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TRENDS IN ACADEMIC MEDIA – OPEN RESEARCHER AND CONTRIBUTOR IDENTIFIER, CONCEPT AND SIGNIFICANCE FOR THE SCIENTIFIC RESEARCH COMMUNITY

TRENDOVI U AKADEMSKIM MREŽAMA – JEDINSTVENI IDENTIFIKATOR AUTORA I SARADNIKA, POJAM I ZNAČAJ ZA NAUČNOISTRAŽIVAČKU ZAJEDNICU

Milica MIRČIĆ and Zorica ĐOKIĆ

Introduction

The contemporary scientific communication occurs in a digital environment and in professional virtual communities the concept of science is gradually transforming into the concept of e-science [1]. All participants of the scientific communication have already accepted the existence of Digital Object Identifier (DOI) number, as a digital content identifier of some object on the internet which is connected with e.g. a book or a journal article, regardless of the fact that their place on the internet may be changed [2]. The DOI is assigned to a scientific paper via the Crossref service, in order to find, cite, link and access scientific articles more easily. Likewise, steps have been taken to establish the identifier of an author's unique identity. Back in 2006, Scopus presented its identification scheme Scopus Author IDENTIFIER (ID), and in 2008, Web of Science (WoS) created its own service called ResearcherID, as a system of unique identification of authors [3, 4]. In October 2012, the service Open Researcher and Contributor ID (ORCID) was presented. The concept of this service has been introduced back in 2009, with the aim to include all of the previous schemes and create a central registry of unique identifiers of all participants in research, education, science, innovation, i.e. a registry of authors/contributors [5]. ORCID is supposed to provide a long-term international digital identity of researchers [6].

The problem of identifying the author

One of the most important reasons for establishing researcher identifiers is to determine the author's name. The international scientific community faced the problem of the author's name with the expansion of scientific papers of Chinese authors. Also, in Serbia, it is not uncommon that several individuals/authors share the same name and surname [6]. The inconsistencies in author names can also be caused by omitting diacritical marks, omitting a hyphen in double-barrelled surnames, permutation of letters, omission of the middle letter, changing the surname, or by adding a surname **(Tables 1 and 2)** [7–9].

Unique author name identification is important for establishing the connection between the author and their scientific papers, affiliations, participation in projects, and financiers [6]. The published scientific papers in journals which are on the International Scientific Index (ISI) list, and their citedness, are a precondition for the evaluation of the scientific paper, and for the progress of the researcher's academic career. Therefore, establishing the author's unique identity is necessary. The Consortium of Serbian Libraries for Coordinated Acquisition (KoBSON) and its service WoS, significantly contribute to correction and unification of names and surnames. This service includes papers of authors referred to in the WoS index database, who cited an institution from Serbia as their affiliation [10]. In connection with the author's affiliation, a problem may occur, for example, if affiliations are not cited consistently, if authors have two affiliations, or if they have one affiliation, but their country has changed its name, etc. The creators of index databases, such as WoS and Scopus, are aware of the problems the authors face, and accordingly they provide the authors the opportunity to directly participate in correcting the errors by sending suggestions about what should be added and corrected [7].

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Abbreviations

DOI – Digital Object Identifier
ID – Identifier
ORCID – Open Researcher and Contributor ID
WoS – Web of Science

ORCID identifier - concept and significance

Another way to overcome the problems mentioned above is to create an ORCID profile. ORCID is a unique and permanent alphanumeric code, a 16-digit number preceded by a web address, which identifies a certain author and is compatible with the International Standard Organization (ISO) standard [6, 11, 12].

Features and advantages of the ORCID profile are the following: a) it is international, available online, and free; b) it protects the author's identity, i.e. the researcher is the owner of the profile, and determines what will be visible or not on his profile; c) ORCID profile which is orderly and continually updated represents a bibliography of researcher's papers which is always ready to be used in applying for projects of the European Commission; d) it is connected with numerous information systems (WoS, Scopus, Crossref, etc.) with which it is interoperable, i.e. it enables importing and exporting metadata [13], saving time needed for filling out data; e) ORCID is on the way of becoming the standard of international scientific information trade [6, 14].

In Croatia, according to data from April 2017, as many as 5.900 authors have created an ORCID profile, and 13.800 scientific papers have been connected with the authors [14]. According to data from October 2017, in Serbia, about 3.000 researchers have created an ORCID profile (of about 12.500 recorded researchers) [13]. Most of these researchers are from the University of Belgrade, and then from the Universities of Novi Sad, Niš, and Kragujevac. The electronic sources which are available and browsed in Serbia, with an ORCID number or its recognizable green ORCID logo are the following index databases: WoS and Scopus, Serbian Citation Index (SCIndeks), National Repository of Dissertations in Serbia – NaRDuS, Repository of the Institute for Biological Research "Siniša Stanković" – RADaR, Digital Archive of Serbian Academy of Sciences and Art; as well as the websites of the University of Belgrade, and the Faculty of Chemistry in Belgrade [15–20].

Certain international scientific journals require mandatory submission of the ORCID identifier, when accepting papers for publication. Such practice has only just started in Serbia, e. g. in the *Military Technical Courier*, a domestic scientific journal, which requires all authors to have their personal ORCID identifier, as well as reviewers and all members of the Editorial Board [21]. With the submission of the ORCID number when publishing scientific papers, the ORCID profile will be automatically updated after the paper is published [4], so in time more and more information about the author's production will be gathered in one place. This way, time is saved, errors are eliminated, and a reliable connection between authors and their published articles is created [22].

Today, researchers in Serbia face more and more challenges. In addition to professional, scientific, and often education engagement, in accordance with the requirements of the relevant Ministry, they need to submit data on their scientific production with the aim to create a comprehensive database of researchers in Serbia [13]. For this purpose, several databases have been created. For example, in Vojvodina, a database on production results of researchers was created on the initiative of the Provincial Secretariat for Higher Education and Scientific Research in order to unify scientific production of researchers in Vojvodina [23], and also, there are activities to fill out the Researchers' Registry of Serbia (RIS) on the request of the Ministry of Education, Science and Technological Development [24]. In this situation, researchers need to fill out multiple forms. Taking into account the complexity of the activities connected with unifying scientific production of every individual, as well as of the scientific community, librarians point out to the existence of the ORCID profile, and to its advantages [25].

The librarians of the Faculty of Medicine in Novi Sad have learnt that some researchers have encountered the concept of the ORCID profile, and created it for the purpose of submitting their papers

Table 1. Surname change, results of author search on the Scopus web site
Tabela 1. *Promena prezimena, rezultati pretrage autora na internet stranici Scopus*

Result Number/*Broj pogodaka*	Author *Autor*	Documents *Dokumenti*	Subject area *Naučna oblast*	Affiliation *Afilijacija*	City *Grad*	Country/Territory *Zemlja/Teritorija*
1	Srdić, Biljana Srdic, Biljana Srdić, B. Srdic, B.	26	Medicine; Biochemistry, Genetics and Molecular Biology; Computer Science ...	University of Novi Sad	Novi Sad	Serbia
2	Srdić Galić, Biljana Srdić Galić, Biljana Srdić-Galić, Biljana Srdic-Galic, Biljana	8	Medicine ; Biochemistry, Genetics and Molecular Biology;	University of Novi Sad	Novi Sad	Serbia

Table 2. Incorrect surname, results of author search on the Scopus web site
Tabela 2. *Pogrešno navođenje prezimena, rezultati pretrage autora na internet stranici Scopus*

Result Number/*Broj pogodaka*	Author *Autor*	Documents *Dokumenti*	Subject area *Naučna oblast*	Affiliation *Afilijacija*	City *Grad*	Country/Territory *Zemlja/Teritorija*
1	Barak, Otto F. Barak, O. Barak, Otto Barak, Oto	43	Medicine; Biochemistry, Genetics and Molecular Biology; Health Professions ...	University of Novi Sad/*Univerzitet u Novom Sadu*	Novi Sad	Serbia/*Srbija*
2	Otto Barak, F.	1	Medicine; Biochemistry, Genetics and Molecular Biology; Neuroscience; ...	University of Novi Sad/*Univerzitet u Novom Sadu*	Novi Sad	Serbia/*Srbija*

to certain international journals. At the Faculty of Medicine in Novi Sad, since the academic year 2017/18, the ORCID number is a part of the Personal Researcher's Record in English; therefore, certain teachers were encouraged to register and get their own ORCID number. The instructions which follow are meant to encourage and help everyone with registration and filling out the ORCID profile.

Instructions for registration and filling out the ORCID profile

It is very simple to open an ORCID account. Open the page https://orcid.org using the Google Chrome or Mozilla Firefox browser, and check first in the search box if there was any previous registration. If your name is not on the list, the registration begins by clicking the **For Researchers** button, and then **Register for an ORCID**. The only required fields for registration are the name, surname and email address, as well as a password that must contain a minimum of 8 characters including at least one number, a capital letter or a symbol. Only the ORCID identifier is always publicly available, all other data do not have to be public and can be changed [26]. It is important to have control over personal information on the Internet, and there is a choice of three visibility options on the ORCID profile [27] - the profile is public, it is partly visible, or it is invisible **(Figure 1)** [28].

After completing the registration form, the first entry of personal data into the profile is accessed. ORCID sends an activation link via email, for accessing the page which contains the ORCID number and fields that need to be filled out and updated. If an email from ORCID is not visible, SPAM or Junk mail should be checked [11]. Every following access to the ORCID profile for adding and updating information is done by clicking **Sign in**, entering the email address and password in the adequate fields. For those who have previously registered but have forgotten the password, there is an option **Forgotten your password**, which can create a new password by using the email address. If there are duplicate ORCID accounts, they can be closed by using the **Account settings** option.

Once the profile is opened, data are edited by clicking on the **Pencil** icon, by clicking the **+ Add** field when new content is added, and the changes are

Figure 1. ORCID registration website
Slika 1. *Internet stranica jedinstvenog identifikatora autora i saradnika za registraciju*

confirmed using the **Save changes** button or **Add to list**. It is recommended to add all forms of names and surnames first, before entering data about the published papers. Since ORCID is an international service, it is best that primary names and surnames are entered in Latin letters, and the other forms of names are entered in the field **Also known as,** for example: Latin letters without diacritical characters, Cyrillic letters, maiden name, two surnames in inversion, with or without a hyphen, foreign names without transliteration, with the first letter of the father's name in the middle, and so on [7]. Adding more options guarantees that papers stored in other databases will be easier to find. These other forms of names need not be publicly visible, and one of three visibility options can be selected for each of the forms separately, as well as the order in which they will be displayed [4]. Among other personal data, it is also desirable to add other email addresses that are in use, such as, for example, an email with the affiliation, if the ORCID account is opened via a private email. Other addresses are added to the primary email address which is used to log in, and to which the ID is connected, and the visibility of each email address can be selected separately.

Education and **Employment** are the fields that should be filled out in English, using the official names of institutions, because this practice significantly facilitates the search of the production of scientific papers within an institution. Many institutions already exist in the database, so their titles can be selected among the offered ones, while all others should be added manually in a consistent way. **Funding** can be completed by using **+ Add manually,** or it can be imported using the **Search & Link** option, if the searched project is found.

After filling in the basic data, from the **Works** field, one switches to retrieving scientific production data from other systems by using one of the three options: **Search & Link**, **Bibtext**, or **+ Add manually**. The first option enables you to select one database at a time: **Scopus to ORCID**, **Researcher ID** (articles from WoS) or **CrossRef Metadata Search** (articles with a DOI number, among which there are papers from some domestic journals, for example, from the SCIndeks). Other articles that were not found, but may be found in some other databases that have metadata, such as Cooperative Online Bibliographic System and Services (COBISS) or World Catalogue (WorldCat), are retrieved in ORCID by using the **Bibtext** option [29]. In that case, paper data should first be transferred to one of the standard forms of metadata using the bibliographic tools *Endnote, Zotero, Mendeley, Reference Manager*, and others, which are otherwise recommended when quoting in scientific papers. In the end, all other data can be entered manually with as much information as possible, even Universal Resource Locator (URL) links of papers or journals, and so on [11].

Conclusion

Open Researcher Contributor Identifier is a unique and permanent alphanumerical code, which enables the creation of a central registry of unique identifiers for researchers and contributors in the scientific-research and education process. This identifier is recommended in the international information trade, and it provides a data infrastructure for the scientific-research community – names, professional biographies, workplaces, published scientific papers and participation in projects. In this way the results of scientific papers become more available and visible. The Open Researcher Contributor ID profile is a kind of constantly updated digital biography and bibliography of researchers. Opening an Open Researcher Contributor ID profile is simple; the application is transparent and enables users to change and add data, as well as to manage the parameters which determine the degree of availability of data on the profile. Furthermore, there are a number of other options which have not been mentioned in this paper because they are not necessary for the registration and primary data input.

Since October 2012, when it was launched, to October 2017, Open Researcher Contributor Identification Initiative has gathered more that 4 million Open Researcher Contributor ID numbers on the international level, and thus demonstrated its readiness to become an integral part and a standard in information systems of the scientific-research process. Since it has so much potential, all researchers from Serbia are encouraged to join this initiative.

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STEREOLOGICAL ANALYSIS OF ANDROGEN RECEPTORS IN PROSTATE CANCER AND BENIGN PROSTATIC HYPERPLASIA

STEREOLOŠKA ANALIZA ANDROGENIH RECEPTORA KOD KARCINOMA I BENIGNE HIPERPLAZIJE PROSTATE

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Summary

Introduction. Through androgen receptors, androgens regulate prostate cellular growth and function, proliferation, differentiation, apoptosis, lipid metabolism and secretory activity, as well as development and progression of prostate cancer. Prostate cancer, and its primary glandular tissue are influenced by hormones, and it is used for therapeutic purposes. Anti-androgen treatment is carried out in patients with metastatic prostatic cancer, in order to block effects of androgens. Immunohistochemical analysis of androgen receptors in the prostate cancer tissue may help us to assume how the tumors will react to the anti-androgen therapy, if they are androgen-positive, -negative, or hormone resistant tumors. Knowledge of the presence of androgen receptors in the tumor tissue may be a prognostic indicator in histopathological analysis. The aim of this study was stereological evaluation of androgen receptor expression in patients with benign prostatic hyperplasia and in patients with prostatic cancer, before therapy. **Material and Methods.** Immunohistochemical analysis was carried out using anti-human androgen receptor monoclonal antibody 441. The presence and intensity of the androgen receptors were evaluated in 195 patients: 165 with benign prostatic hyperplasia and 30 with prostatic cancer using Weibel's multi-purpose M 42 stereological test system. Material was obtained by needle biopsy or transurethral resection of the prostate. **Results.** All secretory cells in patients with benign prostatic hyperplasia were androgen positive, while in patients with prostatic cancer, all tumors were mostly androgen positive, some with foci of negativity. The resulting negative correlation with Gleason score and International Society of Urological Pathology grade was not statistically significant. **Conclusion.** Study results of stereological analysis of androgen receptors indicate that prior the therapy prostate cancer is androgen-dependent, with a high level of androgen receptor expression, although slightly lower compared to benign prostatic hyperplasia.

Key words: Prostatic Neoplasms; Receptors, Androgen; Prostatic Hyperplasia; Immunohistochemistry; Pathology

Sažetak

Uvod. Androgeni preko androgenih receptora utiču na ćelijski rast i funkciju, proliferaciju, diferencijaciju, apoptozu, lipidni metabolizam i sekretornu aktivnost prostate i na razvoj i progresiju karcinoma prostate. Karcinom prostate, kao i njegovo primarno žlezdano tkivo je pod uticajem hormona i ta činjenica je iskorištena u terapijske svrhe. Antiandrogena terapija se sprovodi kod pacijenata sa metastatskim karcinomom prostate, upravo sa ciljem da blokira dejstvo androgena. Imunohistohemijskom analizom androgenih receptora u tkivu prostate sa karcinomom, možemo da pretpostavimo kako će tumor reagovati na datu antiandrogenu terapiju, u odnosu na to da li su androgen-pozitivni i androgen-negativni, ili hormonski rezistentni tumori. Saznanja o zastupljenosti androgenih receptora u tkivu tumora, mogla bi da posluže kao prognostički indikator, u patohistološkoj analizi. Cilj ove studije je stereološka evaluacija ekspresije androgenih receptora kod pacijenata sa karcinomom prostate i benignom hiperplazijom, pre sprovedene terapije. **Materijal i metode.** Imunohistohemijska analiza je sprovedena upotrebom monoklonalnog androgen-receptor441 antitela. Stereološki je procenjivano prisustvo i intenzitet androgenih receptora kod 195 pacijenata sa benignom hiperplazijom (165) i karcinomom prostate (30), uz upotrebu multinamenskog Vajbelovog (*Weibel*) testnog sistema M-42. Materijal je dobijen biopsijom iglom ili transuretralnom resekcijom prostate. **Rezultati.** Sve sekretorne ćelije kod pacijenata sa benignom hiperplazijom su bile androgen-pozitivne, dok su kod pacijenata sa karcinomom prostate, svi tumori mahom bili androgen-pozitivni, ali pojedini sa fokusima negativnosti. Dobijena je negativna korelacija sa Glisonovim skorom (*Gleason*) i *International Society of Urological Pathology* gradus grupom, koja nije statistički značajna. **Zaključak.** Rezultati studije stereološke analize androgenih receptora ukazuje da je karcinom prostate pre terapije androgen-zavisan; ima visok stepen ekspresije androgenih receptora, mada nešto manji u odnosu na benignu hiperplaziju.

Ključne reči: neoplazme prostate; androgeni receptori; hiperplazija prostate; imunohistohemija; patologija

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Abbreviations

AR – androgen receptor
PCa – prostate cancer
BPH – benign prostatic hyperplasia
TURP – transurethral resection of the prostate
ISUP – Integrated Quantitative Gleason Score
Vv – volume density
DHT – 5α-dihydrotestosterone
LHRH – luteinizing-hormone-releasing hormone
IHC – immunohistochemical staining
HE – hematoxylin-eosin
Pt – total number of points in the test system
Pf – total number of points placed on the section
Vvep C – volume density of the epithelium in carcinoma
Vvl C – volume density of lumen in carcinoma
Vvs C – volume density of stroma in carcinoma
Vvep H – volume density of epithelium in hyperplasia
Vvl H – volume density of lumen in hyperplasia
Vvs H – volume density of stroma in hyperplasia
Vvj C – volume density of all nuclei in carcinoma
Vvj+C – volume density of nuclei in carcinoma positive for AR
Vvj-C – volume density of nuclei in carcinoma negative for AR
Vvo C – volume density of other histological elements in carcinoma
Vvj H – volume density of all nuclei in hyperplasia
Vvj+H – volume density of nuclei in hyperplasia positive for AR
Vvj-H – volume density of nuclei in hyperplasia negative for AR
Vvo H – volume density of other histological elements in hyperplasia
GG – grade group

Introduction

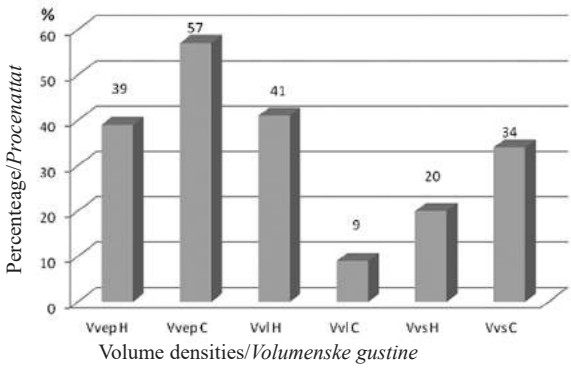
Stereology is a multidisciplinary scientific field that uses two-dimensional sections of bodies or tissues quantitatively, and interprets their inner three-dimensional structure. For stereological measuring of different structures, various test systems are used [1, 2]. There are numerous parameters that can be determined by stereological measuring. Evaluation of volume density (Vv) has its advantages in ease of performance and the fact that the it is a relative stereological unit that shows what part of the total area belongs to the studied phase (volumetric representation of the studied phase within a certain area). Using mathematical calculations, it was established that Vv can be determined from the density of points (Pt) that belong to the tested phase. The Vv is without dimension (mm^0) and if we multiply the obtained values by 100, the value can be expressed in percentages. In this paper, values are presented in percentages. Values of Vv do not depend on the tissue arrangement (anisotropic or isotropic tissue), nor on technical characteristics and power magnification of the microscope, so correction coefficient is not required [1–4].

Prostate, as an accessory gland, is under the influence of hormones, but until today there have been no findings whether accessory sex glands, such as prostate, secrete hormones [5, 6]. Through androgen receptors (ARs) of the prostate, testicular androgens regulate vital aspects of the prostate, such as cellular growth and function, proliferation, differentiation, apoptosis, lipid metabolism and secretory activity. Primary hormonal mediator of benign prostatic hyperplasia (BPH) is 5α-dihydrotestosterone (DHT). This androgen is the main intracellular metabolite of testosterone. It is produced focally in stromal cells from the circulating testosterone, under the influence of the enzyme 5-reductase. The DHT influences stromal cells autocrinally, and epithelial cells paracrinally, increasing their mitotic activity due to binding to the receptors in these cells. The mitotic effect of DHT is about ten times stronger than of testosterone. In addition to DHT, other factors can also influence the mitotic activity in the prostate, i. e. the concentration of estradiol. The effect of estradiol is based on the increase in the number of nuclear receptors for DHT in prostate cells [7].

The BPH is the most common disease in adult men. Prostate cancer (PCa) is one of the most common malignancies and the second leading cause of death among men in industrially developed countries. The development and progression of PCa and its primary glandular tissue, depend on testosterone and dihydrotestosterone. Back in 1941, Huggins and Hodges showed that PCa is under hormonal influence of androgens [8].

Transrectal needle biopsy is the gold standard in the pathohistological diagnosis of PCa, as well as the analysis of the prostatic tissue after transurethral resection of the prostate (TURP) and prostatectomy.

Modern approach to PCa therapy is carried out according to the indications for each stage of the disease separately (monitoring, curative treatment and hormonal therapy) [9, 10]. Endocrine, adjuvant hormone therapy has a goal of inhibiting stimulatory actions of androgens on prostate carcinoma cells. This can be achieved by surgical or pharmacological castration. Administration of luteinizing-hormone-releasing hormone (LHRH) agonists and/or anti-androgen leads to a pharmacological block-



Graph 1. Mean values of volume densities of epithelium (Vvep H, Vvep C), lumen (Vvl H, Vvl C) and stroma (Vvs H, Vvs C) in patients with benign hyperplasia and patients with prostate carcinoma

Grafikon 1. *Srednje vrednosti volumenske gustine epitela (Vvep H, Vvep C), lumena (Vvl H, Vvl C) i strome (Vvs H, Vvs C) kod pacijenata sa benignom hiperplazijom i karcinomom prostate*

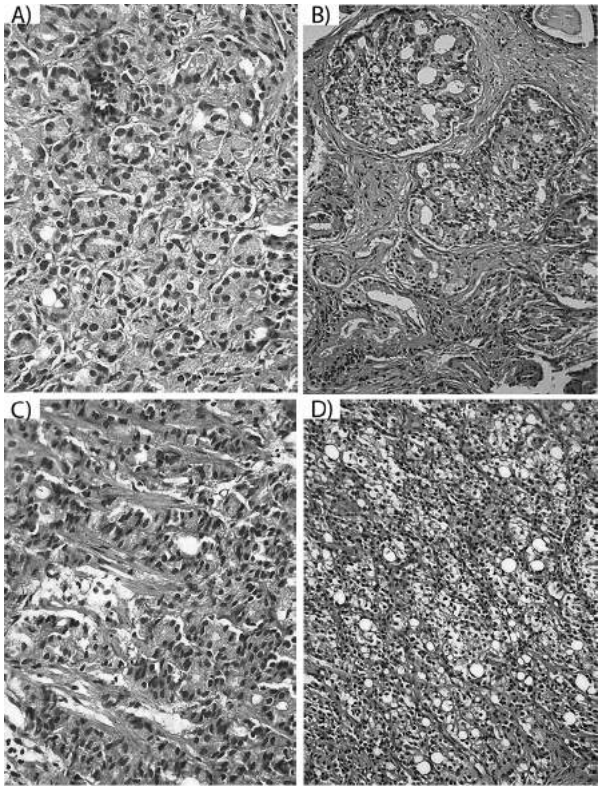


Figure 1. Prostate adenocarcinoma: A) Gleason pattern 2, H&E, x 10; B) Gleason pattern 3, H&E, x 20; C) Gleason pattern 4, H&E, x 40; D) Gleason pattern 5, H&E, x 20
Slika 1. *Adenokarcinom prostate*: *A) Glisonov gradus 2, hematoksilin-eozin (H&E), x10; B) Glisonov gradus 3, H&E, x20; C) Glisonov gradus 4, H&E, x40; D) Glisonov gradus 5, H&E, x20*

ade. Hormone therapy is used to treat metastatic prostate cancer [11, 12].

Using immunohistochemical determination of ARs in patients with PCa, we wished to substantiate the claims that the majority of tumors are androgen-dependent from the beginning, and that the initial anti-androgen therapy is useful. Over time, therapies create clones of androgen resistant cells, which leads to the resistance of the tumor to therapy, to the androgen blockade, both morphologically and immunohistochemically, which prospectively could be proven by the analysis of the material gained by TURP or biopsy, but only in patients who did not undergo prostatectomy. This claim has also been presented by many other authors [13–15].

Using pathohistological analysis after immunohistochemical staining (IHC) ARs were intranuclearly localized, and their determination could prospectively aid as a prognostic indicator for patients with metastatic PCa [16–18].

Material and Methods

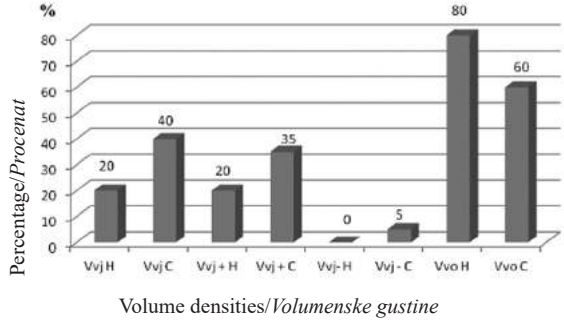
The prospective/retrospective study was carried out at the Centre for Pathology and Histology of the Clinical Centre of Vojvodina in Novi Sad, Republic of Serbia. After transrectal needle biopsies of the prostate and TURP performed at the Clinic for Urology, the bioptic materials of 195 male patients were histopathologically analyzed. The materials were fixed in 4% formalin, embedded in paraffin blocks, cut and stained in a standard way with hematoxylin-eosin (HE), and analyzed immunohistochemically for AR antibodies (DAKO Corp.).

Using histological analysis, patients were divided into two groups: experimental group with histopathologically diagnosed PCa (30 patients) and a control group with histopathologically diagnosed BPH (165 patients).

M-42 Weibel's multipurpose test system was used for stereological determination of the Vv of the prostate slices in both groups, meaning that the total number of test points was 42. M-42 grid has 6 lines in 7 rows, 21 lines and 42 points in total. Lines of the system are parallel and distributed opposite each other. The grid was installed in the eyepiece of the light microscope Galen.

Stereological analysis was performed on 4 fields of view in 4 nonadjacent histological cuts. The first field of view was selected randomly, and the next three adjacent fields of view were selected according to the principles of stereology, or isotropic principle. According to the same isotopic principle, certain elements within a field of view were counted and eliminated.

