible symptoms of a rupture include breast asymmetry, change in the size, shape and firmness of breast, pain, palpable changes, when a rupture is symptomatic. Signs and symptoms of a silicone implant rupture usually develop later, due to slow leaking of silicone due to its higher density and lack of absorption. In most patients a rupture is not accompanied with any major signs and symptoms and is accordingly called a "silent" i.e., asymptomatic rupture [2]. Silicone implants are classified into generations on the basis of development of the external shell and gel material they are filled with.

The first generation was used in the sixties and seventies. These implants had a thick shell and highly viscous gel, resulting in very firm and long-lasting implants. The incidence of ruptures was low, but the incidence of capsular contracture and calcification was high [7]. The second generation was designed with much thinner external shell and less viscous silicone gel. As a result of these design changes the incidence of rupture was much higher and was combined with the "silicone bleeding" phenomenon, i.e., leaking of silicone into the surrounding tissue through the shell itself due to increased fluidity of the implant filling [8, 9]. High incidence of ruptures resulted in discontinuation of use of this generation of implants. The third generation of implants was used from late eighties to 1992 when the Food and Drug Administration (FDA) moratorium on the use of silicone implants came into force [10]. After pertinent trials the moratorium was lifted in 2006 and in the meantime two more generations of breast were developed, which are currently used [7].

In the management of symptomatic and asymptomatic patients several diagnostic modalities can be used in evaluation of a potential implant rupture. These are: (magnetic resonance imaging) MRI, ultrasound, computed tomography, mammography with initial clinical examination. Clinical examination on its own is not an adequate method in assessment of a suspected rupture. MRI is broadly recommended and accepted diagnostic method worldwide. Numerous studies have established its sensitivity and specificity in detection of implant ruptures at 72–94% and 85–100%, respectively [11, 12, 13]. The latest FDA recommendations relating to screening of implant patients specify the following: for asymptomatic patients, the first ultrasound or MRI should be performed at 5–6 years postoperatively, then every 2–3 years thereafter; for symptomatic patients or patients with equivocal ultrasound results for rupture at any time postoperatively, an MRI is recommended [14]. Patients with asymptomatic rupture are presented with a choice between continued periodic imaging or surgical treatment [3], Due to the absence of scientific evidence to clearly support the benefit of removing an asymptomatic ruptured implant, the decision about whether or not to do so should be left to the patient [3]. In case of symptomatic ruptured implant patients should be motivated to undergo surgical treatment in order to eliminate subjective symptoms or additional clinical problems [3]. Surgical treatment implies implant extraction with complete capsulectomy. In the reported case, convincing clinical findings accompanied with ultrasound and mammography were sufficient to suspect ruptures and indicate surgical treatment. The implants were removed on both sides also complete capsulectomy was performed with removal of the surrounding tissue imbibed with silicone. It was also noted that the right breast, preoperatively without signs or symptoms, also had some silicone gel in the capsule, together with connective tissue and macroscopically visible calcification. The patient in this particular case had an almost 40-year-old implant. We believe that these were first generation implants, having the patient's history, age, late occurrence of symptoms of rupture and macroscopic appearance of implants after extraction [7]. We report this case to show that even in almost 40-year-old implants the symptoms of rupture need not necessarily develop, having the macroscopic appearance of her right breast and absence of subjective symptoms relating to the right breast. Also, the absence of symptoms did not correlate with the local and microscopic finding inside the right breast capsule. It remains to be answered how long the patient would remain symptom-free and without any further potential complications if she had not suffered the left breast injury, as described above. The case report supports a possible need for a higher compliance with the United States FDA recommendations relating to periodic screening in order to identify asymptomatic ruptures and other implant-related complications, especially in older generation silicone implants. It is undeniable that throughout the years, breast implant technology has evolved, nevertheless implant rupture with intracapsular and extracapsular silicone leakage continues to be a problem plastic surgeons face in everyday practice. The impact of symptomatic and asymptomatic, particularly extracapsular implant rupture should be investigated further to learn more about development of further complications, overall health of patients alongside with further investigation of diagnostics, screening and management options for such complications.

Conflict of interest: None declared.

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Руптура имплантата 37 година после аугментације груди

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САЖЕТАК

Увод Силиконски имплантати у употреби су од друге половине 20. века. Током тог периода развијено је више генерација имплантата који су се разликовали на основу дебљине капсуле и вискозности силиконског гела. Кроз развој генерација имплантата, мењала се и учесталост компликација. Имплантати прве генерације показали су најмању тендецију ка руптури, али су били склонији капсуларној контрактури и формирању калцификата.

Приказ болесника Пацијенткиња стара 83 године уградила је силиконске имплантате 1983. године, а 2015. године је пала и повредила леву страну грудног коша. Након повреде долази до појаве палпабилног тумефакта у пределу леве дојке праћеног боловима. Пре повређивања негира постојање икаквих субјективних тегоба. Дијагностичким процедурама и клиничким прегледом постављена је сумња на руптуру силиконског имплантата. Оперативним налазом потврђена је руптура оба имплантата, те је учињена екстракција силиконских имплантата, капсулектомија и уклањање околног силиконом имбибираног ткива, а препарати су послати на хистопатолошку анализу.

Закључак Руптура имплантата представља једну од касних компликација аугментације груди. Учесталост руптуре мењала са развојем генерација силиконских имплантата. Мишљења смо да су код наше пацијенткиње уграђени силиконски имплантати прве генерације, узимајући у обзир време протекло од њихове уградње до појаве симптома и макроскопски изглед опне након екстракције. Чињеница је да су се ови имплантати показали као веома издржљиви, али без обзира на изостанак симптома, према савременим препорукама саветује се редован скрининг и евентуална превентивна екстракција, поготову овако старих имплантата.

Кључне речи: руптура имплантата; силиконски имплантати; аугментација груди

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CASE REPORT / ПРИКАЗ БОЛЕСНИКА

Herpes zoster in an immunocompetent infant with a history of varicella in early infancy and after a minor injury – case report and literature review

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SUMMARY

Introduction Chickenpox is a common pediatric disease, while herpes zoster (HZ) is rare among children, especially among infants. HZ in infancy may appear after intrauterine or postnatal infection with varicella-zoster virus (VZV). We report on a case of HZ in an immunocompetent infant who had a history of chickenpox in early infancy.

