YU ISSN 0042-8450

ВОЈНОСАНИТЕТСКИ ПРЕГЛЕД

Часопис лекара и фармацеута Војске Србије

Military Medical and Pharmaceutical Journal of Serbia

Vojnosanitetski pregled

Vojnosanit Pregl 2023; October Vol. 80 (No. 10): pp. 807–894.

2023 October Vol. 80 (No. 10): pp. 807–894.
Vojnosanitetski Pregled

Vol. 80, No. 10, 2023

VOJNOSANITETSKI PREGLED

The first issue of *Vojnosanitetski pregled* was published in September 1944
The Journal continues the tradition of Vojno-sanitetski glasnik which was published between 1930 and 1941

PUBLISHER
Ministry of Defence of the Republic of Serbia, University of Defence, Belgrade, Serbia

ISSN 0042-8450
eISSN 2406-0720

Editorial Office: University of Defence, Faculty of Medicine of the Military Medical Academy, Center for Medical Scientific Information, Crnotravska 17, 11 040 Belgrade, Serbia. E-mail: **vsp@vma.mod.gov.rs**

Papers published in the Vojnosanitetski pregled are indexed in: Science Citation Index Expanded (SCIE), Journal Citation Reports/Science Edition, SCOPUS, Excerpta Medica (EMBASE), Google Scholar, EBSCO, Biomedicina Serbica, Serbian Citation Index (SCIndex), DOAJ. Contents are published in Giornale di Medicine Militare and Revista de Medicina Militara. Reviews of original papers and abstracts of contents are published in International Review of the Armed Forces Medical Services.

The Journal is published monthly. Subscription: Giro Account No. 840-19540845-28, refer to number 122742313338117. To subscribe from abroad phone to +381 11 3608 997. Subscription prices per year: individuals 5,000.00 RSD, institutions 10,000.00 RSD, and foreign subscribers 150 €.

Printed by: Vojna štamparija, Beograd, Resavska 40b

Vol.80, br. 10, 2023

VOJNOSANITETSKI PREGLED

Prvi broj *Vojnosanitetskog pregleda* izašao je septembra meseca 1944. godine
Časopis nastavlja tradiciju *Vojno-sanitetskog glasnika*, koji je izlazio od 1930. do 1941. godine

IZDAVAČ
Ministarstvo odbrane Republike Srbije, Univerzitet odbrane, Beograd, Srbija

ISSN 0042-8450
eISSN 2406-0720

Adresa redakcije: Univerzitet odbrane, Medicinski fakultet Vojnomedicinske akademije, Centar za medicinske naučne informacije, Crnotravska 17, 11 040 Beograd, Srbija. Informacije o pretplati (tel.): +381 11 3608 997. E-mail (redakcija): **vsp@vma.mod.gov.rs**

Radove objavljene u „Vojnosanitetskom pregledu“ indeksiraju: Science Citation Index Expanded (SCIE), Journal Citation Reports/Science Edition, SCOPUS, Excerpta Medica (EMBASE), Google Scholar, EBSCO, Biomedicina Serbica, Srpski citatni indeks (SCIndeks), DOAJ. Sadržaje objavljuju Giornale di Medicine Militare i Revista de Medicina Militara. Prikaze originalnih radova i izvoda iz sadržaja objavljuje International Review of the Armed Forces Medical Services.

Časopis izlazi dvanaest puta godišnje. Pretplate: Žiro račun br. 840-19540845-28, poziv na broj 122742313338117. Za pretplatu iz inostranstva obratiti se službi pretplate na tel. +381 11 3608 997. Godišnja pretplata: 5 000 dinara za građane Srbije, 10 000 dinara za ustanove iz Srbije i 150 € za pretplatnike iz inostranstva. Kopiju uplatnice dostaviti na gornju adresu.

Štampa: Vojna štamparija, Beograd, Resavska 40b

CONTENTS / SADRŽAJ

CASE REPORTS / KAZUISTIKA

The 2023 Nobel Prize winners for Physiology or Medicine are Katalin Karikó (born 17 January 1955) and Drew Weissman (born 07 September 1959). The results of many years of research by these two Nobel laureates have fundamentally changed our understanding of how messenger ribonucleic acid (mRNA) interacts with the immune system. Thanks to these new findings, it was possible to develop efficient mRNA vaccines against COVID-19 at a speed not recorded in modern times. In this issue of the journal, we publish an article on that topic (see pp. 811-813).

Dobitnici Nobelove nagrade za fiziologiju ili medicinu 2023. godine su Katalin Kariko (rođena 17.01.1955. godine) i Dru Vajzman (rođen 07.09.1959. godine). Rezultati višegodišnjih istraživanja ovo dvoje laureata Nobelove nagrade su fundamentalno promenili naše razumevanje o tome kako informaciona ribonukleinska kiselina (iRNK) reaguje sa imunskim sistemom. Zahvaljujući tim novim saznanjima bio je moguć razvoj efikasnih iRNK vakcina protiv COVID-19, brzinom koja nije zabeležena u modernim vremenima. U ovom broju časopisa objavljujemo članak na tu temu (videti str. 811-813).

UDC: 614.47:615.371
DOI: https://doi.org/10.2298/VSP2310811J

RNA vaccines: a milestone toward a new era

RNK vakcine: prekretnica na putu ka novoj eri

Srdja Janković

University Children's Hospital, Department of Hematology and Oncology, Division of Immunology, Belgrade, Serbia

Key words:
RNA; vaccines; biotechnology.

Ključne reči:
RNK; vakcine; biotehnologija.

Introduction

In the beginning was ribonucleic acid (RNA), or, stated slightly less dramatically, the most likely course of events that led to the origin of life on Earth involved an "RNA world" – a prebiotic substrate based on the unique ability of ribonucleic acid to integrate within itself wide-scale catalytic properties (later relegated to proteins) and the coding of heritable information [subsequently taken over by deoxyribonucleic acid (DNA)] [1]. As a lasting legacy of such beginnings, present-day RNA has retained, as one of its main biological functions, the role of a messenger between these two realms – that of genetic information and the myriad structural or active macromolecular forms shaped by its translation into polypeptide sequences [2]. In addition, various types of non-coding RNA play crucial roles in the regulation of gene expression [3]. In the last few decades, mankind has gained immense knowledge about the key roles RNA assumes in biological systems (including RNA viruses, where it has retained, or perhaps regained, its primordial genetic function) [4]. The vast potential of this knowledge to transform the way we safeguard our health and combat disease, however, remained largely untapped until the most recent pandemic caused by an RNA virus (one among hundreds of thousands that inhabit the biosphere, prone to "exploit" any alteration of ecological conditions in order to obtain a new niche for growth, typically by crossing the species barrier [5]) triggered the first application of RNA-based vaccines the world has ever seen.

RNA vaccines in the coronavirus pandemic

High efficacy and safety achieved by both existing mRNA vaccines against coronavirus disease 2019 (COVID-19), BNT162b2 and mRNA-1273, as attested by clinical trials [6, 7], resulted in their emergency use approval by the Food and Drug Administration of the United States of America in 2020, followed by full approval in 2021 and 2022, respectively. Approval by regulatory agencies of most other countries swiftly followed. During the pandemic, these and other vaccines saved millions of lives and prevented serious complications in a great number of people [8]. Vaccination also presumably shortened the time needed to achieve the necessary conditions to ease restrictive epidemiological measures worldwide. The two mRNA vaccines proved capable of inducing both humoral and cellular immunity that was, on the whole, roughly comparable in strength to the immunity conferred by the infection itself (but with the obvious advantage of avoiding the serious risks that the infection entails) [9]. The vaccines also proved to be very safe; severe post-vaccination adverse events are apparently quite rare [10, 11], with partial exception of anaphylactic reactions (estimated frequency was 7.9 *per* million initial doses) [12] and myocarditis (overall incidence rate among men aged 18–25 years was 1.7 *per* 100,000 vaccines for BNT162b2 and 2.2 *per* 100,000 for mRNA-1273) [13]. Both of these adverse events are treatable, and their combined frequency and severity are no match for the dangers of COVID-19 itself. The often-voiced concerns that mRNA vaccines could substantially trigger autoimmunity in susceptible persons failed to materialize [14], which is in accordance with previous assessments that vaccine-induced autoimmunity is generally rare and exceptional [15, 16]. Speculations about the putative risks of mRNA vaccines in pregnancy or their alleged adverse impact on fertility also proved to be void [17], and the widespread myth that mRNA vaccines could interfere with the genetic material of the host had been baseless from the outset since mRNA cannot enter the cell nucleus nor does in any way interact with the host DNA. Finally, even though the actual duration of vaccine-induced humoral immunity (time of retention of

Correspondence to: Srdja Janković, University Children's Hospital, Department of Hematology and Oncology, Division of Immunology, Tiršova 10, 11 000 Belgrade, Serbia. E-mail: srdja.jankovic@udk.bg.ac.rs; ysrdja@gmail.com

neutralizing antibodies), in the long run, did not quite fulfill the initial expectations [18], particularly in view of the rapid evolution of new coronavirus variants in concern [19], both mRNA vaccines retained to date satisfactory effectiveness against the severe, life-threatening, or fatal disease [20]. Indeed, mRNA vaccines offer the additional advantage of being readily adaptable to newly emerging viral variants for the creation of booster vaccine preparations since the appropriate RNA sequence can be selected at will.

To the Nobel Prize and beyond

None of this success, however, would have come to pass without a series of key biotechnological breakthroughs that enabled the development of RNA-based platforms. The Nobel Prize for Physiology or Medicine awarded to Katalin Karikó (born 1955 in Szolnok, Hungary) and Drew Weissman (born 1959 in Lexington, Massachusetts, USA) for the discoveries related to nucleoside base modifications that eventually allowed the successful creation of the first mRNA vaccines [21] should, above all else, be regarded as a recognition of the importance of decades-long efforts in this direction by the laureates as well as many other scientists, including efforts in the development of RNA-based technology directed at the treatment of malignant disorders [22]. First and foremost, in order to make a vaccine, native viral RNA needed to be stabilized and protected against swift destruction *in vivo* by the resident ribonucleases, enzymes with hundreds of millions of years of experience in neutralizing any foreign RNA that found its way into the body. In addition, foreign RNA, in its native form, triggers a strong inflammatory reaction (and is destroyed in the process); as discovered by Karikó, Weissman, and their team, this can be avoided if such RNA is marked by the addition of pseudouridine residues, mimicking its physiological processing and tempering the inflammatory reaction without compromising the ability to activate innate immunity [23, 24]. Furthermore, in order to be used in a vaccine, mRNA needed to be delivered to the immune system, and this required the development of lipid nanoparticle technology [25] – another spectacular advancement that was, sadly, not reflected in this year's Nobel committee decision.

As a brief, but warranted digression, the advent of RNA vaccines will hopefully change the currently prevailing way of thinking: the more advanced or sophisticated a given technology is, the farther from nature it is perceived by the public. Here, we see exactly the opposite – by applying precise molecular modifications guided by deep knowledge of the system in question, we are able to intervene in a way much closer to the natural process itself. That will hopefully aid more people to think beyond the epistemologically dubious – or even spurious – dichotomy of "natural" vs. "artificial" [26].

Returning to RNA vaccines (and looking beyond COVID-19), their ability to efficiently initiate both humoral and cellular immune responses is clearly an immense asset, particularly in the protection against intracellular pathogens (or those with life cycles exhibiting a substantial intracellular phase). Importantly, the ability of RNA vaccines to trigger innate immunity *via* Toll-like receptors and other class-specificity receptors [27] effectively makes such vaccines their own adjuvants, eliminating the need for traditional adjuvants and allowing vaccine designers to control the elicited immune response with unprecedented precision in view of safety and effectiveness.

According to a recent review, twenty-seven RNA-based vaccines are already in clinical trials, while hundreds are undergoing preclinical investigations [28]. Among infectious diseases, the first candidates to be tackled by RNA vaccine technology include some of the greatest global challenges, such as malaria, tuberculosis, and human immunodeficiency virus-caused disease. Platforms based on RNA technology have also demonstrated a wide-scale potential to transform the treatment of many non-infectious disorders, notably autoimmune [29] and malignant disorders [30]. Admittedly (and unfortunately), the onset of this new era appears to be somewhat hampered by the widespread, complex, and highly detrimental psychosocial phenomenon of vaccine hesitancy or resentment [31–37]. Nevertheless, RNA vaccines (and possibly RNA therapeutics) are already promising to become a staple tool of personalized medicine of the future. In spite of some misguided calls to classify RNA vaccines as "gene therapies" (which they are not) and thus make them subject to overregulation [38], it is reasonably safe to say that this future has irrevocably begun.

Conclusion

All of the above testifies that we are indeed on the verge of a new era. In spite of various challenges and obstacles, including the widespread psychosocial phenomenon of vaccine hesitancy or resentment, RNA vaccines (and possibly RNA therapeutics) are likely to become one of the central tools of personalized medicine of the future.

R E F E R E N C E S

1. *Saito H*. The RNA world 'hypothesis'. Nat Rev Mol Cell Biol 2022; 23(9): 582.
2. *Fine JL*, *Pearlman JE*. On the origin of life: an RNA-focused synthesis and narrative. RNA 2023; 29(8): 1085–98.
3. *Panni S*, *Lovering RC*, *Porras P*, *Orchard S*. Non-coding RNA regulatory networks. Biochim Biophys Acta Gene Regul Mech 2020; 1863(6): 194417.
4. *Villarreal L*, *Witzany G*. Self-empowerment of life through RNA networks, cells and viruses. F1000Research 2023; 12: 138.
5. *Kirtipal N*, *Bharadwaj S*, *Kang SG*. From SARS to SARS-CoV-2, insights on structure, pathogenicity and immunity aspects of pandemic human coronaviruses. Infect Genet Evol 2020; 85: 104502.
6. *El Sahly HM*, *Baden LR*, *Essink B*, *Doblecki-Lewis S*, *Martin JM*, *Anderson EJ*, et al. Efficacy of the mRNA-1273 SARS-CoV-2 vaccine at completion of blinded phase. N Engl J Med 2021; 385(19): 1774–85.
7. *Thomas SJ*, *Moreira ED Jr*, *Kitchin N*, *Absalon J*, *Gurtman A*, *Lockhart S*, et al. Safety and efficacy of the BNT162b2 mRNA

COVID-19 vaccine through 6 months. N Engl J Med 2021; 385(19): 1761–73.
8. *Bartsch SM*, *Wedlock PT*, *O'Shea KJ*, *Cox SN*, *Strych U*, *Nuzzo JB*, et al. Lives and costs saved by expanding and expediting coronavirus disease 2019 vaccination. J Infect Dis 2021; 224(6): 938–48.
9. *Noor R*. mRNA vaccines as an efficient approach for the rapid and robust induction of host immunity against SARS-CoV-2. SN Compr Clin Med 2022; 4(1): 88.
10. *Santi Laurini G*, *Montanaro N*, *Broccoli M*, *Bonaldo G*, *Motola D*. Real-life safety profile of mRNA vaccines for COVID-19: An analysis of VAERS database. Vaccine 2023; 41(18): 2879–86.
11. *Blumenthal KG*, *Greenhawt M*, *Phillips EJ*, *Agmon-Levin N*, *Golden DBK*, *Shaker M*. An update in COVID-19 vaccine reactions in 2023: progress and understanding. J Allergy Clin Immunol Pract 2023; S2213–2198(23)00723–7.
12. *Greenhawt M*, *Abrams EM*, *Shaker M*, *Chu DK*, *Khan D*, *Akin C*, et al. The risk of allergic reaction to SARS-CoV-2 vaccines and recommended evaluation and management: a systematic review, meta-analysis, GRADE assessment, and international consensus approach. J Allergy Clin Immunol Pract 2021; 9(10): 3546–67.
13. *Wong HL*, *Hu M*, *Zhou CK*, *Lloyd PC*, *Amend KL*, *Beachler DC*, et al. Risk of myocarditis and pericarditis after the COVID-19 mRNA vaccination in the USA: A cohort study in claims databases. Lancet 2022; 399(10342): 2191–9.
14. *Świerkot J*, *Madej M*, *Szmyrka M*, *Korman L*, *Sokolik R*, *Andrasiak I*, et al. The risk of autoimmunity development following mRNA COVID-19 vaccination. Viruses 2022; 14(12): 2655.
15. *Janković S*. Vaccination and autoimmune phenomena. Central Eur J Paed 2017; 13(1): 12–23.
16. *Toussirot É*, *Bereau M*. Vaccination and induction of autoimmune diseases. Inflamm Allergy Drug Targets 2015; 14(2): 94–8.
17. *Faust JS*, *Rasmussen SA*, *Jamieson DJ*. Pregnancy should be a condition eligible for additional doses of COVID-19 messenger RNA vaccines. Am J Obstet Gynecol MFM 2023; 5(2): 100801.
18. *Goldberg Y*, *Mandel M*, *Bar-On YM*, *Bodenheimer O*, *Freedman L*, *Haas EJ*, et al. Waning immunity after the BNT162b2 vaccine in Israel. N Engl J Med 2021; 385(24): e85.
19. *Wang Z*, *Schmidt F*, *Weisblum Y*, *Muecksch F*, *Barnes CO*, *Finkin S*, et al. mRNA vaccine-elicited antibodies to SARS-CoV-2 and circulating variants. Nature 2021; 592(7855): 616–22.
20. *Ssentongo P*, *Ssentongo AE*, *Voleti N*, *Groff D*, *Sun A*, *Ba DM*, et al. SARS-CoV-2 vaccine effectiveness against infection, symptomatic and severe COVID-19: a systematic review and meta-analysis. BMC Infect Dis 2022; 22(1): 439.
21. *The Nobel Prize*. The Nobel Assembly at Karolinska Instituet: Press release [Internet]. Sweden: Nobel Prize Outreach; 2023 [cited 2023 Oct 12; accessed on 2023 Oct 23]. Available from: https://www.nobelprize.org/prizes/medicine/2023/press-release/
22. *Liang X*, *Li D*, *Leng S*, *Zhu X*. RNA-based pharmacotherapy for tumors: from bench to clinic and back. Biomed Pharmacoter 2020; 125: 109997.
23. *Karikó K*, *Muramatsu H*, *Welsh FA*, *Ludwig J*, *Kato H*, *Akira S*, et al. Incorporation of pseudouridine into mRNA yields superior nonimmunogenic vector with increased translational capacity and biological stability. Mol Ther 2008; 16(11): 1833–40.
24. *Morais P*, *Adachi H*, *Yu YT*. The critical contribution of pseudouridine to mRNA COVID-19 vaccines. Front Cell Dev Biol 2021; 9: 789427.
25. *Schoenmaker L*, *Witzigmann D*, *Kulkarni JA*, *Verbeke R*, *Kersten G*, *Jiskoot W*, et al. mRNA-lipid nanoparticle COVID-19 vaccines: structure and stability. Int J Pharm 2021; 601: 120586.
26. *Ćirković MM*. Natural and artificial: The cosmic Domain of Arnheim. In: *Ćirković MM*, editor. The astrobiological landscape: philosophical foundations of the study of cosmic life. Cambridge: Cambridge University Press; 2012. p. 184–202.
27. *Sadarangani M*, *Marchant A*, *Kollmann TR*. Immunological mechanisms of vaccine-induced protection against COVID-19 in humans. Nat Rev Immunol 2021; 21(8): 475–84.
28. *Matarazzo L*, *Bettencourt PJG*. mRNA vaccines: a new opportunity for malaria, tuberculosis and HIV. Front Immunol 2023; 14: 1172691.
29. *Flemming A*. mRNA vaccine shows promise in autoimmunity. Nat Rev Immunol 2021; 21(2): 72.
30. *Lorentzen CL*, *Haanen JB*, *Met Ö*, *Svane IM*. Clinical advances and ongoing trials of mRNA vaccines for cancer treatment. Lancet Oncol 2022; 23(10): e450–8. Erratum in: Lancet Oncol 2022; 23(11): e492.
31. *Lazarus JV*, *Wyka K*, *White TM*, *Picchio CA*, *Rabin K*, *Ratzan SC*, et al. Revisiting COVID-19 vaccine hesitancy around the world using data from 23 countries in 2021. Nat Commun 2022; 13(1): 3801.
32. *Kafadar AH*, *Tekeli GG*, *Jones KA*, *Stephan B*, *Dening T*. Determinants for COVID-19 vaccine hesitancy in the general population: a systematic review of reviews. Z Gesundh Wiss 2022; 19: 1–17.
33. *Anderson KM*, *Creanza N*. Internal and external factors affecting vaccination coverage: modeling the interactions between vaccine hesitancy, accessibility, and mandates. PLOS Glob Public Health 2023; 3(10): e0001186.
34. *Olivera Mesa D*, *Winskill P*, *Ghani AC*, *Hauck K*. The societal cost of vaccine refusal: a modelling study using measles vaccination as a case study. Vaccine 2023; 41(28): 4129–37.
35. *Coelho P*, *Foster K*, *Nedri M*, *Marques MD*. Increased belief in vaccination conspiracy theories predicts increases in vaccination hesitancy and powerlessness: results from a longitudinal study. Soc Sci Med 2022; 315: 115522.
36. *Janković S*. Anti-vaccine movements and scientific medicine. Biomedicinska istraživanja 2014; 5(1): 59–65. (Serbian)
37. *Janković S*. Childhood vaccination in the twenty-first century: parental concerns and challenges for physicians. Arh Farm 2019; 69: 452–68.
38. *Banoun H*. mRNA: vaccine or gene therapy? The safety regulatory issues. Int J Mol Sci 2023; 24(13): 10514.