During the measurement, the test system overlapped the desired field of view of the observed structure, that is, points of the test system fell on the observed slices of the test tissue. Within the field of view, the total number of points placed on the section (Pf) were counted. Number of points of one phase, e. g. nuclei positive to androgen receptors in PCa, from one field of view were added to other data



Graph 2. Mean values of volume densities of all nuclei (Vvj H, Vvj C), nuclei positive for AR (Vvj+ H, Vvj+ C), negative for AR (Vvj- H, Vvj- C) and other histological elements (Vvo H, Vvo C) in patients with benign prostatic hyperplasia and patients with prostate cancer (%)
Grafikon 2. *Srednje verdnosti volumenske gustine svih jedara (Vvj H, Vvj C), jedara pozitivnih na androgene receptore (Vvj+ H, Vvj+ C), negativnih na androgene receptore (Vvj- H, Vvj- C) i drugih histoloških elemenata (Vvo H, Vvo C) kod pacijenata sa benignom hiperplazijom i karcinomom prostate*

gained from the remaining three fields of view. Then we added up all the hits of one phase in four histological cuts and divided the resultant sub-total by the total number of points in the test system (Pt), i. e. 168 (42 x 4 = 168, because there were four fields of view). In this way we obtained the necessary data for the calculation of the Vv of the Pf according to the well-known formula: Vv=Pf/Pt (Vv - volume density of the tested phase, Pf - total number of points placed on the section, and Pt - total number of points in the test system; number of points of the test system multiplied by the number of analyzed fields of view).

After calculating the Vv of tested phases of one biopsy sample, we determined mean values for both tested groups.

Using stereological analysis we calculated a total of 14 parameters, that we divided in two groups based on staining methods. Based on HE staining: Vv of the epithelium in carcinoma (Vvep C), Vv of lumen in carcinoma (Vvl C), Vv of stroma in carcinoma (Vvs C), Vv of epithelium in hyperplasia (Vvep H), Vv of lumen in hyperplasia (Vvl H), and Vv of stroma in hyperplasia (Vvs H). Based on immunohistochemical staining for ARs: Vv of all nuclei in carcinoma (Vvj C), Vv of nuclei in carcinoma positive for AR (Vvj+C), Vv of nuclei in carcinoma negative for AR (Vvj-C), Vv of other histological elements in carcinoma (Vvo C), Vv of all nuclei in hyperplasia (Vvj H), Vv of nuclei in hyperplasia positive for AR (Vvj+H), Vv of nuclei in hyperplasia negative for AR (Vvj-H), and Vv of other histological elements in hyperplasia (Vvo H). Stereological measurements were performed at 100 x magnification (10oc. x 10 obj.).

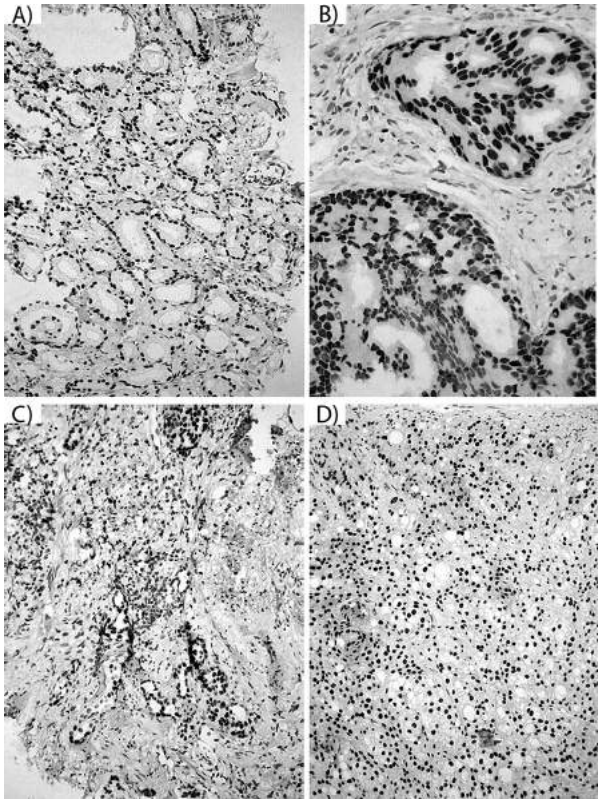


Figure 2. Androgen positive nuclei of prostate carcinoma cells: A) Gleason pattern 2 (+++), x 20; B) Gleason pattern 3 (++), x 40; C) Gleason pattern 4 (++), x 20; D) Gleason pattern 5 (+++), x 20

Slika 2. *Androgen-pozitivna jedra ćelija karcinoma prostate: A) Glisonov gradus 2 (+++), 20; B) Glisonov gradus 3 (++), 20x; C) Glisonov gradus 4 (++), x20; D) Glisonov gradus 5 (+++), x20*

Standard statistical analysis was performed using the computer program Origine, tables and figures using Word and Excel, with calculation of general statistical indicators: mean value, i. e. the arithmetic mean, mode (tyical) value and the median, standard error and standard deviation, minimum and maximum values, T-test and correlation.

Results

The mean age of all (195) patients in both groups was 69.26 ± 0.46, the oldest being 89, and the youngest 51 years old, and the median age of 69. The mean age of patients with PCa was 68.97 ± 1.44, the oldest being 81, and the youngest 51 years old. Most patients with cancer were in the seventh decade of life. The mean age of patients with BPH was 69.3 ± 0.4, the oldest being 89, and the youngest 53 years old. Most patients with hyperplasia were in the sixth decade of life.

The mean values of Vv obtained by stereological measurements based on standard HE staining in BHP and PCa are shown in **Graph 1**. The **Figure 1** shows prostate cancer "pattern" 2 and 3 (**Figure 1A**, **Figure 1B**), "pattern" 4 and 5 (**Figure 1C**, **Figure 1D**) benign hyperplasia.

The mean values of Vv of glandular epithelium in PCa (57%) were percentually higher than the same values in benign hyperplasia (39%), but there was no statistically significant difference in the degree of freedom ($p < 0.05$). The Vv of glandular lumen in PCa (9%) was numerically lower than in BHP (41%), and there was a statistically significant difference in the degree of freedom ($p < 0.05$). The Vv of stroma in PCa (34%) was numerically higher than in hyperplasia (20%), but there was no statistically significant difference in the degree of freedom ($p < 0.05$). Standard deviations (SDs) for Vv of the epithelium, glandular lumen and stroma in patients with BHP were 0.03, 0.01 and 0.03, respectively. In patients with PCa, the SDs for Vv of the epithelium, glandular lumen and stroma were 0.05, 0.06 and 0.04, respectively.

By comparing Integrated Quantitative Gleason Score (ISUP) grade group (GG) and Vv of histological elements in prostate cancer based on HE staining, the following results were obtained: between GG and Vv of the epithelium there was a correlation (0.11), positive and insignificant; between GG and Vv of the lumen there was a correlation (-0.47), negative and low; between GG and Vv

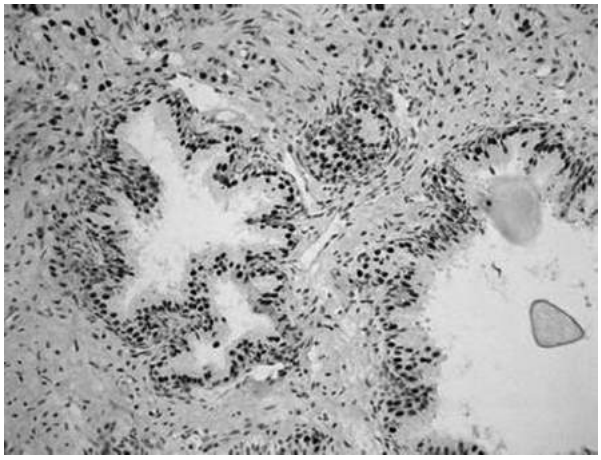


Figure 3. Androgen positive nuclei of secretory cells and negative nuclei of basal cell layer in benign prostatic hyperplasia, x 20
Slika 3. *Androgen-pozitivna jedra sekretornih ćelija i androgen-negativna jedra ćelija bazalnog sloja kod benigne hiperplazije prostate, x 20*

of the stroma there was also a correlation (0.09), positive and insignificant. It can be concluded that the increase in tumor dedifferentiation causes an increase in the amount of epithelium and stroma, and a decreased lumen of adenoid structures.

We also analyzed the mean values of Vv by stereological measurements after immunohistochemical staining for AR, in patients with BHP and PCa (**Graph 2**, as well as **Figure 2**, and **Figure 3**).

All nuclei of glandular epithelium in BHP were positive for AR (Vv of all nuclei equaled Vv of AR+ nuclei). Carcinomas with certain number of nuclei that did not stain for ARs (AR-nuclei) were detected. The mean value of Vv of all nuclei in carcinoma was 40%, and was numerically higher than the Vv of all the nuclei in hyperplasia (20%), with the difference statistically significant in the degree of freedom ($p < 0.05$). The Vv of nuclei negative for AR in carcinoma was 5% and was numerically higher than the same Vv in hyperplasia (0%), but it was not statistically significant in the degree of freedom ($p < 0.05$). The Vv of other histological elements was numericallly higher than in BHP, and the difference was statistically significant in the degree of freedom ($p < 0.05$). The SD for Vv of all nuclei, nuclei positive for AR, nuclei negative for AR and other histological elements in patients with PCa were as follows: 0.03; 0.02; 0.02 and 0.03.

The SD for Vv of all nuclei, nuclei positive for AR, nuclei negative for AR and other histological elements in patients with BPH were as follows: 0.03; 0.03; 0 and 0.03.

By comparing ISUP grading and Vv of histological elements in PCa based on IHC staining for AR, the following results were obtained: between GG and Vv of all nuclei there was a correlation (0.09), positive and insignificant; between GG and Vv of AR + nuclei there was a correlation (0.05), positive and insignificant; between GG and Vv of AR - nuclei there was a correlation (0.09), positive and insignificant; the correlation also existed between GG and Vv of other histological elements, negative and insignificant (-0.25). It can be concluded that increased tumor dedifferentiation caused an increase in the number of nuclei, and a decrease of other histological elements.

Discussion

Formation of two groups of patients, those with benign hyperplasia being the control group and those with PCa being the experimental group, enabled quantitative presentation of histological differences between the two conditions. For stereological measurements of structures such as prostatic parenchyma, a multipurpose test system is recommended, so we used and one of the most frequently used multi-purpose test systems, M-42. In this type of test the entire system has a total of 42 points, and the number of points that correspond to certain phases is divided by 42.

Hematoxylin-eosin staining determined the Vv of the epithelium, glands and stroma lumens, and immunohistochemical staining determined the Vv of: all nuclei, androgen positive, androgen negative nuclei, and other histological elements. The numerical difference between the Vv of epithelium, stroma and lumen between the control and the experimental group was examined, but it was not statistically significant for the degree of freedom ($p < 0.05$). The Vv of the epithelium and stroma in carcinoma was higher than in hyperplasia, unlike Vv of lumen which was lower, corresponding to morphological appearance of changes. Histomorphologically, in prostate cancer, multiplication of glandular, cribriform structures, and formation of chains, strips and solid zones that consist of atypical cells is associated with an increase of the Vv of the epithelium. The same occurs with stroma, for it is important for growth, support, i. e. nutrition of tumors. It can be argued that the increase in Vv of epithelium in carcinoma comes at the expense of reduction of Vv of lumens, and that it is more evident if the tumors are more dedifferentiated [19].

After immunohistochemical staining for AR, it can be concluded that all nuclei in BPH are androgen-dependent, i. e. Vv of all nuclei is equal to Vv of androgen-positive nuclei; however, in PCa there are androgen independent nuclei, which is consistent with literature data [19–24]. The obtained results are consistent with literature data that the majority of prostate adenocarcinomas are AR positive [13, 14, 19–22].

All 30 patients of the experimental group had a histomorphological diagnosis of acinar adenocarcinoma. Other histological types of PCa were not detected, as expected, considering that it accounts for more than 90% of all histological types of PCa [21].

Results of comparison between stereologically evaluated AR and Gleason score were negative with slight correlation (-0.12). Approximate values were obtained by comparing ISUP grade group and semi-

quantitatively evaluated AR (-0.15). It can be argued that the increase in Gleason score and ISUP grade group in certain number of carcinomas lead to the decrease in the number of nuclei with positive AR. The more dedifferentiated the tumor, the more likely it is to have androgen-resistant cells. However, it should not be left out that certain tumors of the same grade had differently evaluated AR, meaning that based only on morphology (HE staining), there is no way to determine the precise extent and intensity of nuclei positive for AR. Our results correlate with results of other authors, who claim that carcinomas with low scores do not have a significantly higher content of AR, than those with high Gleason score. On the other hand, some authors claim otherwise, but one cannot exclude studies that have not determined the existence of correlation between Gleason score and AR, which leaves space for staining of antibodies for AR [13–15, 23–33].

Conclusion

Stereologically calculated volume densities of histological elements in benign prostatic hyperplasia and prostate carcinoma are consistent with morphological appearance of lesions. Compared to benign hyperplasia, prostate carcinoma has higher volume density of the epithelium and stroma.

Volume density of androgen-sensitive nuclei is lower in carcinoma than in benign prostatic hyperplasia; more precisely, all secretory cells in benign prostatic hyperplasia are androgen-sensitive, while in carcinoma, there are also androgen-negative nuclei.

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TROCHLEAR DYSPLASIA – CONGENITAL ANOMALY OR BIOMECHANICAL DEVELOPMENT

TROHLEARNA DISPLAZIJA – KONGENITALNA ANOMALIJA ILI BIOMEHANIČKI RAZVOJ

Zlatko TEMELKOVSKI, Zoran BOŽINOVSKI, Alan ANDONOVSKI and Biljana ANDONOVSKA

Summary

Introduction. The aim of this study was to investigate the appearance of the trochlear groove in infants and to present the possible causes for the development of trochlear dysplasia as one of the most severe pathologic findings in patients with patellar instability. **Material and Methods**. Knee ultrasonography was performed in 200 infants, 3 to 6 months of age. The measurements were made at 30 and 60 degrees of knee flexion, in order to measure the trochlear bone and cartilaginous sulcus angle on the patellar surface of the femur and to determine the degree of trochlear dysplasia. A 7-megahertz probe was used for measurements, which was tangentially placed with the reference to the posterior femoral joint. **Results.** A completely flat trochlear bony sulcus angle was registered in all infants aged 3 to 6 months. The mean cartilaginous sulcus angle was between 149 ± 5.4° and 19 infants had a sulcus angle over 159°. Eleven infants with trochlear dysplasia were in breech presentation at birth. **Conclusion**. Our study showed that the cartilaginous part of the trochlear groove was already well developed at birth. Breech presentation of the fetus could be a predisposing factor for dysplasia of the cartilaginous part of the trochlear groove. The bony part of the trochlear groove is dysplastic in infants and it gradually gets deeper, later getting a shape of the overlying articular cartilage. The influence of the Delpech law, with lower pressure in the trochlear groove, could be the possible mechanical theory explaining the development of the trochlear dysplasia in the later stage of the childhood.

Key words: Joint Instability; Patellofemoral Joint; Ultrasonography; Infant, Newborn; Infant; Breech Presentation; Risk Factors; Models, Theoretical

Sažetak

Uvod. Cilj ove prezentacije je da pokaže pojavu trohlearnog žleba kod novorođenčadi i da predstavi mogući uzrok razvoja trohlearne displazije kao jednog od najtežih patoloških nalaza kod pacijenata sa nestabilnošću patele. **Materijal i metode.** Ultrasonografija je obavljena na 200 novorođenčadi uzrasta od tri i šest meseci. Merenja su izvedena u fleksiji kolena od 30 i 60 stepeni, kako bi se izmerili trohlearni koštani žleb i ugao na hrskavičavoj površini čašice i kako bi se odredio stepen trohlearne displazije. Pre merenja korišćena je 7 MHz linearna sonda koja je postavljena tangencijalno u odnosu na patelofemoralni zglob. **Rezultati.** Kod svakog novorođenčeta uzrasta od tri i šest meseci registrovan je potpuno ravan trohlearni koštani žleb. Prosečan ugao hrskavičavog sulkusa bio je između 149±5,4° a 19 novorođenčadi imalo je ugao sulkusa više od 159°, dok je 11 sa trohlearnom displazijom imalo karlični položaj pri rođenju. **Zaključak.** Naša studija pokazala je da je hrskavični deo trohlearnog žleba već dobro razvijen na rođenju. Karlična prezentacija fetusa može biti predisponujući faktor za displaziju hrskavičnog dela trohlearnog žleba. Koštani deo trohlearnog žleba je displastičan kod novorođenčadi i postepeno dobija dubinu kako bi preuzeo oblik zglobnog hrskavice. Uticaj Delpešovog zakona objašnjen je smanjenim pritiskom u troholarnom žlebu, te je moguće mehaničkom teorijom objasniti razvoj trohlearne displazije u kasnijoj fazi detinjstva.

Ključne reči: nestabilnost zgloba; patelofemoralni zglob; ultrasonografija; novorođenče; odojče; karlični porođaj; faktori rizika; teoretski modeli

Introduction

Patellar instability is a complex orthopedic problem, especially due to the multifactorial etiology. The anatomy of the femoral trochlea is of vital importance for the stability of the patellofemoral joint. Knowing the characteristics of the femoral trochlea in newborns may prove useful when considering the predisposing factors for patellar instability. The dysplastic trochlea was first discovered and described by Richerand in 1802, and its surgical correction performed by elevation of the lateral femoral condyle was described by Albe in 1915 [1]. Brattstorm and Maldague [2, 3] gave the first classification of trochlear dysplasia using axial X-rays of the knee and Dejour H. et al. [4–8] gave the second classification based on the level of the trochlear line crossing with the lines of the two condyles, seen on profile X-rays. According to the studies, there is no consensus about the etiology of the trochlear dysplasia. There is no consensus whether it is genetic in origin [9], caused by imbalanced forces indicating maltracking and re-

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Abbreviations

CT – computerized tomography
MRI – magnetic resonance imaging
SA – sulcus angle

modeling of the trochlea during infancy and growth [10], or due to other unknown factors. Results of some recent studies [11, 12] indicate that dysplasia of the femoral trochlea may be congenital, with a breech presentation of the fetus as a major risk factor for its development. It is likely that excessive lateral pressure during growth and development will modify the shape of the patella as well as the trochlea. This would follow the law of Delpech [9] enunciated in 1829, concerning the influence of compression and tensile forces on an epiphysis. This indicates that dysplasia of the femoral trochlea can also be caused by development.

Axial radiographs do not show cartilaginous structures and therefore give little information about the trochlea shape and patelofemoral articulation in infants and small children [13–15]. Contrast methods are invasive and cumbersome, while computerized tomography (CT) and magnetic resonance imaging (MRI) in small children necessitate sedation or anesthesia. As a method of visualizing the newborn femoral trochlea and the position of the patella, ultrasonography is a reliable tool to define the configuration both the osseous and cartilaginous femoral sulcus. The sulcus angle (SA) is the closed angle defined by the intersection of the lines parallel to the articular cartilage of the medial and lateral femoral facets. It is easy to measure and interpret, so it is commonly used to describe dysplasia of the femoral trochlea. The SA was found to be the most reliable parameter, with an intra-examiner variation of −1°, standard deviation (SD) 2.5 and an inter-examiner variation of 0° (SD 2.1) [11].

The purpose of our study was to investigate the appearance of the trochlear groove in infants using ultrasonography and examine the possible causes for the development of trochlear dysplasia.

Material and Methods

A right knee ultrasonography was performed in 200 infants (120 male and 80 female) aged 3 to 6 months. The measurements were performed at the University Clinic for Orthopedic Surgery in Skopje, with knee flexion at 30 and 60 degrees, in order to measure the bony and cartilaginous SA on the patellar surface of the femur **(Figure 1)**. The measurements were performed using a 7 MHz linear-array probe, which was tangentially placed with the reference to the posterior femoral joint **(Figures 2 and 3).** The osseous and cartilaginous SAs were measured at the level of the most ventral point on the lateral patellar facet of the femur. We took SA of over 159 degrees to indicate trochlear dysplasia.

All data were expressed as mean ± SD and analyzed by statistical package for the social sciences (SPSS) 12.0 software. Group comparison was performed with the Student t-test and $p < 0.05$ was considered statistically significant.

Figure 1. Knee ultrasound examination of a 3-month newborn; the probe is in a perpendicular position to the knee joint
Slika 1. *Ultrazvučni pregled kolena kod tri meseca starog novorođenčeta. Sonda je pod pravim uglom postavljena na koleno za vreme pregleda*

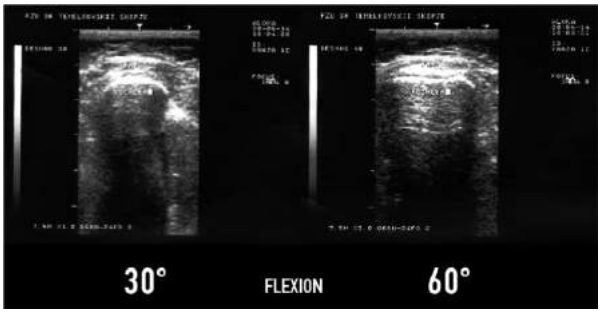

Figure 2. Ultrasonographs of a 3-month infant; the trochlea is flat at 30 and 60 degrees of flexion
Slika 2. *Ultrazvučni pregled tri meseca starog novorođenčeta. Trohlerana površina pod uglom 30 i 60 stepeni savijanja kolena*

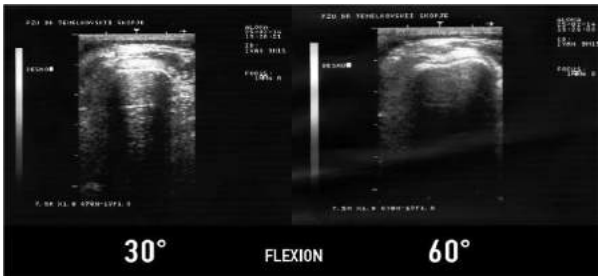

Figure 3. Ultrasonographs of a 6-month infant; the trochlea is still flat at the same degrees of flexion
Slika 3. *Ultrazvučni pregled šest meseci starog novorođenčeta. Trohlerana površina pod istim uglom savijanja kolena*

Results

A completely flat bony SA was registered in all infants. The mean cartilaginous SA was 149 ± 5.4° and 19 infants had a SA over 159°. Eleven infants with trochlear dysplasia were in breech presentation at birth **(Table 1)**. Moreover, we did not observe significant difference in the femoral cartilaginous SA with

Table 1. Presentation of the mean trochlear sulcus angle, number of infants with trochlear dysplasia and number of infants born in breech position with gender distribution
Tabela 1. *Prosečene vrednosti trohleranog ugla, broj dece sa trohleranom displazijom i broj dece rođene karličnim položajem u odnosu na pol*

	Female/*Ženski*	Male/*Muški*	All/*Svi*
Mean ± SD/*Prosek ± SD*	148 ± 6.4	150 ± 5.4	149 ± 5.4
Dysplastic/Normal (%)/*Displastičan/Normalan*	11/120 (9.2%)	8/80 (10%)	20/200 (9.5%)
Presentation/Dysplastic (%)/*Prezentacija ploda/Displastična trohlea*	6/11 (54.5%)	5/8 (62.5%)	11/20 (55%)

respect to various degrees of knee flexion during the measurements.

Discussion

An abnormally shallow trochlear sulcus has been reported to be an important factor in patellar instability [16, 17]. Trochlear dysplasia means that the trochlea is flat or dome shaped and there is no congruence between the patellar and trochlear facets. Dysplasia of the lateral femoral condyle is particularly important, because of the bony support that prevents lateral patellar dislocation. Although cartilage of the patellofemoral joint is visualized in CT scans and MRI, both methods provide images of the centre of the patellar articular cartilage [18]. This makes it difficult to compare measurements of the femoral sulcus, because the vertical position of the patella varies between individuals. The ultrasonographic technique allows the measurement of both osseous and cartilaginous trochlear sulci at a constant and reproducible point of reference, the most of the anterior part of the lateral patellar facet of the femur. Our study showed that cartilaginous trochlear sulcus is well developed at birth. The mean value for the cartilaginous SA in our study correlates with the results presented in other recent studies. Results of our study showed that dysplasia of the femoral trochlea can be also congenital, with a breech presentation of the fetus as a major risk factor for its development. We found about 10% newborns with trochlear dysplasia and more than half of them were in breech presentation at birth. That means that the shape of the fetal femoral trochlea appears to be susceptible to the influence of mechanical forces. A moving knee with normal patellar tracking in the final stage of pregnancy may be of vital importance for normal anatomy [12]. A fetus with space to kick and flex the lower limbs has a good prospect of developing a femoral trochlea with the depth needed to support the patella. There are also studies which show that the anatomic characteristics of the trochlea could be integrated into the genome during the course of evolution. This would be in favor of the genetic origin of trochlear dysplasia [19]. We found flat bony trochlear sulcus in all infants, which means that it gets deeper during growth, getting a shape of the overlying articular cartilage probably by the adolescence.

The basic postulate for biomechanical development of the dysplastic trochlea is the Delpech law, according to which the cartilage of diarthrodial joints transfers decreased pressure and the neighbouring growth cartilage is in reverse proportional hyperactivity. In the human newborn and non-walking children the femoral diaphysis is vertical. As the child starts walking, the femoral obliquity angle develops between 1 and 7 years of age, inducing a secondary valgus of the extensor apparatus, physiological patellofemoral pressure and modifications of the patellofemoral joint. That correlates with the results from the recent experimental studies on animals where patellar malpositioning caused by patellar tendon Z-plasty lengthening or medial patellar soft tissue restraints release leads to more flattened trochlea and predisposition for patellar instability.

Conclusion

Our study showed that the cartilaginous part of the trochlear groove is already well developed at birth. Dysplasia of the femoral trochlea may be congenital, with a breech presentation of the fetus as a major risk factor for its development. Bony part of the trochlear groove is dysplastic in newborns and it gradually gets deeper, in time getting the shape of the overlying articular cartilage. According to the Delpech law, the development of the trochlear dysplasia in the later stage of childhood may be explained by lower pressure in the trochlear groove, in favor of the mechanical theory.

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CONE-BEAM COMPUTED TOMOGRAPHY STUDY OF ROOT NUMBER AND ROOT CANAL CONFIGURATION OF PREMOLARS IN SERBIAN POPULATION

ANALIZA BROJA KORENOVA I KONFIGURACIJE KANALA KORENOVA PREMOLARA KOMPJUTERIZOVANOM TOMOGRAFIJOM SA KONUSNIM SNOPOM ZRAČENJA U SRPSKOJ POPULACIJI

Milica POPOVIĆ, Miloš PAPIĆ, Aleksandar ACOVIĆ, Suzana ŽIVANOVIĆ and Tatjana KANJEVAC

Summary

Introduction. The aim of this study was to establish the number of roots and present the root canal configuration in the maxillary and mandibular premolar teeth, and evaluate the relations among these characteristics with gender and teeth position in the Serbian population using cone-beam computed tomography. **Material and Methods**. Cone-beam computed tomography images of 570 teeth of 150 patients were evaluated. Teeth were classified into the following groups: maxillary first premolars, maxillary second premolars, mandibular first premolars and mandibular second premolars, and the number of roots and root canals per tooth, whereas root canal configurations were examined along with the tooth position and patients' gender. The root canal configuration was classified using Vertucci's classification. Statistical significance was obtained using Chi square test. **Results.** In maxillary first premolars, two roots (53.5%) and two root canals (84.5%) were the most prevalent, as well as type IV configuration (58.9%). In maxillary second premolars, most teeth had one root (88.1%) and one root canal (59.6%). In regard to gender, complex configurations with multiple canals were more prevalent in males. Higher incidence of type IV configuration in maxillary first premolars was present on the right side of the jaw (70.2%). Most mandibular first premolars had one root (98.5%). In mandibular second premolar, all teeth had one root and most had type I configuration (96.2%). Males showed higher incidence of two canals in mandibular first premolars. **Conclusion.** Cone-beam computed tomography is a useful tool for obtaining valuable information on root canal morphology of premolar teeth. Patient's gender should be considered when performing the preoperative assessment of endodontic treatment.