Case outline A seven-month-old male infant was presented with skin changes in the left T1 and T2 dermatomes. Skin changes appeared eight days after the infant had a mild left-arm traction injury. The patient's medical history revealed that he had a mild form of chickenpox at the age of three and a half months. After the clinical diagnosis of HZ was made, he was treated with oral acyclovir 20 mg/kg every six hours for five days and had complete recovery without any sequelae.

Conclusion Risk factors for pediatric HZ are immunosuppression and chickenpox during the first year of life. Local trauma is a reported risk for VZV reactivation among adults. To our best knowledge, our case is the first reported pediatric case in which the injury of the left arm precedes HZ appearance. Routine vaccination against chickenpox may be an important preventive measure because herd immunity will protect infants and immunocompromised children from getting chickenpox and thus HZ.

Keywords: herpes zoster; infants; trauma

INTRODUCTION

Herpes zoster (HZ), also known as shingles, occurs as a result of reactivation of varicella-zoster virus (VZV) which persists in latent form within sensory ganglia after primary infection. HZ is rare in immunocompetent children. In the first year of life, HZ may appear as a consequence of intrauterine infection or after birth due to postnatal infection with VZV. We report a case of HZ in an immunocompetent infant who had a history of chickenpox in early infancy. We also review the literature on risk factors for the development of HZ infection in children.

CASE REPORT

A seven-month-old male infant was presented to the outpatient clinic with a three-day history of skin changes in the left armpit and left upper arm. Skin changes appeared eight days after the child had a mild left-arm traction injury. The injury happened on the same day when the child received the vaccine against hepatitis B. Radial head subluxation was diagnosed by an orthopedic surgeon who performed a reduction maneuver in the outpatient settings after which the infant regained full mobility of the arm. The patient's medical history revealed that he had been a full-term normal delivery with a birth weight of 2.9 kg. At the neonatal ward, complete blood count and routine blood biochemical analysis were performed and the results were as follows (normal range for age is shown in parentheses): leukocyte 18.9×10^9/L ($5–21 \times 10^9$/L), hemoglobin 185 g/L (135–195 g/L), platelet 330×10^9/L ($150–450 \times 10^9$/L). The differential leukocyte count showed 48% of neutrophils (19–49%), 3% of bands (0–15%), 35% of lymphocytes (26–36%), 14% of monocytes (7–18%), 0% of both basophils (0–1%) and eosinophils (0–1%). Blood levels of glucose, urea, creatinine, total protein, albumin, total and direct bilirubin, sodium, chloride, potassium, calcium, aspartate transaminase, alanine transaminase, and C-reactive protein were normal. The infant was breastfed for the first five months of life. He was fully vaccinated according to schedule (including Bacillus Calmette–Guérin vaccine at the neonatal ward, which is part of a routine vaccination program in Serbia) and he did not experience any vaccine side effects. He had a mild form of chickenpox with around 50 skin changes and with no accompanying fever at the age of three and a half months. As an epidemiological risk factor, parents reported indoor gatherings with friends and family two weeks before varicella

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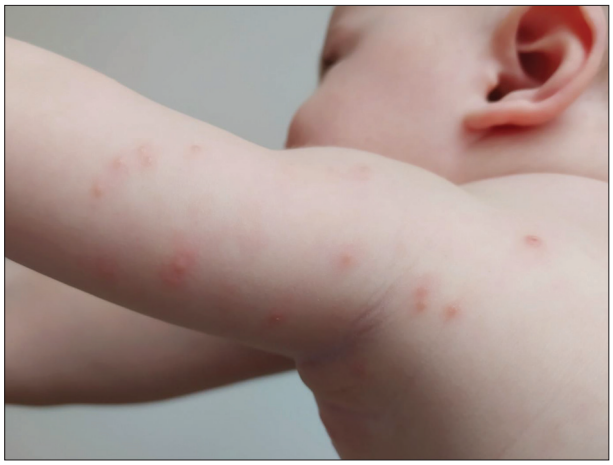

Figure 1. Initial clinical presentation of the patient with papules, vesicles, and crusts within left T1 and T2 dermatomes

eruption, but the definitive source of the infection remains unknown. The infant's mother had chickenpox at age of eight years. Also, the mother was tested for HIV and TORCH pathogens during the first trimester of pregnancy and all tests were negative. The rest of the past medical history was unremarkable.

On physical examination, the infant's weight was 8100 g (at the 40th percentile) and his length was 70 cm (at the 65th percentile). There was polymorphic rash (papules, vesicles, and crusts) in dermatomes T1 and T2 (Figure 1). The infant was slightly irritable while the rest of the examination was normal. At that time, his complete blood count was as follows (normal range for age is shown in parentheses): leukocyte 14.25 × 10^9/L (6–17 × 10^9/L), hemoglobin 116 g/L (109–138 g/L), platelet 412 × 10^9/L (150–450 × 10^9/L). The differential leukocyte count was: neutrophils 18.7% (23–66%), lymphocytes 72.5% (18–60%), monocytes 5.03% (5–13%), basophils 0.37% (≤ 2%), eosinophils 3.4% (≤ 6%). We established the diagnosis of HZ based on clinical presentation and medical history.

The infant received treatment with oral acyclovir 20 mg/kg every six hours for five days. Ten days after the start of treatment all skin changes became crusts (Figure 2). At the next follow-up visit, four weeks after diagnosis, complete resolution was noticed. In the six months follow-up time, there were no sequelae, his psychomotor development was normal and he did not suffer from any other infection during this period.

Parents provided written consent to report the case of their child.

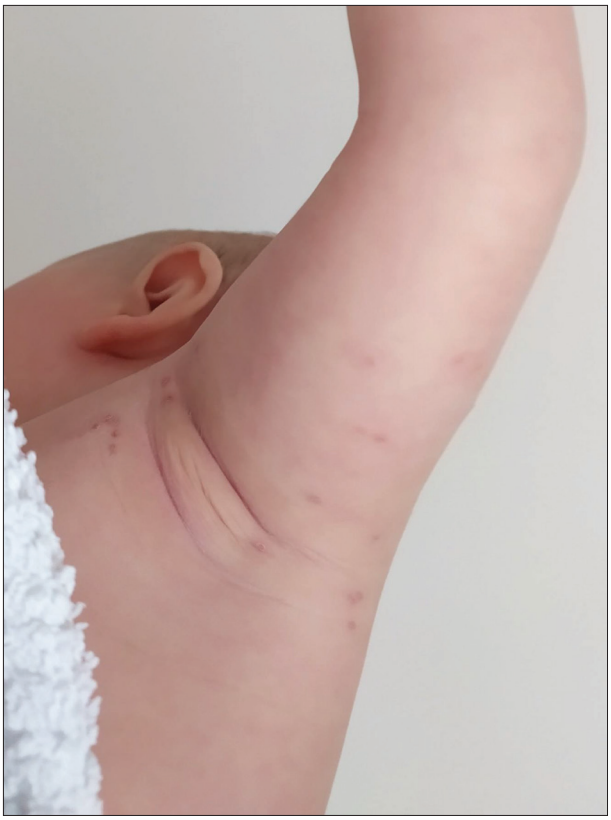

Figure 2. Clinical appearance at the follow-up visit 10 days after the start of treatment