Received on October 16, 2023
Revised on October 20, 2023
Accepted on October 25, 2023
Online First October, 2023

ORIGINAL ARTICLES

UDC: 616-071.3:[611.96:611.83:611.748
DOI: https://doi.org/10.2298/VSP221129025V

Anatomical and functional study of the *musculus psoas major* and *nervus femoralis* in correlation with pelvic diameters

Anatomska i funkcionalna studija slabinskog mišića i butnog živca u korelaciji sa dijametrima karlice

Aleksandar Vojvodić*, Aleksandar Matić†, Jelena Mihailović‡, Predrag Bjelogrlić§, Laslo Puškaš‖, Lazar Stijak‖, Dubravka Aleksić‖, Branka Filipović¶, Biserka Vukomanović-Djurdjević††, Slobodan Kapor‖**

***Clinical Center of Zemun, Department of Orthopedic Surgery, Belgrade, Serbia; †University of Kragujevac, Faculty of Medical Sciences, Department of Orthopedic Surgery, Kragujevac, Serbia; ‡Mayo Clinic, Department of Radiology, Rochester, MN, USA; §University of St Andrews, Faculty of Medicine, Department of Anatomy and Clinical Skills, Scotland, UK; ‖University of Belgrade, Faculty of Medicine, Institute of Anatomy "Niko Miljanić", Belgrade, Serbia; ¶University of Belgrade, Faculty of Medicine, Belgrade, Serbia; ** University Clinical Hospital Center "Dr Dragiša Mišović – Dedinje", Department of Gastroenterology, Belgrade, Serbia; ††University of Defence, Faculty of Medicine of the Military Medical Academy, Belgrade, Serbia**

Abstract

Background/Aim. The iliopsoas muscle [*musculus (m.) iliopsoas*] originates from the Greek word *psóa*, which means loin and represents the only muscle in the body with anatomical preconditions to simultaneously and directly contribute to the stability and movement of the trunk, pelvis, and legs. *M. iliopsoas* belongs to the inner thigh muscle group and forms part of the posterior abdominal wall. This muscle is the major flexor of the hip joint, and it is functionally essential for proper posture, walking, running, and other physical activities. The aim of this study was to determine the relationship between the anatomical parameters of the pelvis and *nervus (n.) femoralis*, as well as the relationship between the same pelvic parameters and *m. psoas major*. **Methods.** The study was conducted at the Faculty of Medicine, University of Belgrade, on cadaveric material of the Institute of Anatomy "Niko Miljanić". For measurement purposes, 14 cadavers were used, seven of which were male and seven female, aged 67–79 years. The measuring instruments used in this study were a ruler and an electronic digital caliper (measuring range 0–500 mm, resolution 0.01 mm). Statistical data processing was performed in the SPSS 11.0 program using the Mann-Whitney *U* test. **Results.** The results of this study indicate a significant statistical difference in pelvic width between male and female cadavers, which was observed in the reduction of the bituberal line in females, while the parameters of the bispinal line showed no significant difference between the two genders. The decrease of the bituberal line in females was followed by an increase in the width of the proximal origin with a statistically significant decrease in the length of the proximal origin of the *m. psoas major*. Furthermore, the vertical distance of *n. femoralis* from the exit point of the muscle to the bispinal line was significantly reduced in the male cadavers. **Conclusion.** Based on our results, we can assume that, in most cases, due to the smaller bituberal and bispinal line or narrower pelvis, a shorter proximal attachment of the *m. psoas major* will occur with greater width (L2–L5 level) in the female than in the male gender, resulting in a longer vertical distance of *n. femoralis*. Such results indicate a close correlation between the anatomical parameters of the *m. psoas major*, which may affect the distance of *n. femoralis* exit from the muscle.

Key words:
anatomy; femoral nerve; pelvis; psoas muscles; sex factors.

Apstrakt

Uvod/Cilj. Slabinsko-bedreni mišić [*musculus (m.) iliopsoas*] vodi poreklo od grčke reči *psóa* što znači slabina i predstavlja jedini mišić u telu koji ima anatomske preduslove da istovremeno i direktno doprinosi stabilnosti i pokretu trupa, karlice i nogu. *M. iliopsoas* pripada unutrašnjoj grupi mišica bedra i čini deo zadnjeg trbušnog zida. Ovaj mišić je glavni pregibač (fleksor) u zglobu kuka i funkcionalno je bitan za pravilno držanje tela, hodanje, trčanje i ostale fizičke

Correspondence to: Slobodan Kapor, University of Belgrade, Faculty of Medicine, Institute of Anatomy "Niko Miljanić", Dr. Subotića starijeg 4, 11 000 Belgrade, Serbia. E-mail: kaporbg@gmail.com

aktivnosti. Cilj rada bio je da se ispita povezanost anatomskih dimenzija karlice i butnog živca [*nervus (n.) femoralis*], kao i odnos tih parametara sa proksimalnim pripojem *m. psoas major*-a. **Metode.** Istraživanje je sprovedeno na Medicinskom fakultetu Univerziteta u Beogradu, na kadaverskom materijalu Instituta za Anatomiju „Niko Miljanić". Za potrebe merenja iskorišćeno je 14 kadavera, sedam kadavera muškog i sedam ženskog pola, starosne dobi od 67–79 godina. Merni instrumenti korišćeni u istraživanju bili su lenjir i elektronski digitalni kaliper (merni opseg 0–500 mm, rezolucija 0,01 mm). Statistička obrada podataka izvršena je u programu SPSS 11.0, primenom Mann-Whitney *U* testa. **Rezultati.** Utvrđena je značajna statistička razlika u širini karlice između kadavera muškog i ženskog pola, koja se manifestovala smanjenjem bituberalne linije kod osoba ženskog pola, dok za dijametar bispinalne linije nije utvrđena značajna razlika između polova. Smanjenje bituberalne linije kod osoba ženskog pola bilo je praćeno povećanjem širine proksimalnog pripoja *m. psoas major*-a, uz statistički značajno smanjenje dužine proksimalnog pripoja. Takođe, vertikalno rastojanje od mesta izlaska *n. femoralis-a* iz samog mišića do bispinalne linije, bilo je značajno smanjeno u ispitivanom uzorku osoba muškog pola. **Zaključak.** Na osnovu prikazanih rezultata, možemo pretpostaviti da će se kod žena češće, u odnosu na osobe muškog pola, zbog manje bituberalne i bispinalne linije, odnosno uže karlice, javljati kraći proksimalni pripoj *m. psoas major*-a, sa većom širinom (nivo L2–L5), a samim tim i dužim vertikalnim rastojanjem izlaska *n. femoralis*-a u odnosu na bispinalnu liniju. Takvi rezultati ukazuju na to da postoji uska povezanost između anatomskih parametara karlice i varijacija proksimalnog pripoja *m. iliopsoas*-a, što može uticati na rastojanje izlaska *n. femoralis*-a iz mišića.

Ključne reči:
anatomija; n.femoralis; karlica; mišići, slabinski; pol, faktor.

Introduction

The iliopsoas muscle [*musculus (m.) iliopsoas*] originates from the Greek word *psóa*, meaning loin, and is the only muscle in the body that has the anatomical prerequisites to simultaneously and directly contribute to the stability and movement of the trunk, pelvis, and legs. It is composed of the large, long muscle (*m. psoas major*) and the thigh muscle (*m. iliacus*), whose muscular fibers converge to form *m. iliopsoas* at the level between L5 and S2 and insert on the lesser trochanter of the femur as the iliopsoas tendon. The other part of *m. iliopsoas* is *m. psoas minor*, which is missing in 40% of cases [1, 2]. It belongs to the inner group of thigh muscles and forms part of the posterior abdominal wall [3]. This muscle is the main flexor in the hip joint and is functionally important for proper posture, walking, running, and other physical activities [2]. The muscular fascia that covers *m. iliopsoas* creates fascial connections that anatomically connect the muscle to the various organs of the abdominal cavity as well as the surrounding structures. In the upper part of the trunk, the muscular fascia merges with the fascia of the chest (*fascia endothoracica*), while the posterior attaches to the arched ligament of the diaphragm (*ligament arcuatum mediale*) and the fascia of the quadratus lumborum muscle (*m. quadratus lumborum*) [3]. Forward and outward, the fascia of *m. iliopsoas* merges with the fascia of the kidneys, pancreas, ascending and descending colon, duodenum, as well as descending aorta and inferior vena cava [4]. According to the previous studies, *m. iliopsoas* was formed from the mesoderm after the eighth week of intrauterine development [4]. The arterial supply of the muscle originates from the common iliac artery as well as its lateral branch, the external iliac artery. *M. psoas major* is supplied by a rich network of arteries derived from the lumbar, iliolumbar, obturator, and femoral artery. The external iliac vein is responsible for venous drainage of blood from *m. iliopsoas*. Nerves for innervation of muscles originate from the lumbar nerve plexus (*plexus lumbalis*), whereby the anterior branches of the lumbar nerves (L1–L3) innervate the *m. psoas major* and *m. psoas minor*, while the femoral nerve [*nervus (n.) femoralis*] or anterior branches of the lumbar nerve (L1–L4) innervate *m. iliacus* [4].

M. psoas major is fusiform in shape, descends from the spinal column to the inguinal ligament (*ligamentum inguinale – Pouparti*), and extends to the lesser trochanter, proximal to the femur [2]. In its central part, *m. psoas major* is round and wide in diameter, while towards the distal attachment, the muscle thins. We can distinguish the superficial and the deep parts of the muscle [3]. The origin of the superficial part is located on the lateral side of the vertebral bodies T12–L5, as well as on the corresponding intervertebral discs. The deep part of the muscle originates from the transverse process of all the lumbar vertebrae [2]. Jelev et al. [5] showed that both lumbar muscles depart from the sacrum. *M. psoas major* is in contact with the organs of the abdominal cavity [kidneys, ureters, lymph nodes, the nerves of the lumbar plexus, sympathetic trunk (*truncus symphaticus*), blood vessels of the kidneys, testicles/ovaries, etc.] as well as with the spinal column and the *quadratus lumborum* muscle [2].

M. iliacus is triangle-shaped and originates in the superior 2/3 of the iliac fossa, posteriorly to the iliac crest, as well as at the iliolumbar ligament (*ligamentum illiolumbale*), where the bundles of *m. iliacus* merge with the bundles of *quadratus lumborum* muscle, and then over the base of the sacrum bone, anterior superior iliac spine, and anterior inferior iliac spine reach the capsule of the hip joint [6]. The bundles of *m. iliacus* are directed towards the tendon of *m. psoas major*, which they join and attach directly at the lesser trochanter [6].

The *n. femoralis* is the largest and most important branch of the lumbar plexus. *N. femoralis* is formed from the anterior branches of the second, third, and fourth lumbar nerve, which then passes between the *m. psoas major* and *m. iliacus*. Upon entering the thigh, it goes beneath the inguinal ligament, after which it passes through the *lacuna musculorum* along with the lateral cutaneous *n. femoralis* and with *m. iliopsoas* [3]. It travels alongside the femoral artery. Beneath the inguinal ligament, it splits into anterior and posterior di-

visions. The anterior branches are responsible for the innervation of the skin of the anteromedial thigh as well as for the innervation of the sartorius muscle. The posterior branches are responsible for innervation of the quadriceps femoris muscle, as well as the knee joint muscle (*m. articularis genus*). The terminal branch of *n. femoralis* is the saphenous nerve. That is the strongest and longest branch responsible for innervation of the skin of the inner part of the lower legs and inner edges of the arch of the feet [7].

Even though numerous studies deal with the variations of the attachment of *m. iliopsoas* and its relationship with the surrounding structures, no research has been done on the relationship between the dimensions of the proximal attachment of *m. psoas major* and the size of the pelvic bones so far. Furthermore, the relationship of *n. femoralis* with the anatomical parameters of the pelvic bones, which is an important factor in the division of the sciatic nerve, has not been examined in detail. Given that the association of the anatomical parameters of the pelvic bones with the division of the sciatic nerve has already been established, the aim of this study was to investigate the relationship between the anatomical dimensions of the pelvis and *n. femoralis* and establish a relationship of these parameters with the proximal attachment of *m. psoas major*.

Methods

Our study was conducted at the Faculty of Medicine, University of Belgrade, Serbia. It was performed on cadaver material of the Institute of Anatomy "Niko Miljanić". Fourteen cadavers, seven of which were male and seven female, aged 67–79 years, were used for measurement purposes. All cadavers used in this study were taken from regular lab dissections during the student lectures, which were held at the Institute of Anatomy. First of all, a visual inspection of each cadaver was carried out to exclude samples with deformities or traces of surgery. After fixing in formalin, the cadavers were subjected to careful dissection, following the standard procedure from the dissection manuals. Afterward, two groups were formed, both of which included the 14 lower extremities. The aim was to define the width and length of the proximal attachment of *m. psoas major*, as well as the vertical distance of the exit of *n. femoralis* from the muscle in relation to the parameters of the width of the pelvis. The width of *m. psoas major* was measured at the proximal attachment of the muscle at the level of the intervertebral discs, between the second and third, third and fourth, and fourth and fifth lumbar vertebrae (L2–L3, L3–L4, L4–L5). The average value at the level of all three intervertebral discs was taken as the final result. As the value of the length of the proximal attachment of the muscle, a vertical distance was taken between the lower edge of the vertebral body, to which the first muscle bundle is attached, and the upper edge of the vertebral body, to where the last muscle bundle attaches. The distance between the right and left anterior superior iliac spine, which we defined as the bispinal line, as well as the distance between the two ischial tuberosity (*tuber ischiadicum*), defined as the bituberal line, were used as parameters of the pelvic width. The vertical distance of *n. femoralis* was measured from the exit point of the nerve from the muscle relative to the bispinal line. All parameters were measured on both sides of the cadaver, especially on the side which was better dissected. The measuring instruments used in the study were a ruler and an electronic digital caliper (measuring range 0–500 mm, resolution 0.01 mm) [8].

The methodology of measurement the tested parameters is presented schematically (Figure 1). All other parameters are shown in Figures 2 and 3. All the data collected is presented in a tabu-

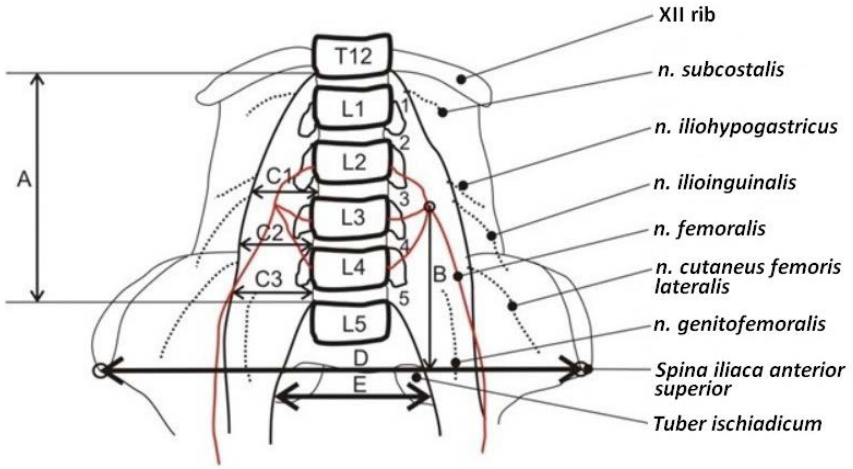


Fig. 1 – An overview of the method of measuring the parameters that were examined in the study. A – distance of the proximal attachment of *musculus (m.) psoas major*; B – vertical distance of the exit of *nervus (n.) femoralis* from the muscle in relation to the bispinal line; C1 – width of the *m. psoas major* at the L2–L3 level of the intervertebral disc; C2 – the width of the *m. psoas major* at the L3–L4 level of the intervertebral disc; C3 – the width of the *m. psoas major* at the L4–L5 level of the intervertebral disc; D – the distance between the two *spinae iliacae anterior superior* (bispinal line); E – the distance between the two ischial tuberosity (bituberal line). The numbers 1–5 show the proximal attachments of *m. psoas major* on the bodies and intervertebral discs of T12–L5.

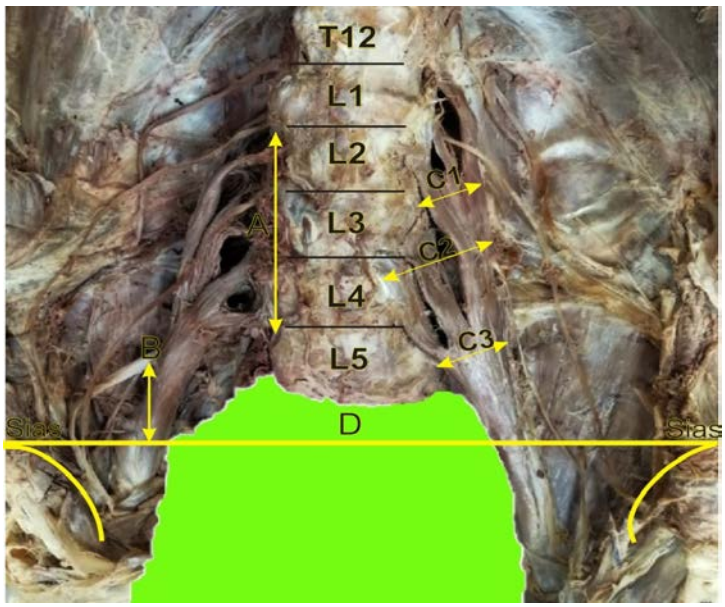


Fig. 2 – An overview of the method of measuring the parameters that were examined in the study. A – distance of the proximal attachment of the *musculus (m.) psoas major*; B – the vertical distance of the exit of *nervus (n.) femoralis* from the muscles relative to the bispinal line; C1 – width of *m. psoas major* at the L2–L3 level of the intervertebral disc; C2 – width of *m. psoas major* at the L3–L4 level of the intervertebral disc; C3 – width of *m. psoas major* at the L4–L5 level of the intervertebral disc; D – the distance between the two *spinae iliacae anterior superior* (bispinal line); Sias – *spina iliaca anterior superior.*

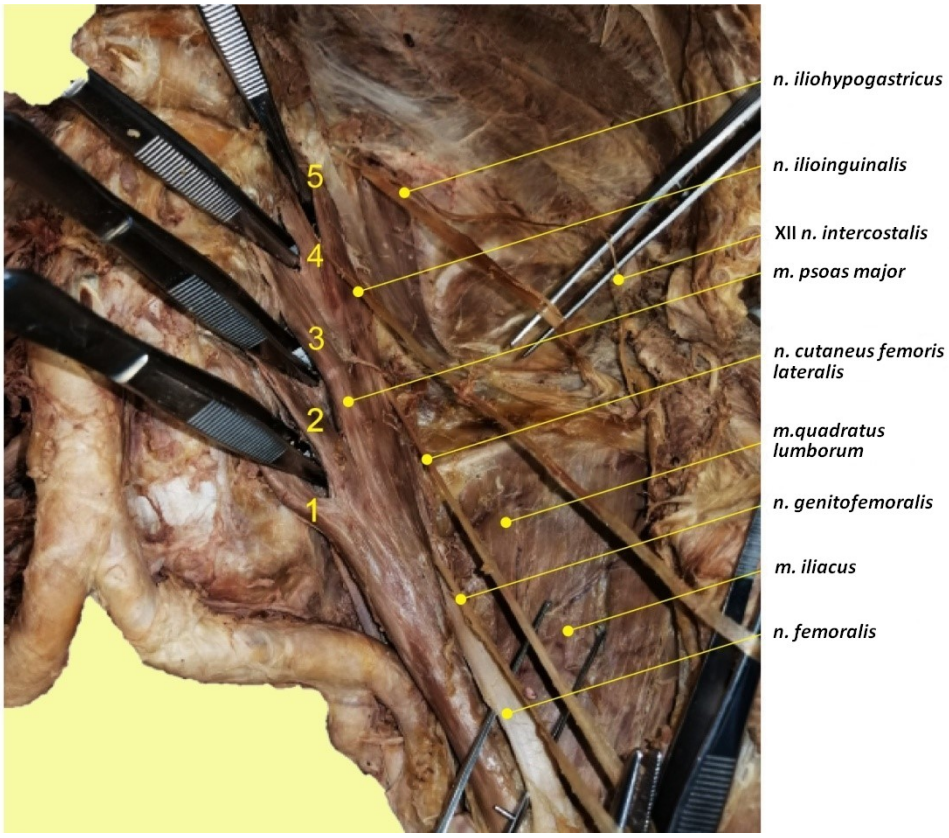


Fig. 3 – Dissection of *musculus (m.) iliopsoas* and nerves (n.) of the lumbar plexus. The numbers 1–5 show the proximal attachment of the bundle of *m. psoas major* on the bodies and intervertebral discs of T12–L5.

Table 1

Mean values of the examined parameters in the male and female group

Parameters	Male	Female
Bispinal line	27.21 ± 3.81	24.50 ± 1.55
Bituberal line	15.79 ± 0.70	14.14 ± 0.80*
Width of the proximal attachment of the *musculus psoas major*	3.69 ± 0.49	4.64 ± 0.81*
Length of the proximal attachment of the *musculus psoas major*	15.36 ± 1.41	13.29 ± 1.32*
Vertical distance of *nervus femoralis* relative to the bispinal line	6.50 ± 0.58	7.86 ± 0.75*

All values (in cm) are expressed as mean ± standard deviation.
*** marked statistically significant differences in female cadavers, $p < 0.05$.**

lar manner (Table 1). All statistical analysis was carried out in the SPSS 11.0 using the Mann-Whitney *U* test, where the accepted level of statistical significance was $p < 0.05$, and $p < 0.001$ was accepted for a highly statistically significant result [8].