Key words: Cone-Beam Computed Tomography; Tooth Root; Dental Pulp Cavity; Bicuspid; Maxilla; Mandible; Sex Characteristics; Population; Serbia

Sažetak

Uvod. Cilj ove studije je da prikaže broj korenova i konfiguraciju kanala korenova gornjih i donjih premolara kao i povezanost ovih karakteristika sa polom i položajem zuba u populaciji Srbije korišćenjem kompjuterizovane tomografije sa konusnim snopom. **Materijal i metode.** Pregledani su snimci kompjuterizovane tomografije sa konusnim snopom 570 zuba od 150 pacijenata. Zubi su klasifikovani po grupama (gornji prvi premolari, gornji drugi premolari, donji prvi premolari i donji drugi premolari); registrovani su broj korenova, kanala korenova po zubu i konfiguracija kanala zajedno sa pozicijom zuba i polom pacijenta. Za klasifikaciju konfiguracija kanala korenova korišćena je klasifikacija po Vertućiju. Statistička značajnost je dobijena korišćenjem hi kvadrat testa. **Rezultati.** U grupi gornjih prvih premolara najčešće su uočena dva korena (53,5%), dva kanala korena (84,5%) i konfiguracija kanala tip IV (58,9%). U grupi gornjih drugih premolara najveći broj zuba imao je jedan koren (88,1%) i jedan kanal korena (59,6%). Postojala je značajna razlika prema polu gde je kompleksnija konfiguracija sa više kanala korena češće uočavana kod muškaraca. Veća učestalost konfiguracije tip IV gornjih prvih premolara uočena je na desnoj strani vilice (70,2%). Najviše donjih prvih premolara imalo je jedan koren (98,5%) i konfiguraciju tip I (83,2%). U grupi donjih drugih premolara, svi zubi su imali jedan koren, a najučestalija je konfiguracija kanala korena tip I (96,2%). Takođe, muškarci su češće imali više kanala korena u grupi donjih prvih premolara. **Zaključak.** Kompjuterizovana tomografija sa konusnim snopom je korisno dijagnostičko sredstvo čijom se primenom dobijaju značajne informacije o morfologiji kanala korenova zuba. Treba imati u vidu pol pacijenta kada se planira endodontski tretman.

Ključne reči: kompjuterizovana tomografija konusnog snopa; koren zuba; kanal zuba; premolar; maksila; mandibula; polne karakteristike; populacija; Srbija

Introduction

Knowledge of the root canal morphology and possible variations in the root canal number and configuration is the basic prerequisite for successful endodontic therapy. One of the most common causes of endodontic therapy failure is omission of one or more root canals, which are overseen. The most common reasons for neglecting the root canal therapy are morphological variations of the root canal system [1, 2]. Previous studies have shown that premolar teeth can vary considerably in the number of roots and root canals. The frequency of three root canals in the maxillary premolar teeth varies from 0% [3] to 10% [4]. Variations in the root number and root canal morphology of premolar teeth are seen among subjects of different race, geo-

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Abbreviations
CBCT – cone-beam computed tomography
FOV – field of view

graphical origin, and gender. A study in Chinese population [5] has demonstrated the incidence of a single root in the maxillary first premolars in 66%, while a study in Saudi population showed an incidence of two roots in 81% [6]. Configurations with multiple root canals in maxillary first premolars vary from 37% to 86% [3, 10]. A single root canal in mandibular first premolars in different populations is seen from 66% to 94% [3, 4, 7–15]. The study of Trope et al. demonstrated that premolar teeth with multiple root canals are more frequently observed in black people [16]. Gender diversity in the number of roots and root canals has also been reported [17]. A study of Ok et al. showed a higher incidence of single-rooted mandibular premolars in females, while two-rooted and tree-rooted mandibular premolars are more common in males [10].

There are various methods used for studying internal root morphology. The most common methods include clearing and staining techniques [4, 9, 11, 15, 18–22], cross-sections [23], radiographic techniques and others [13, 24]. These methods require the use of extracted teeth, and some methods require complete destruction of the observed samples. In the last decade, cone-beam computer tomography (CBCT) has been widely used in the assessment of the root internal morphology [3, 7, 8, 10, 12, 14, 25, 26]. CBCT provides an overview of the object in three dimensions: axial, sagittal and coronal sections without superimposition of anatomical structures. Furthermore, CBCT can be used to evaluate root canal configuration in clinical conditions.

The South-Eastern region of Europe has traditionally been considered as a connecting bridge between the Middle East and Western Europe. Centuries of migrations of different ethnic groups have allocated a specific population of Balkan Peninsula that has specific genetic traces [27]. Thus, due to genetic similarities of many nations of Balkans, it is impossible to separate populations by their ethnicity in order to analyze dental morphology. To the best of our knowledge, there are no studies on the internal configuration of premolar roots by using CBCT in the Serbian population. The aim of this study was to examine the number of roots and the number and configuration of root canals in the maxillary and mandibular first and second premolars, and the relations of these characteristics with two genders and position (left or right side of the jaw) in Serbian population, as a subpopulation of South-Eastern Europe, using CBCT.

Material and Methods

The research was conducted respecting the Declaration of Helsinki and Good Clinical Practice guidelines, as approved by the Ethics Committee of the Faculty of Medical Sciences, University of Kragujevac (No: 01-15942).

The total study sample included CBCT images of 570 teeth, of 150 patients from a pre-existing database. All CBCT images were made at the Radiology Department, Faculty of Medical Sciences, University of Kragujevac, from September 2014 to February 2017. The scans were obtained using Orthophos XG 3D device (Sirona Dental Systems GmbH, Bensheim, Germany), with three-dimensional settings for recording, VOL1 or VOL1 HD, and a voxel size of 160 μm; the layer thickness of 0.16 mm with large field of view (FOV). The reasons for CBCT scanning were different (prosthetic, surgical, orthodontic and endodontic).

The main image inclusion criterion was the existence of at least one premolar in the maxilla or mandible. Other inclusion criteria were as follows: 1) full tooth visibility; 2) complete root growth; 3) no radiographically visible periapical lesions; 4) no radiographically visible external or internal root resorption; 5) no endodontic treatment, and 6) no prosthodontic restoration.

The teeth were classified in groups (maxillary first premolars, maxillary second premolars, mandibular first premolars and mandibular second premolars) and the following parameters were examined:

- The number of roots per tooth
- The number of root canals
- The root canal configuration
- Position of the tooth - left or right side of the jaw
- Patients' gender.

The root canal configuration was classified into eight types of Vertucci's classification (Vertucci FJ, 1984) [9] **(Figure 1)**.

Cone-beam computer tomography images were analyzed using a software program GALAXIS v1.9.4 (Sirona Dental Systems GmbH, Bensheim, Germany), in axial, sagittal, and coronal sections. The examination was conducted using a 23-inch Philips LED monitor, with a resolution of 1920 x 1080 pixels in a room with dim lighting. Brightness and contrast were adjusted using a software program.

Statistical data analysis was performed using a commercial Statistical Package for the Social Sciences (SPSS) software v20.0 (SPSS Inc., Chicago, IL, USA). The number of roots, the number of root canals and root canal morphology were described using descriptive statistics: incidence, percentage, mean (average), median, standard deviation (SD) and scope (range). The incidence of different parameters and possible correlations were analyzed between the two genders and side of the jaw where the tooth was located. Chi-square (χ^2) test was used to compare the incidence of category variables and to demonstrate statistical significance. All results where the probability of null hypothesis was less than 5% ($p < 0.05$) were considered statistically significant.

Results

Scans of 150 patients were analyzed, of whom 78 were male (52%) and 72 female (48%), with a mean age of 39.87 years (SD: 15.86), where the youngest patient was 13, and the oldest was 80 years

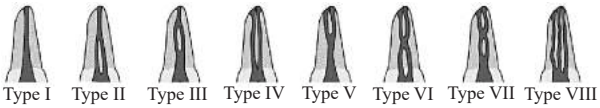

Figure 1. Diagrammatic representation of Vertucci's root canal configurations
Slika 1. *Shematski prikaz konfiguracija kanala korena prema Vertućiju*

old. In regard to the maxillary first premolars, 27% of patients had unilateral, 29% had bilateral, and 44% had no maxillary first premolar teeth. The maxillary second premolars were bilaterally present in 28% of the scans, unilaterally in 14%, and 58% had no second premolars. In regard to the mandibular first premolars, 58% of patients had bilateral, 18% unilateral, and 24% had no mandibular first premolar teeth. The mandibular second premolars were bilaterally present in 36%, unilaterally present in 19%, and missing in 45% of scans. The final sample included 570 teeth (129 maxillary first premolars, 109 maxillary second premolars, 202 mandibular first premolars and 130 mandibular second premolars).

Maxillary First Premolars

Out of the total maxillary first premolars (n= 129), one root was present in 55 teeth (42.6%), two roots in 69 teeth (53.5%) and three roots were present in 5 teeth (3.9%). There was a statistically significant difference in the number of roots between males and females ($p < 0.05$, $p = 0.002$), where single-rooted first premolars were more common in females (63.6%) and two-rooted in males (60.9%). Also, all tree-rooted premolars were observed in males. There was no statistical difference in the number of roots in regard to the teeth position ($p > 0.05$, $p = 0.597$) **(Table 1)**.

The maxillary first premolars with two canals were present in 84.5%, one canal in 10.1%, and three root canals were found in 5.4% **(Figure 2)**. The number of root canals was statistically different between the two genders ($p < 0.05$, $p = 0.012$), where one root canal was more prevalent in females (n = 9, 69.2%) and three canals were significantly more prevalent in males (n = 7, 100%). The tooth position showed no difference in root canal number ($p > 0.05$, $p = 0.076$) **(Table 1)**.

Table 1. Distribution of different number of roots and root canals in first and second maxillary and mandibular premolars
Tabela 1. *Distribucija broja korenova i kanala korenova u grupama prvih i drugih gornjih i donjih premolara*

Tooth/*Zub*	Gender *Pol*	Number of roots/*Broj korenova*			Number of root canals/*Broj kanala*			Number of teeth/*Broj zuba*
		One root/ *Jedan koren*	Two roots/ *Dva korena*	Three roots/ *Tri korena*	One root canal/*Jedan kanal korena*	Two root canals/*Dva kanala korena*	Three root canals/*Tri kanala korena*	
Maxillary first premolar/ *Gornji prvi pretkutnjak*	male *muški*	20 (29.9%)*	42 (62.7%)*	5 (7.5%)	4 (6.0%)*	56 (83.6%)*	7 (10.4%)*	67 (51.9%)
	female *ženski*	35 (56.5%)*	27 (43.5%)*	/	9 (14.5%)*	53 (85.5%)*	/	62 (48.1%)
	total *ukupno*	55 (42.6%)	69 (53.5%)	5 (3.9%)	13 (10.1%)	109 (84.5%)	7 (5.4%)	129 (100%)
Maxillary second premolar/*Gornji drugi pretkutnjak*	male *muški*	35 (74.5%)*	12 (25.5%)*	/	12 (25.5%)*	33 (70.2%)*	2 (4.3%)*	47 (43.1%)
	female *ženski*	61 (98.4%)*	1 (1.6%)*	/	53 (85.5%)*	9 (14.5%)*	/	62 (56.9%)
	total *ukupno*	96 (88.1%)	13 (11.9%)	/	65 (59.6%)	42 (38.5%)	2 (1.8%)	109 (100%)
Mandibular first premolar/ *Donji prvi pretkutnjak*	male *muški*	106 (100%)	/	/	82 (77.4%)*	24 (22.6%)*	/	106 (52.5%)
	female *ženski*	93 (96.9%)	3 (3.1%)	/	86 (89.6%)*	10 (10.4%)*	/	96 (47.5%)
	total *ukupno*	199 (98.5%)	3 (1.5%)	/	168 (83.2%)	34 (16.8%)	/	202 (100%)
Mandibular second premolar/*Donji drugi pretkutnjak*	male *muški*	78 (100%)	/	/	74 (94.9%)	4 (5.1%)	/	78 (60%)
	female *ženski*	52 (100%)	/	/	51 (98.1%)	1 (1.95%)	/	52 (40%)
	total *ukupno*	130 (100%)	/	/	125 (96.2%)	5 (3.8%)	/	130 (100%)

*Statistical significance at the level of $p < 0.05$ (Chi-square test)/**Nivo statističke značajnosti p<0,05 (hi kvadrat test)*

The most common configuration of root canal was type IV (58.9%) followed by type II (20.2%), type I (10.1%), type VIII (5.4%) and other types (5.5%). The gender and tooth position showed a significant difference in different types of Vertucci's classification. Type IV and type VIII were more prevalent in males, while type I was more prevalent in females ($p < 0.05$, $p = 0.033$). On the right side of the jaw most of teeth were type IV (n = 40, 70.2%) ($p < 0.05$, $p = 0.000$) **(Table 2)**.

Maxillary Second Premolars

Of the maxillary second premolars, one root was found in 96 teeth (88.1%), and two roots in 13 teeth (11.9%). There was a statistically significant difference in the number of roots between males and females ($p < 0.05$, $p = 0.00$); single-rooted teeth were more prevalent in females (63.5%), while two-rooted were more prevalent in males (92.3%). The tooth position showed no difference ($p > 0.05$, $p = 0.795$) **(Table 1)**.

Most maxillary second premolars were with one root canal (n = 65, 59.6%), while two root canals were found in 42 maxillary second premolars (38.5%). Three root canals were observed only in two cases (1.8%). Statistical analysis revealed a statistically significant difference between the two genders ($p < 0.05$, $p = 0.000$). Single-canal teeth were more common in females (n = 53, 81.5%), while two canals were more frequent among males (n = 30, 78.6%). The position of the teeth showed no significant difference ($p > 0.05$, $p = 0.225$) **(Table 1)**.

The most common root canal configuration was type I (n = 65, 59.6%), followed by type IV (n = 17, 15.6%), type II and type III (n = 9, 8.3%), and other types in 8.2% (n = 9). There was a statistical difference between the two genders ($p < 0.05$, $p = 0.000$), where type I was more prevalent in females (81.5%), and type IV in males (94.1%) **(Table 2)**. Regarding the teeth position, there was no statistically significant difference ($p > 0.05$, $p = 0.421$).

Mandibular First Premolars

Almost all the mandibular first premolars had one root (n = 199, 98.5%). Two roots were observed only in three teeth (1.6%) with two root canals. Most of the mandibular first premolars had one root canal (n = 168, 83.2%), and 34 teeth had two root canals (16.8%). The number of root canals differed in males and females ($p < 0.05$, $p = 0.02$); two canals were more predominant among males (n = 24, 70.6%). The teeth position showed no difference ($p > 0.05$, $p = 0.347$) **(Table 1)**.

According to the Vertucci's classification, the most common type was type I (n = 168, 83.2%), followed by type V (n = 14, 6.9%), type III (n = 13, 6.4%). and other types (n = 7, 3.5%). Root canal configurations differed between males and females ($p < 0.05$, $p = 0.041$) with more complex root canals in males, while teeth position showed no difference ($p > 0.05$, $p = 0.269$) **(Table 2)**.

Mandibular Second Premolars

All the examined mandibular second premolars had one root (n = 130) and most had one root canal with type I root configuration (n = 125, 96.2%). Two root canals were found in 5 teeth (3.8%) **(Table 1)**. Among the teeth with two root canals, 3 teeth had

Table 2. Root canal configuration according to Vertucci's s classification in groups of first and second maxillary and mandibular premolars

Tabela 2. *Konfiguracija kanala korena prema Vertućijevoj klasifikaciji u grupama prvih i drugih gornjih i donjih premolara*

Tooth *Zub*	Gender *Pol*	Vertucci classification/*Klasifikacija prema Vertućiju*						
		Type I/*Tip I*	Type II/ *Tip II*	Type III/ *Tip III*	Type IV/ *Tip IV*	Type V/ *Tip V*	Type VI/ *Tip VI*	Type VIII/ *Tip VIII*
Maxillary first premolar *Gornji prvi pretkutnjak**	male/*muški*	4 (3.1%)	11 (8.5%)	2 (1.6%)	43 (33.3%)	/	/	7 (5.4%)
	female/*ženski*	9 (7%)	15 (11.6%)	2 (1.6%)	33 (25.6%)	1 (0.8.%)	2 (1.6%)	/
	total/*ukupno*	13 (10.1%)	26 (20.2%)	4 (3.1%)	76 (58.9%)	1 (0.8%)	2 (1.6%)	7 (5.4%)
Maxillary second premolar/*Gornji drugi pretkutnjak**	male/*muški*	12 (11%)	7 (6.4%)	3 (2.8%)	16 (14.7%)	5 (4.6%)	2 (1.8%)	2 (1.8%)
	female/*ženski*	53 (48.6%)	2 (1.8%)	6 (5.5%)	1 (0.9%)	/	/	/
	total/*ukupno*	65 (59.6%)	9 (8.3%)	9 (8.3%)	17 (15.6%)	5 (4.6%)	2 (1.8%)	2 (1.8%)
Mandibular first premolar *Donji prvi pretkutnjak**	male/*muški*	82 (40.6%)	3 (1.5%)	10 (5%)	1 (0.5%)	10 (5%)	/	/
	female/*ženski*	86 (42.6%)	/	3 (1.5%)	3 (1.5%)	4 (2%)	/	/
	total/*ukupno*	168 (82.6%)	3 (1.5%)	13 (6.4%)	4 (2%)	14 (6.9%)	/	/
Mandibular second premolar/*Donji drugi pretkutnjak*	male/*muški*	74 (56.9%)	2 (1.5%)	/	/	2 (.%)	/	/
	female/*ženski*	51 (39.2%)	1 (0.8%)	/	/	/	/	/
	total/*ukupno*	125 (96.2%)	3 (2.3%)	/	/	2 (1.5%)	/	/

*Statistical significance at the level of $p<0.05$ (Chi-square test)/**Nivo statističke značajnosti p<0,05 (hi kvadrat test)*

type II configuration (2.3%), and two had type V configuration (1.5%). No significant difference was seen in regard to the gender or the position of the teeth **(Table 2)**.

Discussion

A lack of knowledge of the anatomy of the pulp cavity may contribute to the failure of endodontic therapy. Root canal therapy of two- and three-rooted teeth is less successful when compared with single-rooted teeth [28]. It is hardly possible to diagnose root configuration with multiple canals just by clinical observation or by using a clinical microscope [29]. Studies of root morphology in different populations provide clinicians with general knowledge of most frequent root configurations.

In previous studies, a variety of methods were used for evaluating the internal morphology of teeth in laboratory and clinical settings [4, 9, 11, 13, 15, 18–24]. Laboratory methods are highly accurate, whereas the clearing and staining technique is considered the gold standard in assessing root canal morphology, but their implementation requires tooth extraction [4]. In the last decade, the CBCT was often used to study anatomical and morphological characteristics of root canals in clinical settings [3, 7, 8, 10, 12, 14, 25, 26]. CBCT is an accurate gold standard (clearing and staining technique) [30]. To our knowledge, this is the first study of the internal morphology of maxillary and mandibular premolars in Serbian population using CBCT. To describe the root canal configuration we used the Vertucci's classification, the same as in most of the previous studies [3, 7–13, 19, 21, 23, 25, 26].

Maxillary Premolars

Using CBCT, the present study showed that maxillary first premolars mostly had two roots (53.5%), while one root was present in 42.6%. Similar results were reported in the study of Loh et al. [20] using the clearing method, where maxillary first premolars with two roots were present in 51%.

Differences in the number of roots and canals are seen in different geographical regions. Previous studies in Serbian population [4, 22] reported a similar incidence of one root in maxillary first premolars as our study (43% and 46.3%, respectively) by using clearing and staining techniques. A study conducted in Spanish population showed similar results, where one root was present in 46% [26]. In Indian population [18], the frequency of one root was 11.7%, whereas the highest prevalence of one root was reported by Tian et al. (66%) who analyzed CBCT scans of the Turkish population [25]. In our study, three roots in the maxillary first premolars were present in 3.9%, which is comparable to the results of Nikolić et al. (5.1%) [4], but higher than Bulut et al. (1%) [3]. Studies of Stošić et al. [22], Loh et al. [20] and Rwenyonyi et al. [19] did not report any maxillary first premolars with three roots.

In the group of maxillary second premolars, our study showed the highest incidence of one root, in 88.1%. Previous studies in different populations have reported similar results ranging from 82.1% to 89.6% [3, 12, 18, 22, 26]. A greater difference was reported by Kartal et al. in Turkish population, where they found the incidence of one root in 69.6% [21]. The incidence of three roots was very low in previous

Table 3. Distribution of root configuration types for maxillary premolars according to other studies
Tabela 3. *Distribucija različitih tipova konfiguracije kanala korenova gornjih premolara u ranijim studijama*

		Method *Metoda*	Sample size/ *Veličina uzorka*	Vertucci classification/*Klasifikacija prema Vertućiju* (%)							
				Type I *Tip I*	Type II/*Tip II*	Type III *Tip III*	Type IV/*Tip IV*	Type V *Tip V*	Type VI/*Tip VI*	Type VII/*Tip VII*	Type VIII/*Tip VIII*
Maxillary first premolar *Gornji prvi pretkutnjak*	Bulut DG. et al.	CBCT	511	62.6	34.1	0.8	1.9	0.6	/	/	/
	Vertucci	Clearing	400	8.0	18.0	/	26.0	62.0	7.0	/	/
	Ok E. et al.	CBCT	1379	9.6	6.5	1.4	76.9	4.6	0.1	/	1.0
	Rwenyonyi CM. et al.	Clearing	210	3.3	2.9	0.5	80.5	0.5	0.5	1.0	2.4
	Kartal N. et al.	Clearing	300	8.7	1.0	/	71.3	14.7	2.3	0.3	1.3
	Tian YY. et al.	CBCT	300	14.0	23.0	4.0	51.0	3.0	2.0	1.0	1.0
	Abella F. et al.	CBCT	430	25.1	10.2	4.4	52.8	1.9	1.6	1.4	2.6
	Present study/*Ova studija*	CBCT	129	10.1	20.2	3.1	58.9	0.8	1.6	/	5.4
Maxillary second premolar *Gornji drugi pretkutnjak*	Bulut DG. et al.	CBCT	476	77.6	12.5	1.33	6.5	1.9	0.17	/	/
	Vertucci	Clearing	200	24.0	11.0	2.5	37.5	5.5	3.0	2.5	1.0
	Ok E. et al.	CBCT	1301	54.5	8.8	3.6	21.9	10.8	/	/	0.3
	Yang L. et al.	CBCT	392	45.4	16.3	11.4	20.2	6.4	/	/	0.3
	Kartal N. et al.	Clearing	300	48.7	6.3	/	38.0	5.7	0.7	/	0.7
	Abella F. et al.	CBCT	374	39.3	22.5	7.2	19.8	4.3	3.2	2.1	
	Present study/*Ova studija*	CBCT	109	59.6	8.3	8.3	15.6	4.6	1.8	/	1.8

CBCT – kompjuterizovana tomografija sa konusnim snopom

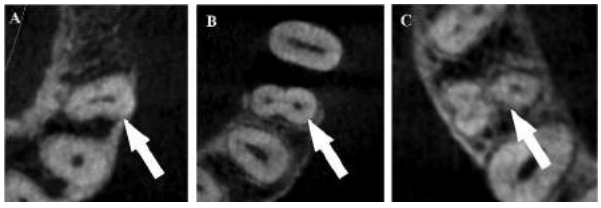

Figure 2. Axial view of root canal number in maxillary premolar teeth. A - one root canal; B - two root canals; C - three root canal

Slika 2. *Aksijalni prikaz različitog broja kanala korenova gornjih premolara. A. Jedan kanal korena. B. Dva kanala korena. C. Tri kanala korena.*

studies (0.6% – 1.6%), while our study showed no three-rooted second premolars [18, 21, 26].

In the present study, we demonstrated a different incidence of root number in maxillary premolars between the two genders. Single-rooted maxillary premolars were more common in females, while multi-rooted were more common in males. All three-rooted maxillary premolars were found in males. Similar differences were reported in previous studies [3, 10].

Most maxillary first premolars in the present study had two root canals (84.5%), while three root canals were found in 5.4%, which is similar to the values shown in the previous studies in the Serbian population [4, 22]. Studies in other populations showed a lower incidence of three root canals (0% – 3.4%) [3, 10, 19, 21, 24]. In the group of maxillary second premolars, one root canal was present in 59.6%, while three root canals were observed only in 1.8%. A similar incidence of one canal was reported in the Turkish population (59.7%) [10], while previous studies in the Serbian population showed a significantly higher incidence of one root canal (79.3%) [4]. Yang et al. [12] reported the highest frequency of two root canals in 54.3%. The incidence of three root canals in previous studies was from 0.3% to 2% [10, 12, 21, 22, 24, 26]. We observed a significantly higher incidence of two root canals in maxillary premolar teeth among males, and one root canal in females. All teeth with three root canals were among males (n = 7). Previous studies reported the same difference between the two genders [3, 10].

In the present study, the most common type of root canal configuration in the maxillary first premolars was type IV (58.9%), followed by type II (20.2%). The Vertucci's classification [9] showed the highest incidence of type V in 62%, which is in contrast to our study (0.8%). This difference could be attributed to methodology since Vertucci used clearing and staining technique, or to the examiner's precision. Since the majority of previous studies, regardless of the methodology, showed the highest frequency of type IV (71.3% – 80%) [10, 19, 21], and studies of Tian et al. [25] and Abella et al. [26], showed the similar incidence of type IV configuration (51% and 52.8%, respectively), we assume that the results differ due to the population. The presence of type VIII in previous studies ranged from 0% to 2.6%, which is lower than in our study (5.4%) [9, 10, 19, 21, 25, 26]. In the group of maxillary second premolars, the most common were type I in 59.6%, followed by type IV in 15.6%. The highest incidence of type I was shown in most of the previous studies [3, 10, 12, 21, 26], while the study of Vertucci [9] showed the highest frequency of type IV (37.5%). The next most common type in our study was type III (8.3%). A similar frequency was shown by Yang et al. [12], in Chinese population (11.4%). Comparison of our results in root configuration with results of other studies for maxillary premolar teeth is shown in **Table 3**. We found difference in the incidence of root canal configuration in maxillary first premolars in regard to both genders and sides of the jaw in which the tooth was located. Also, in the group of maxillary second premolars, we observed a significant difference in relation to both genders, where the type I was higher in females (81.5%), while type IV was higher in males (94.1%). Our results are in accordance with the previous study by Ok et al. [10].

Mandibular Premolars

In previous studies examining the root morphology of mandibular first premolars, the incidence of two roots varied from 1% to 17% [3, 7, 11, 12, 14]. A study of Nikolić et al. [4] observed single-rooted mandibular first premolars in 100% in Serbian population. In the present study, the frequency of single-roots was also high (98.5%). All mandibular second premolars in our study had one root, which is consistent with some of the previous studies [3, 7].

In the present study, the largest number of mandibular first premolar teeth had one root canal (83.2%), which is similar to the results of other studies in Serbian population (78%) [4]. Results of studies in other ethnic groups showed the frequency of one root canal from 66% to 98% [3,7–12, 14]. Most studies reported the presence of three root canals in mandibular first premolars, while in our study three root canals were not found [7–12, 14]. Our research showed that two root canals were more common in males, which was also reported in some of the previous studies [3, 10].

The most common type of Vertucci's classification in mandibular first premolars was type I, which was expected, given that the most teeth had one root canal. Among the other types, the most common were type III and type V in 6.4% and 6.9%, respectively. A study by Llena et al. [8] in Spanish population, also showed the highest incidence of type I followed by type III, but the type V was not observed. In other studies, the frequency of type III was ranging from 0% to 4% [3, 7, 9, 10, 12, 13].