DISCUSSION

Primary infection with VZV causes varicella, also known as chickenpox. Chickenpox is a disease of childhood with > 90% of individuals tested being seropositive by 10 years of age in most countries [1]. In Serbia, where vaccine against VZV is not part of the routine vaccination schedule, the percentage of seropositive results increases from 41.2% in the age group of 1–4 years to 92.7% in the age group of 15 to 19 years [2]. The seroprevalence among infants younger than one year is 75% which is higher than in the age group 1–4 years, probably due to the presence of passively transplacental transfer of maternal antibodies [2]. During the first year of life infection with VZV can be acquired transplacentally (if the mother was seronegative before pregnancy and acquire the infection during pregnancy) or after postnatal exposure. Regarding postnatal exposure, infants born from seropositive mothers are at lower risk to contract varicella in the first few months after birth. However, if an infant develops the disease, he/she will have a mild form of the disease or subclinical infection because of transplacentally transferred maternal VZV-specific antibodies, as was the case in our patient [3].

After primary infection settles down, the virus remains latent in sensory nerve ganglia. The reactivation of the latent virus manifests as HZ. The triggers for reactivation are still not well understood, but declining VZV-specific cell-mediated immunity plays an important role. The known risk factors for the appearance of HZ are immunocompromising states such as malignancy (especially leukemia and lymphoma), HIV infection, organ transplantation, use of immunosuppressive medications (e.g., chemotherapy agents, corticosteroids), and chronic diseases [4, 5].

The overall incidence of HZ among children younger than 18 years of age was estimated to be 74 per 100,000 person years [6]. The incidence among unvaccinated children younger than five years is 20 per 100,000 person years [7]. The incidence of HZ among vaccinated children is 78%

lower than among unvaccinated children and the incidence among immunocompetent children was 5–6 times lower than in immunocompromised children [6]. A higher risk for HZ was found in children who had chickenpox during their first year of life [6]. The probable cause is that immature adaptive T-cell response is less able to withhold VZV in the latent state [8]. In countries where vaccine against chickenpox is a part of the routine vaccination schedule, the trend of decreasing HZ incidence among unvaccinated children was noted. It is likely due to a lack of primary VZV infection resulting from herd immunity in a highly vaccinated population [6]. In children who had chickenpox during the first year of life, the duration of latency ranges 8–142 months, which is significantly longer than 3.5 months of latency as it was in our case [9]. The probable cause of this early virus reactivation was a minor injury to the left arm. Studies on adults showed the association between HZ and trauma at the HZ site and the effect was rapid peaking in the first week after trauma, which is consistent with our case [10, 11]. Although injury-induced VZV reactivation is not well understood, the probable mechanism is excessive stimulation of the sensory nerve which triggers reactivation of the latent virus in the sensory ganglion. Another possible mechanism may be local immunity impairment by direct trauma and subsequent immune escape of latent virus [10]. Reactivations of VZV after different types of vaccines (vaccine against influenza, hepatitis A, rabies, Japanese encephalitis, and COVID-19) were described in adult patients, but to the best of our knowledge HZ after hepatitis B vaccine was not reported yet [12, 13, 14]. Immunomodulation after hepatitis B vaccine was observed, but there is weak evidence that hepatitis B vaccine contributed to VZV reactivation in our case [15].

HZ can be a manifestation of cellular or combined immunodeficiency. Our patient did not fulfill any of the clinical criteria, which would indicate the need for diagnostic evaluation for primary immunodeficiency [16]. Also, he did not have risk factors for acquired immunodeficiency (e.g., immunosuppressive therapy, malignancy).

The most frequent skin localization of HZ in children is thoracic dermatomes, as it was in our patient [17, 18, 19]. Classical clinical presentation of HZ in an immunocompetent child includes the appearance of papules and vesicles within one, rarely two adjacent dermatomes. Later on, crusts develop from papules and vesicles. Pain and/or pruritus in the involved area may precede rash. Systemic manifestations such as fever, lymphadenopathy, and headache may occur. The differential diagnosis of HZ includes impetigo, poison ivy rash, *strophulus infantum*, and *miliaria rubra*. Our patient had a typical clinical presentation, therefore careful history taking, physical examination, and observation of skin changes evolution were sufficient to establish the diagnosis of HZ. In cases with atypical lesions, the diagnosis may be confirmed by detection of multinucleated giant cells in the Tzanck smear of scrapings from the floor of vesicles or by positive VZV PCR in vesicle fluid. Pediatric HZ has a good prognosis. The most common complication among immunocompetent children is a bacterial skin infection, while postherpetic neuralgia, the most common complication in adults, is less frequent in children [20].

Considering that HZ has a mild course in most children with a lower incidence of complications than in adults, antiviral therapy is usually reserved for immunocompromised patients and patients with disseminated disease. We decided to treat our patient with oral acyclovir because it was reported that antiviral therapy shortens the disease course and period of contagiousness, antiviral therapy should be considered in immunocompetent patients of any age with HZ to accelerate healing and prevent further spread among susceptible individuals [19]. The benefit is the most obvious if the therapy is started in the first 72 hours of illness [21].

Children who get chickenpox during the first few months after birth can have a mild form or even clinically unrecognized disease if the mother had immunity to VZV before pregnancy. It could be challenging to diagnose HZ in such cases. Although an injury is a well-known risk factor of HZ in adults, to the best of our knowledge, our patient is the first described pediatric case of injury precipitated HZ. Routine vaccination against chickenpox may be an important preventive measure because herd immunity will protect infants and immunocompromised children from getting chickenpox and thus HZ.

Conflicts of interest: None declared.