Results

The measured parameters are presented in Table 1. The study examined the 14 lower extremities on the right side of the cadaver, with the sample being the seventh male cadaver and the seventh female cadaver. The mean value of the bispinal line in the male group was 27.21 ± 3.81 cm, while in the female group, it measured 24.50 ± 1.55 cm without a statistically significant difference between the groups ($p = 0.259$). The mean value of the bituberal line in the male group was 15.79 ± 0.70 cm, and in the female group, it was 14.14 ± 0.80 cm. Comparing this parameter in the two test groups, we obtained a statistically significant difference ($p = 0.004$). The mean value of the width of the proximal attachment at the levels of L2–L3, L3–L4, and L4–L5 (see the Methods section for more details) in the male group was 3.69 ± 0.49 cm, while the same parameter in the female group was 4.64 ± 0.81 cm. There was an increase in the width of the proximal attachment of *m. psoas major* in the female group with a statistically significant difference ($p = 0.038$) (Table 1). The mean value of the length of the proximal attachment (see the Method section for more details) was 15.36 ± 1.41 cm in the male and 13.29 ± 1.32 cm in the female group. The length of the proximal attachment was lesser in the female group with statistical significance ($p = 0.017$). The vertical distance from the exit point of *n. femoralis*, from *m. psoas major* to the bispinal line, was higher in the female group compared to the male group, with a statistically significant difference ($p = 0.007$) (Table 1). There is a significant statistical difference in pelvic width between male and female cadavers, which is observed in the shorter bituberal line in the female gender, while the diameter of the bispinal line does not make significant differences between the two genders. The shorter bituberal line in the female gender is accompanied by a greater width of the proximal attachment of *m. psoas major* with a statistically significant shorter length of the proximal attachment. Moreover, the distance from the exit point of *n. femoralis* from the muscle itself to the bispinal line was significantly reduced in the male group of the examined sample.

Discussion

Due to its anatomical characteristics, *m. iliopsoas* is described as an axioappendicular muscle that connects the axial and the appendicular skeleton. It is functionally involved in the movements of the trunk, pelvis, and lower extremities and is considered the main flexor in the hip joint [4]. Coactivation of the muscle significantly contributes to the stabilization of the hip joint by pressing the head of the femur into the acetabulum [9]. Neuromuscular diseases of this muscle can lead to dysfunction of the colon, which causes compression of *n. femoralis* and lateral cutaneous *n. femoralis* [10].

M. psoas major contains all three types of muscle fibers that are managed in different directions. The previous study by Johnson et al. [11] found an equal ratio of muscle fibers Type I and Type II (49.2% and 50.08%, respectively). Kimura [12] showed the highest prevalence of Type I fibers (42%) compared to Type II (33%) and Type III (25%). Furthermore, they found that Type I fibers are significantly wider in diameter than Type II fibers. Zheng et al. [13] used histochemical staining of myosin adenosine triphosphate for the fiber typing and described 58% of Type I, 32% of Type II A, and 10% of Type II B fiber. It is believed that there is a difference in muscle fibers in relation to the height of the attachment, whereby in the proximal to the distal attachment, the number of slow muscle fibers decreases (Type II), while the number of fast fibers (Type II and III) increases [13]. This structure can indicate differences in the length of the proximal attachment of the muscle itself as well as the width at the level of L2–L5, which was shown in our study. These results could explain impairment in the dual function of the muscle in males and females due to the different dimensions of the pelvis. The postural function, represented by the proximal part of the muscle, is responsible for stabilizing the lumbar spine and pelvis, while the dynamic function in the distal attachment of the muscle is responsible for movement [14].

Measured bone parameters of the pelvis showed a mean value of the bispinal line (27.21 ± 3.81 cm) and the bituberal line (15.79 ± 0.70 cm), which was higher in the male, compared with the bispinal line (24.50 ± 1.55 cm) and the bituberal line (14.14 ± 0.80 cm) of the female group. We found statistically significant differences in bituberal line values between the male and female groups. The mean width of the proximal attachment of the iliopsoas was lower in the male compared to the female group (3.69 ± 0.49 and 4.64 ± 0.81 cm, respectively). There is also a significant difference ($p = 0.017$) in the length of the proximal muscle attachment,

which was greater in the male group, 15.36 ± 1.41 cm, while in the female group, it was 13.29 ± 1.32 cm. Analyzing the vertical distance of the point where *n. femoralis* exits the muscle to the bispinal line, we found a longer distance in females with $p < 0.007$.

Kepler et al. [15] analyzed the distance between *n. femoralis* and *m. psoas major* from the front of the spinal column. Their results showed that the distance between *n. femoralis* and the front of the spinal column was 3.35 cm at the L3–L4 level, 2.68 cm at the L4–L5 level, and 0.62 cm at the L5–S1 level. The distance of the *m. psoas major* is 0.81 cm at the L1–L2 level, 0.38 cm at the L3–L4 level, 1.16 cm at the L4–L5 level, and 3.15 cm at the L5–S1 level. Another study showed that *n. femoralis* goes lateral to the *m. psoas major*, just below the bifurcation of the common iliac artery, whereby the examined distance between *n. femoralis* and genitofemoral in relation to the common iliac artery showed no statistically significant difference [16]. Regev et al. [17] found safe anatomical zones of the weak lumbar nerves on *m. psoas major*, located at the level of L2–L3. These findings are similar to our results, which show the significant difference in the width of the muscle and, at the same time, the distance of the exit point of *n. femoralis* from the muscle in the two tested groups of cadavers. This safe anatomical zone is of great importance during retroperitoneal surgical procedures, where no damage should be made to the lumbar nerves, especially to *n. femoralis* [17]. Kirchmair et al. [18] showed that in 47 out of 63 cases, *n. femoralis* could be found as a separate nerve, with no lateral branches larger than 0.2 cm in diameter. In all 47 cases, *n. femoralis* was found at the exit point from *m. psoas major*. In 13 out of 63 cases, the division of *n. femoralis* into two or three branches directly emerging from *m. psoas major* was found [18]. The close relationship of *m. iliopsoas* with the largest branch of the lumbar plexus, *n. femoralis*, is of exceptional clinical importance. A detailed study on variations of *m. iliopsoas* and *n. femoralis* itself might be helpful in fasciotomy or the treatment of hematomas and abscesses in the lumbar region [12]. Contractions, tension, and shortening of muscles are also clinically significant as they can lead to a series of disruptions and compression of surrounding structures [10]. A study led by Güvençer et al. [19] on the relationship between the anatomical structures of the pelvic bone and the sciatic nerve was conducted first in the anatomical position and then in the positions that simulate the stretching test in the hip joint, such as 60° flexion, 30° adduction, and 10° internal rotation of the hip joint. It has been shown that the biomechanics of the stretching test lead to the infrapiriform foramen narrowing, the sciatic nerve becoming closer to the ischial spine, and the angle between the sciatic nerve and the transversal plane becoming larger, thus creating conditions in which the nerve is more susceptible to being "stuck". Consequently, in the interpretation and analysis of the results, biomechanics should be considered, which could be the reason for the observed differences in the examined parameters in our study.

Conclusion

Based on our results, we can conclude that females, in most cases, due to a smaller bituberal and bispinal line, i.e., narrower pelvis, will experience a shorter proximal attachment of the *m. psoas major*, with a greater width at the L2–L5 level relative to the males and, therefore, a longer vertical exit distance of *n. femoralis* relative to the bispinal line. Such results indicate a close correlation between the anatomical parameters of the pelvis and variations of the proximal attachment of *m. psoas major*, which may affect the distance of the exit of *n. femoralis* from the muscle.

REFERENCES

1. *Andersson E, Oddsson L, Grundström H, Thorstensson A.* The role of the psoas and iliacus muscles for stability and movement of the lumbar spine, pelvis and hip. Scand J Med Sci Sports 1995; 5(1): 10–6.
2. *Arbanas J, Starčević-Klasan G, Malnar D.* Composition of the psoas major muscle regarding its complex function. Med Flum [Internet] 2012 [accessed on: 2023 June 29]; 48(2): 123–30. Available from: https://hrcak.srce.hr/84186 (Croatian)
3. *Bordoni B, Varacallo M.* Anatomy, Bony Pelvis and Lower Limb, Iliopsoas Muscle. [updated 2023 Apr 24]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2023. [cited 2023 May]. Available from: https://www.ncbi.nlm.nih.gov/books/NBK531508/
4. *Mrvaljević D.* Anatomy of the lower limb. 2nd edition. Belgrade: Faculty of Medicine, University of Belgrade; 2018. p.31–4.
5. *Jelev L, Shivarov V, Surchev L.* Bilateral variations of the psoas major and the iliacus muscles and presence of an undescribed variant muscle--accessory iliopsoas muscle. Ann Anat 2005; 187(3): 281–6.
6. *Siccardi MA, Tariq MA, Valle C.* Anatomy, Bony Pelvis and Lower Limb: Psoas Major. [updated 2022 Aug 8]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2018 [cited 2018 Dec 13]. Available from: https://www.ncbi.nlm.nih.gov/books/NBK535418/
7. *Basinger H, Hogg JP.* Anatomy, Abdomen and Pelvis: Femoral Triangle. [updated 2023 Mar 11]. In StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2019 [cited 2019 Apr 29]. Available from: https://www.ncbi.nlm.nih.gov/books/NBK541140/
8. *Kapor S, Puškaš L, Vojvodić A, Mališ M, Bjelogrlić P, Cezayirli E,* et al. Anatomically high division of sciatic nerve and its clinical significance. Vojnosanit Pregl 2021; 78(10): 1060–4.
9. *Yoshio M, Murakami G, Sato T, Sato S, Noriyasu S.* The function of the psoas major muscle: passive kinetics and morphological studies using donated cadavers. J Orthop Sci 2002; 7(2): 199–207.
10. *Laible C, Swanson D, Garofolo G, Rose DJ.* Iliospoas Syndrome in Dancers. Orthop J Sports Med 2013; 1(3): 2325967113500638.
11. *Johnson MA, Polgar J, Weightman D, Appleton D.* Data on the distribution of fibre types in thirty-six human muscles: An autopsy study. J Neurol Sci 1973; 18(1): 111–29.
12. *Kimura T.* Composition of psoas major muscle fibers compared among humans, orangutans and monkeys. Z Morphol Anthropol 2002; 83(2–3): 305–14.
13. *Zheng A, Rahkila P, Vuori J, Rasi S, Takala T, Väänänen HK.* Quantification of carbonic anhydrase III and myoglobin in different fiber types of human psoas muscle. Histochemistry 1992; 97(1): 77–81.

14. *Arbanas J, Klasan GS, Nikolić M, Jerković R, Miljanović I, Malnar* D. Fibre type composition of the human psoas major muscle with regard to the level of its origin. J Anat 2009; 215(6): 636–41.
15. *Kepler CK, Bogner EA, Herzog RJ, Huand RC.* Anatomy of the psoas muscle and lumbar plexus with respect to the surgical approach for lateral transpsoas interbody fusion. Eur Spine J 2011; 20(4): 550–6.
16. *Mladonado P, Slocum PD, Chin K, Corton M.* Anatomic relationships of psoas muscle: clinical applications to psoas hitch ureteral reimplantation. Am J Obstet Gynecol 2014; 211(5): 563. e1–6.
17. *Regev GJ, Chen L, Dhawan M, Lee YP, Garfin SR, Kim CW*. Morphometric Analysis of the ventral nerve roots and retroperitoneal vessels with respect to the minimally invasive lateral approach in normal and deformed spines. Spine (Phila Pa 1976) 2009; 34(12): 1330–5.
18. *Kirchmair L, Lirk P, Colvin J, Mitterschiffthaler G, Moriggl B.* Lumbar plexus and psoas major muscle: not always as expected. Reg Anesth Pain Med 2008; 33(2): 109–14.
19. *Güvençer M, Akyer P, Iyem C, Tetik S, Naderi S*. Anatomic considerations and the relationship between the piriformis muscle and the sciatic nerve. Surg Radiol Anat 2008; 30(6): 467–74.

Received on November 29, 2022
Revised on April 20, 2023
Accepted on April 25, 2023
Online First April 2023

ORIGINAL ARTICLE

UDC: 616.314
DOI: https://doi.org/10.2298/VSP221017028C

Scanning electron microscopy assessment of tubular penetration depth of root canal sealers combined with different obturation techniques

Procena dubine prodora u dentinske tubule materijala za punjenje kanala korena zuba u kombinaciji sa različitim tehnikama opturacije korišćenjem skenirajuće elektronske mikroskopije

Milica Cvjetićanin, Bojana Ramić, Karolina Vukoje, Milan Drobac, Igor Stojanac, Ljubomir Petrović

University of Novi Sad, Faculty of Medicine, Department of Dental Medicine, Novi Sad, Serbia; Clinic of Dentistry of Vojvodina, Department of Restorative Dentistry and Endodontics, Novi Sad, Serbia

Abstract

Background/Aim. The ability to effectively and consistently penetrate dentinal tubules is considered a favorable factor for the evaluation of root canal sealers (RCSs). The aim of the study was to assess the penetration depth into dentinal tubules of three RCSs combined with four obturation techniques. **Methods.** The mesial canals of 66 extracted human mandibular molars were endodontically prepared and randomly allocated into 12 experimental groups depending on the RCS type used (AH Plus™, EndoREZ™, Sealapex™) as well as the obturation technique applied [cold lateral compaction, cone-fit, carrier-based (with heated gutta-percha), warm vertical compaction]. Using scanning electron microscopy, transversal root cross-sections were analyzed, and the maximum depth of RCS penetration was measured (396 sections, in total, corresponded to the apical, middle, and coronary third). **Results.** Group AH Plus™/warm vertical compaction yielded the highest penetration depth – 1,165 μm, followed by EndoREZ™/cone-fit – 1,154 μm; the lowest depth was measured for EndoREZ™/warm vertical compaction – 502 μm. The mean value of the maximum penetration depth of RCS yielded 1,204 μm in the coronary thirds, 1,005 μm in the middle thirds, and 770 μm in the apical thirds. The AH Plus™ RCS penetrated deeper into dentinal tubules when the obturation techniques with heated gutta-percha were applied, while the opposite findings were obtained for the EndoREZ™ RCS. **Conclusion.** According to our research, the RCS penetration depth appears to be influenced by the RCS type used, as well as the obturation technique applied.

Key words:
microscopy, electron, scanning; root canal filling materials; root canal obturation.

Apstrakt

Uvod/Cilj. Sposobnost efikasnog i konzistentnog prodora u dentinske tubule smatra se favorizujućim faktorom za procenu materijala za punjenje kanala korena zuba (MPKKZ). Cilj rada bio je da se proceni dubina prodora tri MPKKZ u dentinske tubule, u kombinaciji sa četiri različite tehnike opturacije. **Metode.** Mezijalni kanali 66 ekstrahovanih mandibularnih molara su endodontski pripremljeni i nasumično podeljeni u 12 eksperimentalnih grupa, u zavisnosti od vrste upotrebljenog MPKKZ (AH Plus™, EndoREZ™, Sealapex™), kao i od tehnike opturacije (hladna lateralna kompakcija, *cone-fit* tehnika, sa čvrstim nosačem gutaperke, topla vertikalna kompakcija). Upotrebom skenirajuće elektronske mikroskopije analizirani su poprečni preseci korenova zuba i zabeležena je maksimalna dubina prodora MPKKZ za svaku trećinu korena (ukupno 396 preseka koji su odgovarali apikalnoj, srednjoj i kruničnoj trećini korena zuba). **Rezultati.** U grupi AH Plus™/topla vertikalna kompakcija, postignuta je najviša dubina prodora – 1 165 μm, a zatim u grupi EndoREZ™/*cone-fit* – 1 154 μm; najniža dubina prodora izmerena je u grupi EndoREZ™/topla vertikalna kompakcija – 502 μm. Srednja vrednost maksimalne dubine prodora svih MPKKZ iznosila je 1 204 μm u kruničnoj trećini, 1 005 μm u srednjoj trećini i 770 μm u apikalnoj trećini. MPKKZ AH Plus™ prodirao je dublje u dentinske tubule u kombinaciji sa tehnikama opturacije sa zagrejanom gutaperkom, dok su suprotni rezultati zabeleženi kod MPKKZ EndoREZ™. **Zaključak.** Prema rezultatima sprovedenog istraživanja, dubina prodora MPKKZ u dentinske tubule zavisi od vrste MPKKZ, ali i od primenjene tehnike opturacije.

Ključne reči:
mikroskopija, elektronska, skenirajuća; zub, materijali za punjenje korenskog kanala; zub, punjenje korenskog kanala.

Correspondence to: Milica Cvjetićanin, University of Novi Sad, Faculty of Medicine, Department of Dental Medicine, Hajduk Veljkova 3, 21 000 Novi Sad, Serbia. E-mail: milica.cvjeticanin@mf.uns.ac.rs

Introduction

In endodontic therapy, root canal sealers (RCSs) have been used to fill the interface between the core filling material and the root dentinal walls but also the lateral canals, dentinal tubules, and intracanal irregularities. Various obturation techniques have been proposed in order to achieve a three-dimensional canal filling, but the obturation of a complete root canal system is still a challenge for clinicians. The ability to effectively and consistently penetrate dentinal tubules is considered a favorable factor for RCSs [1–4]. Namely, sealer tags into dentinal tubules increase the contact surface area between the filling material and the dentinal wall, improving the retention rate of filling, increasing the fracture resistance of endodontically treated teeth by reinforcing the root, and potentially reducing the risk of microleakage. Sealer into dentinal tubules may entomb the remaining bacteria, while its chemical constituents can exhibit an antibacterial effect [5–7]. Measuring the depth of penetration into dentinal tubules is one of the methods used for evaluating the performance of a sealer [8]. The extent of sealer tags seems to be influenced by the presence of a smear layer, the root canal dimensions, dentine permeability (number and diameter of dentinal tubules), obturation technique applied, temperature, and humidity. The physical and chemical properties of sealers, such as particle size, viscosity, flowability, solubility, surface tension, and contact angle between the sealer and the root dentin, also play an important role in the effectiveness of tubular penetration [1, 5, 9, 10].

Micrographs obtained by scanning electron microscopy (SEM) allow for a detailed observation of dentine tubules and the integrity of sealer tags inside them, as well as precise measurements at a wide range of magnifications. The main disadvantages of this method are the high risk of producing artifacts during specimen preparation and the observation limited only to the specimen's surface [2, 7, 11]. Sealer penetration depths are commonly examined using a confocal laser scanning microscope. This method allows visualization of sealer penetration even below the dentine surface as the sealer is labeled with fluorescent organic dye [3, 6, 12–14]. As Donnermeyer et al. [15] demonstrated, the staining of sealer with a fluorescent dye (Rhodamine B) is not an adequate method for the evaluation of sealer penetrability into dentinal tubules as dye diffuses passively into the tubules and may give incorrect results. Taking all the above into consideration, SEM analysis is preferred for assessing the sealer penetrability into dentinal tubules.

Due to its favorable physical, chemical, and biological properties, AH Plus™ (Dentsply DeTrey, Konstanz, Germany) has become a "gold standard" for evaluating research in endodontics. AH Plus™ is an epoxy resin-based RCS with a setting time of eight hours, allowing long infiltration of dentinal tubules [11, 16]. On the other hand, EndoREZ™ (Ultradent Products Inc., South Jordan, Utah, USA) is a self-priming, methacrylate-resin-based sealer with hydrophilic characteristics and a short setting time (20–30 min). According to the manufacturer, EndoREZ™ needs to be applied to a slightly moistened canal in an oxygen-free environment to prevent inhibition of the polymerization process. The dual nature of the EndoREZ™ setting results in a higher polymerization shrinkage, which may adversely affect material adaptation to the dentinal wall. Sealapex™ (Kerr, Salerno, Italy), as the third sealer examined in this study, is based on calcium hydroxide, has a high biological potential, does not possess adhesive properties, and sets slowly [17, 18].

The aim of the study was to ascertain the degree of sealer penetration into dentinal tubules based on the micrographs obtained by SEM.

Methods

Specimen preparation

The use of extracted human teeth for the study was approved by the Ethics Committee of the Faculty of Medicine, University of Novi Sad, Serbia (approved on 11 March, 2013). The mesiobuccal and mesiolingual canals of permanent first human mandibular molars extracted for orthodontic, periodontal, or prosthetic reasons were used in the research. The selected mesial roots were characterized by a fully formed apex and exhibited type IV anatomic configuration according to the Vertucci [19] classification, along with 10°–30° canal curvature, as measured by the Schneider [20] technique. Endodontically treated teeth, roots with calcifications, cracks, perforations, fractures, resorptions, and immature apices were discarded. Preoperative digital radiographs were taken using a standardized parallel technique (buccolingual and mesiodistal directions) in order to verify the morphology of the canals, discarding the samples with inappropriate features and severe curvatures. Freshly extracted teeth were cleaned using hand curettes and were stored in distilled water at 4 °C until required for the experiment, no longer than a month. As 12 groups were required for the experiments, a sample of 66 mandibular molars with two mesial canals was selected (132 in total), as determined by the total number of ways to choose two different groups out of 12 groups, i.e., 12*11/2.

Prior to instrumentation, the teeth were cut at the cemento-enamel junction using a low-speed diamond disc (Edenta AG, AU/SG, Switzerland) to access the orifices of mesiobuccal and mesiolingual canals; distal roots were discarded. Each root was assigned a unique number; the mesiobuccal canal was marked by a longitudinal groove made along the root buccal surface to distinguish the mesial canals under SEM. Working length (WL) was determined by introducing a #10 K-File (Dentsply Maillefer, Ballaigues, Switzerland) into each canal until the tip of the file was seen at the apical foramen and subtracting 0.5 mm from the measured length. The canals were prepared by a single operator in a crown-down manner, using rotary nickel-titanium endodontic instruments driven by an endodontic motor (X-Smart Plus, Dentsply Maillefer, Ballaigues, Switzerland). Glide paths were created using a #16/02 ProGlider (PG, Dentsply Maillefer, Ballaigues, Switzerland), and canals were shaped with ProTaper Next X1 (17/0.04) and X2 (25/0.06) up to the WL using the recommended settings (300 rpm; 2 Ncm

torque). Each file was lubricated by Glyde (Dentsply Maillefer, Ballaigues, Switzerland); between files, the canals were irrigated with 2 mL of 2.5% sodium hypochlorite (NaOCl) solution delivered through a plastic syringe and 30-G side-vented irrigation needles (KerrHawe Irrigation Probe, KerrHawe SA, Bioggio, Switzerland). Apical patency was maintained by introducing the #10 K-File up to the WL between each file. For smear layer removal, the canals were irrigated with 10 mL of 17% ethylenediaminetetraacetic acid (EDTA, I-dental, Siauliai, Lithuania) and 10 mL of NaOCl; both solutions were activated using EndoActivator (Tips small 15/.02; Dentsply Maillefer, Ballaigues, Switzerland). The canals were finally rinsed with 10 mL of saline and gently dried using absorbent paper points (F2 Paper Points, ProTaper Universal, Dentsply Maillefer, Ballaigues, Switzerland). Throughout the whole process, the roots were kept moist by wrapping them into saline-soaked gauze. The prepared roots were randomly allocated into three experimental groups, depending on the sealer type used, and four further subgroups were created within each, according to the obturation technique applied, resulting in 12 groups, each with 11 canals (Figure 1). Two samples were taken as controls to verify the presence/absence of a smear layer and dentinal permeability obtained after the final rinse.