The present study showed that all mandibular second premolars had one root and most of the teeth had type I configuration (96.2%). In most of the previous studies, a high incidence of type I was also observed [3, 7–10, 15]. A study by Singh and Pawar

Table 4. Distribution of root configuration types for mandibular premolars according to other studies
Tabela 4. *Distribucija različitih tipova konfiguracije kanala korenova donjih pretkutnjaka u ranijim studijama*

		Method *Metoda*	Sample size/ *Veličina uzorka*	Vertucci classification/*Klasifikacija prema Vertućiju* (%)							
				Type I/*Tip I*	Type II/*Tip II*	Type III/ *Tip III*	Type IV/*Tip IV*	Type V/*Tip V*	Type VI/*Tip VI*	Type VII/ *Tip VII*	Type VIII/*Tip VIII*
Mandibular first premolar *Donji prvi pretkutnjak*	Bulut DG. et al.	CBCT	604	94.2	0.6	1.2	0.8	3.2	/	/	/
	Yu H. et al.	CBCT	178	85.0	/	2.0	/	10.0	/	/	1.0
	Llena C et al.	CBCT	73	78.0	8.0	11.0	1.0	/	/	/	/
	Vertucci	Clearing	100	70.0	/	4.0	2.0	24.0	/	/	1.0
	Ok E. et al.	CBCT	1471	93.0	0.5	1.0	1.5	4.5	/	/	0.5
	Singh S. & Pawar M	Clearing	100	80.0	6.0	/	10.0	2.0	/	/	1.0
	Yang H. et al.	CBCT	440	76.0	3.5	2.5	6.5	9.5	/	/	2.0
	Liu N. et al.	Micro CT	115	65.0	/	3.0	/	23.0	/	1.0	/
	Present study/*Ova studija*	CBCT	202	82.6	1.5	6.4	2.0	6.9	/	/	/
Mandibular second premolar *Donji drugi pretkutnjak*	Bulut DG. et al.	CBCT	549	98.9	0.2	0.4	/	0.5	/	/	/
	Yu H. et al.	CBCT	178	97.2	0.55	/	/	1.7	/	/	/
	Llena C et al.	CBCT	53	90.6	1.8	/	/	7.5	/	/	/
	Vertucci	Clearing	400	97.5	/	/	/	2.5	/	/	/
	Ok E. et al.	CBCT	1345	98.5	0.1	0.1	0.6	0.5	/	/	0.2
	Singh S. & Pawar M.	Clearing	66.0	30.0	/	/	/	4.0	/	/	/
	Present study/*Ova studija*	CBCT	130	96.2	2.3	/	/	1.5	/	/	/

CBCT – kompjuterizovana tomografija sa konusnim snopom

[11] showed a significantly lower frequency of Type I (66%) in Indian population, as compared to studies in other ethnic populations. In our study, among teeth with two root canals, we observed only type II and type III configuration, which is similar to previous studies [7, 8, 15]. There are no previous studies showing the configuration of the root canals of mandibular premolars in Serbian population. Comparison of our results in root configuration with results of other studies for mandibular premolar teeth is shown in **Table 4**.

Conclusion

The results of this study showed that maxillary first premolars usually have complex root configuration with two roots, and two root canals. We also reported presence of three-rooted maxillary first premolars in the studied population. Maxillary second premolars usually have one root and little less than a half have configurations with multiple canals. This study also shows that mandibular premolars mostly have type I configuration, and that mandibular first premolars showed higher root canal complexity than second premolars. These findings emphasize the importance of knowledge of variations in root canal morphology, since excluding the possibility of morphological variation can lead to failure in endodontic therapy. Also, patient's gender should be considered when performing the preoperative assessment of endodontic treatment. Our study demonstrated that males have more complex root configurations with higher frequency of multi-rooted teeth and multiple root canals. Cone-beam computed tomography was shown to be a clinically useful tool for the detection of different root canal configurations.

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FUNCTIONAL OUTCOMES OF MIDDLE EAR CHOLESTEATOMA SURGERY

FUNKCIONALNI REZULTATI HIRURŠKOG LEČENJA HOLESTEATOMA SREDNJEG UVA

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Summary

Introduction. Chronic otitis media is defined as a persistent inflammation of the middle ear with signs of an infection lasting for three months or longer. Chronic otitis media may occur either with or without cholesteatoma. For both types of conditions, surgical treatment with closed canal wall-up or open canal wall-down techniques of tympanoplasty are considered. Our aim was to evaluate functional outcomes in two groups of patients (chronic otitis media with cholesteatoma and chronic otitis media without cholesteatoma) treated with various tympanoplasty techniques. **Material and Methods.** This retrospective study included 100 patients who underwent canal wall-down and canal wall-up tympanoplasty for the treatment of chronic otitis media with cholesteatoma and chronic otitis media without cholesteatoma from 2015 to 2016. All study patients underwent routine clinical and audiometric examinations. The study evaluated preoperative and postoperative functional results (evaluation of pure-tone audiogram screening). **Results.** A statistically significant lower incidence ($p < 0.05$) of postoperative air-bone gap < 20 decibels was established in patients with chronic otitis media with cholesteatoma, but there were no statistically significant differences between the two groups. There was a statistically lower incidence ($p < 0.05$) of patients with postoperative pure tone audiometry < 40 decibels in patients with chronic otitis media with cholesteatoma, but the difference between the two groups was not statistically significant. When analyzing the mean postoperative pure tone audiometry and air-bone gap in the study patients, canal wall-up tympanoplasty was found to be statistically more effective ($p < 0.05$). **Conclusion.** Various pathomorphological and pathophysiological changes in the middle ear, presence of extensively different forms of cholesteatomas, the choice of surgical procedures and poor preoperative hearing are in direct correlation with postoperative hearing.

Key words: Cholesteatoma, Middle Ear; Treatment Outcome; Recovery of Function; Tympanoplasty; Otitis Media; Hearing Tests

Sažetak

Uvod. Hronični otitis medija definiše se kao trajna upala srednjeg uva sa znakovima infekcije od tri meseca ili duže. Hronični otitis medija može se ispoljiti sa holesteatomom ili bez holesteatoma. Hirurško lečenje sa zatvorenom (očuvan zid kanala) i otvorenom (uklonjen zid kanala) tehnikom timpanoplastike razmatra se kod oba tipa bolesti. Cilj nam je bio da procenimo funkcionalne rezultate kod dve grupe bolesnika (sa holesteatomom i bez holesteatoma) tretirane različitim tehnikama timpanoplastike. **Materijal i metode.** Retrospektivna studija je obuhvatila 100 bolesnika, operativno tretiranih otvorenom i zatvorenom tehnikom timpanoplastike zbog hroničnog otitis medija sa holesteatomom i hroničnog otitis medija bez holesteatoma u razdoblju od 2015. do 2016. godine. Svi pacijenti su podvrgnuti rutinskim kliničkim i audiometrijskim ispitivanjima. Evaluirani su preoperativni i postoperativni funkcionalni rezultati (evaluacija ispitivanja sluha tonalnim audiogramom). **Rezultati.** U grupi obolelih od hroničnog otitisa medija sa holesteatomom utvrđena je statistički značajno niža incidencija ($p < 0,05$) postoperativnog vazdušno-koštanog zjapa< 20 dB, ali nije bilo statistički značajne razlike između obe grupe. Postojala je statistički značajna manja učestalost ($p < 0,05$) bolesnika s postoperativnim prosečnim pragom sluha < 40 dB u grupi obolelih sa holesteatomom, ali razlika između obe grupe nije bila statistički značajna. Zatvorena timpanoplastika je bila statistički značajno efikasnija ($p < 0,05$) kada je posmatrana srednja vrednost postoperativnog prosečnog praga sluha i vazdušno-koštanog zjapa kod svih bolesnika uključenih u studiju. **Zaključak.** Brojne patomorfološke i patofiziološke promene u srednjem uvu, prisutnost opsežnih oblika holesteatoma, izbor hirurških tehnika i slabiji preoperativni sluh direktno su povezani sa postoperativnim sluhom.

Ključne reči: holesteatom, srednjeg uha; ishod lečenja; oporavak funkcije; timpanoplastika; upala srednjeg uha; ispitivanje sluha

Introduction

Chronic otitis media (COM) is a chronic inflammatory disease of the middle ear and mastoid cavity that often results in partial or total loss of the tympanic membrane and ossicles, leading to conductive hearing loss that can range in severity up to 60 – 70 dB [1].

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Abbreviations
COM – chronic otitis media
COMC – chronic otitis media with cholesteatoma
COMWC– chronic otitis media without cholesteatoma
CWU – canal wall-up
CWD – canal wall-down
ABG – air-bone gap
dB – decibel
PTA – pure-tone audiometry
PORP – partial ossicular replacement prosthesis
TORP – total ossicular replacement prosthesis

The aim of the present study was to evaluate preoperative and postoperative functional results using canal wall-up (CWU) and canal wall-down (CWD) techniques of tympanoplasty.

Material and Methods

This retrospective study included 100 patients of both sexes, aged 16 - 84 years, who underwent surgical treatment for COM from 2015 to 2016. The patients were divided into 2 groups based on the presence or absence of cholesteatoma: 50 COM with cholestatoma (COMC) and 50 COM without cholesteatoma (COMWC). The routine clinical and audiometric tests were performed, as well as CWD and CWU techniques of tympanoplasty.

Preoperative and postoperative assessment of pure-tone audiometry (PTA) screenings were evaluated. The PTA tests were performed 3 months prior to and 6 months after surgery. The tests were performed through air and bone conduction modes. The preoperative and postoperative PTA average was established and evaluated. The PTA scores used in the statistical analysis were based on threshold levels at frequencies of 0.5, 1, 2, and 4 kHz. The air-bone gap (ABG) was calculated from air and bone conduction thresholds of PTA at 0.5 Hz, 1 kHz, 2 kHz at each follow-up.

Audiometric results were interpreted in accordance with the guidelines. We used the criteria recommended by the *Japan Clinical Otology Committee* for calculation of the hearing improvement using the results of patients with postoperative hearing within 40 dB as the first criterion, hearing gain exceeding 15 dB as the second criterion, and postoperative ABG within 20 dB as the third criterion [2, 3].

Patients of both sexes older than 16 years, with COMC or COMWC diagnosed by preoperative and postoperative audiometry and with indication for middle ear surgery were included. Patients who were younger than 16 years, with bilateral COM, sensorineural hearing loss, malignant middle ear disorders, otitis externa, temporal bone fracture, or previous ear surgery, were excluded from the study.

Data analysis was performed using the Statistical Package for the Social Sciences (SPSS) for Windows Version 16. Statistical analysis was performed with Pearson's chi-square test (χ^2) and T-Test for independent and paired samples. Results were considered significant if the p value was < 0.05.

Results

A total of 100 patients were selected for this study. The average age was 51 years with standard deviation of 16.75, with age ranging from 16 to 84 years. Gender distribution was balanced, with 59 male and 54 female patients. The incidence of male patients was statistically higher ($p < 0.05$) in the COMC group but there was no statistically significant gender difference between the two groups.

Of the 100 patients who underwent CWD and CWU tympanoplasty for the treatment of COMC and COMWC, the ossicular chain was preserved in 38 cases. Reconstruction of the ossicular chain was performed in 53 patients. In nine patients, ossiculoplasty was not performed due to active ear infection. The modeled autologous incus body (37/53) and partial ossicular replacement prosthesis (PORP) (9/53) were used if a stapes suprastructure was present. The total ossicular replacement prosthesis (TORP) (7/53) was used when the stapes suprastructure was absent. In case of cholesteatoma with eroded incus, we used a remodeling head of malleus (5/37) and mastoid cortex bone (8/37) for the collumela effect.

In the COMC group, 9 (18%) patients underwent a CWU tympanoplasty and 41(82%) a CWD tympanoplasty. In the COMWC group, 46 (92%) patients underwent a CWU tympanoplasty and 4 (8%) a CWD tympanoplasty. When comparing the mean preoperative and postoperative PTA in both groups and no statistically significant differences were found.

Table 1. The proportion of patients with a postoperative air-bone gap within 20 dB
Tabela 1. *Deo bolesnika sa postoperativnim vazdušno-koštanim zjapom unutar 20 dB*

			Yes/*Da*	No/*Ne*	Total/*Ukupno*
Group/*Grupa*	COMC	N	12	38	50
		%	24*	76	100
	COMWC	N	26	24	50
		%	52	48	100
Total/*Ukupno*		N	38*	62	100
		%	38	62	100

Legend/*Legend*: COMC – chronic otitis media with cholesteatoma *hronični otitis medija sa holesteatomom*; COMWC – chronic otitis media without cholesteatoma/*hronični otitis medija bez holesteatoma*; * p<0,05

Table 2. The proportion of patients with a postoperative improvement in PTA >15 dB
Tabela 2. *Deo bolesnika sa postoperativnim poboljšanjem prosečnog praga sluha > 15 dB*

			PTA>15 dB		Total/*Ukupno*
			Yes/*Da*	No/*Ne*	
Group/*Grupa*	CCOM	N	3	47*	50
		%	6	94	100
	COMWC	N	8	42*	50
		%	16	84	100
Total/*Ukupno*		N	11	89	100
		%	11	89	100

Legend/*Legend*: COMC – chronic otitis media with cholesteatoma/*hronični otitis medija sa holesteatomom*; COMWC – chronic otitis media without cholesteatoma/*hronični otitis medija bez holesteatoma*; * p<0,05

A statistically significant difference ($p < 0.05$) between the mean preoperative and postoperative ABG was found in both groups. However, the difference between the two groups was not statistically significant. In the COMC group, a statistically lower incidence of postoperative ABG < 20 dB was established ($p < 0.05$), but there was no statistically significant difference between the two groups **(Table 1)**.

There was no statistically significant difference in the proportion of patients achieving hearing with postoperative improvement in PTA >15 dB between the two groups **(Table 2)**. There was a statistically lower incidence of patients with a postoperative PTA < 40 dB ($p < 0.05$) in the COMC group, but the difference between the two groups was not statistically significant **(Table 3)**.

In the COMC group, CWD tympanoplasty was statistically more frequent ($p < 0.05$), while the CWU tympanoplasty was statistically more frequent in the COMWC group ($p < 0.05$). The CWU tympanoplasty was statistically more effective ($p < 0.05$) when analyzing the mean postoperative PTA and ABG in the study patients **(Table 4)**.

Discussion

The aim of this study was to compare preoperative and postoperative hearing results in two groups of patients (COMC and COMWC), who underwent CWU or CWD tympanoplasty. There are various prognostic factors that could have a potential impact on functional outcomes of surgical treatment of the middle ear. Poor preoperative hearing, open technique, younger age, bilateral cholesteatoma, oscillatory chain erosion and revision surgery, which are in direct correlation with poor functional hearing results after surgical treatment [4, 5].

Our results showed that there were no statistically significant differences in the main preoperative and postoperative PTAs and ABGs in the two groups. While analyzing the proportion of patients who gained postoperative hearing improvement in PTA >15 dB, we did not find a statistically significant difference between the two groups. A similar finding was also noted by Azevedo AF et al. (2013), who observed that there had been no statistically significant preoperative or postoperative differences in PTA at 500 Hz, 1-2 kHz between CWD and CWU techniques in COM surgical treatment. Kim MB et al. (2010) reported that the proportion of patients with an ABG < 20 dB was 58.6% of CWD patients and 68.4% of CWU patients ($P = 0.25$). The authors concluded that the type of mastoid surgery (CWU and CWD) did not affect the hearing results of chronic suppurative otitis media patients [6, 7].

Our results, however, differ from Shrestha BL et al. (2008), who reported that results after evaluation of the type III tympanoplasty had varied widely, showing statistically significant improve-

Table 3. The proportion of patients with a postoperative PTA<40 dB
Tabela 3. *Deo bolesnika sa postoperativnim prosečnim pragom sluha < 40 dB*

			PTA<40 dB		Total/*Ukupno*
			Yes/*Da*	No/*Ne*	
Group/*Grupa*	CCOM	N	12*	38	50
		%	24	76	100
	COMWC	N	20	30	50
		%	40	60	100
Total/*Ukupno*		N	32	68	100
		%	32	68	100

Legend/*Legend*: CCOM – chronic otitis media with cholesteatoma *hronični otitis medija sa holesteatomom*; COMWC – chronic otitis media without cholesteatoma/*hronični otitis medija bez holesteatoma*; * p<0,05

Table 4. The mean postoperative of PTA and ABG in regard to surgical techniques
Tabela 4. *Srednja postoperativna vrednost PTA i ABG u odnosu na hirurške tehnike*

	Surgical technique	N	Average value	Standard deviation
The mean postoperative PTA *Srednja postoperativna vrednost PTA*	CWU	55	46,038*	20,1037
	CWD	45	65,533	24,9242
The mean postoperative ABG *Srednja postoperativna vrednost ABG*	CWU	55	20,715*	12,7538
	CWD	45	27,551	14,2958

Legend/*Legenda*: PTA – pure tone average/*prosečan prag sluha*; ABG – air-bone gap/*vazdušno-koštani zjap*; * p<0,05, CWV – canal wall-up/*očuvan zid kanala*; CWD – canal wall-down/*uklonjen zid kanala*

ment in mean postoperative PTA-ABG in the range of 15 - 61.2 dB [8]. We noted that in the COMC group a statistically lower incidence of postoperative ABG < 20 dB ($p < 0.05$) was found. Furthermore, there was a statistically lower frequency of patients with postoperative PTA < 40 dB ($p < 0.05$). However, the difference between the two groups was not statistically significant regarding the presence of cholesteatoma.

The choice of surgical technique remains controversial and it is usually decided based on the presence or the absence of cholesteatoma, its location, the state of the middle ear mucosa and auditory thresholds [6]. Both techniques have specific advantages and disadvantages. The CWD technique is superior to the CWU technique, especially in patients with cholesteatoma. In our study, CWD tympanoplasty was statistically more frequent in the COMC group, whereas the CWU tympanoplasty was statistically more frequent in the COMWC group. Our study showed that hearing improvement was possible following surgery with CWU tympanoplasty and ossicular chain reconstruction.

The results of the study demonstrated that there was a statistically significant difference between preoperative and postoperative PTA values and ABG in both groups. Uyar M et al. revealed that postsurgical ABG value was ≤ 20 dB in 27% of CWU patients, and 7.7% of CWD patients. Mean hearing gain of patients with active squamous disease was 3.8 dB in CWU group and 11.9 dB in CWD group ($p < 0.5$) [9]. Lesinskas E et al. (2004) reported that the ABG on pure tone audiogram, 12 months following the surgery, was less than 25 dB in 38.46% of cases after closed tympanoplasty, while there was no hearing improvement after modified radical mastoidectomy [10]. Wilson KF et al. (2012) revealed that closed technique showed better initial hearing results and less morbidity, but a higher recurrence rate, up to 40% [11].

Moreover, other studies have reported no significant differences in hearing outcomes in association with the two techniques. Minovi et al. evaluated the audiometric results after using open cavity tympanomastoidectomy in advanced attic cholesteatoma [12]. A postoperative ABG ≤ 20 dB was achieved in 42.9% of the operated ears, whereas 9.3% (n = 15) of the operated ears showed a postoperative ABG > 30 dB. The authors reported that although most cholesteatomas nowadays could be eradicated with the CWU technique, in far advanced cholesteatomas, the CWD technique may be applied with acceptable postoperative hearing results. Cook JA et al. reported that modified radical mastoidectomy provided relatively safe surgical access for the removal of chronic middle ear and mastoid disease and gave reproducible results [13]. However, it has been suggested that hearing may not be as good as that after "intact canal wall mastoidectomy". The surgery should be tailored regarding the clinical stage and intraoperative findings in each case [12–15].

This study is not without limitations, as it has focused on comparing short-term hearing results following middle ear surgery. Due to a short-time frame, we were not able to follow up potential occurrences of the residual/recurrent cholesteatoma.

Conclusion

In the group of patients with chronic otitis media with cholesteatoma, a statistically significant lower incidence of postoperative air-bone gap < 20 dB ($p < 0.05$) was found. The difference between the two groups was statistically significant ($p < 0.05$). When analyzing the mean postoperative pure tone audiometry and air-bone gap values, the canal wall-up tympanoplasty was found to be statistically more effective than the canal wall-down tympanoplasty.

Various pathomorphological and pathophysiological changes in the middle ear, the presence of extensively different forms of cholesteatomas, the choice of surgical procedures and poor preoperative hearing are in direct correlation with postoperative hearing.

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TREATMENT HISTORY: FACTORS THAT AFFECT THE OUTCOME OF HEPATITIS C VIRUS TREATMENT WITH INTERFERON-ALPHA 2A/B AND RIBAVIRIN

ISTORIJA TERAPIJE: FAKTORI KOJI SU UTICALI NA ISHOD TRETMANA PEGILOVANIM INTERFERONOM ALFA 2A/B I RIBAVIRINOM KOD PACIJENATA SA HEPATITISOM C

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Summary

Introduction. Until the 1990s, there was no available treatment for chronic hepatitis C, but during this decade the benefits of interferon-alfa therapy were reported. At the end of the 1990s, the pegylated interferon-alfa 2a/b has significantly altered the treatment, whereas direct acting antivirals have significantly affected the treatment. The aim of this study was to show the most significant predictive factors of therapy response among patients with chronic hepatitis C treated with pegylated interferon-alfa 2a/b and ribavirin. **Material and Methods.** A non-randomized retrospective study included 292 patients with chronic hepatitis C treated at the Clinic for Infectious Diseases, Clinical Center of Vojvodina, from 2008 to 2015. **Results.** The study showed that therapeutic response was not affected by sex, serum viral load, or if the therapy was applied for the first time or repeated. A sustained virological response was statistically significantly more frequent in younger patients, as well as in patients without extrahepatic manifestations. Cases with higher progression of fibrosis were associated with lower chance for sustained virological response. Genotype 1 showed to be a predictor of adverse response to therapy, and genotype 3 as a predictor of sustained virological response. Steatosis was significantly less frequent in patients with genotype 1 with sustained virological response. Patients with a shorter duration of infection were more prone to sustained virological response. **Conclusion.** A positive response to pegylated interferon-alfa 2a/b and ribavirin was found in 70.20% of patients with chronic hepatitis C. Elderly age, late detection of the infection, hepatitis C virus 1 genotype, fibrosis progression, presence of hepatic steatosis, and extrahepatic manifestations were risk factors for poor treatment outcome.

Key words: Treatment Outcome; Interferon-alpha; Ribavirin; Hepatitis C; Drug Users; Risk Factors

Sažetak

Uvod. Do početka devedesetih nije postojao dostupan tretman hroničnog hepatitisa C, ali tokom te decenije uočene su koristi interferon-alfa terapije. Pegilovani interferon-alpha 2a/b krajem devedesetih godina uspešno je promenio tretman pacijenata, a značajan napredak poslednjih godina doneli su direktni antiviralni lekovi. Cilj rada je prikazivanje najznačajnijih prediktivnih faktora koji su uticali na ishod lečenja pegilovanim interferonom-alfa 2a/b i ribavirinom kod pacijenata sa hroničnim hepatitisim C. **Materijal i metode.** Retrospektivna studija uključila je 292 pacijenta sa hroničnim hepatitisom C lečenih 2008-2015 godine na Klinici za infektivne bolesti Kliničkog Centra Vojvodine. **Rezultati**. Na vrstu terapijskog odgovora ne utiče pol, nivo virusnih partikula u serumu i da li je terapija primenjena prvi put ili ne. Kod mlađih pacijenata stabilan virusološki odgovor statistički je bio značajno češći. Kod pacijenata bez ekstrahepatičkih manifestacija postignut je stabilan virusološki odgovor, statistički značajan. U slučajevima sa većom progresijom fibroze dokazana je manja šansa za stabilan virusološki odgovor . Genotip 1 se istakao kao prediktor nepovoljnog odgovora na terapiju, a genotip 3 prediktor stabilnog virusološkog odgovora. Steatoza je bila značajno manje prisutna kod pacijenata sa genotipom 1 koji su postigli stabilan virusološki odgovor. Pacijenti sa kraćim trajanjem infekcije pre početka terapije težili su stabilnom virusološkom odgovoru. **Zaključak.** Studija je utvrdila pozitivan odgovor na terapiju kod 70,20% pacijenata sa hroničnim hepatitisom C virusa i kao faktore rizika za neuspeh lečenja pegilovanim interferonom-alfa 2a/b i ribavirinom označila stariji uzrast pacijenta, duži tok infekcije pre započinjanja terapije, genotip 1 hepatitis C virusa, progresiju fibroze, prisustvo steatoze jetre i ekstrahepatičkih manifestacija.

Ključne reči: ishod lečenja; interferon alfa; ribavirin; hepatitis C; narkomani; faktori rizika

Introduction

Hepatitis C virus (HCV) is the leading cause of chronic liver disease worldwide [1, 2]. Epidemic transmission of HCV in the twentieth century has reached its peak by mid-1980s among intravenous drug users [3]. After an incubation period of 2 weeks to 6 months, approximately 80% of people do not exhibit any symptoms. After the acute phase of infection, about 15–45% of infected persons spontaneously clear HCV within 6 months, while 55 - 85% of infected people develop chronic HCV infection [4, 5]. Research studies have shown a faster rate of disease progression among people who got infected at older

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Abbreviations

HCV	– hepatitis C virus
PEG-IFN	– pegylated interferon
CHC	– chronic hepatitis C
SVR	– sustained virological response
HBV	– hepatitis B virus
HIV	– human immunodeficiency virus
DAAs	– direct acting antivirals
WHO	– World Health Organization
PegINF-Alfa2/Rbv	– PEG-INF-alpha 2a/b with ribavirin
RNA	– ribonucleic acid
BMI	– body mass index
ALT	– alanine aminotransferase
LB	– liver biopsy
VL	– viral load
PCR	– polymerase chain reaction
F0	– without liver fibrosis
F1	– mild fibrosis
F2	– moderate fibrosis
EHMs	– extrahepatic manifestations

age, predominantly among men, those who consume alcohol as well as hepatitis B virus (HBV), hepatitis D virus (HDV) or human immunodeficiency virus (HIV) positive patients [6]. Because the infection remains asymptomatic until decades after infection, chronic hepatitis C (CHC) is often undiagnosed despite being the most common cause of chronic liver disease. HCV infection is usually diagnosed based on abnormal liver function tests. The second most common way of diagnosis is to test patients who inject drugs or use intranasal drugs, recipients of blood transfusion, person who engage high-risk sexual behavior, etc [7]. Anti-HCV prevalence in the general population of Vojvodina is 1.5% and the diagnosis of HCV infection is usually made by detecting anti-HCV antibodies among blood donors [8].

Until the early 1990s, there was no available treatment for CHC, but during this decade the benefits of interferon-alfa therapy were reported, and development of pegylated interferon (PEG-IFN)-alfa 2a and 2b at the end of the 1990s has significantly altered the treatment for CHC. The treatment for CHC has advanced significantly in the last few years with the appearance of first-generation direct acting antivirals (DAAs) in 2011 [9]. In May 2016, the World Health Assembly adopted the first "Global Health Sector Strategy on Viral Hepatitis, 2016 - 2021". The strategy has a vision of eliminating viral hepatitis as a public health problem with reducing new viral hepatitis infections by 90% and reducing deaths due to viral hepatitis by 65% by 2030 [10]. The World Health Organization (WHO) recommends therapy regimens based on DAAs of the newest generation [10–15]. With the emergence of therapies able to cure HCV in almost all instances, WHO set the goal of global elimination of HCV by 2030. However, in order to achieve this, there are a number of barriers such as high cost of DAAs, expensive and complex polymerase chain reaction (PCR)-based testing, etc.

Previous standard therapy by PEG-INF-alpha 2a/b in combination with ribavirin (PegINF-Alfa2/Rbv) is still current therapy in Serbia. Therapy success is defined by the non-presence of HCV-ribonucleic acid (RNA) in the serum 6 months after completing therapy, sustained virological response (SVR). Unfortunately, this therapy has not shown definite success. This therapy achieves a SVR rate of 50% to 80%. About 99% of treated patients remain PCR HCV RNA negative. Many studies have been conducted in order to improve therapy outcomes and to identify predictive factors for successful therapy response. Some of the predictive factors are fibrosis score, body mass index (BMI), steatosis, age of patients, co-infection with HBV, HIV, alcohol consumption, therapy adherence, etc [16, 17]. The therapy success rate most likely depends on genetic barriers within the virus itself on one, and the human host on the other side.