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Херпес зостер код имунокомпетентног одојчета после лаке повреде са анамнезом овчијих богиња у раном одојеначком узрасту – приказ болесника и преглед литературе

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САЖЕТАК

Увод Овчије богиње су честа болест у педијатријском узрасту, док је херпес зостер (ХЗ) редак код деце, а изузетно редак код одојчади. У одојеначком периоду ХЗ се може јавити као последица интраутерине или постнаталне инфекције вирусом варичела-зостер. Приказали смо случај ХЗ код имунокомпетентног одојчета које је прележало овчије богиње у раном одојеначком периоду.

Приказ случаја Мушко одојче узраста седам месеци прегледано је због кожних промена у регији Т1 и Т2 дерматома лево. Кожне промене које одговарају ХЗ су се појавиле осам дана након што је одојче имало мању тракциону повреду леве руке. Из личне анамнезе сазнали смо да је одојче имало овчије богиње у узрасту од три и по месеца. Након што је клинички постављена дијагноза ХЗ, одојче је лечено оралном применом ацикловира у дози 20 *mg/kg* на шест сати током пет дана и потпуно се опоравило, без икаквих последица.

Закључак Фактори ризика за појаву ХЗ код деце су стање имуносупресије и овчије богиње у првој години живота. Код одраслих се као један од факора ризика наводи претходна траума у регији ХЗ. Према нашим сазнањима, ово је први описан случај ХЗ у одојеначком узрасту где је повреда леве руке претходила појави ХЗ и вероватно била окидач за реактивацију вирус варичела-зостер. Рутинска вакцинација против вируса варичела-зостер би била важна превентивна мера, јер би стицањем колективног имунитета имунокомпромитовани и одојчад били заштићени од оболевања од овчијих богиња, а самим тим и ХЗ.

Кључне речи: херпес зостер; одојчад; повреда

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PRELIMINARY AND SHORT COMMUNICATION / ПРЕТХОДНО И КРАТКО САОПШТЕЊЕ

Routes and types of microbial infection in the pathology of pancreatic adenocarcinoma

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SUMMARY

Introduction/Objective Pancreatic cancer may be accompanied by infections caused by various microorganisms. It is uncertain wheatear pancreatic infection precedes the development of cancer or vice versa. The aim of this study is to analyze routes of infections from the duodenum through the pancreatic duct to determine what types of microorganisms can get through this duct into the pancreas and nearby tissue.

Methods In patients subjected to cephalic duodenopancreatectomy (Whipple procedure) due to adenocarcinoma of the ampulla of Vater, the duodenum or head of the pancreas, swabs from duodenal mucosa, pancreatic duct, and pancreatic tissue at the line of the resection were taken. Microscopic slides were prepared directly from the patients' specimens as well as from colonies on culture plates, and both were Gram stained.

Results *Candida* was present in all three types of swabs (duodenum, pancreatic duct, and tissue), while bacteria, depending on the species (*Pseudomonas aeruginosa*, α-hemolytic *Streptococcus*, coagulase-negative *Staphylococcus*, *Enterococcus spp*., *Serratia spp.)*, were present in pancreatic duct or tissue, but not in the duodenum.

Conclusion There is a connection between the presence of microorganisms and pathology of the pancreatic adenocarcinoma. Results show that *Candida* infection originates from the duodenum, while bacterial infections originate directly from blood or tissue injuries.

Keywords: *Candida albicans*; *Pseudomonas aeruginosa*; pancreatic adenocarcinoma; pancreas infections; duodenum

INTRODUCTION

Pancreatic cancer belongs to the group of cancer with high lethal outcome. Symptoms are often detected very late and cure rates are very low. Pancreatic cancer development is often preceded by inflammation, such as pancreatitis, which increases the chance of tumor development. Infections can worsen acute pancreatitis by inflammation expansion and tissue necrosis [1].

It is well known that certain pathological conditions such as acute pancreatitis, necrotic pancreatitis, and cysts are caused by Gram-positive bacteria (74%), Gram-negative bacteria (21%), *Enterobacter spp*. (up to 58%), and *Candida albicans* (5–24%). Pancreatic infections can also be caused by infected venous catheter, urinary tract, tracheal mucosa, and bile. The fact that *Candida* infection of pancreatic pseudocysts can lead to sepsis or even death due to organ failure shows the perils of infection with this organism [2]. Infections with *Candida spp*. caused by spontaneous perforation or surgical opening of the gastrointestinal tract may increase general mortality [3].

Pancreatic infections mostly originate from the duodenum. Some studies showed infections of the pancreatic duct with *Candida* [4, 5]. Data show that microorganisms from the intestine can access the pancreas in three ways: directly from the duodenum via the pancreatic duct, penetration into the body cavity due to injuries, and via blood [1, 4].

So, how do microorganism from the intestine get to the pancreas. There are some pathological conditions that facilitate penetration of microorganisms from the intestine into the pancreas such as decreased secretion of pancreatic juice (in acute and chronic pancreatitis), weakening of the sphincter of Oddi (often present in elderly populations), anatomical changes in the gastrointestinal tract, tumors, or abundant and frequent food intake that may cause stretching of the stomach and intestine, thus leaving the sphincter of Oddi opened or dilated [6, 7].

The main aim of this study is to analyze routes of infections from the duodenum through the pancreatic duct to determine what types of microorganism can gain access through this duct into the pancreas and nearby tissue. Another aim is to establish a possible connection between infection and the pathology of pancreatic adenocarcinoma.

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METHODS

In patients subjected to Whipple's procedure (duodenopancreatectomy) due to adenocarcinoma of the ampulla of Vater, duodenum, or head of the pancreas, swabs from the duodenal mucosa at the line of the resection, from the pancreatic duct, and pancreatic tissue were taken [8]. All surgical procedures were performed at the First Surgical Clinic, University Clinical Center of Serbia. All subjects enrolled in this research responded to an informed consent form approved by the institutional Ethics Committee on Human Research and this protocol was found acceptable by them. From January to June 2018, 24 patients were operated on – 16 men and eight women, aged 58–65 years. The patients were from the city of Belgrade. Microbiological analyses were performed according to the standard procedure at the Institute for Microbiology, Faculty of Medicine, University of Belgrade. Microbiology isolates were identified on the basis of microscopic, cultural, and biochemical properties. Microscopy slides were prepared directly from patient specimens as well as from colonies on culture plates, and then Gram stained. We used Columbia blood agar plates (7% sheep blood), MacConkey agar and XLD agar plates, and Sabouraud dextrose agar. Depending on the cultural and morphological characteristics of the microorganisms isolated, we prepared a small series of biochemical tests.