Obturation techniques were performed in an incubator at 37 °C environment as described previously [21]. Three RCSs were used in the study (Table 1). Components of AH Plus™ and EndoREZ™ were packed in double syringes and automatically mixed; Sealapex™ was mixed according to the manufacturer's instructions using a precise weighing scale that allowed the extrusion of equal amounts of both components. AH Plus™ and Sealapex™ were introduced into the canals using a #20 K-File coated with the chosen sealer by a counter-clockwise rotation, inserting a length 1 mm shorter than the WL. According to the manufacturer, EndoREZ™ was dispensed into canals through a narrow syringe (Skini™ syringe) connected to a fine-tipped cannula (NaviTip™), placing 2–3 mm less than the WL while withdrawing the syringe until the sealer level reaches the canal orifice. The excess material was removed with dry paper points (F1, ProTaper, Dentsply Maillefer, Ballaigues, Switzerland).

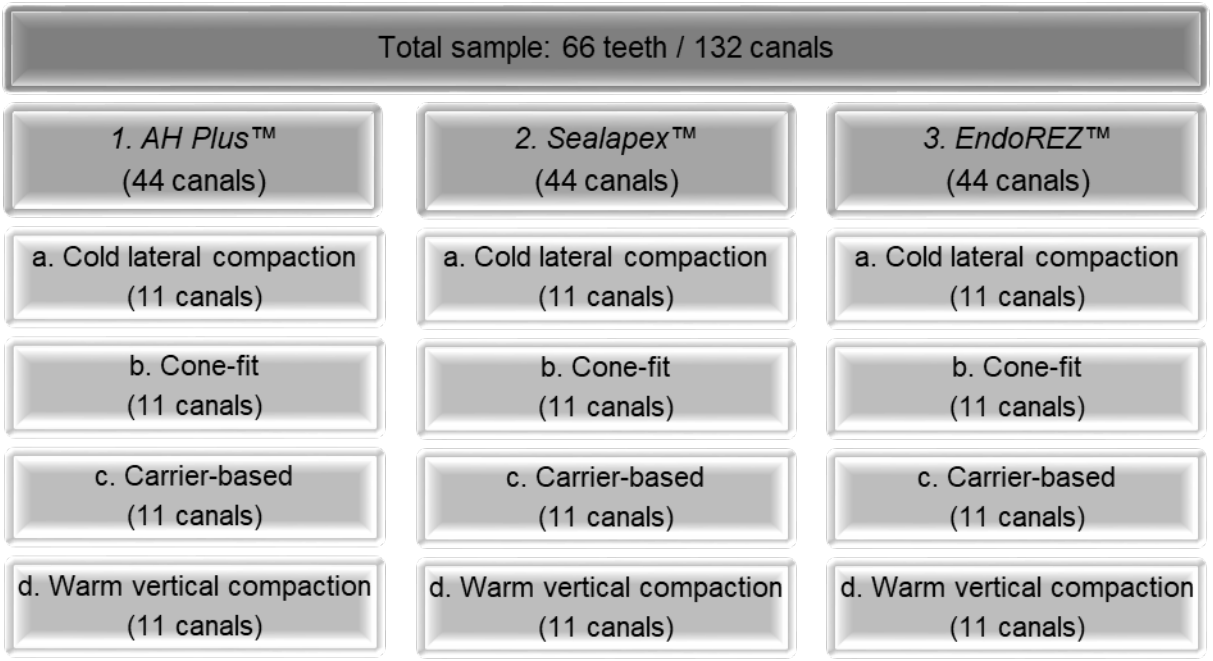


Fig. 1 – Schematic diagram of the experimental groups.

Table 1

The chemical composition of root canal sealers

Material	Type	Chemical composition	Manufacturer
AH Plus™	Epoxy resin	Paste A: epoxy resin, zirconium oxide, calcium tungstate, iron oxide pigments, aerosil Paste B: amines, zirconium oxide, calcium tungstate, silicone oil, aerosil	Dentsply De Trey, Konstanz, Germany
Sealapex™	Calcium hydroxide polymeric resin	Base: N-ethyl toluene sulfonamide, zinc oxide, silica, calcium oxide Catalyst: Isobutyl salicylate, methyl salicylate, polymethyl methacrylate, silica, titanium dioxide, bismuth trioxide	Kerr, Salerno, Italy
EndoREZ™	Methacrylate resin	Base: Diurethane dimethacrylate, benzoyl peroxide Catalyst: 2,2'- diethanol, triethylene glycol dimethacrylate	Ultradent, South Jordan, Utah, USA

In this study, four obturation techniques were used. The first technique used was cold lateral compaction. Master gutta-percha (GP) cone (ISO 25, Gutta-percha Points; Dentsply Maillefer, Ballaigues, Switzerland), fitted to the WL with apical tug-back, was inserted into a root canal previously coated with the chosen sealer. The remaining intracanal space was filled by lateral compaction of accessory, non-standardized GP cones using a #20 finger spreader (Dentsply Maillefer, Ballaigues, Switzerland). The excess GP was removed by a heated instrument. The second technique used was the cone-fit technique. Master GP cone (F2 ProTaper Universal, Dentsply Maillefer, Ballaigues, Switzerland), fitted to the WL with apical tug-back, was inserted into a root canal previously coated with the chosen sealer. The excess GP was removed by a heated instrument. The third technique used was the carrier-based obturation technique (Thermafil). Upon heating in ThermaPrep 2 Oven (Dentsply Maillefer, Ballaigues, Switzerland) for 20 sec, GP obturator (F2 ProTaper Universal Thermafil Obturator, Dentsply Maillefer, Ballaigues, Switzerland) was inserted into the canal with a constant apical pressure up to the WL. The coronal part of the obturator was manually stabilized and removed with a Thermacut bur (#12; Dentsply Maillefer, Ballaigues, Switzerland). Finally, the fourth technique used was the warm vertical compaction. Master GP cone (F2 ProTaper Universal Gutta-Percha Points, Dentsply Maillefer, Ballaigues, Switzerland) was inserted into a root canal previously coated with the chosen sealer, 0.5 mm shorter than the WL. The down-packing phase was performed with DiaPen (Pen type size – fine; DiaDent, Korea) heated at 200 °C (medium temperature setting) for 1–2 sec, inserting length 4 mm shorter than the WL. Back-filling was performed with DiaGun (DiaDent, Korea), filling the remaining intracanal space with melted GP (200 °C) using intermittent compaction with Heat Carrier plugger (No 1/2; Dentsply Maillefer, Ballaigues, Switzerland).

After obturation, the flowable composite (Filtek ™ Supreme Ultra Flow, 3M/ESPE, St. Paul, MN, USA) was applied over canal orifices and light-polymerized for 20 sec (Radii Plus, SDI, Bayswater, Victoria, Australia). The roots were stored at 37 °C and 100% humidity for 14 days to allow the material to set.

Scanning electron microscope analysis

All roots were sectioned perpendicular to the long axis at 3, 5, and 8 mm from the anatomic apex using a low-speed diamond disc under water-cooling (Edenta AG, AU/SG, Switzerland). Three cross-sections per root were created, with a thickness of 1 mm corresponding to the apical, middle, and coronary third, producing 396 sections in total. The coronal surfaces of cross-sections were chosen for analysis and were immersed in 17% EDTA solution (I-dental, Siauliai, Lithuania) for 10 min, followed by 4% NaOCl for 10 min in order to remove any residues of organic components around the sealer tags. The sections were finally rinsed with distilled water and gently dried. The prepared sections were dehydrated, mounted onto aluminum stubs, and sputter-coated with a gold coating (SCD050 Sputter Coater, BAL-TEC, PA, USA) under low-vacuum conditions.

Using micrographs obtained by an SEM (JEOL-JSM-6460LV, Tokyo, Japan), the depth of sealer penetration into dentinal tubules was measured. First, using an overall view obtained at low magnification (×50, ×100), the area with maximum sealer tag density was selected. Next, in this area at higher magnification (≥ ×400), the maximum sealer penetration depth was identified and marked digitally. Upon reducing magnification until the root canal wall was visualized, the image was captured, and, using a calibrated measuring tool (NIH Image Analyser), the distance from the marked point and root canal wall was measured (Figure 2). The depth of sealer tags was calculated in μm, but also in relative measure as a percentage of the total distance: inner canal dentinal wall – the outer root surface. The measurements were conducted by two operators blinded to the group being tested.

Data analysis comprised descriptive and comparative statistical methods. Although the values of the examined features are continuous, some samples did not meet the requirements for applying parametric comparative tests (Student's *t*-test, ANOVA), and, in those cases, non-parametric comparative tests were used (Mann-Whitney *U* test, Kruskal-Wallis ANOVA). Tukey HSD test for groups of different sizes was performed as a *post-hoc* test in ANOVA, whereas Multiple Comparisons of mean ranks were adopted as a non-

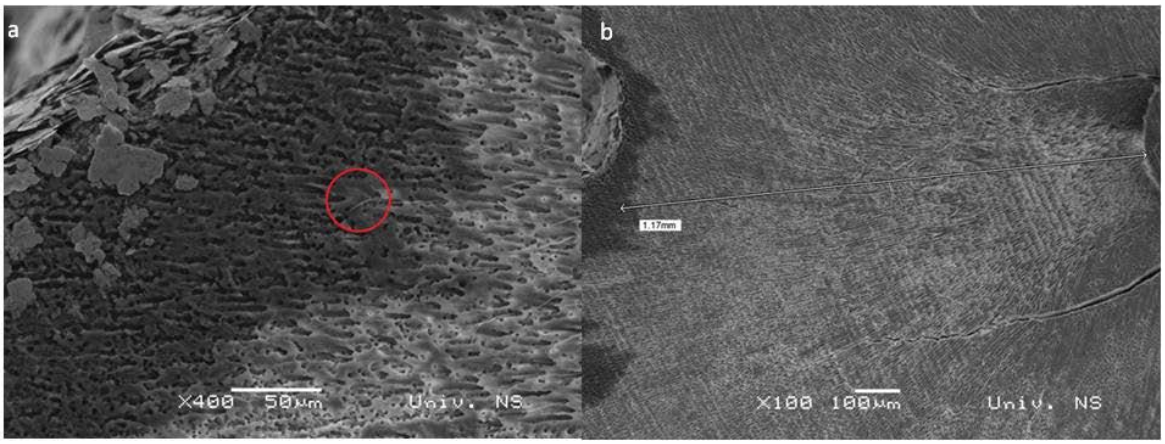


Fig. 2 – Scanning electron microscopy micrograph of sealer penetration into dentinal tubules: a) The deepest detected sealer tag; b) The deepest sealer tag measured from the dentinal wall.

parametric *post-hoc* test in Kruskal-Wallis ANOVA. The results were considered statistically significant if the corresponding *p*-value was below 0.05.

Results

The basic indicators of the descriptive statistics for the depth of sealer tags in all twelve groups are shown in Table 2. Presented data refer to the arithmetic mean of the maximum depth of sealer penetration into dentinal tubules for each group (coronary, middle, and apical third together) shown in percentage. Among all groups, statistically significant differences in the mean values of the variable were noted. Namely, group AH Plus™/warm vertical compaction yielded the highest penetration depth – 85.4% (1,165 μm), followed by groups EndoREZ™/cone-fit technique – 80.6% (1,154 μm), and AH Plus™/Thermafil – 77.6% (1,229 μm). The lowest penetration depth was measured for group EndoREZ™/warm vertical compaction – 46.2% (502 μm) (Table 2).

The results yielded by the analysis of each sealer separately are given in the following text. Using AH Plus™, a statistically significant difference was noted between the mean values of maximum penetration depth achieved *via* different obturation techniques ($p = 0.0000$). AH Plus™, in combination with warm vertical compaction, showed a significantly higher penetration depth compared to groups 1a ($p = 0.000008$), 1b ($p = 0.000008$), and 1c ($p = 0.00043$), while no significant differences were noted among other pairs. Sealapex™, in combination with warm vertical compaction, resulted in a significantly lower penetration depth into dentinal tubules compared to the combinations involving cold lateral compaction ($p = 0.005631$) and carrier-based techniques ($p = 0.003658$), while no significant differences were noted among other pairs. EndoREZ™, in combination with warm vertical compaction, had a significantly lower mean maximum depth of penetration compared to the combinations with cold lateral compaction ($p = 0.000138$), cone-fit technique ($p = 0.00014$), and carrier-based technique ($p = 0.000148$). In combination with the cone-fit technique, EndoREZ™ had a significantly higher depth of penetration compared to the combination with the carrier-based technique ($p = 0.004883$). There were no significant differences between the other pairs.

The results obtained when obturation techniques were analyzed separately were discussed in the following text. There were no significant differences between the mean maximum depth of tubular penetration of AH Plus™, Sealapex™, and EndoREZ™ using cold lateral compaction and carrier-based obturation technique. Using the cone-fit technique, a significantly higher penetration depth into dentinal tubules was noted for EndoREZ™ compared to AH Plus™ ($p = 0.006759$) and Sealapex™ ($p = 0.000167$). The penetration depth of all tested sealers differed significantly using warm vertical compaction. The highest penetration depth was measured for AH Plus™, followed by Sealapex™, and finally EndoREZ™ (for 1d and 2d, $p = 0.00011$; 1d and 3d, $p = 0.000002$; 2d and 3d, $p = 000011$).

The mean value of the maximum penetration depth of AH Plus™, Sealapex™, and EndoREZ™ yielded 79.3% (1,204 μm) in the coronary thirds, 73.2% (1,005 μm) in the middle thirds, and 64% (770 μm) in the apical thirds. By analyzing each group, statistically significant differences in the maximum penetration depth between coronary, middle, and apical thirds of the root canals can be observed. In almost all groups, higher penetration depth in the coronary thirds compared to the middle thirds was noted, while the lowest value was measured in the apical thirds. The results are presented in detail in Table 3.

The SEM images of the control group showed complete smear layer removal and open dentinal tubules produced by the final irrigation protocol (Figure 3). The representative SEM micrographs (Figures 4–8) depict the dentinal tubular infiltration of AH Plus™, Sealapex™, and EndoREZ™ achieved in different groups.

Table 2

Basic indicators of descriptive statistics for the penetration depth of AH Plus™, Sealapex™, and EndoREZ™ into dentinal tubules for all experimental groups

Group	Arithmetic mean		95% CI limit		SD	Min–Max	Median
	%	μm	lower	upper			
RCS AH Plus™							
a	75.02	1,098.00	71.86	78.19	8.78	59.30–89.20	75.65
b	73.76	1,183.00	70.17	77.34	9.61	55.30–93.70	75.95
c	77.59	1,229.00	74.79	80.40	7.77	60.70–90.80	78.15
d	85.43	1,165.00	83.79	87.07	4.55	76.50–92.00	85.70
RCS Sealapex™							
a	74.70	1,113.00	71.68	77.71	8.22	59.30–93.10	75.10
b	70.81	997.00	67.79	73.83	8.10	53.40–85.40	70.80
c	75.03	985.00	71.07	79.00	10.42	50.70–90.10	79.10
d	66.35	778.00	62.57	70.13	9.55	52.20–81.60	65.70
RCS EndoREZ™							
a	75.07	1,010.00	71.58	78.56	9.85	58.30–93.20	78.80
b	80.63	1,154.00	77.87	83.39	7.78	65.00–92.30	81.10
c	73.89	883.00	70.79	77.00	8.16	55.80–84.50	75.30
d	46.21	502.00	40.99	51.42	10.15	21.30–62.70	48.10
Total	74.10	1,008.00	72.90	75.29	11.48	21.30–93.70	76.90

RCS – root canal sealer; a – cold lateral compaction technique; b – cone-fit technique; c – carrier-based technique; d – warm vertical compaction technique; CI – confidence interval; SD – standard deviation; Min – minimum; Max – maximum.

Table 3

Results of the Kruskal-Wallis ANOVA test for the penetration depth of AH Plus™, Sealapex™, and EndoREZ™ into dentinal tubules in the coronary (1), middle (2), and apical third (3) of the canal

Group	H-statistics	Degrees of freedom	*p*-value	*Post-hoc* test
RCS AH Plus™				
a	17.0501	2	0.0002	level 1 is significantly different from levels 2 and 3
b	15.786	2	0.0004	level 1 is significantly different from levels 2 and 3
c	15.8505	2	0.0004	level 3 is significantly different from levels 1 and 2
d	14.4999	2	0.0007	level 1 is significantly different from level 3
RCS Sealapex™				
a	22.5219	2	0.0000	level 3 is significantly different from levels 1 and 2
b	17.3372	2	0.0002	level 1 is significantly different from levels 2 and 3
c	13.7759	2	0.001	level 1 is significantly different from level 3
d	19.5621	2	0.0001	level 1 is significantly different from levels 2 and 3
RCS EndoREZ™				
a	17.9072	2	0.0001	level 3 is significantly different from levels 1 and 2
b	23.8586	2	0.0000	level 1 is significantly different from levels 2 and 3
c	13.6763	2	0.001	level 1 is significantly different from levels 2 and 3
d	6.3137	2	0.04	level 3 is significantly different from level 1

ANOVA – analysis of variance. For abbreviations of other terms, see Table 2.

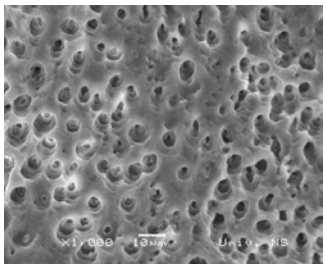


Fig. 3 – Control group: removed smear layer and open dentinal tubules.

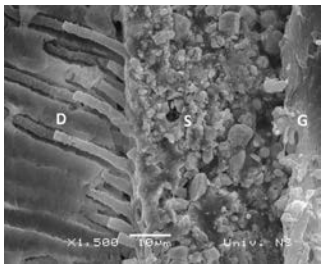


Fig. 4 – The penetration of AH Plus™ sealer with cone-fit technique at the middle third of the root; sealer tags are visible penetrating dentinal tubules. D – dentin; S – sealer; G – gutta-percha.

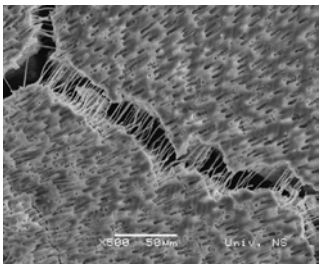


Fig. 5 – The penetration of AH Plus™ sealer with Thermafil at the middle third of the root; a micro crack can be seen as a result of the tooth specimen preparation for scanning electron microscopy; sealer tags are visible inside the crack.

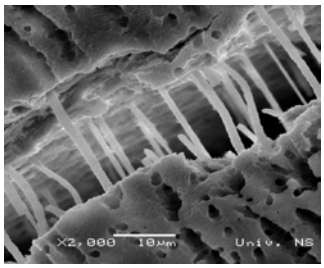


Fig. 6 – The penetration of EndoREZ™ sealer with cone-fit technique at the coronary third of the canal; a micro crack filled with sealer tags can be seen.

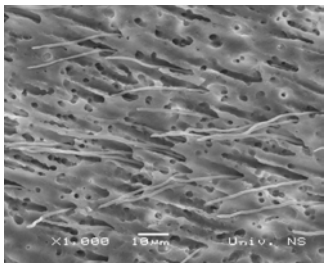


Fig. 7 – The penetration of Sealapex™ sealer using Thermafil at the apical third of the canal; extensive sealer penetration into dentinal tubules can be observed.

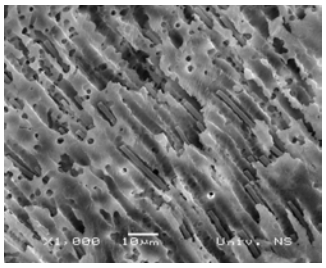


Fig. 8 – The penetration of AH Plus™ sealer with cone-fit technique at the middle third of the root; sealer tags penetrate deep into dentinal tubules.

Discussion

In the present study, the tubular penetration depth of RCSs was evaluated using SEM. Statistically significant differences were obtained between the groups. The sample standardization in the present study was achieved by focusing on mandibular molars with two mesial canals obturated using different combinations of sealers and techniques, whereby a sample of 66 mesial roots with two canals was segregated into 12 groups, allowing pairwise comparison between groups, as 12*11/2 = 66 represents the total number of ways to choose two different groups out of 12. In this way, the influence of factors such as internal root anatomy and dental permeability on the research results was minimized, i.e., all tested sealers had the same conditions to penetrate into dentinal tubules. The results were presented in μm, but also in percentages, i.e., the maximum sealer penetration depth with respect to the total distance: canal wall – root cement. This method eliminated any influence of factors such as the tooth size (i.e., the cross-section diameter) on the measurement results because, as can be seen in Table 2, the comparative values of the measurement results expressed in μm do not correspond exactly to the values expressed in percentages.