The aim of this research was to show the impact of the most significant predictive factors of the therapy response among patients with chronic hepatitis C treated with a combination of interferon and ribavirin.

Material and Methods

A non-randomized retrospective study included 292 patients with CHC treated at the Clinic for Infectious Diseases of the Clinical Center of Vojvodina from January 2008 to December 2015. All patients fulfilled the criteria of the National Health Insurance Fund of the Republic of Serbia for a combination antiviral treatment regimen (PegIFN–Alfa 2/Rbv) for chronic HCV infection: 18 years old or above, anti–HCV positive longer than six months, high level serum alanine aminotransferase (ALT), hepatic fibrosis confirmed by liver biopsy or fibro scan, did not use alcohol or psychoactive substances over a longer period than one year. The patients with multiple HCV genotypes were not included in the study.

The data used in the study included:

- Medical history data: gender, age, path of infection, duration of infection, medical records of patients with CHC who were not treated with PegIFN–Alfa 2/Rbv for the first time;
- Laboratory data: pre-therapy serum ALT level (kinetic UV test, Olympus AU 400 at the Laboratory Diagnostics Center of the Clinical Center of Vojvodina);
- Liver biopsy (LB): the degree of fibrosis and steatosis (percutaneous LB using Hepafix needle of 1.2 or 1.4 mm; B Braun Melsungen AG, Germany);
- Pathohistological analysis was conducted at the Pathology and Histology Center of the Clinical Center of Vojvodina. Metavir classification for staging hepatitis C liver disease was used;
- The presence of extrahepatic manifestations (arthritis, diabetes mellitus, thyroiditis, organ nonspecific auto-antibodies) was verified by clinical checkup and laboratory tests;
- Genotype of the virus was determined by Linear Array HCV Genotyping test (Roche Diagnostics Systems, Basel, Switzerland);

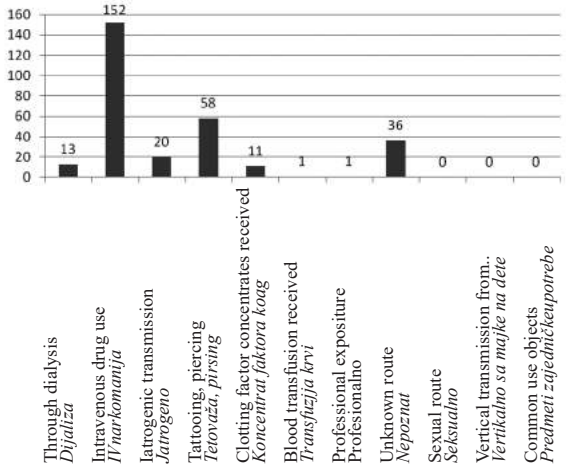
- Serum viral load (VL) and active virus replication were determined by real-time PCR on the COBAS® AmpliPrep/COBAS® TaqMan® (Roche Diagnostics Systems, Basel, Switzerland), analytic sensitivity 5x103 to 1x104 copies HCV/ml.

The patients were divided into three groups based on the response to antivirus treatment: 1) patients with SVR - PCR HCV RNA below the level of detection at the end of treatment and 6 months later; 2) non-responders (NR) – patients who didn't respond to antivirus treatment - PCR HCV RNA positive after 12-week treatment or at the end of the treatment; 3) relapse (RL) patients with the activation of the virus during the period of monitoring - PCR HCV RNA negative at the end of the treatment, but positive at the end of the monitoring period. The influence of social, demographic and clinical characteristics (gender, age, path of infection, duration of infection, fibrosis degree, steatosis, presence of extrahepatic manifestations, repeated treatments, virus genotype and serum VL) on the success of antiviral therapy for CHC was examined.

The data were analyzed by descriptive and inferential statistics using the GraphPad Prism Software. The difference in the frequency of attributive characteristics was tested by χ^2 and differences between the values in these groups by unpaired t-test. The $p < 0.05$ was taken as statistically significant. The results were shown in tables and graphs.

Results

A total of 292 patients included 190 (65.07%) male and 102 (34.93%) female patients, with a male to female ratio of 1,86 : 1. The patients' age ranged from 19 to 64 years. The average age of patients was 38.54 years (SD ± 11.30). The average age of males (37.78) was by 2.18 years lower than in females (39.96). The SVR was achieved in 70.20% (205/292) of patients: in 71.05% (135/190) of males and 68.63% (70/102) of females **(Table 1)**. Gender did not show to be a statistically significant predictive factor for reaching SVR (χ^2 test, p = 0,665).



Graph 1. The route of transmission of HCV among the patients in the study

Grafikon 1. *Način prenosa hepatitis C virusa kod pacijenata uključenih u studiju*

In the group of patients over 40 years of age (average age 49.95) SVR was achieved in 44.85% (61/136) whereas in the group of patients younger than 40 years (average age 29.69) SVR was achieved in 92.31% (144/156). These results showed a high statistical significance of SVR at younger age (χ^2 test, p = 0.0001).

The most frequent paths of infection were intravenous drug addiction (52.05%) and tattoo and piercing (19.86%). The path of infection could not be determined in 12.33% of patients **(Graph 1)**.

The HCV genotype 1 was found in 183 patients (62.67%), genotype 2 in 98 (33.56%), genotype 3 in 10 (3.43%), and genotype 4 only in one patient (0.34%). The SVR was achieved in 61.20% (112/183) of patients with genotype 1; 85.71% (84/98) of patients with genotype 3; 80% (8/10) of patients with genotype 2, and in one patient (1/1) with genotype 4. The SVR was statistically significantly higher in patients with HCV genotype 3 compared with patients with genotype 1 (χ^2 test, $p < 0.0001$).

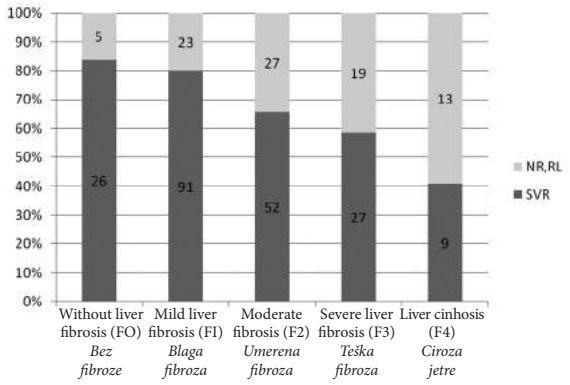
Before the beginning of treatment, normal levels of ALT were found in 0.34% (17/292) of patients. The rest of patients had elevated ALT levels, from 1 to 10 times (average 2.86) higher comparing to the upper reference limit. The ALT activity was not considered as a factor that influenced the treatment response.

The assessment of the stage of hepatic fibrosis in patients after LB showed that there were 31 patients (10.62%) without liver fibrosis (F0), 114 patients (39.04%) with mild fibrosis (F1), 79 patients (27.05 %) with moderate fibrosis (F2), 46 patients (15.75%) with severe fibrosis (F3), and 22 patients (7.54%) with liver cirrhosis (F4). There were 224 (76.71%) patients with F0 - F2 fibrosis. The SVR was achieved in 75.45% (169/224) of patients in the group F0 - F2 and in 52.94% (36/68) in the group F3 - F4 **(Graph 2)**. Therefore, the stage of liver fibrosis showed to be a significant predictive factor for achieving SVR (χ^2 test, p = 0.0004).

Steatosis was found in 18.15% (53/292) of patients with CHC. In this group of patients, the SVR was achieved in 52.83% (28/53). In the group of patients without steatosis, the SVR was achieved in 74.06% (177/239). This difference (18.15% vs. 74.06%) was highly statistically significant (χ^2 test, p=0.0022).

Among patients with HCV genotype 1, the SVR was achieved in 40% (14/35) of patients with steatosis, compared to 66.22% (98/148) of those without steatosis. Steatosis showed to have a statistically significant (Fisher's test, p=0.0013) impact on the treatment outcome in genotype 1. Among patients with HCV genotype 3, the SVR was achieved in 76.47% (13/17) of patients with steatosis, compared to 87.65% (71/81) of those without steatosis. In HCV genotype 3, steatosis showed to be statistically insignificant (Fisher's test, p=0.2164) regarding the treatment outcome.

Extrahepatic manifestations were determined in 43.49% (127/292) of patients. Out of them, 63.78% (81/127) achieved SVR. Among 56.51% (165/292)



Graph 2. Treatment outcome in the patients with CHC in regard to hepatic fibrosis
Grafikon 2. *Ishod terapije kod pacijenata sa hroničnim hepatitsom C u odnosu na fibrozu jetre*

of patients without extra hepatic manifestations, the SVR was achieved in 75.15% (124/165) of patients. The study confirmed that extrahepatic manifestations showed a statistically significant impact (χ^2 test, p= 0.0352) on the treatment response.

Among 85.27% (249/292) of patients being treated for hepatitis C with PegIFN-Alfa2/Rbv for the first time, the SVR was achieved in 72.29% (180/249) of patients. Of 14.73% (43/292) of patients previously treated with PegIFN-Alfa2/Rbv, the SVR was achieved in 58.14% (25/43). The study showed no statistically significant difference in the treatment response between patients being treated for hepatitis C for the first time and those who have previously been treated (χ^2 test, p = 0.0610).

The supposed duration of infection before treatment ranged from to 2 to 50 years, 16.38 years on average (SD ± 10.41). The average duration of HCV infection was 14.47 years (SD ± 10.16) in patients with SVR and 18.57 (SD ± 8.87) in patients who had poor treatment outcomes. The difference between these two groups was statistically significant in regard to the treatment outcome (unpaired t-test, p = 0.0012).

The serum VL in patients with CHC ranged from 11421 to 111100000 IU/ml, average 6297698.09 (SD ± 11781304.79). The average serum VL in patients with SVR was 5833121.36 IU/ml (SD ± 10438542.61) while in patients with poor treatment outcomes it was 7392390.38 IU/ml (SD ± 14583823.48). The influence of VL on the treatment outcome in patients with CHC was statistically insignificant (unpaired t-test, p = 0.3034).

Discussion

The study shows that CHC affects males more than females, that gender does not affect the response to PegIFN-Alfa2/Rbv therapy, as well as the serum VL. The study has also shown that there was no difference in the treatment response between patients being treated for hepatitis C for the first time or have previously been treated. It was found that intravenous drug use was the most frequent (52.05%) transmission path. Statistically significantly higher SVR was found in younger patients, patients without extrahepatic manifestations, as well as in patients with shorter duration of untreated illness. Findings from this study suggest that early stages of liver fibrosis were associated with a greater chance for SVR. Genotype 1 has been identified as a predictor of unfavorable treatment response, and genotype 3 as a positive predictor of SVR to PegIFN-Alfa2/Rbv therapy in CHC patients. Absence of liver steatosis in patients with SVR suggests that it may play an important role in determining response rates to PegIFN-Alfa2/Rbv treatment.

In 2016, WHO defined the latest recommendations about the importance of treating CHC, treatment protocols, and desired effects of the therapy [10]. In settings where DAAs are not available, the combination of PegIFN-Alfa2/Rbv remains acceptable. However, a significant number of patients who did not achieve SVR nor had side effects show that the research of predictive factors (by the virus and a host) of the treatment outcome in CHC patients is still a subject of interest. In 2012, Nathan Ford and his associates conducted a meta-analysis to assess treatment success in less developed and middle developed countries. Their study showed that SVR was achieved in 52 % of patients and that in patients infected by HCV genotype 1, after 48 weeks of treatment with PegIFN-Alfa2/Rbv, SVR ranged from 45–48% [18]. The results of our study showed that SVR was achieved in 70% of patients and in 61% of patients infected with HCV genotype 1.

The male to female ratio in our sample of patients suffering from CHC showed male predominance. However, there were no significant differences between

Table 1. Sex distribution and treatment outcome in patients with CHC
Tabela 1. *Polna duistribucija i ishod terapije kod pacijenata sa hroničnim hepatitisom C*

The treatment outcome/*Ishod terapije*	Gender/*Pol*		Total/*Ukupno* (%)
	Male/*Muški* (%)	Female/*Ženski* (%)	
NR/*BO*	36 (18.95)	21 (20.59)	57 (19.52)
SVR/*SVO*	135 (71.05)	70 (68.63)	205 (70.21)
RL/*RL*	19 (10.00)	11 (10.78)	30 (10.27)
Total/*Ukupno*	190 (100)	102 (100)	292 (100)

Legend: NR – non-responders; SVR - sustained virological response; RL – relapse
Legenda: BO – bez odgovora; SVO – stabilan virusološki odgovor; RL - relaps

male and female participants in regard to the therapeutic response. The results of the study are in accordance with previous studies about gender as a possible predictive factor for treatment success with PegIFN-Alfa2/Rbv [6, 16].

The researches show increasing incidence of HCV infection in patients aged 18 – 30 years, because they visit doctors more often [19]. The reported data are in accordance with the fact that 53.42% of patients included in our study were younger than 40 years of age. Our study found that the average age of patients was 38.54 years, as well as that the younger patients achieved SVR statistically significantly more often. A previous study reported that patients infected at an older age (>40 years) could have faster progression of liver disease [4]. Unfavorable treatment outcomes of older patients with CHC can be explained by a greater prevalence of comorbid states at old age, as well as higher cumulative exposition to hepatotoxins from the surroundings during their lives [20].

The Annual Epidemiological Report for 2016 of the European Center for Disease Prevention and Control, suggested that in Europe hepatitis C is more frequent in males (male to female ratio: 1.8 : 1). The HCV seroprevalence was found to be 51.3% in 25 and 44 years olds, 8.0% in patients under the age of 25 years, and 40.7% in patients over 45 years [21]. In patients with CHC in the Republic of Serbia age distribution corresponds to that in Southern Europe with two peaks. The first peak occurs in patients over 55 years of age (HCV transmission through blood transfusion, before introducing a regular screening of blood donors) and the second peak occurs in patients over 35 years of age (infected by contaminated set for intravenous drug use) [22, 23]. In our study, the average age of patients was 38.54 years. The research confirmed the Public Service Announcement that the intravenous addiction was the most frequent path of transmission (52.05%).

In a study including almost 440 patients treated with PegIFN-Alfa2/Rbv, Delić and colleagues demonstrated SVR in 70.5% of patients [24]. The effect of the combined treatment was much better in patients under the age of 40 years, without liver cirrhosis, not affected by HCV genotype 1, and if the recommended regimen of antiviral therapy for CHC was respected.

Mild increases in ALT levels (30 - 100 IU/l) are commonly found and could be associated with viral hepatitis [25]. Our study showed that 99.66% of patients had increased ALT levels, by one to ten times than the upper normal limit before therapy. Although the ALT level before therapy was not considered as a factor that affects the treatment response, the study draws attention to the need for anti-HCV testing of all patients with increased ALT levels.

The fibrosis may progress or regress during the treatment with PegIFN-alfa2/Rbv. In patients who did not achieve SVR, regression of fibrosis can be mostly explained by the action of interferon. Progression of fibrosis can be explained as a consequence of homogenization quasi species under pressure of immunological system and selective overgrowing of aggressive virus alternatives [26, 27]. The present study has found fibrosis stage F0 - F2 in 76.71% of patients with hepatitis C, and SVR was achieved in 52.94%. It has statistically been confirmed that patients with lower fibrosis progression had a bigger chance of achieving SVR, and fibrosis was found to be a significant predictive factor for treatment response in patients with hepatitis C.

Steatosis is more frequently associated with obesity, diabetes mellitus, CHC, metabolic syndrome. In their research, Preveden and colleagues found steatosis in 35% of patients with CHC and statistically confirmed its negative effects to the efficiency of antivirus therapy in patients with hepatitis C genotype 3 [28]. Our findings showed steatosis in 18% of patients with CHC and statistically confirmed significant absence of steatosis in patients with hepatitis C genotype 1 with SVR.

Adaptation of HCV for replication outside hepatocytes causes proliferation of B lymphocytes and appearance of extrahepatic manifestations (EHMs). EHMs are frequent and polymorphous [29]. The study focuses on four extrahepatic manifestations (arthritis, diabetes mellitus, thyreoiditis, and presence of organically nonspecific auto antibodies). The presence of EHMs was found in 43% of patients in the study. Although 60% of patients with SVR did not have EHMs, effects of EHMs on the treatment response were not statistically confirmed.

The study included 85.27% naïve patients (who received PegIFN-Alfa2/Rbv for the first time). The SVR of 72.29% was achieved in this group of patients. In the group of previously treated patients, SVR of 58.14% was achieved. The difference in the treatment response was not statistically confirmed so the data of this study are consistent with the published data [13, 14].

In our study, HCV genotype 1 (62.67%) and HCV genotype 3 (33.56%) were most frequent. Similar findings of wide spreading of HCV genotype 1 and 3 were observed in a study conducted in Europe [2]. The results of the study also confirmed significant influence of HCV genotype on treatment response: SVR was achieved in 61.20% of patients with genotype 1 and in 85.71% of patients with genotype 3. The SVR achieved in this study (70.20%) is consistent with publish data [18, 24, 27].

Our research showed that patients with shorter duration of infection before therapy aimed towards SVR, whereas those with longer infection duration aimed towards unfavorable treatment response. Other research studies reported that the duration of infection before treatment represents a significant predictive factor of treatment response [5, 6]. Nevertheless, our study did not confirm that the serum VL before therapy significantly affected the treatment response.

Conclusion

The knowledge about demographic and clinical characteristics of patients which may have major effects on treatment outcomes is useful for researchers

in searching for opportunities to achieve positive response therapy. The results of this study show that positive therapy response in pegylated interferon-alpha 2a/b with ribavirin has been found among 70% of patients with chronic hepatitis C. Circumstances of failure have been identified in elderly patients, longer duration of infection before the therapy, hepatitis C virus genotype 1, advanced fibrosis, presence of liver steatosis and extrahepatic manifestations.

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CASE REPORTS
PRIKAZI SLUČAJEVA

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PRIMARY DIFFUSE LARGE B-CELL LYMPHOMA OF THE ORAL CAVITY – A CASE REPORT

PRIMARNI DIFUZNI KRUPNOĆELIJSKI B-ĆELIJSKI LIMFOM USNE DUPLJE – PRIKAZ SLUČAJA

Aleksandra ILIĆ[1], Ivanka SAVIĆ[2], Aleksandra FEJSA LEVAKOV[1,3] and Branislav BAJKIN[3,4]

Summary

Introduction. Lymphomas are a group of neoplasms of the lymphatic and reticuloendothelial system. The two main types are Hodgkin lymphoma and non-Hodgkin lymphoma, depending on the immunohistological characteristics of malignant lymphocytes. B-cell lymphomas are more frequent than T-cell lymphomas. In this case report we present a male patient with diffuse large B-cell lymphoma localized in the maxilla. **Case Report.** A 59-year-old man presented with a painless swelling in the right posterior region of the maxilla. A solid tumor was found in the right posterior maxillary region by intraoral examination, firstly suspected as a dental infection. Since the symptoms did not improve a month after the first dental treatment, he was referred for further diagnostic evaluation. The established diagnosis was diffuse large B–cell lymphoma. The patient received standard therapy for diffuse large B–cell lymphoma – rituximab-cyclophosphamide, hydroxydaunomycin, oncovin, prednisolone, six cycles and regression of the oral lesion was noticed. **Conclusion.** Lymphoma of the oral cavity usually presents as a swelling and may mimic odontogenic infection. Dentists should be aware of this possibility especially if the swelling is painless and if the initial treatment has failed.

Key words: Lymphoma, Large B-Cell, Diffuse; Maxilla; Diagnosis, Differential; Mouth Neoplasms; Signs and Symptoms; Immunotherapy

Sažetak

Uvod. Limfomi su neoplazme limfnog i retikuloendotelnog sistema. Dva osnovna tipa su Hočkinov limfom i Non-Hočkinov limfom na osnovu imunohistohemijskih karakteristika malignih limfocita. B-ćelijski limfomi su mnogo češći od T-ćelijskih limfoma. U ovom prikazu slučaja predstavljen je pacijent muškog pola sa difuznim krupnoćelijskim B-ćelijskim limfomom sa lokalizacijom u gornjoj vilici. **Prikaz slučaja.** Muškarac star 59 godina se javio zbog bezbolnog otoka u desnom zadnjem regionu gornje vilice. Intraoralnim pregledom nađena je čvrsta tumorska formacija u desnom zadnjem regionu gornje vilice, za koju se prvobitno sumnjalo da je dentalna infekcija. Kako simptomi nisu prolazili ni mesec dana nakon stomatološkog tretmana, pacijent je upućen na dalju dijagnostiku. Postavljena je dijagnoza difuznog krupnoćelijskog B-ćelijskog limfoma. Pacijent je primio standardnu terapiju za difuzni krupnoćelijski B-ćelijski limfom – šest ciklusa rituksimab-ciklofosfamida, hidroksidaunomicina, onkovina, prednizolona i uočena je regresija lezije u usnoj duplji. **Zaključak.** Limfom usne duplje se obično prezentuje kao otok i može da imitira odonogenu infekciju. Važno je da stomatolog bude svestan ove mogućnosti, naročito ako je otok bezbolan i ako inicijalni tretman ne uspe.

Ključne reči: difuzni B ćelijski limfom; maksila; diferencijalna dijagnoza; neoplazme oralne šupljine; znaci i simptomi; imunoterapija

Introduction

Lymphomas are a group of neoplasms of the lymphatic and reticuloendothelial system. The two main types are Hodgkin lymphoma (HL) and non-Hodgkin lymphoma (NHL), depending on the immunohistological characteristics of malignant lymphocytes. NHL arises from mature B-cells and natural killer (NK) T-cells. B-cell lymphomas are more frequent than T-cell lymphomas, accounting for about 85% of all NHL [1, 2].

Except nodal, extranodal localization is more specific for NHL than HL, predominantly involving the gastrointestinal tract, then the head and neck, and rarely develops in the oral cavity and jaws [3, 4]. About 30–40% of all cases of NHL are diffuse large B-cell lymphomas (DLBCL) and the subtype called not otherwise specified (NOS) accounts for

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Abbreviations

HL – Hodgkin lymphoma
NHL – non-Hodgkin lymphoma
DLBCL – diffuse large B–cell lymphoma
R-CHOP – rituximab - cyclophosphamide, hydroxydaunomycin, oncovin, prednisolone
NK – natural killer
NOS – not otherwise specified
NGC – non germinal center

80–85% of DLBCL. DLBCL accounts for approximately 31% of all NHL in Western Countries and 37% of B-cell tumors worldwide [2].

The standard therapy regimen for DLBCL is immnunochemotherapy, a combination of rituximab and cyclophosphamide, hydroxydaunomycin, oncovin, prednisolone (R-CHOP) [5].

In this case report we present a male patient with DLBCL localized in the maxilla.

Case Report

A 59-year-old man presented with a painless swelling in the right posterior region of the maxilla. He consulted a dentist who suspected a dental infection and extracted the first upper right molar. After the extraction, the patient received antibiotics. The swelling remained unchanged, so the patient was referred to the oral surgeon for intraoral excision. Since symptoms did not improve a month after the first dental treatment, he was referred for further diagnostic evaluation.

Clinical examination showed a swelling in the right maxillary region. The intraoral examination showed a solid tumor formation in the right posterior maxillary region **(Figure 1)**. A panoramic radiograph revealed a diffuse radiolucent lesion in the right maxilla **(Figure 2)**. An incisional biopsy was performed, and the specimen was sent for histological and immunohistochemical examination.

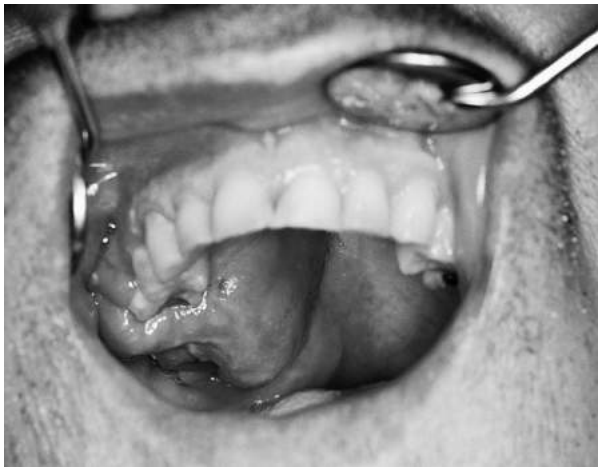

Figure 1. Gross appearance of the diffuse B-cell lymphoma
Slika 1. *Makroskopski izgled difuznog krupnoćelijskog B-ćelijskog limfoma*

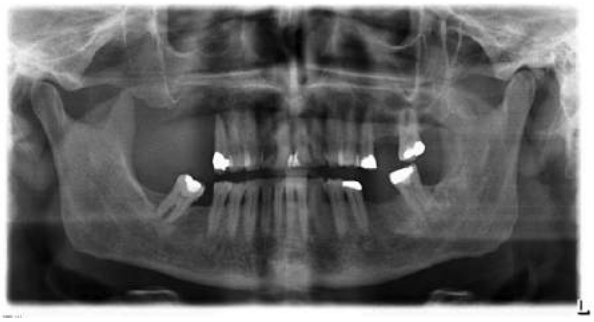

Figure 2. Panoramic radiograph – a diffuse radiolucent lesion in the right maxilla
Slika 2. *Ortopan – Difuzno rasvetljenje u gornjoj vilici sa desne strane*

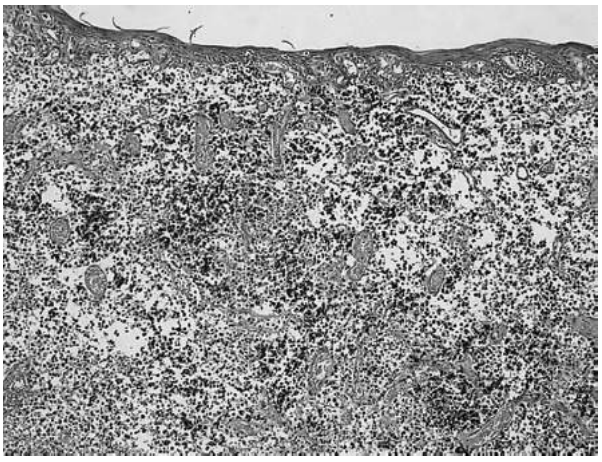

Figure 3. Photomicrophraph of the diffuse B-cell lymphoma (HE, 10 x)
Slika 3. *Mikrofotografija difuznog krupnoćelijskog B-ćelijskog limfoma (HE, 10x)*

The hematoxylin–eosin stained sections revealed a lymphoid neoplasm partly covered with squamous epithelium, with necrotic debris and neutrophils. Bone tissue fragments were also present in the tumor. The lymphoma cells were large, arranged in a diffuse pattern, with round to oval or vesicular nuclei and basophilic cytoplasm. Most nucleoli were prominent, often peripherally located adjacent to the nuclear membrane (centroblasts), with few large vesicular nucleoli, solitary and centrally located (immunoblasts).

A medium number of small T-lymphocytes were found in the background (CD3+/CD5+) and also a small number of plasma cells. The tumor mitotic index was moderate.

Tumor cells showed immunoreactivity for CD 79α, CD20, bcl-2, bcl-6, MUM-1, and negativity for CK, EMA, CD10, CD3, CD5, CD56, CD38, CD138, ALK-1 **(Figures 3, 4)**. Ki-67 proliferative index of the tumor was more than 90%.

The established diagnosis was DLBCL, NOS, centroblastic variant, non germinal center (NGC) and the patient was referred to a hematologist for further diagnosis and therapy.