RESULTS

The results are shown in Table 1. Swab analysis showed no pathogenic microorganisms in the duodenum except for *Candida* in 16.7% of the cases. The pancreatic duct was infected with α-hemolytic *Streptococcus*, coagulase-negative *Staphylococcus*, *Enterococcus spp.*, *Rhodotorula rubra*, and *Serratia spp*. (8.3%), and *Candida albicans* was detected in 25%. In pancreatic tissue, the presence of α-hemolytic *Streptococcus*, coagulase-negative *Staphylococcus*, *Enterococcus spp*. and *Serratia spp*. was established (8.3%). The majority of the cases were infected with *Pseudomonas aeruginosa* (33.3%) and *Candida albicans* (25%).

Table 1. Results of swabs of the duodenum, pancreatic duct and pancreatic tissues; microorganisms were detected in only one swab taken from the site indicated in the table; where there are more positive results, they are expressed as a percentage of the total number of swabs taken

Swabs	I	II	III
Location	Duodenum	Duct	Tissue
Pseudomonas aeruginosa	-	-	33%+
α-hemolytic *Streptococcus*	-	+	+
Coagulase-negative *Staphylococcus*	-	+	+
Enterococcus spp.	-	+	+
Candida albicans	16.7%+	25%+	25%+
Rhodotorula rubra	-	+	-
Serratia spp.	-	+	+

DISCUSION

Results presented here suggest that pancreatic infection with *Candida spp*. originates from the duodenum, as it was detected in all three areas swabbed. Similar results from other studies support this finding [4, 5]. *Candida albicans* has high adaptability as illustrated by the possession of different adhesins, as well as the presence of hyphae, pseudohyphae, and yeast-like cells that allow colonization and infection of almost all body sites.

Candida albicans is a commensal and a constituent of the normal microflora in 80% of the human population. It predominately colonizes the mucosal surfaces of the gastrointestinal tract, genitourinary tract and, to a lesser extent, the skin. It can cause superficial but also systemic and potentially life-threating infections, especially in immunocompromised patients (e.g., cancer chemotherapy, AIDS) or after long-term antibiotic therapy [9, 10].

Immunological response to *Candida* infection in humans is unclear. It is not likely that the yeast growth correlates directly with activation of gut-associated lymphoid tissue. Saprophytic bacteria normally present in the intestine have considerable influence and antibiotic use can disrupt this relationship, decreasing the number of saprophytic bacteria that normally do not allow propagation and overgrowth of *Candida*, hence reducing the chance of entry to the duodenum.

A recent study revealed that the prevalence of fungi increases up to 3000 times in pancreatic ductal adenocarcinoma (PDA) in humans compared to normal pancreatic tissue [11]. *Candida*, *Saccharomyces*, and *Malassezia* predominate in the mycobiome. These fungi were isolated and transferred to experimental mice causing PDA. Ablation of mycobiomes in mice slowed the growth of invasive forms of the cancer with the simultaneous use of certain chemotherapy. Although the research points out *Malessia spp*. as a possible oncogenic factor, the data are not clear. Proposed hypothesis is that fungal MBL axis (ligation of mannose-binding lectins) promotes PDA progression by complement activation.

Regarding the presence of bacteria in pancreatic cancer, *Pseudomonas aeruginosa* was present only in pancreatic tissue. This may suggest that bacteria gained entry to the pancreas through injury or through the blood. It is possible that bacteria are retained only in pancreatic tissue and not in the duodenum or pancreatic duct, which are not suitable for their growth. Presence of *Rodotorula rubra* and *Enterococcus* and *Serratia* only in the pancreatic duct does not mean that they will not spread to pancreatic tissue.

Penetration of bacteria from the intestine into the bloodstream is best illustrated in patients who consume excess alcohol. Heavy alcohol use affects gut microflora composition, leading to increased permeability of the intestine, thus permitting pathobionts to gain access to the bloodstream and other, distant organs. For example, alcohol consumption, a common cause of chronic pancreatitis, is related to dysfunction of the intestinal barrier and overgrowth of Gram-negative bacteria. Regular alcohol consumption also affects the normal function of the sphincter of Oddi, preventing its closure.

Commensal microbiota may play a role in the onset of pancreatic inflammation [12]. Moreover, intestinal microbiota has a synergistic, interactive effect during inflammation.

Damage to the pancreas increases intestine permeability, causing ischemia as well as overgrowth of intestinal bacteria and their translocation in the pancreas, where they provoke secondary infections [13]. Furthermore, infection of necrotic pancreatic tissue is one of the main causes of mortality in acute pancreatitis [14, 15].

Substantial numbers of preclinical and clinical results suggest that bacterial influence on this process is by activation of immune receptors and prolongation of cancer-associated inflammation. The most recent research suggests that disruption in the commensal bacterial population can affect the inflammatory process and some diseases, including carcinogenesis [16, 17]. *Chlamydia trachomatis* infection has been associated with an increased risk of the development of invasive cervical carcinoma [18]. Bacteremia and endocarditis due to *Streptococcus bovis* have likewise been linked with malignancies in the colon, and *Helicobacter pylori* infection is considered a causative agent for both gastric adenocarcinoma and mucosa-associated lymphoid tissue lymphomas [19, 20, 21]. Moreover, several mechanisms by which different bacteria may play a role in cancer development have been proposed, such as through the induction of chronic inflammation, by interference, either directly or indirectly, with eukaryotic cell cycle and signaling pathways [22, 23, 24].

CONCLUSIONS

Based on the data presented here, we can assume that microorganisms isolated in this study have a role in the development of pancreatic adenocarcinoma. It is also possible that infections are consequences of pancreatic diseases such as pancreatic cancer. However, further research of these issues is required in order to provide support for these hypotheses.

There are some indications that the presence of certain microorganisms may play a role in the pathogenesis of pancreatic adenocarcinoma. Based on the results presented here, we can conclude that the origin of infection in pancreatic cancer is from the duodenum (i.e., *Candida* infection), through body injuries, or via the bloodstream (bacterial infections).

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Путеви и типови микробиолошке инфекције у патологији аденокарцинома панкреаса

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САЖЕТАК

Увод/Циљ Рак панкреаса може бити праћен инфекцијама изазваним различитим микроорганизмима. Није сигурно да ли инфекција панкреаса претходи развоју рака или обрнуто. Циљ ове студије је да анализира путеве инфекција из дванаестопалачног црева кроз канал панкреаса да би се утврдило које врсте микроорганизама могу да прођу кроз овај канал у панкреас и оближње ткиво.