The influence of a smear layer on the sealer penetration depth was examined by many authors whose findings indicate that its effective removal results in deeper penetration [7, 9, 22]. On the other hand, sealer tubular penetration can serve as an indicator of the degree of the smear layer removal [23]. In the present study, the smear layer was removed by flushing with 17% EDTA, followed by 1% NaOCl and finally saline solution, which is consistent with the methods adopted in other studies and is in line with the recommendations of American Association of Endodontics for the work in clinical endodontics [1, 5, 24].

Dentinal tubules can be observed on longitudinal or transversal root sections. In teeth with curved canals, e.g., maxillary and mandibular molars, the longitudinal cross-section is difficult to perform, increasing the risk of producing artifacts, especially in the apical third [1, 2, 5, 25]. In the present study, transversal cross-sections of the roots were used, providing insight into the complete circumference of the corresponding third of the canal. Regional variations in the sealer penetration depth have been established by many authors, with a common finding that the lowest penetration depth is achieved in the apical third of the root canal [8, 11, 16]. The findings obtained in the present study are in line with these observations, as a significantly lower penetration depth of all tested sealers was measured in the apical third, while the variations between the coronary and middle thirds of the root were not significantly different. The apical third of the root is anatomically the most variable region, having fewer dentinal tubules of a smaller diameter, which are often closed or occluded with cement tissue, so the lowest measured depth of sealer tags was expected [5, 16].

Although manufacturers claim that RCSs are convenient for all obturation techniques, it is undeniable that the physicochemical properties of the sealer are affected by the heat application [2, 10, 26]. It was demonstrated in a previous study that the rheological properties of RCSs are highly temperature-dependent. Namely, the complex viscosity of AH Plus™ was significantly decreased with temperature increase, while the complex viscosity of Sealapex™ and EndoREZ™ behaved the opposite [10]. These findings might explain the higher penetration depth of AH Plus™ in combination with heated GP obturation techniques and the lower values obtained with Sealapex™ and EndoREZ™. Low-viscosity materials have the potential to penetrate deeper into the dentinal tubules. It was also confirmed in the recent study that AH Plus™ is more suitable for thermal endodontic obturation techniques compared to EndoREZ™ and other tested sealers [27]. Viapiana et al. [2] noted that heat affects the amino groups of AH Plus™ sealer, resulting in lower compressive strength, while in another study, it was found that film thickness and the setting time of resin-based RCSs were affected by the heat [28]. Baldi et al. [29] showed that the physicochemical properties of epoxy-based sealers, such as flowability and setting time, depend on the tube segment (initial, middle, and final) from which the materials were squeezed and mixed. All of these findings may be the reason for the different behavior of the materials after the application of heat.

The depth of sealer penetration into dentinal tubules has been measured by many authors, yielding inconsistent findings. While the results obtained in the majority of these studies are aligned with those presented in this work, in terms of measured length [1, 7, 30], a significantly lower measured depth was noted in others [5, 11, 25]. These conflicting research results can be attributed to a wide range of factors, most notably differences in sample selection, sample size calculation, the methods of smear layer removal, obturation technique applied, and sample preparation for microscope analysis. A lack of validation of the experimental method is certainly a contributing factor to the inconsistency of the results [31]. It should be kept in mind that the maximum penetration depth was measured and compared in the present study; if the average values were calculated and compared, which is extremely difficult to measure, the penetration depth results would certainly be lower. The results yielded by the present study pertaining to the tubular penetration of AH Plus™ and EndoREZ™ are in agreement with those reported by Mamootil and Messer [1], who used the same evaluation methods as those adopted in this work, but also confirmed the sealer penetration into dentinal tubules *in vivo* getting similar results: over 1,000 μm for epoxy resin-based RCS.

Conclusion

Within the limitations of this study, it can be concluded that the penetration depth of RCSs into dentinal tubules varies depending on the sealer type used, as well as its handling, i.e., combination with different obturation techniques. SEM is a suitable tool for the analysis of sealer penetrability as it provides precise measurement at a wide range of magnifications and allows detailed observation of ultrastructural morphology within the root dentin. Clinically, the results obtained might be applicable in the material selection for endodontic therapy and its proper handling.

R E F E R E N C E S

1. *Mamootil K, Messer HH.* Penetration of dentinal tubules by endodontic sealer cements in extracted teeth and in vivo. Int Endod J 2007; 40(11): 873–81.
2. *Viapiana R, Guerreiro-Tanomaru J, Tanomaru-Filho M, Camilleri J.* Interface of dentine to root canal sealers. J Dent 2014; 42(3): 336–50.
3. *Heran J, Khalid S, Albaaj F, Tomson PL, Camilleri J.* The single cone obturation technique with a modified warm filler. J Dent 2019; 89: 103181.
4. *Long J, Kreft JU, Camilleri J.* Antimicrobial and ultrastructural properties of root canal filling materials exposed to bacterial challenge. J Dent 2020; 93: 103283.
5. *Balguerie E, van der Sluis L, Vallaeys K, Gurgel-Georgelin M, Diemer F.* Sealer penetration and adaptation in the dentinal tubules: a scanning electron microscopic study. J Endod 2011; 37(11): 1576–9.
6. *Collares FM, Leitune VCB, Portella FF, Santos PD, Balbinot GS, Dos Santos LA,* et al. Methacrylate-based root canal sealer containing chlorexidine and α-tricalcium phosphate. J Biomed Mater Res B Appl Biomater 2018; 106(4): 1439–43.
7. *Turkyilmaz A, Erdemir A.* Comparison of dentin penetration ability of different root canal sealers used with different obturation methods. Microsc Res Tech 2020; 83(12): 1544–51.
8. *McMichael GE, Primus CM, Opperman LA.* Dentinal Tubule Penetration of Tricalcium Silicate Sealers. J Endod 2016; 42(4): 632–6.
9. *Kuçi A, Alaçam T, Yavaş O, Ergul-Ulger Z, Kayaoglu G.* Sealer penetration into dentinal tubules in the presence or absence of smear layer: a confocal laser scanning microscopic study. J Endod 2014; 40(10): 1627–31.
10. *Cvjeticanin M, Zorica D, Krstonošić V, Hadnađev M, Stojanac I, Ramic B,* et al. The Influence of Temperature on Rheological Properties of Three Root Canal Sealers. Mater Plastice 2022; 59(2): 174–82.
11. *Schmidt S, Schäfer E, Bürklein S, Rohrbach A, Donnermeyer D.* Minimal Dentinal Tubule Penetration of Endodontic Sealers in Warm Vertical Compaction by Direct Detection via SEM Analysis. J Clin Med 2021; 10(19): 4440.
12. *Küçük M, Kermeoğlu F.* Efficacy of different irrigation methods on dentinal tubule penetration of Chlorhexidine, QMix and Irritrol: A confocal laser scanning microscopy study. Aust Endod J 2019; 45(2): 202–8.
13. *Reynolds JZ, Augsburger RA, Svoboda KKH, Jalali P.* Comparing dentinal tubule penetration of conventional and "HiFlow" bioceramic sealers with resin-based sealer: An in vitro study. Aust Endod J 2020; 46(3): 387–93.
14. *Ramić BD, Stojanac IL, Drobac MR, Kantardžić IR, Maletin AZ, Cvjetićanin MT,* et al. Application of Scanning Electron Microscopy in the observation of dentin-adhesive interface. Microsc Res Tech 2021; 84(4): 602–7.
15. *Donnermeyer D, Schmidt S, Rohrbach A, Berlandi J, Bürklein S, Schäfer E.* Debunking the Concept of Dentinal Tubule Penetration of Endodontic Sealers: Sealer Staining with Rhodamine B Fluorescent Dye Is an Inadequate Method. Materials (Basel) 2021; 14(12): 3211.
16. *Caceres C, Larrain MR, Monsalve M, Peña Bengoa F.* Dentinal Tubule Penetration and Adaptation of Bio-C Sealer and AH-Plus: A Comparative SEM Evaluation. Eur Endod J 2021; 6(2): 216–20.
17. *Allan NA, Walton RC, Schaeffer MA.* Setting times for endodontic sealers under clinical usage and in vitro conditions. J Endod 2001; 27(6): 421–3. Erratum in: J Endod 2001; 27(10): 626.
18. *AlEisa K, Al-Dwairi ZN, Lynch E, Lynch CD.* In vitro evaluation of the effect of different endodontic sealers on retentive strength of fiber posts. Oper Dent 2013; 38(5): 539–44.
19. *Vertucci FJ.* Root canal anatomy of the human permanent teeth. Oral Surg Oral Med Oral Pathol 1984; 58(5): 589–99.
20. *Schneider SW.* A comparison of canal preparations in straight and curved root canals. Oral Surg Oral Med Oral Pathol 1971; 32(2): 271–5.
21. *Donnermeyer D, Schäfer E, Bürklein S.* Real-time Intracanal Temperature Measurement During Different Obturation Techniques. J Endod 2018; 44(12): 1832–6.
22. *Ordinola-Zapata R, Bramante CM, Bernardineli N, Graeff MS, Garcia RB, de Moraes IG,* et al. A preliminary study of the percentage of sealer penetration in roots obturated with the Thermafil and RealSeal-1 obturation techniques in mesial root canals of mandibular molars. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2009; 108(6): 961–8.
23. *Moon YM, Shon WJ, Baek SH, Bae KS, Kum KY, Lee W.* Effect of final irrigation regimen on sealer penetration in curved root canals. J Endod 2010; 36(4): 732–6.
24. *American Association of Endodontists.* Endodontics: Colleagues for Excellence. Obturation of root canal systems [Internet]. 2009 [accessed on 2023 July 13]. Available from: https://besteendoglenview.com/wp-content/uploads/2022/07/Obturation-of-root-canal-systems.pdf
25. *Viapiana R, Guerreiro-Tanomaru JM, Tanomaru-Filho M, Camilleri J.* Investigation of the effect of sealer use on the heat generated at the external root surface during root canal obturation using warm vertical compaction technique with System B heat source. J Endod 2014; 40(4): 555–61.
26. *Camilleri J.* Sealers and warm gutta-percha obturation techniques. J Endod 2015; 41(1): 72–8.
27. *Chavarria-Bolanos D, Komabayashi T, Shen I, Vega-Baudrit J, Gandolfi MG, Prati C,* et al. Effects of heat on seven endodontic sealers. J Oral Sci 2022; 64(1): 33–9.
28. *Rai RU, Singhal KP, Parekh V.* The effect of temperature on rheological properties of endodontic sealers. J Conserv Dent 2016; 19(2): 116–9.
29. *Baldi JV, Bernardes RA, Duarte MA, Ordinola-Zapata R, Cavenago BC, Moraes JC,* et al. Variability of physicochemical properties of an epoxy resin sealer taken from different parts of the same tube. Int Endod J 2012; 45(10): 915–20.
30. *Moradi S, Ghoddusi J, Forghani M.* Evaluation of dentinal tubule penetration after the use of dentin bonding agent as a root canal sealer. J Endod 2009; 35(11): 1563–6.
31. *Furtado TC, de Bem LA, Machado LS, Pereira JR, Só MVR, da Rosa RA.* Intratubular penetration of endodontic sealers depends on the fluorophore used for CLSM assessment. Microsc Res Tech 2021; 84(2): 305–12.

Received on October 17, 2022
Accepted on May 9, 2023
Online First May 2023

ORIGINAL ARTICLE

UDC: 616.314-089.843
DOI: https://doi.org/10.2298/VSP221110032D

Cone beam computed tomography analysis of maxillary vestibular bone thickness in the aesthetic region

Debljina vestibularne koštane lamele maksile u estetskoj regiji analizirana primenom kompjuterizovane tomografije konusnog zraka

Milica Djurdjević*, Marija Bubalo[†‡], Ana Luković[§], Ana Igić[‖], Aleksandar Acović*, Tatjana Kanjevac[¶]

University of Kragujevac, Faculty of Medical Sciences, *Department of Dentistry, [§]Department of Surgery, Kragujevac, Serbia; [†]Military Medical Academy, Dental Clinic, Belgrade, Serbia; [‡]University of Defence, Faculty of Medicine of the Medical Military Academy, Belgrade, Serbia; [‖]University of Niš, Faculty of Medicine, Preventive and Pediatric Dentistry, Niš, Serbia; [¶]Institute of Dentistry, Kragujevac, Serbia

Abstract

Background/Aim. Insufficient buccal bone thickness (thickness less than 2 mm) frequently leads to fenestration and dehiscence, and their consequences are additional bone resorption. That represents an additional problem during implant placement. Cone beam computed tomography (CBCT) is becoming a priority in the diagnosis of bone thickness needed for implant placement since it has proven to be an accurate and largely reliable diagnostic tool in the image of morphology and buccal wall thickness. The aim of this study was to measure the vestibular bone thickness of the anterior maxillary region in the Serbian population and compare the difference between men and women, as well as between the left and right sides of the jaw. **Methods.** CBCT images of 68 patients were examined from the existing database. The length from the cementoenamel junction to the beginning of the alveolar bone was measured, followed by the thickness of the vestibular bone at various clinically relevant locations. The data were statistically processed and analyzed. **Results.** A total of 373 teeth of the frontal region of the upper jaw, including 128 central incisors, 124 lateral incisors, and 121 canines, were analyzed. The thickness of the buccal bone in more than 88% of cases was less than 1.5 mm at all reference points, with mean values from 0.72 to 1.02 mm. **Conclusion.** A very small number of maxillary teeth have a vestibular bone thickness greater than 2 mm; therefore, the criterion to provide at least 2 mm of thickness needed for implant placement is difficult to meet. That increases the use of auxiliary methods of bone augmentation during immediate implant placement.

Key words:
alveolar bone loss; cone-beam computed tomography; dental implantation; maxilla; serbia.

Apstrakt

Uvod/Cilj. Nedovoljna debljina bukalne kosti (debljina manja od 2 mm) često dovodi do fenestracije i dehiscencije, a njihove posledice su dodatna resorpcija kosti. To predstavlja dodatni problem prilikom ugradnje implantata. Kompjuterizovana tomografija konusnog zraka (KTKZ) postaje prioritet prilikom dijagnostikovanja debljine kosti i planiranju ugradnje implantata, jer se u pogledu morfologije i debljine vestibularne lamele pokazala kao precizan i u velikoj meri pouzdan dijagnostički alat. Cilj rada bio je da se izmeri debljina vestibularne koštane lamele prednjih maksilarnih zuba u populaciji Srbije i uporedi razlika između muškaraca i žena, kao i između leve i desne strane vilice. **Metode.** Analizirani su snimci KTKZ 68 ispitanika, iz postojeće baze podataka. Izmerena je udaljenost gleđno-cementne granice od vrha alveolarnog grebena, a zatim i debljina vestibularne lamele na različitim, klinički relevantnim tačkama. Podaci su statistički obrađeni i analizirani. **Rezultati.** Analizirano je ukupno 373 zuba frontalne regije gornje vilice, uključujući 128 centralnih sekutića, 124 lateralnih sekutića i 121 očnjak. Debljina vestibularne lamele kod više od 88% slučajeva bila je manja od 1,5 mm na svim referentnim tačkama, sa srednjim vrednostima od 0,72 do 1,02 mm. **Zaključak.** Veoma mali broj maksilarnih frontalnih zuba ima debljinu vestibularne lamele veću od 2 mm; stoga je teško ispuniti kriterijum da se obezbedi najmanje 2 mm debljine kosti potrebne za implantaciju. Ovim se povećava potreba za primenom pomoćnih metoda uvećanja kosti prilikom neposredne ugradnje implantata.

Ključne reči:
alveolna kost, gubitak; kompjuterizovana tomografija konusnog zraka; implantacija, stomatološka; maksila; srbija.

Correspondence to: Aleksandar Acović, University of Kragujevac, Faculty of Medical Sciences, Department of Dentistry, Svetozara Markovica 69, 34 000 Kragujevac, Serbia. E-mail: dr.acovic115@gmail.com

Introduction

The anterior maxillary region is the segment of the maxilla in which the four upper incisors and two canines are located. It encompasses the area between the right to the left first premolars and is also often referred to as the "aesthetic zone" [1]. The thickness of the cortical bone of both the maxilla and mandible varies; palatally and lingually, it is usually thicker compared to the vestibular areas [2]. Previous studies have noted that insufficient bone thickness (thickness less than 2 mm) leads to frequent fenestration and dehiscence [3]. The immediate implant placement has primacy when placing teeth in the "aesthetic zone", considering that implants serve as replacements for frontal teeth, and the therapeutic approach is most influenced by the factors examined in this study, but also the shape of the face, the inclination of the teeth, and the type of malocclusion [3, 4].

There is an increasing interest in immediate implant placement [5, 6]. The existing vestibular cortical bone will determine the application technique, more precisely, the thickness and height of the bone itself [6–8]. As previously mentioned, at least 2 mm of buccal bone (BB) thickness is necessary after the formation of the implant bed to achieve gum support and reduce additional bone resorption [9]. Bone augmentation procedures are recommended to obtain adequate bone contour when it is impossible to provide sufficient BB thickness [9, 10].

Up to now, several retrospective studies analyzed the thickness of the BB; in the majority of studies, the data show that this thickness is below 1 mm, in some cases even less than 0.5 mm, while the tooth is still present in the alveolus [11–13]. Loss of teeth will lead to additional bone resorption. Many authors suggest placing the implant at a greater distance from the BB to prevent changes in dimension [3].

Since its introduction, cone beam computed tomography (CBCT) has found a place in many branches of dentistry due to its advantages over conventional radiographic techniques [14]. CBCT is becoming a priority in the diagnosis and planning of oral and maxillofacial surgeries, as well as implant placement. Besides the three-dimensional representation and noninvasiveness, CBCT has a low radiation dose and high resolution. It has proven to be an accurate and largely reliable diagnostic tool in the image of morphology and buccal wall thickness [15]. Therefore, the CBCT is suitable for this type of research. The aim of this study was to measure the vestibular (buccal) bone thickness of anterior maxillary teeth in the Serbian population and compare the difference between men and women, as well as between the left and right sides of the jaw.

Methods

Sample selection

Images of 68 patients (36 women and 32 men, mean age 42 years, range 20–70 years) were examined with CBCT from the existing database. All recordings were made at the Department of Dentistry at the Medical Faculty of the University of Kragujevac, Serbia from October 25, 2014, to December 20, 2019. Ethical approval was obtained from the local Ethics Committee (No. 01-8735).

Patients were subjected to recording only in indicated cases, not for analysis of recordings for research purposes. The inclusion criteria were CBCT scans made during the diagnosis, therapy planning, and treatment in the cases of prosthetic, surgical, or orthodontic indications. The exclusion criteria were pregnancy, patients on chemotherapy, and radiation therapy. Patients with advanced periodontitis or a history of periodontitis were also excluded from the study. Teeth with large fillings, metal and ceramic crowns, endodontic treatment, and chronic inflammatory diseases of the periapex were not analyzed. Images that did not show the frontal teeth completely, on which the region of the examined teeth was cut or distorted for technical reasons, were excluded from the analysis.

Image analysis

The scans were done on an Orthophos XG 3D device (Sirona Dental Systems GmbH, Bensheim, Germany). All measurements were analyzed on a standard monitor (Philips LED monitor, 23 inches, 1,920 × 1,080 pixels); the analyzed field size was 8 × 8 cm. In the frontal region (the area of central incisors, lateral incisors, and canines), the thickness of the BB was analyzed, and the images were placed so that the cross-section of the sagittal and transversal plane passes through the longitudinal axis of the tooth, while the vertical plane adjusts to the set sections. The length from the cementoenamel junction (CEJ) to the beginning of the alveolar bone was measured, followed by the thickness of the BB at various clinically relevant locations. Two researchers analyzed the collected data. Using the "measure distance" tool and "measure along path" tool on the sagittal section, the following values were measured: 1) the distance of the CEJ from the beginning of the bone crest (BC), CEJ-BC; 2) the thickness of the facial plate at the beginning of BC; 3) the thickness of the facial plate at 2, 5, and 8 mm from BC (BC-2, BC-5, BC-8) (Figure 1).

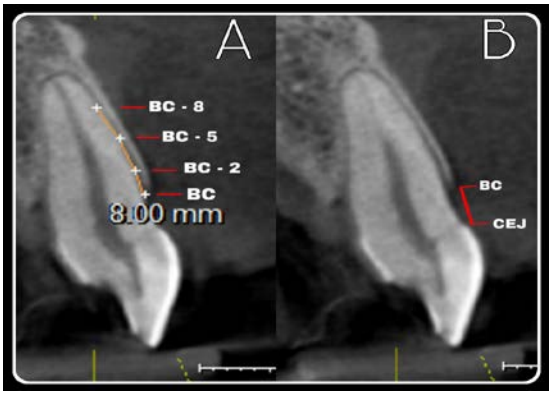


Fig. 1 – A) "Measure along path" tool was used to make points at the bone crest (BC), 2 mm, 5 mm, and 8 mm from BC. B) The distance of the cementoenamel junction (CEJ) from the beginning of BC ("measure distance" tool was used).

Statistical analysis

All the data were statistically analyzed with both descriptive and analytical tests using the software SPSS v20.0 (SPSS Inc., Chicago, IL, USA). Mann–Whitney *U* test, Friedman's test, and Wilcoxon signed-rank test were used to compare differences between measurements. The significance level was set at $p \leq 0.05$.

Results

The data from 68 patients were statistically processed, analyzing a total of 373 maxillary teeth of the frontal region, including 128 central incisors, 124 lateral incisors, and 121 canines. The mean value of the distance of the CEJ from the beginning of the bone was 2 mm, with the highest value observed on the lateral incisors, without a statistically significant difference between the groups (Table 1).

Table 2 shows the mean values of the distance of the CEJ from the beginning of the alveolar bone and the thickness of the BB of each analyzed tooth on the left and right sides. A significantly higher value between the left and right sides was observed on the left central incisors at BC ($p = 0.000$), BC-2 mm ($p = 0.001$), and BC-5 mm ($p = 0.003$). No statistically significant difference in BB thickness or the distance of the CEJ from the beginning of the alveolar bone was observed on the remaining teeth.