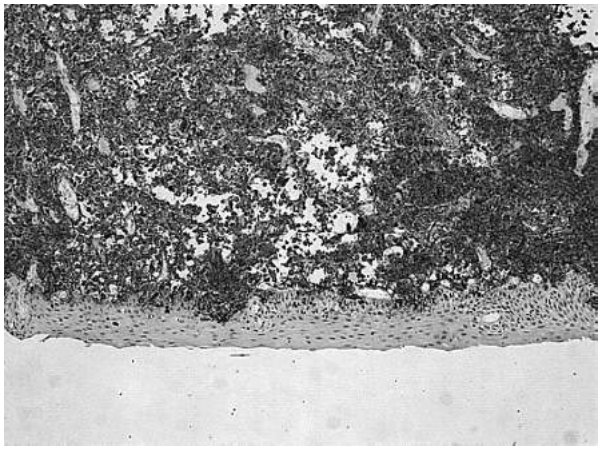

Figure 4. Photomicrograph of the CD20 immunoreactivity of the diffuse B-cell lymphoma (CD20, 10x)
Slika 4. *Mikrofotografija CD20 pozitivnosti difuznog krupnoćelijskog B-ćelijskog limfoma (CD20, 10x)*

The patient had no B symptoms for lymphoproliferative disease such as night sweats, weight loss more than 10% during the last 6 months, had no appetite loss, no fever over 38°C for more than 5 days, excluding infection. Laboratory test results pointing to this kind of disease – lactate dehydrogenase (LDH), beta 2 microglobulin, high sedimentation rate, pathological ratio of serum immunoglobulins and proteins and cytopenia, were absent. The whole body computer tomography (CT) presented no dissemination of DLBCL. Sternal punction and bone marrow biopsy presented no lymphoma infiltration. The patient received standard therapy for DLBCL - R-CHOP, VI cycles and regression of the oral lesion was noticed.

Discussion

Lymphomas are malignancies which affect lymphocytes and their precursor cells. Depending on the histological characteristics, there are two main types –HL and NHL [1].

Commonly, HLs originate in lymph nodes, whereas NHL may involve both extranodal and nodal sites, but have predilection for extranodal tissues, arising from mature B-cells and NK/T-cells [1, 6].

The most common oral cavity neoplasms include squamous cell carcinoma and salivary gland malignancies, followed by lymphomas in 3.5% of all cases, predominantly of B-cell lineage. T-cell lymphomas are rarely seen in the oral cavity. Head and neck localized lymphomas mostly affect Waldeyer's ring, salivary glands and palate. Jaw lesions are rare and if they occur, the lesions are more often localized in the posterior region of maxilla than in mandible [6 - 8]. The presented case of DLBCL involved the right posterior maxillary region.

The incidence of lymphoma increases in the elderly, with the median age of 50 - 55 years and is slightly predominant in males. However, there are no significant age-related differences in tumor characteristics [3, 9]. The presented patient is a 59-year-old man, which is in accordance with the literature.

There are no specific symptoms associated with jaw lymphomas. The patients usually report swelling, tooth mobility, pain and neurological disturbances. They also report sudden enlargement of the lymph nodes or limited extranodal lesions [3, 10, 11]. In this case, the main symptom was painless maxillary swelling without any other symptoms. The panoramic radiograph revealed a diffuse radiolucent lesion in the right maxilla.

Histologically, in DLBCL normal tissue architecture is replaced by large cells, centroblasts and immunoblasts, growing in a diffuse pattern. Their nuclei are twice bigger than small lymphocytes, nucleoli are prominent and the cytoplasm is basophilic [12]. In this case DLBCL presented with these morphologic characteristics. Based on the immunoreactivity for CD10, BCL6, MUM-1, immunohistochemical analysis confirmed the diagnosis of DLBCL, NOS, centroblastic variant, and NGC.

The standard therapy regimen for DLBCL is immnunochemotherapy – a combination of rituximab, cyclophosphamide, doxorubicin, vincristine and prednisone. The therapy success rate is 60 – 70%, whereas 50% of patients may be cured by immnunochemotherapy combined with surgery or radiotherapy [5].

According to López-Guillermo A. et al., no differences were found in the treatment outcome in patients with primary nodal and those with extranodal lymphomas. Their study included 382 patients with diagnosed DLBCL. Complete response rate was 63%, 61% in primary nodal and 68% in extranodal cases [13].

Conclusion

Lymphoma of the oral cavity usually presents as a swelling and can mimic odontogenic infection. It is important for dentists to be aware of this possibility, especially if the swelling is painless and if the initial treatment has failed. Biopsy is used to establish the accurate diagnosis of the tumor and to prevent further delays in therapy.

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A LARGE IDIOPATHIC PSEUDOANEURYSM OF THE POPLITEAL ARTERY – A CASE REPORT

VELIKA IDIOPATSKA PSEUDOANEURIZMA POPLITEALNE ARTERIJE – PRIKAZ SLUČAJA

Slavko BUDINSKI, Dragan NIKOLIĆ and Janko PASTERNAK

Summary

Introduction. Pseudoaneurysm, also known as a false aneurysm, is a collection of blood which passes through individual layers of the blood vessel or through all layers, but it is retained in the surrounding soft tissues. **Case Report.** We report presents a 41-year-old patient with a large idiopathic pseudaneurysm of the second segment of the popliteal artery, referred by an orthopedist with a magnetic resonance image of the left leg. The patient's medical history showed that the pain started three weeks ago, the swelling in the left popliteal fossa about 6 months ago, and a large, painful, non-pulsatile tumefaction was found during the examination, resulting in a 90 degrees flexion contracture of the left knee. The magnetic resonance showed a pseudoaneurysm of the left popliteal artery, and an emergency computed tomography angiography was performed; it confirmed a pseudoaneurysm of the left leg second segment of the left popliteal artery without signs of obliterative diseases. The posterior approach and exposition of the popliteal artery was applied; autovenous graft interposition with a great saphenous vein of the right leg was done. **Conclusion**. Early diagnosis plays an important role in avoiding temporary and permanent complications. Arterial reconstruction with autovein grafting is a gold standard and a method of choice in the surgery of the pseudaneurysm of the popliteal artery. The temporary setting of occlusive Fogarty catheters reduces damage to the surrounding tissue, accelerates bleeding control, which together shortens the time of surgery.

Key words: Aneurysm, False; Popliteal Artery; Endovascular Procedures; Blood Vessel Prosthesis Implantation; Signs and Symptoms; Diagnosis

Sažetak

Uvod. Pseudoaneurizma, poznata i kao "lažna" aneurizma, je kada krv prolazi kroz pojedine slojeve krvnog suda ili kroz sve slojeve, ali je zadržana okolnim mekim tkivom. Najčešći uzrok pseudoaneurizme je trauma, infekcija i prethodne medicinske procedure, ali postoje retki slučajevi kada uzrok nije pronađen. **Prikaz slučaja.** Ovaj rad je prikaz rekonstruktivne hirugije u slučaju velike idiopatske pseudaneurizme II segmenta poplitealne arterije kod 41-godišnjeg pacijenta, kojeg je uputio ortoped sa urađenom magnetnom rezonancijom leve noge. Anamnestički sa pojavom bola unazad tri nedelje, a otoka tkiva u levoj zatkolenoj jami unazad oko 6 meseci, a u kliničkom nalazu u toku pregleda je dominirala velika, bolna, nepulsatilna tumorska masa u levoj zatkolenoj jami koja je dovela do fleksne kontrakture kolena od 90 stepeni. Magnetna rezonancija je prikazala pseudoaneurizmu leve poplitealne arterije, te je hitno načinjena kompjuterizovana tomografska angiografija koja je potvrdila da je reč o pseudoaneurizmi drugog segmenta poplitealne arterije leve noge, bez znakova obliterativne bolesti. Intraoperativno je primenjen zadnji pristup i ekspozicija poplitealne arterije, a sa velikom safenskom venom desne noge načinjen je autovenski interponat. **Zaključak**. Rana dijagnostika ima važnu ulogu u sprečavanju privremenih i trajnih komplikacija. Rekonstrukcija arterije sa autovenskim graftom je zlatni standard i metoda izbora u hirurgiji pseudaneurizme poplitealne arterije. Privremeno postavljanje okluzivnih Fogartijevih katetera smanjuje oštećenje okolnog tkiva, ubrzava kontrolu krvarenja što zajedno skraćuje i vreme operacije.

Ključne reči: pseudoaneurizma; poplitealna arterija; endovaskularne procedure; implantacija vaskularne proteze; znaci i simptomi; dijagnoza

Introduction

Pseudoaneurysm, also known as a false aneurysm, is a collection of blood which passes through individual layers of the blood vessel or through all layers, but it is retained in the surrounding soft tissues [1].

Pseudoaneurysm may be pulsatile and resemble a real aneurysm. A true aneurysm is a permanent, localized dilation of all three layers of a blood vessel, by more than 50% of the usual diameter for the viewed segment [1].

The common causes of the pseudoaneurysms of the femoropopliteal segment include penetrating traumas [2], blunt traumas [3], fractures [4], previous medical procedures [5], infections [6], and in rare cases exostosis [7] and cystic adventitial diseases [8]. Since these rare lesions can be corrected, early detection and appropriate treatment should be a priority [9].

The main cause of false aneurysm is trauma and in the literature it has been reported that about 72.5% of limb loss is due to the trauma of the pop-

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Abbreviations

LL – latero-lateral
AP – anteroposterior
KK – cranio-candal
US – ultrasound
MRI – magnetic resonance imaging
CT – computeriuzed tomography
GSV – great saphenous vein
DUS – duplex ultrasonography
PAD – peripheral artery disease

liteal artery. In our case, there were no anamnestic indications about injuries [10].

Traditionally, pseudoaneurysms were treated with surgical repair, but traditional surgical treatment is invasive and is often associated with significantly higher morbidity and mortality rates [11]. Over the past few years, minimally invasive radiologic treatments have been developed as alternatives to surgery, including ultrasound (US)-guided compression [12], direct percutaneous management (including US-guided thrombin injection) [13], and endoluminal management [14]. Larger and symptomatic pseudoaneurysms should be repaired [11].

There are still delays in the diagnosis and treatment, so we present a case of a pseudoaneurysm with slow progression caused by a disease of unknown etiology, and large nonpulsatile tumor mass that has not been recorded so far, with successful treatment of a large pseudoaneurysm of the second segment of the popliteal artery of the left leg.

Case Report

A 41-year-old patient was admitted to the Emergency Department, referred by an orthopedist where he was first sent for a suspected Baker's cyst. He had a magnetic resonance image of the left leg, and this was his first and only diagnostic method until then.

The patient's medical history showed that the pain behind the left knee started three weeks ago, and the tissue swelling about 6 months ago, and before that he had no problems with this leg. The clinical examination showed a large, painful, nonpulsatile tumor mass, resulting in a 90 degrees flexion contracture of the knee.

Baseline laboratory test results showed: leukocytes - 12x10⁹/l; neutrophils - 9.5x10⁹/l ;blood glucose - 5,2 mmol/l; urea - 6mmol/l; creatinine - 84 mmol/l; international normalized ratio (INR) - 0.96.

The patient is a smoker, without a history of trauma or serious illnesses, and previously had only an appendectomy. Magnetic resonance imaging (MRI) (Siemens Avanto 1.5T) showed a pseudoaneurysm of the left popliteal artery **(Figure 1)**.

The computeriuzed tomography (CT) scan (Siemens Somatom Sensation 16) was performed urgently in the Emergency Department, which confirmed a pseudoaneurysm with 9.7 x 8 x 15 cm/ latero-lateral (LL) x anteroposterio (AP) x craniocandal (KK) diameters of the second segment of the

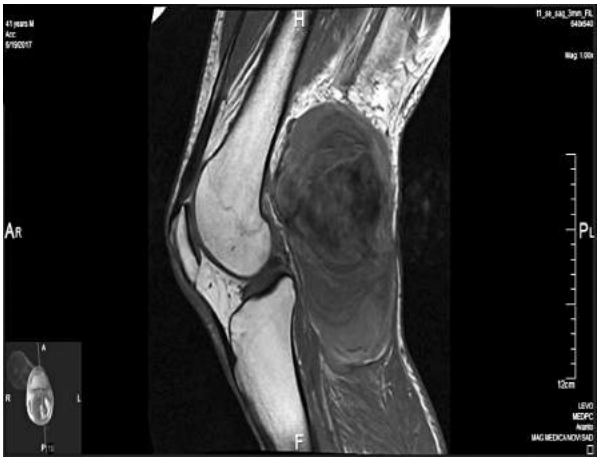

Figure 1. Magnetic resonance image of the left knee region shows a large, clearly demarcated aneurysm, which has a significant compressive effect on the surrounding soft tissues

Slika 1. *Magnentna rezonancija regije levog kolena koja opisuje veliku aneurizmu, jasno demarkiranu, koja ima izražen kompresivni efekat na okolna meka tkiva*

left popliteal artery without signs of peripheral arterial obliterative disease, so digital subtraction angiography (DSA) was not performed **(Figure 2)**.

After adequate preoperative preparation and assessment of general operability, the patient underwent surgery. Standard anesthetic protocol, orotracheal intubation, and a posterior approach to the popliteal artery were performed, with pronounced periarterial inflammatory infiltrate, excessive fibrosis, but without signs of infection.

After pseudoaneurysmectomy and evacuation, occlusive Fogarty catheters were temporarily placed

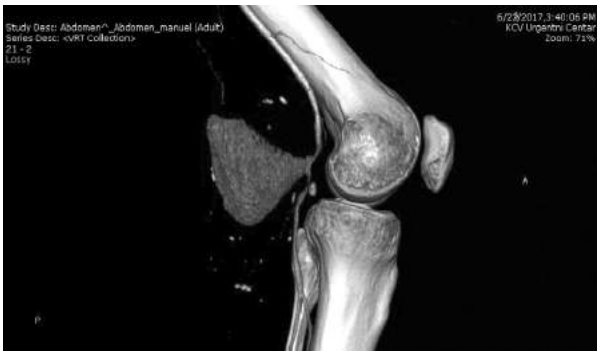

Figure 2. CT angiography of the left knee region (In space supreme vascular reconstruction) confirms the diagnosis of a large pseudoaneurysm 9.7 x 8 x 15cm/ LLxAPxKK in diameter, with a clear position of the popliteal artery laceration

Slika 2. *Kompjuterizovana tomografska angiografija regije levog kolena (In Space Supreme Vascular rekonstrukcija) koja potvrđuje dijagnozu velike pseudoaneurizme 9.7 x 8 x 15 cm/LL x AP x KK dijametra, sa uočljivim položajem defekta odnosno laceracije poplitealne arterije*

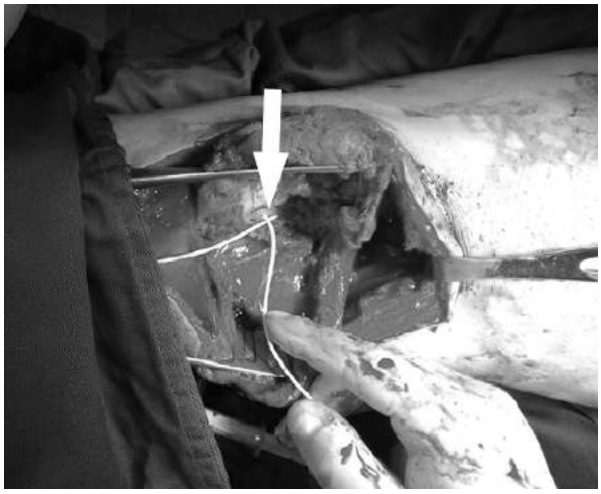

Figure 3. Intraoperative finding showing Fogarty occlusion catheters in the artery lumen proximally and distally to the artery defect
Slika 3. *Intraoperativni nalaz sa postavljenim okluzivnim Fogartijevim kateterima u lumenu arterije proksimalno i distalno od defekta arterije*

to control bleeding, and a 2 cm rupture (laceration) of the popliteal artery was verified **(Figure 3)**.

Due to the length of the laceration, a decision to create an autovenous graft interposition was made, followed by preparation of the proximal segment of the great saphenous vein (GSV) of the opposite leg.

Interposition of an autovenous graft was performed, without technical difficulties and complications **(Figure 4)**. A part of the pseudoaneurysm wall was taken for pathohistological analysis. After correction of hemostasis, two active Redon drains and one passive ribbed drain were placed **(Figure 5)**. The operation lasted 100 minutes. The post-operative period at the Clinic for Vascular Surgery and Transplantation Surgery was uneventful, with palpable peripheral pulses and good revascularization effect. The passive ribbed drain was extracted on the second, and the active drains on the third postoperative day.

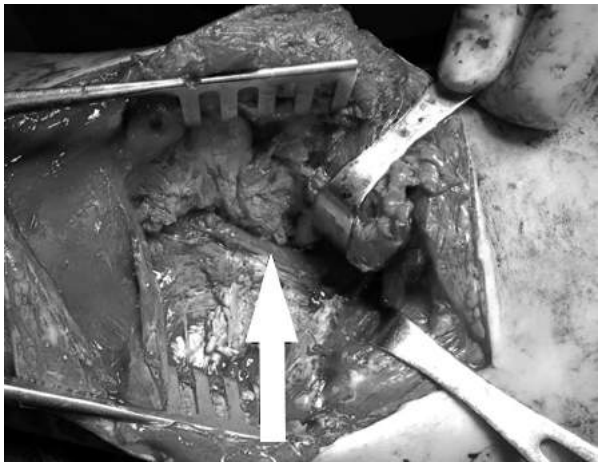

Figure 4. Intraoperative finding (pseudoaneurysmectomy of the left popliteal artery and autovenous interposition using the right leg GSV)
Slika 4. *Intraoperativni nalaz (tehnika pseudoaneurizmektomije leve poplitealne arterije i autovenski interponat sa vene safene magne desne noge)*

Because of reduced knee mobility due to contracture, during hospitalization the patient was examined by a physiotherapist who carried out physical treatment to establish the full extent of movement in the joint.

The patient was discharged from hospital on the 10^{th} postoperative day, mobile, in good general condition, with palpable distal pulses and equal ankle–brachial indices in the lower extremities, receiving an oral anticoagulant (Sintrom) and antiaggregation therapy (Aspirin).

The diagnosis of pseudoaneurysm was confirmed pathohistologically, without expected adventitial wall degeneration **(Figure 6)**.

On the last checkup, six months after surgery, a control Duplex scan ultrasonography showed regular flow in the autovenous graft, the leg was without any recurrent vascular lesions, with palpable peripheral pulses, and with maximum mobility in the knee joint. The surgical wound healed per primam intentionem.

Discussion

The incidence of popliteal artery pseudoaneurysms is low, estimated to be 0~3.5% of all popliteal artery aneurysms of various etiologies, most commonly caused by trauma, but it can easily lead to instability [2, 15, 16].

A detailed medical history is of great importance, followed by physical examination. If the lesion is large or superficially located, it is generally palpable. The diagnostic procedures are important in order to set the final diagnosis. Duplex ultrasonography (DUS) should be considered as the first diagnostic method for early diagnosis. This technique is successfully used for detection and monitoring of pseudoaneurysms, but does not provide full visualization of the whole area and has limited accuracy [9].

Digital subtraction angiography has been the traditional first-line imaging technique in patients with peripheral artery disease (PAD), but in our case it was not indicated because PAD was not present [9].

MRI is useful and non-invasive, but it is expensive and does not provide such a good insight into the lumen of the artery like CT angiography, so CT remains the diagnostic procedure of choice for pseudoaneurysms [9].

In the diagnosis of the disease, the examiner must strictly follow the rules of the medical methodology for the diagnosis, which requires strict adherence to a certain order of examination of certain tissues, systems and organs. After medical history taking and physical examination, the next step is

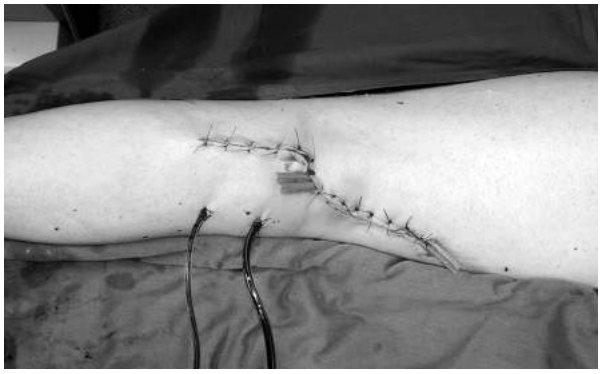

Figure 5. Final result after surgery showing two active and one passive drain
Slika 5. *Konačni rezultat nakon operacije gde se uočavaju dva aktivna drena i jedan pasivni dren*

DUS, after which the need for further diagnostics is considered [9]. Masahiro and associates published a case with a small popliteal aneurysm and the first diagnostic procedure was radiographic examination, where they noticed exostosis of the femur bone, and after a CT was performed, a pseudoaneurysm was found [8]. Janssen and associates described a case of a patient with pain in the popliteal fossa, and Doppler examination was their first diagnostic procedure, after which MR-angiography revealed a pseudoaneurysm of the popliteal artery [17]. Woolgar and associates described a delayed diagnosis and treatment (24 months), in our case it was six months [2].

If the cause of the pseudoaneurysm is unknown, the pathohistological analysis of the wall verifies the cystic changes [8], inflammatory process in the artery wall and presence of giant cells [6] or complete lack of muscle layer in the media [18]. In our case, we did not find such changes, so the etiology remained unknown. Our opinion is that adventitial cystic disease is the cause of this false aneurysm, which may be a result of (1) repeated microtrauma; (2) ectopic ganglion cyst migrating from the adjacent joint; (3) systemic myxomatous degeneration; and (4) developmental rests of mucin-secreting mesenchymal cells derived from adjacent joints and development of obliterative disease, or it can rupture and cause a pseudoaneurysm, which may have happened in our patient [8].

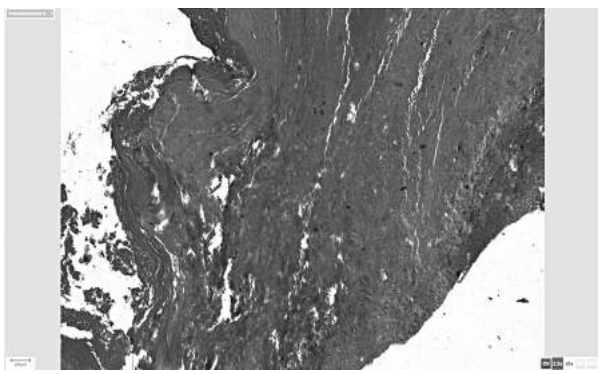

Figure 6. Pathohistological finding shows fragments of necrotic masses, with some fresh blood and smaller fragments of connective tissue
Slika 6. *Patohistološki se uočavaju fragmenti nekrotičnih masa prožete svežom krvi; se nalaze manji fragmenti vezivnog tkiva*

Accepted procedures for resolving popliteal pseudoaneurysms are percutaneous induction of thrombin [13], stent grafting [14], Duplex ultrasound-guided compression [12], as well as surgical excision by placing a patch, graft or ligature with interposition and creating a bypass [11].

Earlier investigations of pseudoaneurysms show that the patients are mostly males, like in our case [2, 5, 6, 17], aged from 55 – 60 years [6, 18], and our patient was 41 years of age. Although pulsatile masses were found in all cases, mostly up to 6 cm in diameter, with knee contracture up to 40% on the right leg, described by Woolgar and associates [2], in our case there was a non-pulsatile mass, 9 cm in diameter, with knee contracture of 90% in the left leg.

A small number of cases were reported with different reconstruction techniques, but as in our case, the approach was posterior and GSV from the opposite leg was used. The final diagnosis was made using CT and MR, like in our case. The most important information is the size of arterial defects and its location, followed by the decision of reconstruction [7, 17, 19].

Jansen and associates published a case where an autovenous bypass between supra-genicular popliteal artery and tibio-peroneal trunk was created with GSV from the opposite leg, because of a large arterial defect and deep location [17]. In our case this was not necessary, because of a minor defect and a good exposure of the II segment of the popliteal artery.

Masahiro and associates performed an embolectomy of the distal popliteal artery because of a 5 mm diameter defect on the popliteal artery, and using this hole, an autovenous patch with GSV was created [7]. In our case it was not possible because of a bigger defect of the artery.

Perez and associates decided to perform a direct suture due to a 1mm artery defect, and a resection of the artery was not necessary [19]. As in the previous case, direct suture was not possible in our case because of a bigger defect of the artery.

Postoperative complications are possible after each operation, so Kirkpatrick and associates published a case with symptomatic enlargement of the aneurysm, with continued expansion [20], whereas Halidday and associates published a case with bove-knee amputation because of a distal arterial occlusion [21]. Huang and associates published a case with perioperative myocardial infarctions and graft thrombosis [19]. We did not have any complications.

Because of anamnestic and physical findings we assumed that the cystic adventitial disease was the cause of the pseudoaneurysm, but the pathohistological analysis showed no cysts, so the etiology remained unknown. The etiology of this disease is unclear. Four hypotheses have been proposed, including developmental, ganglionic, microtraumatic and degenerative causes [8].

After establishing control of the proximal part of the femoral and distal parts of the popliteal artery, intraoperatively we entered the pseudoaneurysm. Two occlusive Fogarty balloon catheters were used to occlude blood vessels, and repaired the artery. This technique offers numerous advantages: 1) adherent surrounding structures are not damaged; 2) veins and arterial branches remain untouched, thereby reducing the risk of bleeding; 3) the best possible anastomosis is provided because the lumen is not surrounded by pressure; 4) the balloon catheter provides a dry operating field and shortens the duration of the surgical procedure; and 5) thrombectomy of the distal blood vessel with a balloon catheter can be performed to prevent acute ischemia [23].

Conclusion

Early diagnosis plays an important role in avoiding temporary and permanent complications. Arterial reconstruction with autovein grafting is a gold standard and a method of choice in the surgery of the pseudaneurysm of the popliteal artery. The temporary setting of occlusive Fogarty catheters reduces damage to the surrounding tissue, accelerates bleeding control, which together shortens the time of surgery.

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METEOROPATHY AND METEOROSENSITIVE PERSONS

METEOROPATIJA I METEOROPATE

Milorad ŽIKIĆ[1] and Tamara RABI ŽIKIĆ[2, 3]

Summary

Introduction. The authors are dealing with the questions often asked by their patients: how weather changes, oscillations in humidity, air pressure and other climate factors affect functioning of the human body. **Meteorology and Biometeorology.** Definitions of meteorology and biometeorology are given, describing some of the most important characteristics, such as the types of disorders and the reasons for their occurrence, frequency, gender and age factors, etc. with particular reference to disorders related to the mental and nervous functions. **Discussion.** The possibilities of prevention, the importance of monitoring biometeorology forecasts and the questionable need for drug use during the seasonal shifts, which cause fatigue, irritability, poor concentration and apathy, hypersensitivity to pain, headache, dizziness, anxiety, head buzzing, etc. are discussed.

Key words: Meteorology; Weather; Acclimatization; Temperature; Risk Factors; Signs and Symptoms; Adaptation, Physiological; Therapeutics

Sažetak

Uvod. Autori daju odgovore na pitanja koja se često postavljaju lekarima u praksi – kako promene vremena kao što su oscilacije u temperaturi, vlažnosti, atmosferskom pritisku i drugim klimatskim činiocima, utiču na funkcije ljudskog organizma. **Meteorologija i biometeorologija.** Definišu se pojmovi meteorologije i biometeorologije, opisuju neke od njihovih važnijih karakteristika kao što su vrste poremećaja i razlozi njihove pojave, učestalost, zastupljenost prema polu, uzrastu i druge, sa posebnim osvrtom na poremećaje u vezi sa mentalnim i nervnim funkcijama. **Diskusija**. Raspravlja se o mogućnostima prevencije, važnosti praćenja biometeoroloških prognoza i upitne potrebe za upotrebom lekova tokom smene godišnjih doba, kada su izraženi: umor, razdražljivost, loša koncentracija i apatija, preosetljivost na bol, glavobolja, vrtoglavica, nervoza, anksioznost, zujanje u glavi i drugo.