Методе Код болесника подвргнутих цефаличној дуоденопанкреатектомији (Виплова процедура) због аденокарцинома папиле Ватери, дванаестопалачног црева или главе панкреаса, узети су брисеви слузнице дванаестопалачног црева, канала панкреаса и панкреаса на линији ресекције. Микроскопски дијапозитиви су припремљени директно од узорака пацијената, а такође и из колонија на плочама за узгој, а оба су обојена по Граму.

Резултати Кандида је била присутна у све три врсте брисева (дванаестопалачном цреву, каналу панкреаса и ткиву), док су бактерије, зависно од врсте (*Pseudomonas aeruginosa*, *a-hemolytic Streptococcus, coagulase-negative Staphylococcus, Enterococcus spp*., *Serratia spp*.) биле присутне у панкреасном каналу или ткиву, али не и у дванаестопалачном цреву.

Закључак Постоји веза између присуства микроорганизама и патологије аденокарцинома панкреаса. Резултати показују да инфекција кандидом потиче из дуоденума, док бактеријске инфекције потичу директно из крви или из ткивних повреда.

Кључне речи: *Candida albicans*; *Pseudomonas aeruginosa*; аденокарцином панкреаса; инфекције панкреаса; дуоденум

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PRELIMINARY AND SHORT COMMUNICATION / ПРЕТХОДНО И КРАТКО САОПШТЕЊЕ

Surgical treatment of verrucous carcinoma of the vulva – 15-year experience and literature review

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SUMMARY
This paper seeks to present surgical procedures, results and complications of treatment of verrucous carcinoma (VC) of the vulva treated at the clinics for gynecology and obstetrics within the Faculty of Medicine, University of Novi Sad (Serbia), as well as a literature review of surgical treatment of VC. During a period of 15 years (2005–2019), we performed 76 surgeries of vulvar cancer, nine (11.8%) due to VC of the vulva. In surgical treatment of vulva VC, we performed complete surgical excision of the tumor (three), complete surgical excision of the tumor with defect coverage using VY fasciocutaneous skin flap (two), simplex vulvectomy (two), radical hemivulvectomy (one), and radical vulvectomy (one). We came across two main complications (22.2%): suture bleeding within 12 hours after excision in one patient (11.1%) and lower extremity lymphoedema after inguinofemoral lymphadenectomy with ligation of the great saphenous vein in one patient (11.1%). Out of the total number of nine treated patients, survival rate was 88.8% (eight patients) with death rate of 11.1% (one patient) within 12 months after surgery. In three patients (33.3%), the disease returned after surgery: one residual tumor after surgery, one relapse on the other side one year after surgery, one newly developed invasive squamous cell carcinoma 10 years after primary surgery.
Keywords: verrucous carcinoma; vulva; vulvar cancer; vulvectomy

INTRODUCTION

Verrucous carcinoma (VC) was first described as a separate histomorphological entity in 1948 [1]. The most common localization of VC is in the nasopharynx and oropharynx but it also occurs in the organs of the anogenital region: anus, rectum, bladder, scrotum, vulva, vagina, and cervix [2]. VC is a locally destructive malignant neoplasm with low metastatic potential and has a long growth period [3]. The pathogenesis of VC is directly related to the mutation of the type 6 human papillomavirus, which causes benign condylomas [4, 5]. Prognosis of VC of the vulva depends on the size of the tumor and the presence of local invasion of the surrounding organs of the anogenital region [6, 7]. Before any procedure, in addition to the evaluation of the external genital organs it is necessary to evaluate organs of the lower genital system (vagina, cervix, urethra, and anus). Complete surgical excision of tumors with histopathologically-confirmed negative edges (minimum 5–8 mm) is a standard therapeutic procedure used in the treatment of vulva VC as it provides good local control of the disease [2, 4]. The most common complications of surgical treatment are bleeding, infection, and skin necrosis, as well as dehiscence of the wound, which can prolong hospitalization and occur more frequently in tumor masses that engulf large areas of the skin or infiltrate surrounding organs [8, 9, 10].

The aim of this work is to present the surgical procedures, results and complications of surgical treatment of vulva VC in a 15-year period (2005–2019) at the Clinic for Gynecology and Obstetrics and the Institute of Oncology of Vojvodina within the Faculty of Medicine, University of Novi Sad (Serbia) as well as a literature review related to the surgical treatment of verrucous vulvar carcinoma.

METHODS

We summarized different techniques, results, complications, and patient outcomes of surgical treatment of vulva VC over a 15-year period (2005–2019) at the Clinic for Gynecology and Obstetrics and Oncology Institute of Vojvodina.

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Written consent to publish all shown material was obtained from the patients.

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Table 1. Verrucous carcinoma – tumor localization in the vulva region, recurrence, surgical procedure, complications, and treatment outcome

Case N°	Localization on the vulva	Surgical procedure	Relapse	Complication	Treatment outcome
1	Labia majora	Complete surgical excision to healthy tissue	None	None	7 years alive
2	Perineum	Complete surgical excision + V-Y fasciocutaneous skin flap	None	None	3 years alive
3	Whole area of vulva	Complete surgical excision to healthy tissue	Residual tumor 6 weeks after surgery	Bleeding 12 hours after the surgery	12 years alive
4	Labia minora	Radical vulvectomy with bilateral inguinofemoral lymphadenectomy	Development of new invasive squamocellular cancer 10 years later	Lower limb lymphedema	15 years alive
5	Whole area of vulva	Complete surgical excision to healthy tissue	None	None	8 years alive
6	The right half of the vulva	Complete surgical excision+ V-Y fasciocutaneous skin fglap	Recurrence on the other side 12 months later	None	1 year alive
7	The right half of the vulva	Radical hemivulvectomy	None	None	1 year alive Loss to follow-up after 1 year
8	The left side of the labia majora	Simplex vulvectomy	None	None	4 years alive
9	The right side of the labia majora	Simplex vulvectomy	None	Local vulva tissue infiltration	Deceased from colonic cancer after 1 year