Table 3 shows the number and percentage of teeth in three categories (< 1.5 mm, 1.5–2.0 mm, and > 2 mm) concerning the BB thickness at defined reference points. The largest number of teeth (86–93%) had a BB thickness of less than 1.5 mm at all reference levels. The lowest prevalence of central incisors, lateral incisors, and canines was observed in the group > 2 mm, with the largest number of teeth within the group having a buccal wall thickness greater than 2 mm at the reference point BC-8 mm on lateral incisors (7.3%) and canines (3.3%).

In all groups (central incisors, lateral incisors, and canines), a significantly smaller thickness of the buccal wall on the BC point was shown compared to other measurement points ($p < 0.05$). At the BC-2 mm reference point, significantly less BB thickness was observed compared to BC-5 mm and BC-8 mm on the central incisors, and BC-5 mm on the lateral incisors (Table 4).

A comparison between the genders showed that the thickness of the buccal wall at all reference points was greater in men. The statistically most significant difference between men and women ($p = 0.000$) was present at BC-5 mm on the lateral incisors and BC-8 mm on the canines (Table 5).

Table 6 shows the values of Pearson and Spearman correlation coefficients. No correlation was found between the age of the subjects and the thickness of the buccal lamella of the upper jaw in the area of the front teeth.

Table 1

Distance between cementoenamel junction (CEJ) and bone crest (BC) and results of comparison between groups

Parameter	Central incisors	Lateral incisors	Canines
Number of teeth used	128	124	121
CEJ-BC, mm	2.04 ± 0.80	2.05 ± 0.88	2.1 ± 0.81

Values are presented as mean ± standard deviation.
***Statistically not significant ($p > 0.05$, *t*-tests).**

Table 2

The buccal bone thicknesses of the upper anterior teeth and results of comparison between the left and right side

Parameter	Central incisors		Lateral incisors		Canines	
	left side	right side	left side	right side	left side	right side
Tooth number	11	21	12	22	13	23
Number of teeth used	65	63	63	61	63	58
CEJ-BC distance	1.98 ± 0.92	2.04 ± 0.77	2.15 ± 0.92	2.01 ± 0.81	2.16 ± 0.88	2.07 ± 0.72
BC	0.63 ± 0.20	0.81 ± 0.39*	0.7 ± 0.35	0.81 ± 0.39	0.78 ± 0.35	0.81 ± 0.28
BC-2 mm	0.75 ± 0.30	0.86 ± 0.26*	0.97 ± 0.42	1.08 ± 1.09	0.91 ± 0.4	0.96 ± 0.5
BC-5 mm	0.76 ± 0.36	0.87 ± 0.35*	0.81 ± 0.44	0.87 ± 0.46	0.86 ± 0.38	0.92 ± 0.6
BC-8 mm	0.94 ± 0.57	1.00 ± 0.5	0.93 ± 0.52	1.04 ± 0.64	0.85 ± 0.36	0.95 ± 0.61

CEJ – cementoenamel junction; BC – bone crest. Values (in mm) are presented as mean ± standard deviation.
***Statistically significant difference between right and left side, $p \leq 0.05$ (Mann–Whitney *U* test).**

Table 3

The buccal bone thickness within three categories at different levels

Parameter	Central incisors			Lateral incisors			Canines		
	< 1.5	1.5–2	> 2	< 1.5	1.5–2	> 2	<1.5	1.5–2	> 2
BC	125 (97.7)	2 (1.6)	1 (0.8)	120 (96.8)	1 (0.8)	3 (2.4)	119 (98.3)	1 (0.8)	1 (0.8)
BC-2 mm	125 (97.7)	3 (2.3)	0 (0)	110 (88.7)	12 (9.7)	2 (1.6)	108 (89.3)	10 (8.3)	3 (2.5)
BC-5 mm	119 (93)	8 (6.1)	1 (0.8)	110 (88.7)	11 (8.9)	3 (2.4)	111 (91.7)	9 (7.4)	1 (0.8)
BC-8 mm	119 (93)	8 (6.1)	1 (0.8)	107 (86.3)	8 (6.5)	9 (7.3)	109 (90.1)	8 (6.0)	4 (3.3)
Total	122 (95.3)	6 (4.4)	0 (0)	116 (93.4)	6 (4.4)	2 (1.6)	116 (95.9)	4 (3.3)	1 (0.8)

BC – bone crest. < 1.5 – less than the required thickness; 1.5–2 – minimally required thickness; > 2 – preferable required thickness. All values (in mm) are expressed as numbers (percentages).

Table 4

Results of comparison between buccal bone thickness at different levels

Parameter	Central incisors	Lateral incisors	Canines
Number of teeth used	128	124	121
BC	0.72 ± 0.32[a]	0.76 ± 0.37[a]	0.79 ± 0.32[a]
BC-2 mm	0.81 ± 0.29[b]	1.02 ± 0.83[b]	0.94 ± 0.45[b]
BC-5 mm	0.82 ± 0.36[c]	0.86 ± 0.43[c]	0.89 ± 0.50[b]
BC-8 mm	0.99 ± 0.54[d]	0.97 ± 0.59[b]	0.90 ± 0.50[b]
**p*-value	≤ 0.001	≤ 0.001	≤ 0.005

BC – bone crest. Values (in mm) are presented as mean ± standard deviation.
***Significant at $p \leq 0.05$ (Friedman's test, Wilcoxon signed-rank test, Bonferroni's correction). Different letters in the same column represent statistically significant differences among groups (BC, BC-2, BC-5, BC-8). A statistically significant difference exists between the groups for each tooth when they are labeled with distinct letters in the table. If the groups are marked with the same letter, there is no statistically significant difference between them.**

Table 5

Thickness of buccal plate of maxillary anterior and results of comparison between males and females

Parameter	Females	Males	*p*-value
Central incisors			
number of teeth used	66	62	
BC	0.67 ± 0.20	0.78 ± 0.41	0.217
BC-2 mm	0.80 ± 0.31	0.82 ± 0.27	0.539
BC-5 mm	0.79 ± 0.37	0.86 ± 0.34	0.044*
BC-8 mm	0.94 ± 0.55	1.03 ± 0.52	0.299
Lateral incisors			
number of teeth used	68	56	
BC	0.70 ± 0.33	0.83 ± 0.41	0.013*
BC-2 mm	0.86 ± 0.30	1.22 ± 1.15	0.002*
BC-5 mm	0.70 ± 0.30	1.06 ± 0.49	0.000*
BC-8 mm	0.83 ± 0.48	1.14 ± 0.66	0.001*
Canines			
number of teeth used	63	58	
BC	0.77 ± 0.35	0.83 ± 0.28	0.084
BC-2 mm	0.83 ± 0.41	1.05 ± 0.47	0.002*
BC-5 mm	0.81 ± 0.57	0.99 ± 0.39	0.001*
BC-8 mm	0.78 ± 0.51	1.03 ± 0.45	0.000*

BC – bone crest. Values (in mm) are presented as mean ± standard deviation.
***Significant at $p \leq 0.05$ (Mann-Whitney *U* test).**

Table 6

Correlation coefficients between age and the buccal bone thickness of the alveolar bone measurement points in the maxillary anterior teeth

Measurement points	Age	
	Spearman's rho	*p*-value*
Central incisors		
BC	0.69	0.733
BC-2 mm	0.128	0.475
BC-5 mm	0.59	0.914
BC-8 mm	-0.201	0.112
Lateral incisors		
BC	0.372	0.497
BC-2 mm	0.247	0.090
BC-5 mm	0.131	0.324
BC-8 mm	0.11	0.673
Canines		
BC	-0.13	0.898
BC-2 mm	0.33	0.909
BC-5 mm	-0.003	0.969
BC-8 mm	-0.62	0.737

BC – bone crest. *Pearson correlation is significant at 0.01 level.

Discussion

It is known that in 80% of cases, the tooth roots are located next to the buccal plate, in the anterior maxillary region, and in 87% of cases, its thickness is less than 1 mm (average value is 0.8 mm), while in only 3% of cases, the thickness is 2 mm. Dehiscence or fenestration of the buccal plate due to vertical root fracture, endodontic complications, or tooth extraction is common. A thin or damaged buccal plate, 0.5–0.6 mm thick, is predominantly present in the extraction sockets of the anterior maxillary region [3, 16–18]. In patients where immediate implants should be placed, the buccal plate is missing in 24–57% of cases, which correlates with a greater gingival recession [3].

Since the buccal plate is essential for the long-term stability of the gingival margin, placing the implant in sockets with an insufficient buccal plate, without bone augmentation, would result in a gingival recession [19].

Regardless of the diversity of the implant system, the correct three-dimensional position is the most important in terms of the aesthetic outcome of the treatment. Satisfactory height and width of facial bones can ensure the implant's long-term stability [20, 21]. In addition to stability, the height and the width of the buccal alveolar bone have an impact on the soft tissues that cover them, especially the interdental papilla [22–26]. A better aesthetic effect and lower frequency of gingival recession were observed after implantation in sockets with higher alveolar bone height and in thicker bone biotypes [27].

According to the results of this study, the distance of the CEJ from the beginning of the alveolar bone was 2–3 mm, confirming the statement that the head of the implant should be placed 3 mm below the imaginary line that joins the CEJ of adjacent teeth [28, 29].

The analysis of this study showed that the thickness of the BB in the anterior maxilla, in more than 88% of cases, was less than 1.5 mm at all reference points, with mean values from 0.72 to 1.02 mm. Similar results were found by Sheerah et al. [3], wherein the group of less than 1.5 mm were 88.7% of central incisors, 83.3% of lateral incisors, and 95.2% of canines. Slightly lower values were shown by Rangari et al. [10], who found 32–53% of central and lateral incisors and 36–58% of canines in the group < 1 mm.

BB thickness of less than 1 mm was observed in the area of central incisors, lateral incisors, and canines on all teeth except at reference point BC-8, where the value was slightly above 1 mm. Similar values were observed in other studies, and, in these cases, placing the implant more palatal is suggested, as the BB plate is thin. It is important to place the implant shaft to match the incisal edges of adjacent teeth or to place it more palatal in relation to the mentioned landmark, which leads to the possibility of bone perforation [28, 29].

In this study, the analysis showed that thicker BB (mean thickness of > 2 mm) was present in only 0–0.8% of central incisors, 1.6–2.4% at all reference points except BC-8 where thicker bone was found in as much as 7.3%, while on the canines it occurred in 0.8–3.3%. Similar results have been shown in other studies [6, 30]. Compared to the available literature, a higher prevalence was observed in the study of Fuentes et al. [31] in central incisors (14.4%), lateral incisors (6.2%), and canines (9%); Ghassemian et al. [32] presented similar results. Different results may be due to differences in the surveyed populations, the number of respondents, and many other factors. On the other hand, a minimum buccal plate thickness of 2 mm proved to be an essential feature for maintaining the vertical dimension after tooth extraction [33]. Studies examining gender and age differences have shown that these factors might be important when planning immediate implant placement [34, 35], while the effect of systemic diseases was not statistically significant at the reference point 8 mm from the alveolar bone crest [36]. In this study, a statistically significant difference between men and women was also confirmed, and the women had a significantly smaller BB plate thickness than males.

A statistically significant difference between the left and right sides was present between the central incisors at the alveolar bone crest and the distance of 2 mm and 5 mm from it. There were no statistically significant differences between the left and right sides of the remaining teeth, which coincides with the data from the literature [6, 37]. Similar to the results of other studies, there was no correlation between age and buccal plate thickness of the aesthetic region [6, 30].

A limitation in the present study, and one common to similar studies using CBCT measurements, is the unreliability of the images when measuring bone plate thinner than 1 mm. Behnia et al. [38] evaluated that CBCT is very accurate when measuring bone thickness greater than 1 mm, while radiographic measurement of smaller dimensions more often overestimates bone thickness compared to direct measurement using calipers. Another potential limitation is the small sample size. A larger number of patients is needed to obtain more valid results, considering that a certain number of patients did not have all the teeth in the aesthetic region.

Conclusion

This study shows that a very small number of maxillary teeth have a BB thickness > 2 mm, and thus, it can be concluded that the bone in the area of the anterior maxillary teeth is mostly thin. Therefore, the criterion to provide at least 2 mm of thickness is difficult to meet, which increases the use of auxiliary methods of bone augmentation during immediate implant placement. Consequently, CBCT scans are highly recommended for implantation planning to minimize complications and observe the critical dimensions of the BB before starting surgical therapy.

R E F E R E N C E S

1. *Trevizan M, Consolaro A.* Premaxilla: an independent bone that can base therapeutics for middle third growth! Dental Press J Orthod 2017; 22(2): 21–6.
2. *Garib DG, Yatabe MS, Ozawa TO, da Silva Filho OG.* Alveolar bone morphology under the perspective of the computed tomography: Defining the biological limits of tooth movement. Dental Press J Orthod 2010; 15(5): 192–205.
3. *Sheerah H, Othman B, Jaafar A, Alsharif A.* Alveolar bone plate measurements of maxillary anterior teeth: a retrospective cone beam computed tomography study, AlMadianh, Saudi Arabia. Saudi Dent J 2019; 31(4): 437–44.
4. *Morad G, Behnia H, Motamedian SR, Shahab S, Gholamin P, Khosraviani K,* et al. Thickness of labial alveolar bone overlying healthy maxillary and mandibular anterior teeth. J Craniofac Surg 2014; 25(6): 1985–91.
5. *Naghibi N, Fatemi K, Hoseini-Zarch SH, Sadeghi B, Fasihi Ramandi M.* CBCT evaluation of buccal bone thickness in the aesthetic zone of menopausal women: A cross-sectional study. Clin Exp Dent Res 2022; 8(5): 1076–81.
6. *London RM.* The esthetic effects of implant platform selection. Compend Contin Educ Dent 2001; 22(8): 675–82; quiz 683.
7. *Zhou Z, Chen W, Shen M, Sun C, Li J, Chen N.* Cone beam computed tomographic analyses of alveolar bone anatomy at the maxillary anterior region in Chinese adults. J Biomed Res 2014; 28(6): 498–505.
8. *Zhang X, Li Y, Ge Z, Zhao H, Miao L, Pan Y.* The dimension and morphology of alveolar bone at maxillary anterior teeth in periodontitis: a retrospective analysis-using CBCT. Int J Oral Sci 2020; 12(1): 4.
9. *Becker W, Sennerby L, Bedrossian E, Becker BE, Lucchini JP.* Implant stability measurements for implants placed at the time of extraction: a cohort, prospective clinical trial. J Periodontol 2005; 76(3): 391–7.
10. *Rangari P, Singh U, Singh N, Dewangan A.* A Retrospective Study Evaluating the Anterior Bone Wall Thickness Using Cone Beam Computed Tomography Images with a Three Dimensional Software. J Med Sci Clin Res 2018; 6(4): 849–54.
11. *Arango E, Plaza-Ruíz SP, Barrero I, Villegas C.* Age differences in relation to bone thickness and length of the zygomatic process of the maxilla, infrazygomatic crest, and buccal shelf area. Am J Orthod Dentofacial Orthop 2022; 161(4): 510–8. e1.
12. *El Nahass H, N Naiem S.* Analysis of the dimensions of the labial bone wall in the anterior maxilla: a cone-beam computed tomography study. Clin Oral Implants Res 2015; 26(4): e57–61.
13. *Al-Haj Husain A, Stadlinger B, Özcan M, Schönegg D, Winklhofer S, Al-Haj Husain N,* et al. Buccal bone thickness assessment for immediate anterior dental implant planning: A pilot study comparing cone-beam computed tomography and 3D double-echo steady-state MRI. Clin Implant Dent Relat Res 2023; 25(1): 35–45.
14. *Venkatesh E, Elluru SV.* Cone beam computed tomography: basics and applications in dentistry. J Istanb Univ Fac Dent 2017; 51(3 Suppl 1): S102–21.
15. *Khoury J, Ghosn N, Mokbel N, Naaman N.* Buccal Bone Thickness Overlying Maxillary Anterior Teeth: A Clinical and Radiographic Prospective Human Study. Implant Dent 2016; 25(4): 525–31.
16. *Braut V, Bornstein MM, Belser U, Buser D.* Thickness of the anterior maxillary facial bone wall- a retrospective radiographic study using cone beam computed tomography. Int J Periodontics Restorative Dent 2011; 31(2): 125–31.
17. *Vera C, De Kok IJ, Reinhold D, Limpiphipatanakorn P, Yap AK, Tyndall D,* et al. Evaluation of buccal alveolar bone dimension of maxillary anterior and premolar teeth: a cone beam computed tomography investigation. Int J Oral Maxillofac Implants 2012; 27(6): 1514–9.
18. *Chen ST, Darby I.* The relationship between facial bone wall defects and dimensional alterations of the ridge following flapless tooth extraction in the anterior maxilla. Clin Oral Implants Res 2017; 28(8): 931–7.
19. *Kuchler U, Chappuis V, Gruber R, Lang NP, Salvi GE.* Immediate implant placement with simultaneous guided bone regeneration in the esthetic zone: 10-year clinical and radiographic outcomes. Clin Oral Implants Res 2016; 27(2): 253–7.
20. *Belser UC, Buser D, Hess D, Schmid B, Bernard JP, Lang NP.* Aesthetic implant restorations in partially edentulous patients - a critical appraisal. Periodontol 2000 1998; 17: 132–50.
21. *Buser D, von Arx T.* Surgical procedures in partially edentulous patients with ITI implants. Clin Oral Implants Res 2000; 11(Suppl 1): 83–100.
22. *Tarnow DP, Magner AW, Fletcher P.* The effect of the distance from the contact point to the crest of bone on the presence or absence of the interproximal dental papilla. J Periodontol 1992; 63(12): 995–6.
23. *Choquet V, Hermans M, Adriaenssens P, Daelemans P, Tarnow DP, Malevez C.* Clinical and radiographic evaluation of the papilla level adjacent to single-tooth dental implants. A retrospective study in the maxillary anterior region. J Periodontol 2001; 72(10): 1364–71.
24. *Ryser MR, Block MS, Mercante DE.* Correlation of papilla to crestal bone levels around single tooth implants in immediate or delayed crown protocols. J Oral Maxillofac Surg 2005; 63(8): 1184–95.
25. *Palmer RM, Farkondeh N, Palmer PJ, Wilson RF.* Astra Tech single-tooth implants: an audit of patient satisfaction and soft tissue form. J Clin Periodontol 2007; 34(7): 633–8.
26. *Lops D, Chiapasco M, Rossi A, Bressan E, Romeo E.* Incidence of inter-proximal papilla between a tooth and an adjacent immediate implant placed into a fresh extraction socket: 1-year prospective study. Clin Oral Implants Res 2008; 19(11): 1135–40.
27. *Le BT, Borzabadi-Farahani A.* Labial Bone Thickness in Area of Anterior Maxillary Implants Associated with Crestal Labial Soft Tissue Thickness. Implant Dent 2012; 21(5): 406–10.
28. *Langer B, Sullivan DY.* Osseointegration: its impact on the interrelationship of periodontics and restorative dentistry. Part 3. Periodontal prosthesis redefined. Int J Periodontics Restorative Dent 1989; 9(4): 240–61.
29. *Lee SL, Kim HJ, Son MK, Chung CH.* Anthropometric analysis of maxillary anterior buccal bone of Korean adults using cone-beam CT. J Adv Prosthodont 2010; 2(3): 92–6.
30. *Nowzari H, Molayem S, Chiu CH, Rich SK.* Cone beam computed tomographic measurement of maxillary central incisors to determine prevalence of facial alveolar bone width ≥2 mm. Clin Implant Dent Relat Res 2012; 14(4): 595–602.
31. *Fuentes R, Flores T, Navarro P, Salamanca C, Beltrán V, Borie E.* Assessment of buccal bone thickness of aesthetic maxillary region: a cone-beam computed tomography study. J Periodontal Implant Sci 2015; 45(5): 162–8.
32. *Ghassemian M, Nowzari N, Lajolo C, Verdugo F, Pirronti T, D'Addona A.* The thickness of facial alveolar bone overlying healthy maxillary anterior teeth. J. Periodontol 2012; 83(2): 187–97.
33. *Belser U, Martin W, Jung R, Hämmerle C, Schmid B, Morton D,* et al. Implant therapy in the esthetic zone: single-tooth replacements. In: *Buser D, Belser U, Wismeijer D*, editors. ITI Treatment Guide Series, Volume 1. Berlin (DE): Quintessence Publishing Co. Ltd.; 2006. p. 268.
34. *Boskey AL, Coleman R.* Aging and bone. J Dent Res 2010; 89(12): 1333–48.

35. *Kelly PJ, Twomey L, Sambrook PN, Eisman JA*. Sex differences in peak adult bone mineral density. J Bone Miner Res 1990; 5(11): 1169–75.
36. *Farahamnd A, Sarlati F, Eslami S, Ghassemian M, Youssefi N, Jafarzadeh Esfahani B*. Evaluation of Impacting Factors on Facial Bone Thickness in the Anterior Maxillary Region. J Craniofac Surg 2017; 28(3): 700–5.
37. *Zekry A, Wang R, Chau AC, Lang NP*. Facial alveolar bone wall width - a cone-beam computed tomography study in Asians. Clin Oral Implants Res 2014; 25(2): 194–206.
38. *Behnia H, Motamedian SR, Kiani MT, Morad G, Khojasteh A*. Accuracy and reliability of cone beam computed tomographic measurements of the bone labial and palatal to the maxillary anterior teeth. Int J Oral Maxillofac Implants 2015; 30(6): 1249–55.

Received on November 10, 2022
Revised on May 12, 2023
Accepted on May 23, 2023
Online First June 2023

ORIGINAL ARTICLE
UDC: 616.13-089-037
DOI: https://doi.org/10.2298/VSP230516033S

Virtual ankle-brachial index – can the immediate outcome of femorodistal bypass surgery be predicted?