Ključne reči: meteorologija; vreme; aklimatizacija; temperatura; faktori rizika; znaci i simptomi; fiziološka adaptacija; terapija

Meteoropathy - History, Concept, Definition and Causes

As part of nature and its product, human beings respond to all the events in their environment, including meteorological factors. Those who cannot adjust to changes experience manifestations and problems that are called meteoropathic. The Ancient Greeks emphasized the interactions between the weather and human health more than 2.500 years ago, pointing to the importance of weather conditions and their impact on the human health. Today, they are generally accepted and are getting more and more attention.

The term meteoropathy comes from the Greek word meteoron - a celestial phenomenon and pathos - disease. Difficulties associated with weather changes, including air temperature, humidity and wind, air pressure, ionization, ultraviolet radiation, atmospheric precipitation and other meteorological factors, are a set of symptoms and reactions of people whose organisms cannot adjust. According to the stated, generally accepted definition, meteorosensitive persons are individuals who experience health problems or deterioration of chronic diseases associated with bad weather conditions and weather changes. Meteoropathy symptoms usually occur 24 to 48 hours before weather change, usually last a day or two and pass when the organism is adjusted to new weather conditions or they withdraw [1–4].

Many contemporary studies, as a recent study on heart attacks conducted in Sweden, revealed that the incidence of heart attacks varies by month and day, that summer months are safer than winter months, and Monday is more risky than Saturday [5]. A previous research performed at the Neurology Clinic in Novi Sad suggests that the time of winter and spring holidays, preceded by excessive exposure to food, alcohol consumption, smoking

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Abbreviations
ACTH – adrenocorticotropic hormone
MS – multiple sclerosis
SAD – seasonal affective disorder

and prolonged sitting, are periods with the highest incidence of strokes [6].

We are witnessing a systematic public warning by media about health risks that all or certain groups of people are exposed to during extreme or less intense changes of meteorological factors. Today we know that almost all people respond in a certain way to adverse weather conditions that cause electromagnetic waves affecting the hypothalamus, indirectly enhancing the secretion of stress hormone, adrenocorticotropic hormone (ACTH) and reducing hormone of happiness (endorphin), which leads to increased anxiety, headache and other meteorological symptoms.

In psychophysically unstable persons and those susceptible to weather changes, stress can be a trigger for changes in the body that can make them vulnerable to various physical and mental disorders. Increase in stress levels also enhances the brain cells activity of the amygdala nucleus, part of the brain responsible for emotions. Thus, at any time, it may coincide with an increase in the level of stress hormones in the organism triggered by return to work on Monday, from vacation, during winter and spring holidays, and encourages the bone marrow to produce more immune cells for the defense from stress. This causes inflammation that can damage the arterial vessels responsible for the brain function [3, 5, 7] and so in the natural environment of the living world, it is a vicious circle from the beginning to the end, the chain of events continues to occur repeatedly.

One of the causes of meteoropathy, especially in the urban population, is human alienation from nature, which results in poorer ability to adapt to weather changes [7]. The description of modern life of people "living in concrete cities looking like graveyards with traffic lights" vividly depicts the above assumption.

Is meteoropathy a disease of individuals at risk, or can it affect anyone?

Meteoropathy is not a disease, but rather a condition, an overreaction to weather changes. Despite developments of medicine as a science, it has not yet been fully explained what exactly occurs in the body reacting to this overwhelming phenomenon. This certainly is not a reason to deny that weather affects human health. Meteoropathy exists, many are susceptible to it, and it is assumed that it affects about 30% of the general population, which makes it very common. If the symptoms occur in young and healthy people, they are mild and almost imperceptible, but in older people and chronic patients it can be severe, so they require special treatment. In some cases these patients need hospital treatment. It is especially dangerous for people with severe illnesses, because they can seriously deteriorate their health, even lead to fatal outcome.

The most vulnerable categories are middle-aged people, women more than men, especially menopausal women but everybody, even children, can suffer from meteoropathy. Meteoropathy symptoms often occur before or after the weather change, rather than at the time of change [3, 7].

Which meteoropathy symptoms are associated with neurology and/or psychiatry?

Meteoropathy symptoms induce responses of the nervous system that are various and represent disorders associated with any psychic or nerve function - thinking, emotions, attention, memory, will, instincts, observations, sensory and motor functions, coordination of movements especially when changing position, balance maintenance, etc. [8].

Weather changes in meteorosensitive persons cause headaches, especially migraines; buzzing in the head, imbalance, weakness, irritability, insomnia, concentration and will deficit, decrease in work and life energy, etc. These symptoms last till the weather conditions improve, and the symptoms decrease and gradually disappear.

In healthy and younger people, symptoms include psychophysical fatigue, mood swings, reduced concentration, apathy, and pain at the site of old injuries and scars may occur.

Symptoms of chronic mental and neurological disorders are exacerbated or are associated with other organic diseases. They include hypersensitivity to pain, headache, dizziness, buzzing in the ears, nervousness, anxiety, depression, rapid heart rate and breathing difficulties [7, 8].

It is known that solar flares, coronal mass ejections and solar energetic particles are driven by the solar magnetic field have great influence on the Earth, which protects itself by its magnetic field. However, a certain amount of radiation comes to the Earth and affects human health, especially in the first three days after the beginning. It is associated with nervous system problems – in some persons conditioned reflexes get slower, resulting in an increased number of traffic accident others have a heavy leg feeling, accelerated pulse, poor vision, etc. There are people who can anticipate solar flares, while others have problems a few days later [7].

How do meteorosensitive persons react to sudden drop in temperature and changes in atmospheric pressure?

The nervous system and sensory organs register external influences on physical and mental functions responding to everything, most of all to turbulent meteorological changes, to which they react in various ways.

Sudden weather changes are not pleasant for anybody, but sensitive people experience serious problems. At temperatures above 30°C, or when humidity exceeds 70%, the thermoregulation system tries to cool the body, which is an additional effort for the cardiovascular system. This also threatens to supply the brain with nutrients and gaseous substances from arterial blood, because high temperatures cause spreading of the blood vessels and drop of blood pressure. In order to cool the body, sweating is increased and fluid loss occurs [3, 9, 10].

Residential air conditioning with a difference between indoor and outdoor temperature over 7° C is another health risk for meteorosensitive individuals due to sudden cooling. The organism responds to such a fall in temperature by contraction of blood vessels in order to reduce the body temperature, resulting in an increase in cardiac and vascular resistance [4].

Recent studies in Germany and the United States have shown that cloudy and cold weather may cause bad mood, but also that in general population the connection is not clear. This means that low temperatures, rain and wind have much less impact on the mood than it has been previously thought. Chilly weather, however, increases the incidence of angina attacks, strokes and heart attacks as blood becomes denser and increases the tendency to blood clotting [9, 10].

In contrast, the symptoms of multiple sclerosis (MS) are exacerbated when the external temperatures are high, and the patients feel too hot, so the summer rains and cooler weather are a relief. For example, therapeutic benefits of "Atomic Spa" in Gornja Trepča (near Čačak, Serbia) in MS patients may be attributed to the low-radioactive water, but also lower temperatures. The accuracy of this assumption is indicated by exacerbation of MS patients if they are taking warm baths.

When the atmospheric pressure drops, the body fluids and gases are spreading, and it results in pains, as the pressure of fluid and air inside the joints of the inflamed tissues or injuries causes increased pain. In the same way, sinus headaches increase.

And so, it is a vicious circle; the previous problem causes another one due to the consequent state opposite to the one that produced it in the first place. The problems will pass when the circumstances are back to normal, to which the organism is adapted [3, 7].

How to help meteorosensitive individuals?

We are witnessing rapid climate changes with significant daily temperature differences, and people's interest in weather and its effects on health is growing around the planet. A new branch of meteorology has been developed – biometeorology that deals with the influences of weather factors on the whole living world. Bio-meteorological forecast contains a description of the meteorological situation and announces weather conditions that may adversely affect the health of particular groups of people.

The mass media inform us about the bio-meteorological forecast on daily basis e. g. how long the changes will take place, about their intensity, as well as whether there will be any harmful risks for weather-sensitive persons. The purpose of these information is to take preventive measures regarding exposure to environmental changes, stay indoors in air-conditioned spaces till the air temperatures and intensity of ultraviolet radiation are harmful to health; follow a healthy diet regime - low calorie diets, avoid all harmful forms of behavior, mandatory use of preventive doses of drugs to prevent the aggravation of chronic diseases and so on [1].

People should regularly follow the bio-meteorological forecasts, so that they can prepare and avoid upcoming difficulties. The proposed measures should preferably be applied at least three days before the expected occurrence of climate changes. All of the above turbulences represent the so-called "meteorological stress" that can aggravate the general state of chronic patients. However, the statistics show that unfavorable outcomes are more common in the period from October to May than during the summer months [10].

How long does it take to adapt to the changing weather conditions?

When the weather suddenly changes or the adverse weather conditions last longer, like during cold or heat waves, in days of increased humidity and consequently lower air pressure, the pituitary gland increases secretion of the stress hormone, adrenocorticotropic hormone (ACTH) that causes anxiety and irritation, and due to the simultaneous reduction, the secretion of endorphins (natural analgesics) the threshold of pain decreases and the subjective feeling of pain increases.

The second reason for amplified pain is explained by the laws of physics: due to the fall of atmospheric pressure, fluids and gases are spreading in the body and their increased pressure on the inflamed tissues or injurie increases the painful sensation.

The importance of this issue was long ago seen in the Dubrovnik Republic and some other Mediterranean countries in a provision about never deliberating and making decisions when the southwind (Jugo), was blowing. It was seen as a mitigating circumstance when judged on crimes committed during Jugo [7, 10].

Is the treatment of meteoropathy symptoms different from the treatment of other disorders?

There is an increasing awareness of weather conditions affecting the health of people, especially in chronic patients. Many developed countries have established specialized institutions and centers to study these impacts, such as the Research Center in Bioclimatology, Biotechnology and Natural Medi-

cine of the University of Milan, which studies these effects and, accordingly, prepares specific weather forecasts for meteorosensitive individuals. The elimination of negative weather conditions certainly helps to follow the instructions derived from biometeorological forecasts. Their goal is to alert sensitive people in a timely manner and reduce the impact of adverse weather conditions on their health to the smallest possible extent.

The preconditions for good health that diminish the impact of weather changes are primarily: healthy diet of foods rich in magnesium and vitamins, especially B-complex or supplements based on these nutrients, with fresh and varied fruits and vegetables, avoidance of processed and fatty foods, alcoholic beverages and cigarette smoking, adequate daily fluid intake and sufficient sleep, less psychophysical stress, avoidance of being outdoors during periods of unfavorable circumstances and staying in ventilated, naturally-lit rooms. In addition, all and especially meteorosensitive persons should spend at least half an hour a day walking in fresh air far from traffic jams, favorably in the morning and in the evening, in hot days take showers a few times a day, alternating hot and cold water, follow the diet regimes and avoid taking synthetic medications. As an example among many, we would like to mention that diet based on soy or lecithin, herbal teas - lemon balm, chamomile, hawthorn and valerian, especially if taken one or two days prior to announced weather changes, as well as regular physical and mental exercises, represent a good choice when fighting against weather disturbances [7, 9].

When should meteorosensitive individuals use medications?

As a model of treatment, the same treatment as in patients with seasonal affective disorder (SAD) is recommended. It is a type of depression that is related to changes in seasons, associated with the following symptoms: significant lack of energy, mood swings when the outside temperatures are lower, when the patients sleep longer (in summer about 7 hours on average, during autumn and winter longer than 10 - 12 hours, and they still feel a lack of sleep) and they are hard to wake, have low concentration, no interest in everyday activities, they are lifeless, avoid contact with other people, prone to weight gain because they need foods rich in carbohydrates and are sexually inactive, but all of these symptoms disappear with the arrival of spring. Persons with a mild form, should spend more time outdoors or, if that is not possible, use every opportunity to sit in bright places, best at the window. Those who suffer from severe forms, need medical therapy. Overusing medications should be avodied, because good results are achieved by using light therapy, and if it does not lead to satisfactory results, antidepressant and psychotherapy are prescribed. Apart from the described SAD, characteristic for cold season, there is an opposite disorder, when people feel good in the winter and as the days become warmer their mood becomes worse and they have symptoms that are contrary to those that characterize winter affective disorders.

People who are sensitive to these and other weather changes should have three vegetable and two fruit meals a day. Spring and summer are ideal for this recommendation, because at high temperatures, nothing can replace refreshing meals. Easy to digest foods with lots of fresh vegetables, refreshing salads, seasonal fruits are ideal for the hottest part of the year, because they satisfy hunger and thirst at the same time. Nutrition based on fresh fruits, some vegetables and sufficient fluid intake is an irreplaceable advice to meteorosensitive persons. Fats and fatty foods increase the circulation in the digestive tract and "steal" it from the general circulation, which is practically analogous to the already harmful physical effort; these persons should quit smoking, alcohol drinking, and artificial sweeteners, very cold, carbonated drinks and coffee, whereas short morning and evening walks are very useful.

As mentioned above, one should pay attention to the use of air conditioners, sun exposure from 15 minutes to half an hour is safe (until 10 am, after 5 pm in the afternoon), in the shade rather than direct exposition. Sudden peripheral cooling is associated with centralization of blood circulation, higher heart load and higher energy consumption, and if bathing is planned, it is necessary to gradually adapt to the water temperature. The greater the difference between the water and air temperature, the more caution is needed. All of the above applies also to people who spend time in the mountains, because at high altitude the partial pressure of oxygen is lower and consequently the supply of necessary oxygen to the cells of the nervous system is lower [1, 7, 9, 11].

Why is self-medication dangerous?

We will explain this in case of a summer vacation in a very warm place, where the adaptation period is the most dangerous e.g. in the first four days in the new environment. The optimal time for such a holiday is at least three weeks - the first for adjustment, the other two for relaxation and enjoyment. During such holidays, the prevention of disorders implies avoiding major psychophysical efforts, staying in the sun during strong UV radiation, and adherence to the already stated measures of the dietary regime. As you can see, no medications are mentioned, especially self-medication. Drug use is justified only if there have been accompanying symptoms, such as unconsciousness and dizziness due to high temperatures, blood vessel spreading and blood pressure drop.

Excessive exposure to heat leads to the redistribution of blood from the central circulation into the peripheral, e.g. from the diseased to the healthy segments of the damaged organ, where the spread of

the arteries is not uniform because the damaged blood vessels are less spread and brain blood supply is insufficient. These problems can be avoided by regular health control before exposure to weather changes, regardless of where they take place, and only if necessary, revision of the previous therapy is performed, but by the doctor, not on our own. It specifically refers to patients who are taking psychoactive drugs, diuretics and calcium channel blockers whose mechanisms of action, side effects and complications are not known by most meteorosensitive individuals. Drug doses should generally be reduced, because on the one hand blood vessels are expanding for thermoregulation, and on the other hand, these are the effects of some drugs, for example doses of diuretics should be decreased, as further loss of fluids by sweating is expected. Non-compliance with these instructions may cause extremely dangerous consequences, as well as severe nervous, mental, and cardiovascular disorders requiring hospital treatment or emergency ambulatory intervention [1, 9].

It is good to live according to the laws of nature before weather changes as well!

Let's just repeat and highlight the above points: meteorosensitive persons, like other people, should spend half an hour per day in the fresh air, walking as the most appropriate physical activity, at least 30 minutes a day, 3 - 4 hours per week. It is beneficial to accelerate the circulation, by taking hot and cold showers, and spend time in well ventilated and bright rooms. Hydration with recommended daily fluid intake, preferably water, is of utmost importance; if one cannot consume natural mineral and vitamin-rich foods, he should use synthetic dietary supplements on regular basis, best during the meals, and it is especially important to eliminate all unhealthy habits, primarily smoking and alcohol drinking, which increase the body temperature and affect the loss of fluids [4, 10, 11].

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HISTORY OF MEDICINE
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ENVIRONMENTAL NOISE IN NOVI SAD 1985 − 2016

BUKA U ŽIVOTNOJ SREDINI U NOVOM SADU 1985–2016

Emil ŽIVADINOVIĆ[1], Marija JEVTIĆ[1, 2] and Sanja BIJELOVIĆ[1, 2]

Summary

Introduction. Noise is recognized as a physical hazard in the environment, and if it causes adverse effects to human health, it is recognized as a risk. Also, it is a harmful outdoor sound created by human activity. The aim of this paper was to present the history of environmental noise measurements in Novi Sad, as well as the indicators, methods, standards and results during three decades. **Material and Methods.** From 1985 to 2011, the Institute of Public Health of Vojvodina conducted noise measurements presented as the equivalent continuous sound pressure levels, which were, at the same time, the rating equivalent continuous levels, due to the representativity of the measurement conditions and measurement sites selection. Since 2011, the measurements have included the basic noise indicators - daily, evening, night and total noise, while the measurement sites were ranked in accordance to the European Environmental Noise Directive. **Results.** There is a lot of available data about environmental noise in Novi Sad from 1985 − 2016, but only the data from five representative measurement sites are presented in the paper. The linear trends of daily and night noise from all the measurement sites show a fall, but it does not mean that the environmental noise is reduced. All the data show that the minimum and maximum values are getting close to each other, which indicates that environmental noise is evenly distributed and is present everywhere. **Conclusion.** Based on the 30-year results, always using modern methodology and equipment, as well as expert knowledge, it may be concluded that the environmental noise in Novi Sad presents a long-lasting physical hazard.

Key words: Cities; Noise; Environment; Risk Factors; Public Health; Equipment and Supplies; History of Medicine

Sažetak

Uvod. Buka je prepoznata kao fizička opasnost koja se, ukoliko ugrožava zdravlje i dovodi do obolevanja stanovništva, prepoznaje kao rizik iz životne sredine, odnosno buka u životnoj sredini je neželjeni/štetni zvuk stvoren ljudskom aktivnošću. Cilj rada je da se prikažu: istorijat merenja buke u životnoj sredini u Novom Sadu, korišćeni pokazatelji, metode, normativi i rezultati merenja za prethodne tri decenije. **Materijali i metode.** Od 1985. do 2011. godine, rezultati merenja koja je sprovodio Institut za javno zdravlje Vojvodine prikazivani su kao vrednosti ekvivalentnog nivoa buke, koji je bio istovremeno i merodavni nivo buke usled reprezentativnosti uslova merenja i izbora mernih mesta. Od 2011. godine određuju se vrednosti osnovnih indikatora buke − indikatora dnevne, večernje, noćne i ukupne buke, a merna mesta se rangiraju u skladu sa evropskom direktivom. **Rezultati.** Od izobilja raspoloživih podataka o merenju buke u Novom Sadu u periodu 1985−2016. godine, u radu su prikazani samo podaci sa pet reprezentativnih mernih mesta. Linerarni trendovi dnevne i noćne buke na svim mernim mestima pokazuju pad, ali to ne znači da se buka u životnoj sredini smanjuje. Istorijski podaci pokazuju da se minimalne i maksimalne vrednosti približavaju jedne drugima, što ukazuje na to da se buka u gradu „ravnomerno raspoređuje" i da je svuda prisutna. **Zaključak.** Na osnovu 30-godišnjih rezultata merenja, korišćenjem uvek aktuelne metodologije i opreme, na osnovu istraživanja stručnih lica i aktuelnih stručnih saznanja, zaključuje se da buka u životnoj sredini Novog Sada predstavlja dugotrajno prisutnu fizičku opasnost.

Ključne reči: gradovi; buka; životna sredina; faktori rizika; javno zdravlje; oprema i materijali; istorija medicine

Introduction

Noise is recognized as a physical hazard and if it is threatening the health and leading to diseases in the population, it is recognized as an environmental risk; in other words, environmental noise is a harmful sound created by human activities, including road, rail and air traffic noise and noise

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Abbreviations

WHO	– World Health Organization
EEA	– European Environment Agency
EPA	– Environmental Protection Agency
IPHV	– Institute of Public Health of Vojvodina
SEL	– sound exposure level
TNI	– traffic noise index
Lday	– noise level during the day
Levening	– noise level during the evening
Lnight	– noise level during the night
Lden	– noise level during the day, evening and night
LReqT	– rating equivalent continuous level
NMT	– noise monitoring terminal
LAeq	– equivalent continuous noise levels
LAmin	– minimum values of the average noise levels
LAmax	– maximum values of the average noise levels
L10, L50, L90	– average noise levels at selected intervals

originating from industrial plants [1]. Noise, or sound, is a wave of air pressure, and the human ear is sensitive to such waves in the range of 20 − 20.000 Hz. The speed of sound through the air is approximately 343 m/s. Sounds are emitted by natural (thunder, wind, for example) and artificial sources (human activity, mainly traffic and industry). Sounds are not limited to any one point in space, but are present all around the sound source, so we refer to a sound field in which the sound intensity or sound waves diminish with the increase of distance relative to the sound source, due to spreading and absorption. How do we hear sounds? Air vibration consequently causes eardrum vibration, which is then transmitted to the auditory nerve, and the nervous system translates the vibration into sound information. Sounds can be loud (a high amplitude wave) or quiet (a low amplitude wave); they can be broadband (a mix of audio information from a sound field) or tonal (when one audio component audibly stands out from general noise).

ANoise level meter Type 2205 Brtiel & Kjasr. used in the period 1985–1989./*Merač zvuka tipa 2205 Brtiel i Kjasr, korišćen u periodu 1985–1989*

ANMT installed in Futoška Street, Novi Sad/*NMT postavljen u Futoškoj ulici, Novi Sad.*

A Briiel & Kjajr 4427 and an octave filter Briiel & Kjar 1625, used in the period 1990–2002/ *Briiel & Kjajr 4427 i oktavni filter 1625, korišćen 1990–2002*

A Briiel & Kjar type 2250, Brüel & Kjar type 3535-A protective case set (with the noise meter inside) and a Briiel & KjarOutdoor Microphone type 4952/*Briiel & Kjar tip 2250 i tip 3535-A zaštitno kućište (sa bukometrom unutar njega)*

Figure 1. Noise monitoring equipment in Futoška Street, Novi Sad

Slika 1. *Oprema za monitoring buke u Futoškoj ulici u Novom Sadu*

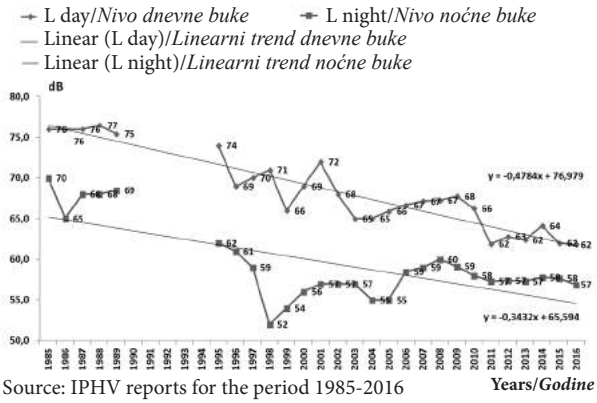
According to contemporary scientific insights of the World Health Organization (WHO), the European Environment Agency (EEA) and the Environmental Protection Agency (EPA), as well as researches conducted in our country [2−6] environmental noise is recognized as a factor that leads to anxiety, hearing disorders, sleep disturbances, cognitive disorders in children, and cardiovascular diseases [7], and is considered a stressogenic factor that affects human mental health as well [8]. Besides the above mentioned, environmental noise presents a challenge for modern urban planning, and certainly closely related human health and the issues of people's exposure to noise, and is unavoidable in all action plans and strategies for human health and environmental protection [9, 10]. Considering the fact that environmental noise is a contributing factor to the appearance of the above mentioned diseases, noise measurement and assessment of its impact on the health of the population has been in the focus of medical profession for a number of years. Data on the determined environmental noise levels collected for several decades that are at the disposal of public health institutes and institutions enable a detailed analysis and historical review of the environmental noise level trends [11].

The aim of this study is to present the history of environmental noise measurements in Novi Sad, together with the indicators, methods and standards used and to offer a review of the measurement results for the previous three decades.

Material and Methods

Based on the data available, the first noise measurements in Serbia were conducted in Belgrade in 1957, using a phonometer, General Radio Co [12], while environmental noise in Niš has been monitored since 1995 [13, 14]. The oldest available reports on the measurement of environmental noise in Novi Sad, stored in the archives of the Institute of Public Health of Vojvodina (IPHV) date back to April 1985.

Measurements of "communal noise" (commonly used terminology in the 80s and 90s) were carried out each month, three times a day (at 06:30 AM, 14:00 PM, and at 17:00 PM) on two measurement sites, in the course of a single day. Night-time measurements were conducted during September at 0:30 and 03:30 AM, on the same measurement sites as the day-time measurements. There were a total of 15 - 18 measurement sites, covering representative city areas. With minor changes (e.g. in 2010, night noise was measured four times a year - in spring, winter, summer, and autumn), this methodology was maintained until March 2011. The new history of measuring environmental noise in the City of Novi Sad



Source: IPHV reports for the period 1985-2016
Izvor: Izveštaji IZJZV za period 1985-2016
•data for1990-1994 are not available/•*podaci za 1990-1994 nisu dostupni*

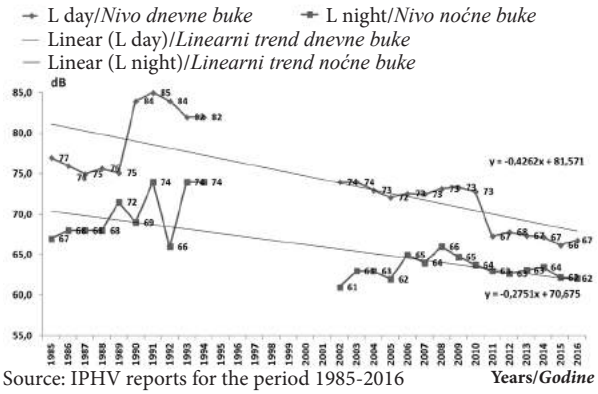
Graph 1. Mean annual equivalent noise levels (1985 – 2010)/day/night time indicators (2011 – 2016) in leasure and recreational areas/hospital zone

Grafikon 1. *Srednje godišnje vrednosti ekvivalentnog nivoa buke (1985–2010)/ indikatora dnevne i noćne buke (2011–2016) u područjima za odmor i rekreaciju/ bolničkoj zoni*

started in April 2011. The 24-hour measurements have been conducted since then, because hardware and software development made it possible.

In regard to equipment used for measuring noise levels, records show that in the period 1985 – 1989, the Brüel & Kjær Sound Level Meter Type 2205 was used together with the Octave Filter Type 1613 by the same manufacturer, sampling 300 audio levels per each measurement, and a frequency analysis was done for each time of the day.

From 1990 to April 2002, the Brüel & Kjær Sound Level Meter Type 4427 was used, which sampled 64 sound levels per second, and generated data about Leq, L0, L10, L50, L90, L100, traffic noise



Source: IPHV reports for the period 1985-2016
Izvor: Izveštaji IZJZV za period 1985-2016
•data for1995-2001are not available/•*podaci za 1995-2001 nisu dostupni*

Graph 2. Mean annual equivalent noise valuse (1985 – 2010)/ day/night time indicators (2011 – 2016) in business-residential areas

Grafikon 2. *Srednje godišnje vrednosti ekvivalentnog nivoa buke (1985–2010)/ indikatora dnevne i noćne buke (2011–2016) u poslovno-stambenim područjima*

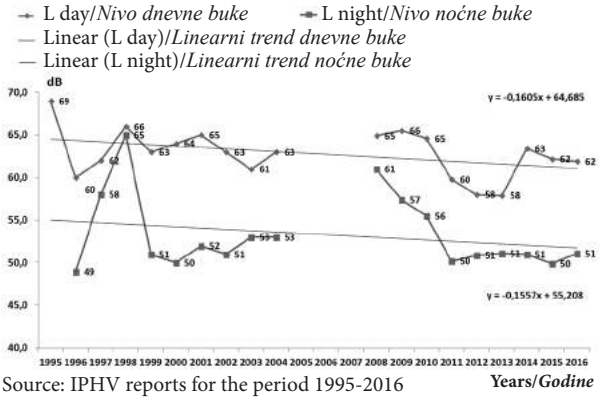
index (TNI) and short sound exposure level (SEL) (noise level in decibels that can produce a current sound event lasting one second). The microphone windscreen polyethylene foam cover was also used in order to prevent possible wind caused noise, as well as to protect the microphone from dirt, dust, rain or snowfall. From 1995, the Brüel & Kjær Octave Filter 1625 was used, as an addition to the Brüel & Kjær Noise meter 4427, for frequency analysis in the frequency range from 31.5 Hz to 8000 Hz.