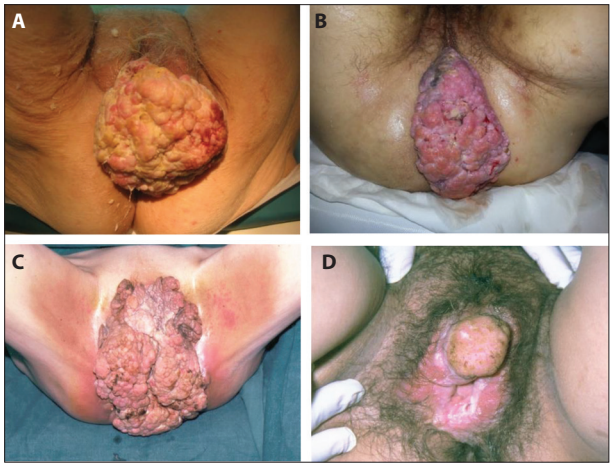

Figure 1. Different localization of verrucous carcinoma in the vulva: A – labia majora on the left; B – perineum; C – entire region of the vulva; D – labia minora on the right

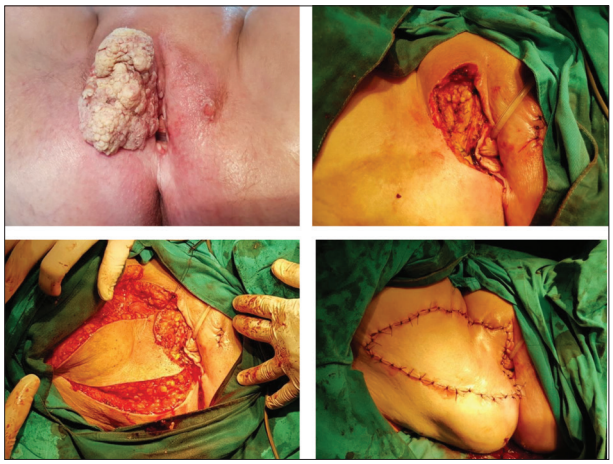

Figure 2. Radical surgical excision of the right ventricular verrucous carcinoma with defect coverage using a V-Y fasciocutaneous skin flap

RESULTS

In the said period of 15 years, approximately 15,000 gynecological surgeries were performed at the Clinic for Gynecology and Obstetrics and the Institute of Oncology of Vojvodina within the Faculty of Medicine in Novi Sad, of which 76 (0.5%) were due to vulvar cancer. In the group of surgically treated women with vulvar cancer, nine (11.8%) cases were due to VC. All the patients were operated on after the usual preoperative preparation, which included bowel preparation 24 hours before surgery, administration of 0.3–0.6 IU SC nadroparin two hours before surgery and lower extremity bandage. Before surgery, 1–2 g of cephalosporin was administered and a Foley urinary catheter was placed. Preoperatively, for all the patients, two transfusion units of decanted erythrocytes were reserved. After surgery, the tumor tissue was sent for histopathological analysis. The patients' age range was 27–79 years. Table 1 shows the localization of VC in the vulva region, the type of used surgical procedure, the occurrence of recurrence, complications, and the outcome of treatment. Figure 1 shows different localizations of VC in the vulva region. Application of a local fasciocutaneous skin flap to cover the defect after extensive surgical excision of labia majora VC is shown in Figures 2 and 3 (A) shows a residual tumor six weeks after complete surgical excision of vulvar cancer. Figure 3 (B) shows relapse of VC on the other side of the vulva 12 months after radical surgical excision using V-Y fasciocutaneous skin flap.

DISCUSSION

Depending on the localization, the magnitude of the change, and the pathohistological finding of tumor tissue

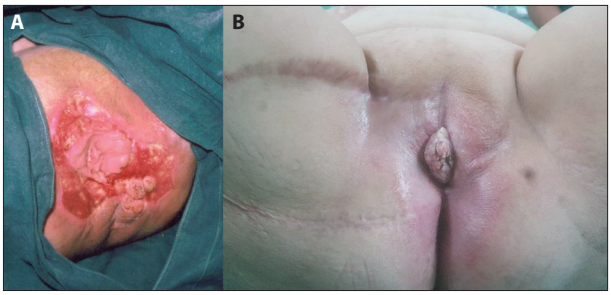

Figure 3. A – Residual tumor six weeks after complete surgical excision of vulvar carcinoma; B – recurrence of verrucous carcinoma on the other side of the vulva 12 months after radical excision and coverage of the defect with a V-Y skin flap

biopsies, a surgical procedure is individually planned for each patient. Basic surgical principle for the treatment of vulva VC involves complete removal of the tumor with histopathologically confirmed negative edges (minimum 5–8 mm) [3]. This is not always easy to achieve, especially if the surrounding organs (rectum, urethra, vagina) are involved. In these cases, different skin-muscle flaps are applied to cover the skin defects on the vulva by the principles of plastic and reconstructive surgery [8]. Brătilă et al. [9] describe the coverage of a surgical defect in the perineum by applying a skin graft after the removal of VC. A paper by Campaner et al. [2] shows radical surgical excision of vulva VC with V-Y fasciocutaneous flap without postoperative complications. Minor dehiscence and necrosis of the vulva after excision of the vulva VC have been reported in the literature [8]. In our practice, we applied the fasciocutaneous V-Y skin flap in two (22.2%) patients and no complications were observed in the postoperative period. Using other surgical techniques, one (11.1%) patient underwent radical hemivulvectomy, simplex vulvectomy was performed in two (22.2%) patients, and three (33.3%) patients underwent complete surgical excision of healthy area VC. In one (11.1%) patient, radical vulvectomy was performed with bilateral inguinal-femoral lymphadenectomy, because of the suspicion of invasive squamocellular carcinoma of the vulva in histopathological examination of the biopsy specimen prior to surgery, which again was not confirmed at the definitive pathohistological examination. Dissection of lymph nodes in VC is still controversial. In a review of 50 surgically treated patients with VC, 17 (34%) lymphadenectomies were performed and lymph nodes were without metastases in all the cases [10]. Similar results are presented in other studies. Liu et al. [4] in their paper show the results of treatment of 24 patients with vulva VC who underwent unilateral or bilateral lymphadenectomy, also with negative lymph nodes. In one (11.1%) patient, radical vulvectomy was performed with bilateral inguinofemoral lymphadenectomy, because it was suspected to be an invasive squamocellular carcinoma of the vulva on the histopathological examination of the preoperative biopsy, which was not confirmed at the definitive pathohistological examination. The same patient developed a new invasive squamocellular carcinoma of the vulva 10 years later, affecting the perineum and anus, and treatment included radiotherapy treatment that led to complete tumor regression. In one (11.1%) patient there was a residual tumor that was re-treated with extensive electroexcision without recurrence in the period of six weeks after treatment. In one (11.1%) patient 12 months after radical surgical excision and covering the defect with the V-Y fasciocutaneous flap, a relapse developed on the other side of the vulva resulting in repeated surgical excision. Of complications, we noted bleeding 12 hours after VC excision between stitches in one (11.1%) patient and lower extremity lymphedema after inguinal lymphadenectomy with ligation of the saphenous vein, also in one (11.1%) patient.