Virtuelni brahijalni indeks gležnja – može li se predvideti neposredni ishod femorodistalne bajpas hirurgije?

Dragan B. Sekulić*, Aleksandar P. Tomić*†, Andreja D. Dimić‡§, Aleksandar C. Mitrović‡, Lazar B. Davidović‡§, Dragana S. Paunović*, Dalibor D. Nikolić‖, Uroš M. Miladinović¶, Igor M. Sekulić†¶, Nemanja K. Rančić†¶ , Momir M. Šarac*†, Ivan R. Marjanović*†, Ivan R. Leković*†, Boško I. Milev†††**

Military Medical Academy, *Clinic for Vascular and Endovascular Surgery, ¶Institute for Radiology, **Center for Clinical Pharmacology, ††Clinic for General Surgery, Belgrade, Serbia; †University of Defence, Faculty of Medicine of the Military Medical Academy, Belgrade, Serbia; ‡University Clinical Center of Serbia, Clinic for Vascular and Endovascular Surgery, Belgrade, Serbia; §University of Belgrade, Faculty of Medicine, Belgrade, Serbia; ‖University of Kragujevac, Faculty of Engineering Sciences, Kragujevac, Serbia

Abstract

Background/Aim. The best treatment for the occlusion of the largest artery in the thigh is a femorodistal (FD) bypass. Ankle-brachial index (ABI) and multidetector computed tomographic (MDCT) angiography are the gold standards for diagnosing peripheral arterial occlusive disease. The finite element analysis (FEA) method can help measure the quantity of blood flow and arterial pressure in the arteries in the leg. The aim of this study was to examine the possibility of using the FEA method in predicting the outcome of FD bypass surgery. **Methods.** The study involved 45 patients indicated for FD arterial reconstruction from December 1, 2021, to March 31, 2023. Each patient underwent pre- and postoperative MDCT angiography of the arteries of the lower extremities, on the basis of which, with the use of FEA, models were made for measuring ABI. All patients had their ABI measured preoperatively and postoperatively using the Doppler ultrasound and sphygmomanometer. Based on the findings of the preoperative MDCT angiography, postoperative virtual surgical models were created using the FEA method, on which ABI were also measured. The values of ABI were divided into five groups: ABI measured preoperatively (ABI pre-op), ABI measured postoperatively (ABI post-op), ABI measured on FEA models based on the MDCT findings [ABI (sim) pre-op], ABI sim post-op, and ABI measured on virtual surgery model [ABI sim post-op (virtual)]. The ABI of the models were statistically compared with preoperative and postoperative measurements done on patients. **Results.** The values based on the virtual ABI model did not show significant differences compared to the values obtained on patients and values obtained with the FEA method using MDCT angiography ($p < 0.001$). A strong statistically significant correlation was shown between the virtual ABI and the values obtained by the other two methods, measured on the postoperative MDCT angiography model and virtual postoperative model ($p < 0.001$). **Conclusion.** Virtual simulation based on the MDCT angiography parameters of peripheral blood vessels can be successfully used to predict the immediate outcome of the FD bypass surgery.

Key words:
arterial occlusive diseases; ankle brachial index; computed tomography angiography; finite element analysis; image interpretation, computer-assisted; leg; prognosis; ultrasonography.

Apstrakt

Uvod/Cilj. Najbolji način lečenja okluzije površne femoralne arterije je femorodistalni (FD) bajpas. Brahijalni indeks gležnja (BIG) i angiografija primenom metode multidetektorske kompjuterizovane tomografije (MDKT) predstavljaju „zlatni standard" u dijagnostici periferne okluzivne bolesti arterija. Analiza konačnih elemenata (AKE) može pomoći u merenju količine protoka krvi i arterijskog pritiska u arterijama donjih ekstremiteta. Cilj rada bio je da se ispita mogućnost korišćenja AKE u predviđanju ishoda FD bajpas hirurgije. **Metode.** Istraživanjem je

Correspondence to: Dragan B. Sekulić, Military Medical Academy, Clinic for Vascular and Endovascular Surgery, Crnotravska 17, 11 000 Belgrade, Serbia. E-mail: doktorsekula@gmail.com

obuhvaćeno 45 bolesnika kojima je indikovana FD arterijska rekonstrukcija u periodu od 01. decembra 2021. do 31. marta 2023. godine. Svakom bolesniku je preoperativno i postoperativno urađena angiografija arterija donjih ekstremiteta primenom MDKT, na osnovu koje su, uz korišćenje AKE, napravljeni modeli na kojima su mereni BIG. Svim bolesnicima su mereni BIG preoperativno i postoperativno, korišćenjem Doppler ultrazvuka i sfigmomanometra. Na osnovu preoperativne MDKT angiografije, korišćenjem metode AKE, napravljeni su postoperativni virtuelni hirurški modeli, na kojima su takođe mereni BIG. Vrednosti BIG raspoređene su u pet grupa: BIG meren preoperativno (BIG *pre-op*), BIG meren postoperativno (BIG *post-op*), BIG meren na modelima konačnih elemenata dobijenim primenom MDKT [BIG (*sim*) *pre-op*], BIG *sim post-op*, i BIG dobijen merenjem na virtuelnom hirurškom modelu [BIG *sim post-op* (*virtual*)]. Statistički su upoređivane vrednosti BIG dobijene na modelima sa vrednostima dobijenim merenjem na bolesnicima. **Rezultati.** Vrednosti dobijene na osnovu virtuelnih BIG modela nisu pokazale značajnu razliku u poređenju sa vrednostima dobijenim merenjem na bolesnicima i vrednostima dobijenim primenom AKE uz MDKT angiografiju ($p < 0{,}001$). Značajna statistička korelacija pokazana je između vrednosti virtuelnih BIG i vrednosti dobijenih primenom druge dve metode, merenim na postoperativnom modelu MDKT angiografije i na virtuelnom postoperativnom modelu ($p < 0{,}001$). **Zaključak.** Virtuelna simulacija parametara dobijenih primenom MDKT angiografije perifernih krvnih sudova može se uspešno koristiti za predviđanje neposrednog ishoda FD bajpas hirurgije.

Ključne reči:
arterije, okluzione bolesti; brahijalni indeks gležnja; angiografija, tomografska, kompjuterizovana; analiza konačnih elemenata; kompjuterski asistirano tumačenje slika; noga; prognoza; ultrasonografija.

Introduction

The blockage of the largest artery in the thigh caused by peripheral arterial occlusive disease (PAOD) of the lower limbs affects 12–14% of the general population, and the prevalence increases with age [1]. Nowadays, the best treatment is femoropopliteal or femorocrural bypass, where the blocked portion of the artery is bypassed by adding a piece of great saphenous vein or a synthetic vascular graft [2]. The diagnostic assessment of PAOD is based on the measurement of the ankle-brachial index (ABI), i.e., the ratio of the systolic pressure measured at the ankle and the systolic pressure measured at the brachial artery (BA). To calculate ABI_{ATP}, systolic pressure is measured in the *arteria tibialis posterior* (ATP), and to calculate $ABI_{ADP,}$ it is measured in the *arteria dorsalis pedis* (ADP). Multidetector computed tomographic (MDCT) angiography is becoming more popular because of the increased speed of imaging acquisition and the volume of coverage, decreased contrast dose, and improved spatial resolution [3]. Finite element analysis (FEA) is used to divide the fluid domain in an arterial blood vessel into many small, finite elements. For each of these elements, it is possible to accurately calculate the flow *per* unit of time using mathematical equations and measuring the corresponding physical quantities [4–6].

Our previous study proposed using computationally derived ABI – a virtual ABI [7, 8], as a non-invasive procedure that could assist clinicians in finding the optimal bypass strategy for a particular patient. The key contribution of the study was a novel approach to prescribing boundary conditions (BC) by combining non-invasive preoperative measurements and medical imaging. The validation of the study showed the high reliability of the proposed approach to prescribing BC by combining clinical measurements of blood pressure and blood flow rates with the values obtained using computational fluid dynamics.

The aim of the study was to examine existing procedures for computing the ABI (virtual ABI) to predict the outcome of femorodistal (FD) bypass surgery.

Methods

The observational study with mathematical modeling based on data obtained by measurements was carried out at the Clinic for Vascular and Endovascular Surgery of the Military Medical Academy and University Clinical Center of Serbia in Belgrade, Serbia from December 1, 2021, to March 31, 2023.

The study was approved by the Ethics Committee of the Military Medical Academy, at the meeting held on November 1, 2021 (Decision No. 25/2021).

It involved 45 patients with PAOD with recommended FD arterial reconstruction. The MDCT angiography of the arteries of the lower extremities was performed both preoperatively and postoperatively (third or fourth day after surgery), based on which FEA models were made (preoperative and real surgery model) (Figure 1).

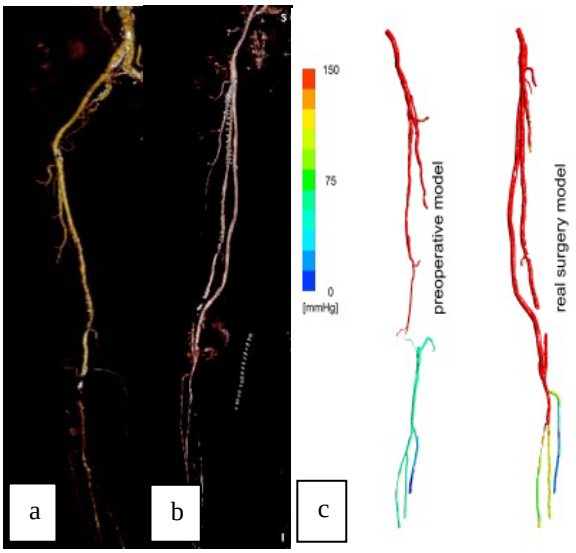


Fig. 1 – a) Preoperative multidetector computed tomographic (MDCT) angiography; b) Postoperative MDCT angiography; c) Finite element analysis models.

All patients had their ABI measured daily preoperatively and on the third or fourth day after the surgery (ATP pre-op, ATP post-op, ADP pre-op, ADP post-op). The ABI was measured on preoperative and postoperative FEA models based on the MDCT findings (ATP sim pre-op, ATP sim post-op, ADP sim pre-op, ADP sim post-op). Based on the findings of the preoperative MDCT angiography, a postoperative model was made using the FEA (virtual surgery model), and the ABI was calculated (ATP sim post-op virtual, ADP sim post-op virtual) (Figure 2). In the virtual surgical model, the same diameter and length of the graft were used as in the surgery. Where a vein graft was used, the vein was measured at 2 cm distal to the proximal anastomosis, and this size was used for the virtual bypass.

Fig. 2 – Postoperative finite element analysis model based on the findings of the preoperative multidetector computed tomographic angiography.

The ABI values measured on models were statistically compared with the values measured on patients preoperatively and postoperatively. A sphygmomanometer and a stethoscope were used to measure the pressure of the BA to calculate the ABI. The pressure in the ankle joint area was measured using Doppler sonography (Esaote MyLabGamma 4790) and a sphygmomanometer, and Atys medical BASIC 3.4. Then, the quotient was calculated, and the ABI values were obtained [9]. The MDCT angiography was performed using the Toshiba Prime Aquilion (CGGT-032A and CGGT 030A, Toshiba Medical System Corporation, Japan).

The FEA models were based on three-dimensional MDCT angiography imaging. The blood flow was calculated using the Navier-Stokes equation with the set initial and BC obtained through measurement.

A virtual bypass was created based on a preoperative model using the Solidworks 2018 software. To achieve adequate mesh density, the models were discretized into unstructured 3D finite elements using TetGen mesh parameters [10]. The "dfemtoolz" software was used to generate the hexahedral mesh and impose the BC [11].

All patients older than 18 years who were recommended FD arterial reconstruction due to PAOD with preserved renal function and who had signed informed consent for the research were included in the study. Patients who were excluded from the study were the ones who were recommended FD arterial reconstruction due to other conditions (aneurysms of peripheral arteries), who had had operations on the iliac or other arteries of the lower extremities, a bypass surgery on the arteries of the lower extremities, endovascular procedures on the aorta, iliac or lower limb arteries, patients diagnosed with malignancy, patients with renal insufficiency, and patients who did not sign a consent for the research.

Statistical analysis

The research defined two groups of data. The first was the values of ABI obtained by the FEA method based on preoperative and postoperative MDCT angiography, as well as by the FEA method of virtual surgery using only preoperative MDCT angiography, while the second group of data was the same data but obtained by experimental measurement on patients. For comparison, these two data group *t*-test independent samples were used. These two groups of data were measured before and after surgery. Student's *t*-test for dependent samples was used to compare the same group before and after intervention. The computer software SPSS version 26.0 (IBM, USA, 2019) was used for statistical data processing. The Kolmogorov-Smirnov test was used to test the normality of the data distribution. A value of $p < 0.05$ was considered significant during all analyses.

Results

The ABI was significantly higher after surgery compared to the preoperative value. In both cases, it was measured using Doppler sonography and MDCT angiography (Tables 1 and 2, Figures 1 and 2).

Virtual ABI was simulated for each patient. The values based on the virtual ABI model did not show significant differences compared to the values measured using Doppler sonography and MDCT angiography (Tables 1 and 2, Figures 3 and 4).

There was a strong, statistically significant correlation between the virtual ABI values and the values obtained using the other two methods (Table 3). There was a strong positive correlation between the ATP sim post-op virtual, the ATP post-op (Pearson correlation $r = 0.912$, $p < 0.001$), and the ATP sim post-op (Pearson correlation r = 0.925, $p < 0.001$). Furthermore, there was a strong positive correlation between the ADP sim post-op virtual, the ADP post-op (Pearson correlation r = 0.900, $p < 0.001$), and the ADP sim post-op (Pearson correlation $r = 0.987$, $p < 0.001$).

Table 1

The ankle-brachial index (ABI) in the *arteria tibialis posterior* (ATP) before and after surgery

Parameter	mean ± SD	*p*-value
ATP pre-op	0.406 ± 0.211	< 0.001
ATP post-op	0.782 ± 0.205	
ATP sim pre-op	0.416 ± 0.213	< 0.001
ATP sim post-op	0.788 ± 0.193	
ATP sim post-op virtual	0.782 ± 0.196	
ATP post-op vs. ATP sim post-op virtual		1.000
ATP sim post-op vs. ATP sim post-op virtual		0.569

ATP pre-op – ABI preoperative value on ATP measured on the patient; ATP post-op – ABI postoperative value on ATP measured on the patient; ATP sim pre-op – ABI preoperative value on ATP measured on preoperative finite element analysis (FEA) models based on the multidetector computed tomographic (MDCT) findings; ATP sim post-op – ABI postoperative value on ATP measured on postoperative FEA models based on the MDCT findings; ATP sim post-op virtual – ABI postoperative value on ATP measured on virtual surgery model; SD – standard deviation.
The Paired Samples *t*-test was applied.

Table 2

The ankle-brachial index (ABI) of the *arteria dorsalis pedis* (ADP) before and after surgery

Parameter	mean ± SD	*p*-value
ADP pre-op	0.393 ± 0.193	< 0.001
ADP post-op	0.759 ± 0.198	
ADP sim pre-op	0.397 ± 0.194	< 0.001
ADP sim post-op	0.744 ± 0.193	
ADP sim post-op virtual	0.749 ± 0.197	
ADP post-op vs. ADP sim post-op virtual		= 0.461
ADP sim post-op vs. ADP sim post-op virtual		= 0.232

ADP pre-op – ABI preoperative value on ADP measured on patient; ADP post-op – ABI postoperative value on ADP measured on patient; ADP sim pre-op – ABI preoperative value on ADP measured on preoperative finite element analysis (FEA) models based on the multidetector computed tomographic (MDCT) findings; ADP sim post-op – ABI postoperative value on ADP measured on postoperative FEA models based on the MDCT findings; ADP sim post-op virtual – ABI postoperative value on ADP measured on virtual surgery model; SD – standard deviation.
The Paired Samples *t*-test was applied.

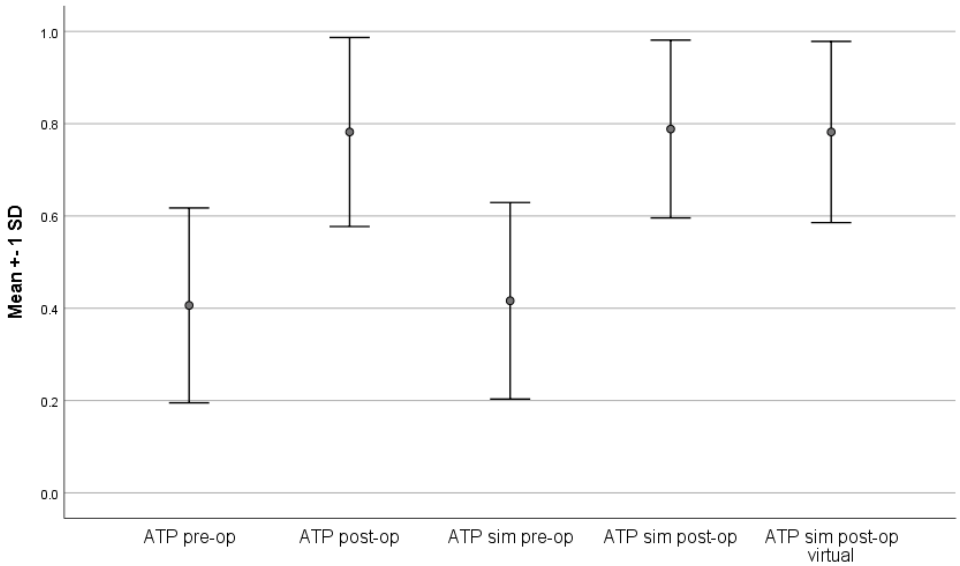


Fig. 3 – The average values of the ankle-brachial index (ABI) in the *arteria tibialis posterior* (ATP) before and after surgery, measured using Doppler, multidetector computed tomographic angiography, and virtual ABI.
SD – standard deviation; For other abbreviations, see Table 1.

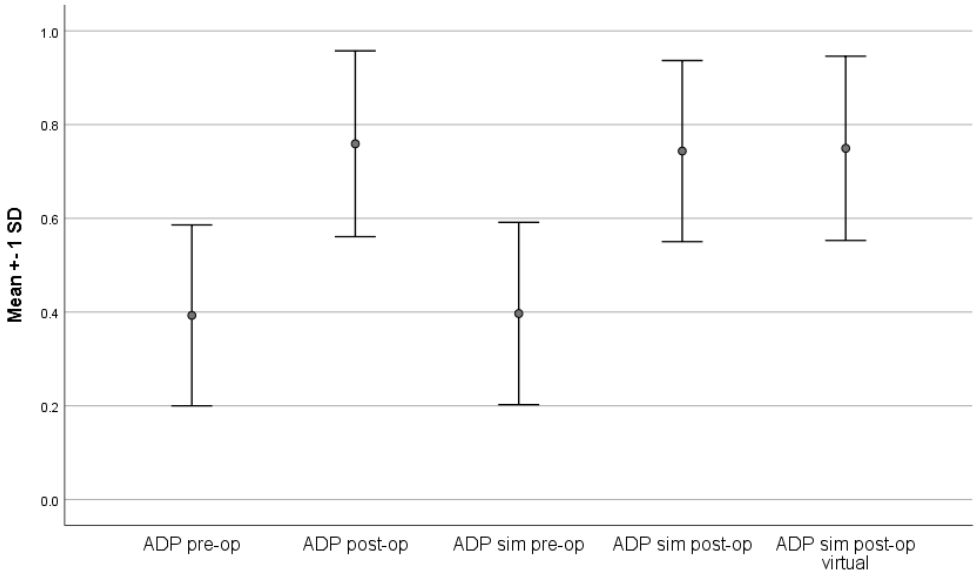


Fig. 4 – The average values of the ankle-brachial index (ABI) of the *arteria dorsalis pedis* artery (ADP) before and after surgery, measured using Doppler, multidetector computed tomographic angiography, and virtual ABI.
SD – standard deviation; For other abbreviations, see Table 2.

Table 3

Correlation of the ankle-brachial index (ABI) of the *arteria tibialis posterior* (ATP) and the *arteria dorsalis pedis* (ADP) before and after surgery

Parameter		ATP pre-op	ADP pre-op	ATP post-op	ADP post-op	ATP sim pre-op	ADP sim pre-op	ATP sim post-op	ADP sim post-op	ATP sim post-op virtual	ADP sim post-op virtual
ATP pre-op	r	1									
	p										
ADP pre-op	r	**0.489**	1								
	p	**0.001**									
ATP post-op	r	**0.508**	0.179	1							
	p	**< 0.001**	0.239								
ADP post-op	r	-0.021	**0.302**	**0.336**	1						
	p	0.891	**0.044**	**0.024**							
ATP sim pre-op	r	**0.971**	**0.498**	**0.462**	0.001	1					
	p	**< 0.001**	**< 0.001**	**0.001**	0.993						
ADP sim pre-op	r	**0.461**	**0.982**	0.181	**0.327**	**0.493**	1				
	p	**0.001**	**< 0.001**	0.233	**0.028**	**0.001**					
ATP sim post-op	r	**0.520**	0.161	**0.986**	**0.320**	0.499	0.168	1			
	p	**< 0.001**	0.291	**< 0.001**	**0.032**	< 0.001	0.269				
ADP sim post-op	r	-0.009	**0.322**	**0.328**	**0.920**	-0.054	**0.333**	0.277	1		
	p	0.953	**0.031**	**0.028**	**<0.001**	0.727	**0.025**	0.065			
ATP sim post-op virtual	r	**0.478**	0.099	**0.912**	**0.305**	**0.455**	0.103	**0.925**	0.267	1	
	p	**0.001**	0.516	**< 0.001**	**0.041**	**0.002**	0.500	**< 0.001**	0.076		
ADP sim post-op virtual	r	-0.061	**0.330**	0.277	**0.900**	-0.100	**0.338**	0.229	**0.987**	0.233	1
	p	0.692	**0.027**	0.066	**< 0.001**	0.511	**0.023**	0.130	**< 0.001**	0.124	

For abbreviations, see Tables 1 and 2. Statistically significant values are bolded (Pearson correlation was applied).