From April 2002 to March 2009, the Brüel & Kjær 2260 was used, together with the 4231 type calibrator, condenser microphone type 4189, and the Software Noise Explorer Type 7815 Version 4.15. In 2005, the IPHV was certified for the first time, fulfilling the requirements of the JUS ISO 9001:2001 and JUS ISO 14001:1997 standards, and accredited in accordance with the requirements of the JUS ISO/IEC 17025 standard in 2010.

Since April 2009, the IPHV has been using a system for 24-hour noise level measurement and determination of the value of the basic environmental noise indicators (noise level during the day - L_{day}, noise level during the day, evening and night - L_{den}, noise level during the evening - $L_{evening}$, noise level during the night - L_{night}) and the supplementary noise indicator (rating equivalent continuous level -L_{ReqT}), which consists of: The Brüel & Kjær Noise meter type 2250, Brüel & Kjær type 3535-A (a protective case that acts as physical protection equipment), Brüel & Kjær Outdoor Microphone type 4952, BZ 5503 Utility Software and the Noise Explorer Type 7815 Software.

Since 2012, the IPHV has been using the Brüel & Kjær Noise Monitoring Terminal (NMT) 3639–E–103 with the accompanying Environmental Noise Management System Software Type 7843, which, is used only at two measurement sites: 121 Futoška Street and Partizanska Street, due to its immobility **(Figure 1)**.

The methodology of measuring environmental noise has always been prescribed by applicable national and international standards. The applicable regulation in the 1980s was the Rulebook on the allowed noise levels in the environment, Official Gazette SRS, No. 57/82. The regulations applied in the period 1992 to 2010 were: Law on Environmental Protection, Official Gazette of RS No. 66/91 [15], Rulebook on permitted environmental noise levels, Official Gazette of RS No. 54/92 [16], Methods for measuring environmental noise, Official Gazette of RS No. 54/92 [17], as well as standards for Measuring noise in the communal environment, SRPS U.J6.090 [18] and Acoustic Zoning, JUS U.J6.205 [19]. Since the 2010s, national standards have been harmonized with the European Directive 2002/49/EC [20]. Since then, the following have been in effect: Law on Public Health, Official Gazette of RS No. 72/2009 [21], Law on Environmental Noise Protection, Official Gazette of RS No. 36/2009 [22], Regulation on noise indicators, limit values, noise indicators assessment methods, annoyance and harmful effects of environmental noise, Official



Source: IPHV reports for the period 1995-2016
Izvor: Izveštaji IZJZV za period 1995-2016
•data for2005-2007 are not available/•*podaci za 2005-2007 nisu dostupni*

Graph 3. Mean annual equivalent continuous noise levels (1995 – 2010)/day/night time noise indicators (2011 – 2016) in residential areas
Grafikon 3. *Srednje godišnje vrednosti ekvivalentnog nivoa buke (1995–2010)/indikatora dnevne i noćne buke (2011–2016) u stambenim područjima*

Gazette No. 75/2010 [23], Rulebook on the methods of noise measurement, content and scope of the noise measurement report, Official Gazette of the RS 72/2010 [24], as well as the SRPS ISO 1996-1:2010 [25] and SRPIS ISO 1996-2:2010 [26].

The results of the measurements were presented differently in different periods. From 1985 to 2011, measurement results were shown as values of the equivalent continuous noise levels (L_{Aeq}), which was, in principle, also a rating equivalent continuous level (L_{ReqT}) due to representativeness of the duration of measurement, measurement conditions and choice of measuring sites. Reports also showed the minimum (L_{Amin}) and maximum values (L_{Amax}), as well as the average noise level in the selected interval, such as L_{10}, L_{50}, L_{90}.

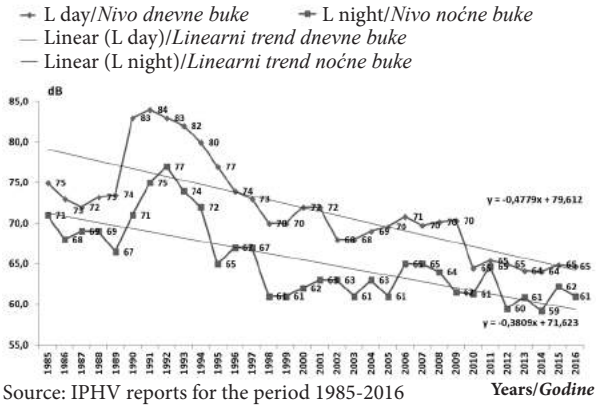
Since 2011, the values are determined by the basic noise indicators - daily, evening, night and overall noise (L_{day}, L_{den}, $L_{evening}$, L_{night}) (an indicator is a physical value that describes environmental noise, which is associated with adverse effects on human health), while the measurement sites are ranked by measured values in accordance with the European Directive.

There is a question of comparing different methodologies. It is important to point out that comparison between the "old" (rating equivalent continuous level) and "new" (basic noise indicators) noise indicators will be reported in a new paper, and the authors of this paper intend to do it in the future. The task, due to complexity, exceeds the historical survey of noise measurements during 30 years in Novi Sad.

Results

From the abundance of available data collected during the noise measurement in Novi Sad in the period 1985 – 2016 (**Graphs 1**–**5**), this paper presents data only from five (5) measurement sites, which were used both in the 1980s and in the last decade, and are not only representative measurement sites, but representative zones for a longer period of time. In retrospect, while keeping in mind the defined acoustic zones of Novi Sad, the measurement sites are shown in accordance with the purpose of the areas as defined by the Spatial Plan of the City of Novi Sad [26] and the City Noise Measurement Program [27, 28]. In the period 1985 – 2013, the Institute determined environmental noise in Novi Sad at the network of measurement sites, designed in the Institute. Measurement sites in the City of Novi Sad presented in the study and classified according to the purpose of the given area were: Novo Naselje in Residential Areas, Bulevar oslobođenja/across the "Dnevnik" and Bulevar Mihajla Pupina in Zone of City Roads, Salajka (Partizanska Street) in Business-residential Areas, Close to hospital and Towards Sports Center Sajmište in Leisure and Recreation Areas/Hospital Zone, and Petrovaradin (vicinity of Elementary School Jovan Dučić) in School Zone.

The legal environmental noise limit values are: 50 dB for day/evening and 40 dB for night in Leisure and Recreation Areas/Hospital Zone; 50 dB for day/evening and 45 dB for night in School Zone; 55 dB for day/evening and 45 dB for night in Residential Areas; 60 dB for day/evening and 50 dB for night in Business-residential Areas; 65 dB for day/evening and 55 dB for night in Zone of City Roads. The environmental noise limit values have been exceeded in all decades and in all acoustic zones in Novi Sad, regardless of the methodology or measuring equipment used, which indicates that noise monitoring and actions for noise reduction should be the priority for the community.



Source: IPHV reports for the period 1985-2016
Izvor: Izveštaji IZJZV za period 1985-2016

Graph 4. Mean annual equivalent continuous noise levels (1985 – 2010)/day and night time noise indicators (2011 – 2016) in the zone of city transportation
Grafikon 4. *Srednje godišnje vrednosti ekvivalentnog nivoa buke (1985–2010)/indikatora dnevne i noćne buke (2011–2016) u zoni gradskog saobraćaja*

The Linear trends for daytime and nighttime noise, based on thirty years of monitoring results at the measuring sites, show a decline (**Graphs 1 – 5**). However, this does not mean that there is a decrease in environmental noise. In fact, historical data from 1985 to 2016 show that the minimum and maximum values are getting closer to each other, which indicates that the noise in the city is "evenly distributed" and that it is omnipresent, which has been corroborated by studies of IPHV experts [29, 30].

Discussion

What has changed since the 1980s in regard to measuring environmental noise and the interpretation of the noise impact on human health?

In our country, over the past decades, only public health and scientific institutions have been concerned with environmental noise measurement. However, gradual changes of regulations and their interpretation, together with a developing need for monitoring environmental factors, have led to the fact that monitoring environmental noise is no longer the sole responsibility of healthcare institutions, although there is a strong public feeling that it primarily still is. New legislative solutions [31] "are returning" noise to the sphere of public-health issues, in regard to interpretation of the impact of noise on human health.

Secondly, the terminology has changed. Although it is referred to as "environmental noise" today, the term "communal noise" is being "reinstituted" through the new version of the SRPS ISO 1996-1:2016 standard, which defines "noise" in the community ("community noise"). The reports have, of course, changed over time, and the quality system and the National Accreditation Body (NAB) have contributed to the advances in measurement and a greater reliability of measurement results.

Thirdly, measurement technologies have developed, which has been described above in detail.

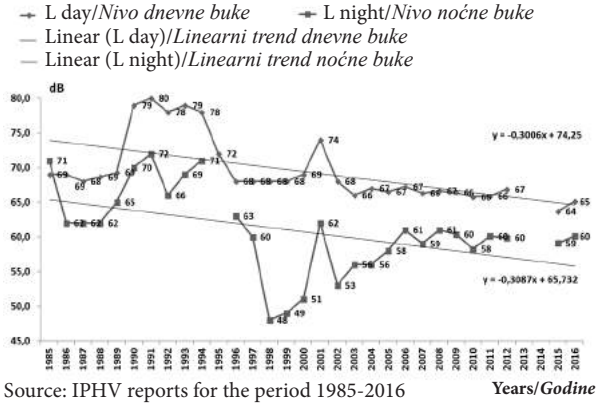
Fourthly, the contemporary era, the concept of general availability of data, the use of open source, as well as the fact that anyone can measure noise using a smartphone (such measurements are not reliable, but they offer an insight into the approximate noise level) and to share the measurement information with others through various open information systems, have made the issue available and interesting to the general public. Saying that, for example, the noise level measured at a certain place is 70 dB, is becoming less interesting for the public, so data are displayed in the form of various indexes (for example, by red colour, an index type "5 of 7" or an angry "emoji" face, etc.). The future will definitely see an increase in additional environmental noise monitoring - by the noise modelling method, as technological progress and an increasing accuracy of models are followed by a development of new softwares.

Today, there are software solutions that generate noise maps, for example, "Lima", which is in possession of IPHV since 2011. The software produces a noise map based on the entered data on the measured noise levels.

What has not changed since the 1980s in regard to measuring environmental noise and the interpretation of the noise impact on human health?

The sources of noise have remained the same: traffic, particularly road traffic noise, human activities, and industrial noise. The following passage from a report dating back to 1990 shows that when environmental noise is concerned, not much has changed over time: "In urban areas, there are three main sources of noise: traffic, daily living activities, and the industry. The most important source of noise in inhabited places is traffic, as this type of noise accounts for about 80% of the overall noise. The intensity of traffic noise in inhabited areas is affected by: the number and types of vehicles, use of sound signals, speed of vehicles, street width, arrangement of buildings and number of floors, street greenery, as well as the time of the day and year [32]."

The purpose of measuring noise levels has remained the same - to monitor the effect of noise on human health (particularly the contribution of noise to cardiovascular diseases) and the study of auditory and extra-auditory effects of noise. Back in 1990, experts from the IPHV wrote in their reports about "health aspects of communal noise: The main purpose of determining (measuring) the intensity of communal noise is its impact on human health. The effects of noise depend on the intensity, frequency and duration of noise, sensitivity to noise and changes in noise [32]."



Source: IPHV reports for the period 1985-2016
Izvor: Izveštaji IZJZV za period 1985-2016
•data for1995, 2013-2014 are not available/•*podaci za 1995, 2013-2014 nisu dostupni*

Graph 5. Mean annual equivalent continuous noise levels (1985 – 2010)/day and night time noise indicators (2011 – 2016) in the school zone

Grafikon 5. *Srednje godišnje vrednosti ekvivalentnog nivoa buke (1985–2010)/indikatora dnevne i noćne buke (2011–2016) u školskoj zoni*

Proposals for reducing noise are still the same, and the public is still interested in this issue. According to historical data, proposals for noise reduction included the following: "Due to adverse effects of noise on human health, it is necessary to reduce the intensity of communal noise. Primarily, town zoning needs to be carried out in terms of traffic rerouting, particularly heavy vehicle traffic, into the parts outside the residential zones or outside of the town. Followed by proper urban solutions, in terms of building streets of sufficient width and planting greenery, which can reduce the intensity of noise up to 10–15 dB (A). In apartment buildings, by proper architectural solutions in terms of room layout (putting rooms where people spend more time on the side opposite the roads). By constructive solutions, in terms of the choice of building materials and methods of construction, street noise transfer into homes and work spaces can be affected. By administrative solutions – introduce time limits for driving for all vehicles or heavy vehicles particularly on the roads vulnerable to noise [32]."

Conclusion

Based on the 30 years' measurement results, collected always using current, up-to-date methodology and equipment, based on the research of the experts of the Institute of Public Health of Vojvodina and current scientific knowledge, it is concluded that the environmental noise in Novi Sad presents a long-lasting physical hazard.

Taking preventive measures, ranging from those in the field of urban planning, through transportation to individual measures, in particular in the areas of the city with sensitive population (kindergartens, schools, healthcare facilities), should be considered as one of the priorities of the urban development of the City of Novi Sad. It is important to point out that comparison between the "old" (rating equivalent continuous level) and "new" (basic noise indicators) noise indicators will be studied in another paper, and the authors of this paper intend to do it in the future.

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Radovi u časopisima

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Ginsberg JS, Bates SM. Management of venous thromboembolism during pregnancy. J Thromb Haemost 2003;1:1435-42.

∗ Organizacija kao autor

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∗ Bez autora

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∗ Sažetak u časopisu

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Knjige i druge monografije

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∗ Disertacija

Borkowski MM. Infant sleep and feeding: a telephone survey of Hispanic Americans [dissertation]. Mount Pleasant (MI): Central Michigan University; 2002.

Elektronski materijal

∗ Članak iz časopisa u elektronskom formatu

Abood S. Quality improvement initiative in nursing homes: the ANA acts in an advisory role. Am J Nurs [Internet]. 2002 Jun [cited 2002 Aug 12];102(6):[about 1 p.]. Available from: http://www.nursingworld.org/AJN/2002/june/Wawatch.htmArticle

∗ Monografija u elektronskom formatu

CDI, clinical dermatology illustrated [monograph on CD-ROM]. Reevs JRT, Maibach H. CMEA Multimedia Group, producers. 2nd ed. Version 2.0. San Diego:CMEA;1995.

∗ Kompjuterska datoteka

Hemodynamics III: the ups and downs of hemodynamics [computer program]. Version 2.2. Orlando (FL): Computerized Educational Systems; 1993.

5. Prilozi (tabele, grafikoni, sheme i slike)

BROJ PRILOGA NE SME BITI VEĆI OD ŠEST!

Tabele, grafikoni, sheme i slike se postavljaju kao posebni dokumenti.

– Tabele i grafikone bi trebalo pripremiti u formatu koji je kompatibilan programu u kojem je napisan tekst rada. Slike bi trebalo poslati u jednom od sledećih oblika: *JPG*, *GIF*, *TIFF*, *EPS*.

– Svaki prilog mora biti obeležen arapskim brojem prema redosledu po kojem se navodi u tekstu rada.

– Naslovi, tekst u tabelama, grafikonima, shemama i legende slika bi trebalo da budu napisani na srpskom i engleskom jeziku.

– Nestandardne priloge označiti u fusnoti uz korišćenje sledećih simbola: *, †, ‡, §, | |, ¶, **, † †, ‡ ‡ .

– U legendi slika trebalo bi napisati korišćeno uveličanje okulara i objektiva mikroskopa. Svaka fotografija treba da ima vidljivu skalu.

– Ako su tabele, grafikoni, sheme ili slike već objavljene, navesti originalni izvor i priložiti pisano odobrenje autora za njihovo korišćenje.

– Svi prilozi će biti štampani kao crno-bele slike. Ukoliko autori žele da se prilozi štampaju u boji, obavezno treba da plate dodatne troškove.

6. Dodatne obaveze

AUTORI I SVI KOAUTORI RADA OBAVEZNO TREBA DA PLATE GODIŠNJU PRETPLATU ZA ČASOPIS *MEDICINSKI PREGLED*. U PROTIVNOM, RAD NEĆE BITI ŠTAMPAN U ČASOPISU.

INFORMATION FOR AUTHORS

Medical Review publishes papers (previously neither published in nor submitted to any other journals) from various fields of biomedicine intended for broad circles of doctors.

Since January 1th, 2013 the Medical Review has been using the service e-Ur: Electronic Journal Editing. All users of the Registration system, i.e. authors, reviewers, and editors have to be registered users with only one e-mail address. Registration should be made on the web address:

http://aseestant.ceon.rs/index.php/medpreg/user/register.

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http://aseestant.ceon.rs/index.php/medpreg/

A SUPPLEMENTARY FILE, WITH THE STATEMENT THAT THE PAPER HAS NOT BEEN SUBMITTED OR ACCEPTED FOR PUBLICATION ELSEWHERE AND A CONSENT SIGNED BY ALL AUTHORS, HAVE TO BE ENCLOSED WITH THE MANUSCRIPT.

Authors may not send the same manuscript to more than one journal concurrently. If this occurs, the Editor may return the paper without reviewing it, reject the paper, contact the Editor of the other journal(s) in question and/or contact the author's employers.

Papers should be written in English language, with an abstract and title page in English, as well as in Serbian language.

All papers submitted to ***Medical Review*** are seen by one or more members of the Editorial Board. Suitable articles are sent to at least two experts to be reviewed, thier reports are returned to the assigned member of the Editorial Board and the Editor. Revision of an article gives no guarantee of acceptance and in some cases revised articles are rejected if the improvements are not sufficient or new issues have arisen. Material submitted to *the Journal* remains confidential while being reviewed and peer-reviewers' identities are protected unless they elect to lose anonymity.

Medical Review publishes the following types of articles: editorials, original studies, preliminary reports, review articles, professional articles, case reports, articles from history of medicine and other types of publications.

1. Editorials – up to 5 pages – convey opinions or discussions on a subject relevant for the Journal. Editorials are commonly written by one author by invitation.

2. Original studies – up to 12 pages – present the authors' own investigations and their interpretations. They should contain data which could be the basis to check the obtained results and reproduce the investigative procedure.

3. Review articles – up to 10 pages – provide a condensed, comprehensive and critical review of a problem on the basis of the published material being analyzed and discussed, reflecting the current situation in one area of research. Papers of this type will be accepted for publication provided that the authors confirm their expertise in the relevant area by citing at least 5 self-citations.

4. Preliminary reports – up to 4 pages – contain scientific results of significant importance requiring urgent publishing; however, it need not provide detailed description for repeating the obtained results. It presents new scientific data without a detailed explanation of methods and results. It contains all parts of an original study in an abridged form.

5. Professional articles – up to 10 pages – examine or reproduce previous investigation and represent a valuable source of knowledge and adaption of original investigations for the needs of current science and practice.

6. Case reports – up to 6 pages – deal with rare casuistry from practice important for doctors in direct charge of patients and are similar to professional articles. They emphasize unusual characteristics and course of a disease, unexpected reactions to a therapy, application of new diagnostic procedures and describe a rare or new disease.

7. History of medicine – up to 10 pages – deals with history with the aim of providing continuity of medical and health care culture. They have the character of professional articles.

8. Other types of publications – The journal also publishes feuilletons, book reviews, extracts from foreign literature, reports from congresses and professional meetings, communications on activities of certain medical institutions, branches and sections, announcements of the Editorial Board, letters to the Editorial Board, novelties in medicine, questions and answers, professional and vocational news and In memoriam.

Preparation of the manuscript

The complete manuscript, including the text, all supplementary material and covering letter, is to be sent to the web address above.

The covering letter:

– It must contain the proof given by the author that the paper represents an original work that it has neither been previously published in other journals nor is under consideration to be published in other journals.

– It must confirm that all the authors meet criteria set for the authorship of the paper, that they agree completely with the text and that there is no conflict of interest.

– It must state the type of the paper submitted (an original study, a review article, a preliminary report, a professional article, a case report, history of medicine).

The manuscript:

General instructions.

Use Microsoft Word for Windows to type the text. The text must be typed in font *Times New Roman*, page format A4, space 1.5 (for tables as well), margins set to 2.5 cm and font size 12pt. All measurements should be reported in the metric system of the International System of Units – SI. Temperature should be expressed in Celsius degrees (°C) and pressure in mmHg.

The manuscript should contain the following elements:

1. The title page.

The title page should contain a concise and clear title of the paper, without abbreviations, then a short title (up to 40 characters), full names and surnames of the authors (not more than 6) indexed by numbers corresponding to those given in the heading along with the full name and place of the institutions they work for. Contact information including the academic degree(s), full address, e-mail and number of phone or fax of the corresponding author (the author responsible for correspondence) are to be given at the bottom of this page.

2. Summary.

The summary should contain up to 250 words, without abbreviations, with the precise review of problems, objectives, methods, important results and conclusions. It should be structured into the paragraphs as follows:

– Original and professional papers should have the introduction (with the objective of the paper), materials and methods, results and conclusion

– Case reports should have the introduction, case report and conclusion

– Review papers should have the introduction, subtitles corresponding to those in the paper and conclusion.

The authors should provide up to 10 keywords below the summary. These keywords will assist indexers in cross-indexing the article and will be published with the summary, but the authors' keywords could be changed in accordance with the list of Medical Subject Headings, MeSH of the American National Medical Library.

The summary should be written in both languages, English as well as Serbian. The summary in Serbian language should be the translation of the summary in English; therefore, it has to contain the same paragraphs.

3. The text of the paper.

The text of original studies must contain the following: introduction (with the clearly defined objective of the study), materials and methods, results, discussion, conclusion, list of abbreviations (if used in the text) and not necessarily, the acknowledgment mentioning those who have helped in the investigation and preparation of the paper.

The text of a case report should contain the following: introduction (with clearly defined objective of the study), case report, discussion and conclusion.

Introduction contains clearly defined problem dealt with in the study (its nature and importance), with the relevant references and clearly defined objective of the investigation and hypothesis.

Materials and methods should contain data on design of the study (prospective/retrospective, eligibility and exclusion criteria, duration, demographic data, follow-up period). Statistical methods applied should be clear and described in details.

Results give a detailed review of data obtained during the study. All tables, graphs, schemes and figures must be cited in the text and numbered consecutively in the order of their first citation in the text.

Discussion should be concise and clear, interpreting the basic findings of the study in comparison with the results of relevant studies published in international and national literature. It should be stated whether the hypothesis has been confirmed or denied. Merits and demerits of the study should be mentioned.

Conclusion must deny or confirm the attitude towards the 0based solely on the author's own results, corroborating them. Avoid generalized and unnecessary conclusions. Conclusions in the text must be in accordance with those given in the summary.

4. References are to be given in the text under Arabic numerals in parentheses consecutively in the order of their first citation. Avoid a large number of citations in the text. The title of journals should be abbreviated according to the style used in Index Medicus (http://www.nlm.nih.gov/tsd/serials/lji.html). Apply Vancouver Group's Criteria, which define the order of data and punctuation marks separating them. Examples of correct forms of references are given below. List all authors, but if the number exceeds six, give the names of six authors followed by 'et al'.

Articles in journals

∗ *A standard article*

Ginsberg JS, Bates SM. Management of venous thromboembolism during pregnancy. J Thromb Haemost 2003;1:1435-42.

∗ *An organization as the author*

Diabetes Prevention Program Research Group. Hypertension, insulin, and proinsulin in participants with impaired glucose tolerance. Hypertension 2002;40(5):679-86.

∗ *No author given*

21st century heart solution may have a sting in the tail. BMJ. 2002;325(7357):184.

∗ *A volume with supplement*

Magni F, Rossoni G, Berti F. BN-52021 protects guinea pig from heart anaphylaxix. Pharmacol Res Commun 1988;20 Suppl 5:75-8.

∗ *An issue with supplement*

Gardos G, Cole JO, Haskell D, Marby D, Pame SS, Moore P. The natural history of tardive dyskinesia. J Clin Psychopharmacol 1988;8(4 Suppl):31S-37S.

∗ *A summary in a journal*

Fuhrman SA, Joiner KA. Binding of the third component of complement C3 by Toxoplasma gondi [abstract]. Clin Res 1987;35:475A.

Books and other monographs

∗ *One or more authors*

Murray PR, Rosenthal KS, Kobayashi GS, Pfaller MA. Medical microbiology. 4th ed. St. Louis: Mosby; 2002.

∗ *Editor(s) as author(s)*

Danset J, Colombani J, eds. Histocompatibility testing 1972. Copenhagen: Munksgaard, 1973:12-8.

∗ *A chapter in a book*

Weinstein L, Shwartz MN. Pathologic properties of invading microorganisms. In: Soderman WA Jr, Soderman WA, eds. Pathologic physiology: mechanisms of disease. Philadelphia: Saunders; 1974. p. 457-72.

∗ *A conference paper*

Christensen S, Oppacher F. An analysis of Koza's computational effort statistic for genetic programming. In: Foster JA, Lutton E, Miller J, Ryan C, Tettamanzi AG, editors. Genetic programming. EuroGP 2002: Proceedings of the 5th European Conference on Genetic Programming; 2002 Apr 3-5; Kinsdale, Ireland. Berlin: Springer; 2002. p. 182-91.

∗ *A dissertation and theses*

Borkowski MM. Infant sleep and feeding: a telephone survey of Hispanic Americans [dissertation]. Mount Pleasant (MI): Central Michigan University; 2002.

Electronic material

∗ *A journal article in electronic format*

Abood S. Quality improvement initiative in nursing homes: the ANA acts in an advisory role. Am J Nurs [Internet]. 2002 Jun [cited 2002 Aug 12];102(6):[about 1 p.]. Available from: http://www.nursingworld.org/AJN/2002/june/Wawatch.htmArticle

∗ *Monographs in electronic format*

CDI, clinical dermatology illustrated [monograph on CD-ROM]. Reevs JRT, Maibach H. CMEA Multimedia Group, producers. 2nd ed. Version 2.0. San Diego:CMEA;1995.

∗ *A computer file*

Hemodynamics III: the ups and downs of hemodynamics [computer program]. Version 2.2. Orlando (FL): Computerized Educational Systems; 1993.

5. Attachments (tables, graphs, schemes and photographs).

THE MAXIMUM NUMBER OF ATTACHMENTS ALLOWED IS SIX!

– Tables, graphs, schemes and photographs are to be submitted as separate documents, on separate pages.

– Tables and graphs are to be prepared in the format compatible with Microsoft Word for Windows programme. Photographs are to be prepared in JPG, GIF, TIFF, EPS or similar format.

– Each attachment must be numbered by Arabic numerals consecutively in the order of their appearance in the text

– The title, text in tables, graphs, schemes and legends must be given in both Serbian and English languages.

– Explain all non-standard abbreviations in footnotes using the following symbols *, †, ‡, §, | |, ¶, **, † †, ‡ ‡ .

– State the type of color used and microscope magnification in the legends of photomicrographs. Photomicrographs should have internal scale markers.

– If a table, graph, scheme or figure has been previously published, acknowledge the original source and submit written permission from the copyright holder to reproduce it.

– All attachments will be printed in black and white. If the authors wish to have the attachments in color, they will have to pay additional cost.

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