CONCLUSION

The following surgical procedures were applied in the surgical treatment of VC vulva: complete surgical excision of the tumor (three), complete surgical excision of the tumor with defect using the V-Y fasciocutaneous flap (two), simplex vulvectomy (two), radical hemivulvectomy (one), and radical vulvectomy with inguinofemoral lymphadenectomy (one). From the record of 12 months after surgery of all nine (100%) patients who were operated on, survival rate was 88.8% (eight patients), while death rate was 11.1% (one patient). In three (33.3%) patients the disease returned after surgery (one residual tumor after surgery, one relapse on the other side one year after surgery, one newly developed invasive squamous cell carcinoma 10 years after primary surgery). In two patients (22.2%) with residual VC and relapse, we applied re-surgical treatment, while in the patient with newly acquired invasive cancer, radiotherapy treatment was applied.

Conflict of interest: None declared.

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Хируршко лечење верукозног карцинома вулве – 15 година искуства и преглед литературе

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САЖЕТАК

Овај рад настоји да представи хируршке захвате, резултате и компликације хируршког лечења верукозног карцинома (ВК) вулве лечене на гинеколошким и акушерским клиникама Медицинског факултета Универзитета у Новом Саду (Србија), као и преглед литературе о хируршким интервенцијама у лечењу ВК вулве. Током периода од 15 година (2005–2019) обавили смо 76 операција карцинома вулве, девет (11,8%) због ВК вулве. У хируршком третману ВК вулве спроводили смо потпуну хируршку ексцизију тумора (3), потпуну хируршку ексцизију тумора са корекцијом дефекта помоћу *V-Y* фасциокутаног режња (2), симплекс вулвектомију (2), радикалну хемивулвектомију (1) или радикалну вулвектомију (1). Сусрели смо се са две главне компликације (22,2%): крварење из шава у року од 12 сати после ексцизије код једног болесника (11,1%) и лимфедем доњег екстремитета после ингвинофеморалне лимфаденектомије са лигацијом велике вене сафене код једног болесника (11,1%). У укупном броју од девет лечених болесника стопа преживљавања била је 88,8% (осам болесника) са стопом смрти од 11,1% (један болесник) у року од 12 месеци после операције. Код три болесника (33,3%) после операције болест се вратила: један резидуални тумор после операције, један рецидив с друге стране годину дана после операције, један новоразвијени инвазивни планоцелуларни карцином 10 година после примарне операције.

Кључне речи: верукозни карцином; вулва; карцином вулве; вулвектомија

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 радови, претходна и кратка саопштења, прикази болесника и случајева, видео-чланци, слике из клиничке медицине, прегледни радови, актуелне теме, радови за праксу, радови из историје медицине и језика медицине, медицинске етике, регулаторних стандарда у медицини, извештаји са конгреса и научних скупова, лични ставови, наручени коментари, писма уреднику, прикази књига, стручне вести, *In memoriam* и други прилози. Оригинални радови, претходна и кратка саопштења, прикази болесника и случајева, видео-чланци, слике из клиничке медицине, прегледни радови и актуелне теме, 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.

ПЛАГИЈАРИЗАМ. Од 1. јануара 2019. године сви рукописи подвргавају се провери на плагијаризам/аутоплагијаризам преко *SCIndeks Assistant* – Cross Check (iThenticate). Радови код којих се докаже плагијаризам/аутоплагијаризам биће одбијени, а аутори санкционисани.

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, 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 сажетак треба да има следећу структуру: Увод/Циљ рада, Методе рада, 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, потребно је превести називе прилога (табела, графикона, слика, схема) уколико их има, целокупни текст у њима и легенду на енглески језик.

СТРУКТУРА РАДА. Сви поднаслови се пишу великим масним словима (болд). Оригинални рад и 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 и актуелну тему чине: Увод, одговарајући поднаслови, Закључак, Литература. Првоименовани аутор 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, рад из историје медицине и преглед литературе до 5000 речи, а за претходно и кратко саопштење, приказ болесника, актуелну тему, рад за праксу, едукативни чланак и рад за рубрику „Језик медицине“ до 3000 речи; радови за остале рубрике могу имати највише 1500 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.

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 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 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.

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, и у метаанализи, где их је дозвољено 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 публи-

кације 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. Примере 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) и измирити накнаду за обраду чланака (*Article Processing Charge*) у износу од 3000 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).

СЛАЊЕ РУКОПИСА. Рукопис рада и сви прилози уз рад достављају се искључиво електронски преко система за пријављивање на интернет-страници часописа: *http://www.srpskiarhiv.rs*

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 доле наведене адресе и број телефона.

АДРЕСА:
Српско лекарско друштво
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The papers are always submitted with Summary in both English and Serbian, included in the manuscript file. The text of the manuscript should be typed in *MS Word* using the *Times New Roman* typeface, and font size 12 pt. The text should be prepared with margins set to 25 mm and onto A4 paper size, with double line spacing, aligned left and the initial lines of all paragraphs indented 10 mm, without hyphenation. Tabs and successive blank spaces are not to be used for text alignment; instead, ruler alignment control tool and *Toolbars* are suggested. In order to start a new page within the document, *Page Break* option should be used instead of consecutive enters. Only one space follows after any punctuation mark. If special signs (symbols) are used in the text, use the *Symbol* font. References cited in the text are numbered with Arabic numerals within parenthesis (for example: [1, 2]), in order of appearance in the text. Pages are numbered consecutively in the right bottom corner, beginning from the title page.

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., 99Tc, IL-6, O2, B12, 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.

PLAGIARISM. Since January 1, 2019 all manuscripts have been submitted via SCIndeks Assistant to Cross Check (software iThenticate) for plagiarism and auto-plagiarism control. The manuscripts with approved plagiarism/auto-plagiarism will be rejected and authors will not be welcome to publish in Serbian Achieves of Medicine.

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

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

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

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

STRUCTURE OF THE MANUSCRIPT. All section headings should be in capital letters using boldface. Original articles 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 and current topic include: Introduction, corresponding section headings, Conclusion, References. The firstly named author of 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.

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