Discussion

The key result of this study is that the measurement properties for the Virtual ABI method compared to the Doppler and MDCT angiography simulation techniques were generally good. The precision of the virtual ABI measurement was also markedly good. There was a strong correlation between the virtual ABI and the values obtained using the other two methods.

The basic diagnostic procedure for PAOD is Color Doppler, which provides direct visualization of stenoses and arterial plaques, as well as the indirect analysis of the Doppler signal proximal and distal to the stenosis, which can determine the degree of stenosis [12]. The basic diagnostic screening method is the calculation of the ABI. In our study, the ABI was measured on real models (patients) using Doppler, and it was compared with the models based on the MDCT angiography findings.

The ABI is a sensitive and inexpensive method defined as a quotient of the systolic pressure at the level of the ankle joint (ADP and ATP) and the systolic pressure measured at the level of the BA. The ABI values between 0.4 and 0.9 indicate a mild to moderate PAOD, while the ABI values lower than 0.4 indicate significant PAOD [13]. However, this method cannot be used in patients with severely calcified arteries because the ABI cannot be adequately measured [14]. MDCT angiography or magnetic resonance of the blood vessels of the lower extremities is useful in diagnosing the site of significant stenosis on the arterial blood vessel [15, 16].

Today, efforts are being made to find different virtual models for assessing ABI as an indicator of PAOD. Diagnosis of PAOD is largely based on the recorded ABI. The equipment that automatically determines the ABI could facilitate diagnosing PAOD [17]. In our previous study, the measurement properties of an automated oscillometric ABI measuring device were tested against reference ABI values measured manually in patients with and without PAOD [7]. Generally, the automated device tended to overestimate lower ABI values while underestimating the higher ones, potentially leading to underdiagnosing PAOD. Using the FEA method, other physical quantities can be measured: shear stress, oscillatory shear stress, particle tracking, and velocity [18–20]. These physical quantities were not examined in this study, but they were published in our earlier study [21]. The focus of this study was on arterial pressure values, i.e., ABI, which we also use in clinical practice, and the immediate outcome of FD bypass surgery. In the following studies, these unused values could be measured for the purpose of testing long-term patency or reasons for the failure of the FD bypass surgery.

Our previous study proposed using computationally derived ABI – a virtual ABI [7], as a non-invasive procedure that could assist clinicians in finding the optimal bypass strategy for a particular patient. The validation of the study indicated the high reliability of the proposed approach.

Conclusion

The study showed that the virtual simulation based on the MDCT parameters of peripheral blood vessels could be successfully used to predict the outcome of the disease. However, considering the relatively small cohort available for this study, a larger study group is needed before the virtual ABI could be established as a diagnostic procedure.

Declaration of Competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

This study was scientifically supported by the medical staff of the Military Medical Academy in Belgrade, Serbia.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

REFERENCES

1. *Shammas NW*. Epidemiology, classification, and modifiable risk factors of peripheral arterial disease. Vasc Health Risk Manag 2007; 3(2): 229–34.
2. *Collins TC, Nelson D, Ahluwalia JS*. Mortality following operations for lower extremity peripheral arterial disease. Vasc Health Risk Manag 2010; 6: 287–96.
3. *Chhetri PK, Thapa K*. CT Angiography in Patients with Peripheral Arterial Disease. J Coll Med Sci-Nepal 2020; 16(2): 78–82.
4. *Filipović N, Milašinović D, Jagić N, Miloradović V, Hetteriche H, Rieber J*. Numerical simulation of the flow field and mass transport pattern within the coronary artery. Comput Methods Biomech Biomed Engin 2011; 14(4): 379–88.
5. *Zhang H, Liu H, Dong Y, Wang J, Zao Y, Cui Y*, et al. Low carotid wall shear stress independently accelerates the progression of cognitive impairment and white matter lesions in the elderly. Oncotarget 2017; 9(13): 11402–13.
6. *Colombo M, Luraghi G, Cestariolo L, Ravasi M, Airoldi A, Chiastra C*, et al. Impact of lower limb movement on the hemodynamics of femoropopliteal arteries: A computational study. Med Eng Phys 2020; 81: 105–17.
7. *Milašinović DZ, Sekulić DB, Nikolić DD, Vukićević AM, Tomić AP, Miladinović UM*, et al. Virtual ABI: A computationally derived ABI index for noninvasive assessment of femoropopliteal bypass surgery outcome. Comput Methods Programs Biomed 2021; 208: 106242.
8. *Nikolić DD, Sekulić DB, MIlašinović DZ, Paunović DS, Sekulić IM, Saveljić IB*, et al. Hemodynamics of Femoro-Popliteal "Bi-Pass" Surgery using FEA Methods. In: 2021 IEEE 21st International Conference on Bioinformatics and Bioengineering (BIBE), 2021: Kragujevac, Serbia; pp. 1–5.
9. *Aboyans V, Criqui MH, Abraham P, Allison MA, Creager MA, Diehm C*, et al. Measurement and Interpretation of the Ankle-

Brachial Index. A Scientific Statement From the American Heart Association. Circulation 2012; 126(24): 2890–909.
10. *Si H*. TetGen, a Delaunay-Based Quality Tetrahedral Mesh Generator. ACM Trans Math Softw 2015; 41(2): Article No. 11; pp. 1–36.
11. *Milašinović D, Vukićević A, Filipović N*. Dfemtoolz: An open-source C ++ framework for efficient imposition of material and boundary conditions in finite element biomedical simulations. Comput Phys Commun 2020; 249: 106996.
12. *Hwang JY*. Doppler ultrasonography of the lower extremity arteries: anatomy and scanning guidelines. Ultrasonography 2017; 36(2): 111–9.
13. *Rac-Albu M, Iliuta L, Guberna SM, Sinescu C*. The role of ankle-brachial index for predicting peripheral arterial disease. Maedica (Bucur) 2014; 9(3): 295–302.
14. *Hoek GA, Zwaknberg SR, Elders PJM, de Jong PA, Spiering W, Bartstra JW*, et al. An elevated ankle-brachial index is not a valid proxy for peripheral medial arterial calcification. Atherosclerosis 2021; 323: 13–9.
15. *Burbelko M, Augsten M, Kalinowski MO, Heverhagen JT*. Comparison of contrast-enhanced multi-station MR angiography and digital subtraction angiography of the lower extremity arterial disease. J Magn Reson Imaging 2013; 37(6): 1427–35.
16. *Shareghi S, Gopal A, Gul K, Matchinson JC, Wong CB, Weinberg N*, et al. Diagnostic accuracy of 64 multidetector computed tomographic angiography in peripheral vascular disease. Catheter Cardiovasc Interv 2010; 75(1): 23–31.
17. *Zebari F, Amlani V, Langenskiöld M, Nordanstig J*. Validation of an automated measurement method for determination of the ankle-brachial index. Scand Cardiovasc J 2022; 56(1): 73–8.
18. *Zhang X, Gao Y, Huo B*. Finite Element Analysis on Wall Fluid Shear Stress on Cells under Oscillatory Flow. Appl Sci 2021; 11(21): 10021.
19. *Herschlag G, Gounley J, Roychowdhury S, Draeger EW, Randles A*. Multi-physics simulations of particle tracking in arterial geometries with a scalable moving window algorithm. In: 2019 IEEE International Conference on Cluster Computing (CLUSTER), 2019; Albuquerque, NM (USA). pp. 1–11.
20. *Athanasiou LS, Fotaidis DI, Michalis LK*. Structure and Mechancal Behavior of Atherosclerotic Plaque. In: Atherosclerotic Plaque Characterization Methods Based on Coronary Imaging. Academic Press; 2017; 181–98.
21. *Sekulić DB, Tomić AP, Milašinović DZ, Nikolić DD, Paunović DS, Miladinović UM*, et al. Haemodynamics of femoropopliteal bypass surgery using finite element analysis method. Med čas 2021; 55(2): 66–70. (Serbian)

Received on May 16, 2023
Revised on June 1 2023
Accepted on June 6, 2023
Online First June 2023

ORIGINAL ARTICLE

UDC: 616.12-089
DOI: https://doi.org/10.2298/VSP230306036S

Early clinical outcomes of surgical myocardial revascularization in patients with preoperative platelet dysfunction

Neposredni ishodi hirurške revaskularizacije miokarda kod bolesnika sa preoperativnom disfunkcijom trombocita

Vladimir Stojiljković*, Aleksandar Kamenov*, Milan Lazarević*†, Mladjan Golubović*†, Velimir Perić*†, Marija Stošić*†, Saša Živić*, Dragan Milić*†

***University Clinical Center of Niš, Department of Cardiac Surgery, Niš, Serbia;**
†University of Niš, Faculty of Medicine, Niš, Serbia

Abstract

Background/Aim. Coronary artery bypass grafting (CABG) is the treatment of choice for a significant number of patients with ischemic heart disease. Some of the postoperative complications are closely linked with the preoperative antiplatelet therapy (APT). The aim of this study was to compare the early clinical outcomes of CABG in patients with preserved platelet (PLT) function and patients with PLT function impaired by the residual therapeutic effect of APT. **Methods.** A total of 181 patients with isolated CABG were enrolled in this prospective, nonrandomized, observational study. Patients were divided into four groups: control group (arachidonic acid-dependent PLT aggregation group), with aspirin-induced platelet inhibition (ASPI) test ≥ 790 aggregation units (AU)/min; mild (M) acetylsalicylic acid (ASA) effect (MASAE) group, with ASPI test = 410–789 AU/min; pronounced (P) ASA effect (PASAE) group, with ASPI test ≤ 409 AU/min; dual (D) APT (DAPT) group, with ASPI test ≤ 789 AU/min and adenosine diphosphate (ADP) test ≤ 405 AU/min. Preoperative data, intraoperative characteristics, and postoperative outcomes were obtained and compared between the groups. **Results.** A significant difference was found regarding the average time of APT cessation between groups ($p < 0.001$). The DAPT group had a significantly higher frequency of drainage compared to the control ($p = 0.004$), MASAE ($p = 0.001$), and PASAE ($p = 0.006$) groups. The PASAE group had a significantly higher rate of chest reexploration compared to the MASAE group ($p = 0.032$). The DAPT group required significantly more packed red blood cells (PRBC) compared to the control ($p < 0.001$) and MASAE ($p = 0.009$) groups. The PASAE group received significantly more PRBC compared to the control ($p < 0.001$) and MASAE ($p = 0.019$) groups. The DAPT group required higher amounts of PLTs compared to the control ($p < 0.001$), MASAE ($p = 0.002$), and PASAE ($p < 0.001$) groups. The DAPT group received higher amounts of cryoprecipitate compared to the control ($p = 0.002$), MASAE ($p = 0.009$), and PASAE ($p = 0.016$) groups. **Conclusion.** Patients with a residual effect of DAPT, as well as patients with a pronounced residual effect of ASA, have a higher risk of postoperative bleeding and chest reexploration, as well as increased transfusion demands.

Key words:
coronary artery bypass; coronary disease; platelet aggregation; platelet aggregation inhibitors; treatment outcome.

Apstrakt

Uvod/Cilj. Hirurška revaskularizacija miokarda (*coronary artery bypass grafting* – CABG) kod značajnog broja bolesnika je osnovni vid lečenja ishemijske bolesti srca. Neke od postoperativnih komplikacija su blisko povezane sa preoperativnom antiagregacionom terapijom (AAT). Cilj rada bio je da se uporede neposredni ishodi CABG kod bolesnika sa očuvanom funkcijom trombocita i bolesnika kod kojih je funkcija trombocita bila narušena rezidualnim efektom AAT. **Metode.** Prospektivnom, opsrevacionom, nerandomizovanom studijom obuhvaćeno je 181 bolesnika sa izolovanim CABG. Bolesnici su bili podeljeni na četiri grupe: kontrolnu grupu (agregacija trombocita zavisna od arahidonske kiseline), sa *aspirin-induced platelet inhibition* (ASPI) testom ≥ 790 agregacionih jedinica (*aggregation units* – AU)/min; grupu bolesnika sa blagim efektom (BE) acetilsalicilne kiseline (ASK) (BEASK), sa ASPI testom = 410–789 AU/min; grupu bolesnika sa izraženim efektom (IE) ASK (IEASK), sa ASPI testom ≤ 409 AU/min; grupu bolesnika sa dvojnom (D) AAT (DAAT), sa ASPI testom ≤ 789 AU/min i adenozin difosfat (ADF) testom ≤ 405 AU/min. Preoperativni podaci, intraoperativne karakteristike i postoperativni ishodi upoređeni su među grupama. **Rezultati.** Ispitivane grupe su se značajno razlikovale u odnosu na prosečno vreme obustave AAT

Correspondence to: Vladimir Stojiljković, University Clinical Center of Niš, Department of Cardiac Surgery, Blrd Dr. Zorana Đinđića 48, 18 000 Niš. E-mail: serbvlada@yahoo.com

($p < 0,001$). DAAT grupa imala je značajno češću drenažu u poređenju sa kontrolnom ($p = 0,004$), BEASK ($p = 0,001$) i IEASK ($p = 0,006$) grupom. Grupa IEASK imala je značajno veću učestalost reeksploracije grudnog koša u poređenju sa BEASK grupom ($p = 0,032$). Bolesnici iz DAAT grupe primili su značajno više koncentrovanih eritrocita u poređenju sa kontrolnom ($p < 0,001$) i BEASK ($p = 0,009$) grupom. Bolesnici iz IEASK grupe primili su značajno više koncentrovanih eritrocita u poređenju sa kontrolnom ($p < 0,001$) i BEASK ($p = 0,019$) grupom. DAAT grupa primila je značajno više trombocita u poređenju sa kontrolnom ($p < 0,001$), BEASK ($p = 0,002$) i IEASK ($p < 0,001$) grupom. Transfuzije krioprecipitata bile su značajno češće u DAAT grupi u poređenju sa kontrolnom ($p = 0,002$), BEASK ($p = 0,009$) i IEASK ($p < 0,016$) grupom. **Zaključak**. Bolesnici sa rezidualnim efektom DAAT, kao i bolesnici sa izraženim rezidualnim efektom ASK imaju povišen rizik od postoperativnog krvarenja i reeksploracije grudnog koša, kao i povećanu potrebu za transfuzijama.

Ključne reči:
aortokoronarno premošćavanje; koronarna bolest; trombociti, agregacija; antiagregaciona sredstva; lečenje, ishod.

Introduction

Surgical myocardial revascularization is the treatment of choice for patients with ischemic heart disease who are not candidates for percutaneous coronary interventions (PCI) [1]. Even though coronary artery bypass grafting (CABG) has been one of the most performed surgical procedures, operative mortality is still high, about 2.8%. The incidence of postoperative complications after CABG surgery ranges between 30% and 40%, depending on the study and type of complication [2, 3].

Perioperative coronary events and bleeding are common and important complications after CABG, and both of them are strongly influenced by the management of preoperative antiplatelet therapy (APT) [4].

The incidence of perioperative myocardial infarction (PMI) ranges from 2–12%, depending on the definition and methodology used in detection [5]. Studies conducted so far have shown that PMI is a significant predictor of increased mortality in the first 30 days and the first six months after surgery [6].

Bleeding is the most important cause of chest reexploration (2–5% of patients) after CABG. In patients with this complication, there is a significantly higher in-hospital mortality (3.3% vs. 9.5%), a twice as long stay in the intensive care unit, more frequent need for inotropic therapy, as well as frequent need for an expensive broad spectrum antibiotic as meropenem active against gram-positive and gram-negative bacteria [7]. Hemorrhagic events are associated with more transfusions of blood products and thus increase operative mortality and morbidity and compromise the long-term benefits of CABG [8, 9].

According to current guidelines, dual APT (DAPT) is recommended for all patients after acute coronary syndrome and after percutaneous myocardial revascularization. In patients with stable coronary disease, routine use of DAPT is not indicated [10]. The main problem with DAPT is establishing a balance between adverse ischemic events and hemorrhagic complications, which are frequent in patients on antiplatelet drugs. That is especially pronounced in patients in whom both CABG and DAPT are indicated, such as patients who require more urgent CABG in the early period after acute coronary syndrome, which is about 5–10% of all patients with acute coronary syndrome [11].

The results of the studies conducted so far, which refer to the preoperative use of acetylsalicylic acid (ASA), are largely contradictory, and the recommendations related to the use of ASA in cardiac surgery patients have often changed. Most recent studies indicate better short-term and long-term survival, lower incidence of adverse cardiovascular events, and better long-term patency of the vein graft in patients treated preoperatively with ASA without a significant increase in adverse hemorrhagic events [12, 13].

Therefore, according to current guidelines, stopping ASA before cardiac surgery is not recommended [14].

Based on our clinical experience [15] and what has been confirmed in a large number of studies, the preoperative administration of platelet (PLT) $P2Y_{12}$ receptor ($P2Y_{12}R$) antagonists is associated with excessive blood loss, a higher frequency of chest reexploration, and increased transfusion requirements in the postoperative period [16].

According to current guidelines, clopidogrel should be stopped at least five days before surgery and ticagrelor three days before surgery. In patients in whom urgent myocardial revascularization is indicated, $P2Y_{12}R$ antagonists should be stopped at least 24 hours before surgery [14].

The aim of this study was to compare the early clinical outcomes of surgical myocardial revascularization in patients with preserved PLT function and patients with PLT function impaired by the residual therapeutic effect of APT.

Methods

Study design

The study was conducted as a prospective, nonrandomized, observational study and included 181 patients. Patients were treated at the Department of Cardiac Surgery, University Clinical Center of Niš, Serbia from June 2021 to October 2022.

The research was approved by the Ethics Committee of the University Clinical Center of Niš (No. 3830/6, from February 4, 2020) and by the Ethics Committee of the Faculty of Medicine of Niš (No. 12-15637-2/9, from December 24, 2019) and was conducted following the ethical standards specified in the Declaration of Helsinki (1964) and subsequent amendments of the declaration.

All patients older than 18 years who underwent isolated, elective surgical myocardial revascularization with extracorporeal circulation and cardioplegic arrest were included in the study. Exclusion criteria were the following: patients with elevated preoperative values of cardiac enzymes [high sensitivity troponin I (hsTnI) and creatine kinase-MB fraction (CK-MB)]; myocardial revascularization with "off-pump" technique; patients who underwent combined myocardial revascularization and heart valve surgery or aortic surgery; emergency patients operated in a cardiogenic shock; patients whose biological material was inadequately sampled.

Clinical methodology

Upon admission to the hospital, all patients underwent a physical examination with anthropometric parameters measurement. The 12-channel electrocardiogram (ECG) and an echocardiographic examination, focusing on the ejection fraction (Teicholz) and the kinetics of the left ventricular walls, were performed.

The patients underwent standard surgical revascularization of the myocardium under general endotracheal anesthesia, with extracorporeal circulation (ECC) and cardioplegic arrest. Cold crystalloid cardioplegia was used, and surgical procedures were performed under conditions of mild hypothermia 32–35 °C. The left internal thoracic artery and the great saphenous vein were used as grafts. The Cell Saver model Xtra device (LivaNova, United Kingdom) was used for intraoperative blood saving.

All patients received a single bolus dose of 30 mg/kg of tranexamic acid after heparin administration before ECC. Heparin reversal was achieved with protamine in a 1:1 fixed dose ratio. Effective heparin reversal was defined as post-reversal activated coagulation time (ACT) within 10% of the baseline ACT value.

Every postoperative day, patients underwent a 12-channel ECG. Echocardiography was performed on the second postoperative day and more often if necessary.

Transfusions were administered according to the clinic's established protocol, based on the blood count, the results of impedance aggregometry, and rotational thrombelastometry (ROTEM, Delta Roche, Germany). For rotational thrombelastometry, 4 mL of whole blood was sampled in a test tube with sodium citrate.

The indication for intraoperative packed red blood cells (PRBC) transfusion was a hematocrit value < 22% during ECC. After surgery, the decision for transfusions was based on postoperative hemodynamics, laboratory parameters, and chest tube drainage.

The decision to perform chest reexploration was based on conventional guidelines, the amount of blood loss according to the Kirklin and Barratt-Boyes criteria: 1) drainage of more than 500 mL during the first hr, more than 400 mL during each of the first 2 hrs, more than 300 mL during each of the first 3 hrs, more than 1,000 mL in total during the first 4 hrs, and more than 1,200 mL in total during the first 5 hrs; 2) excessive bleeding that restarts; 3) sudden massive bleeding; 4) hemodynamic status of the patient and laboratory parameters.

Laboratory analysis

To assess PLT function, a whole blood impedance aggregometry (MULTIPLATE, Roch, Germany) was used. Blood for analysis was sampled upon admission and 2 hrs before the start of the operation in a 4 mL test tube with the anticoagulant lithium-heparin. All analyses were carried out within 30 min from the moment of sampling. The values of the parameters of the multiplate test that indicate a disorder of PLT function were defined based on the manufacturer's recommendations and guides: adenosine diphosphate (ADP) test reference range (RR) values 406–1,130 aggregation units per min (AU/min); arachidonic acid-dependent PLT aggregation (ASPI) test RR values 790–1,490 AU/min.

Cardiac enzymes (hsTnI and CK-MB) were measured: 24 hrs before surgery, blood sampling from a peripheral vein; perioperative – 10 min after releasing the aortic clamp, blood sampling from the arterial line; 24 hrs after surgery, blood sampling from the arterial line. For analysis, 4 mL of whole blood was sampled in a test tube with ethylenediaminetetraacetic acid (EDTA) anticoagulant. RR values for hsTnI were 0.00–0.04 ng/mL, and for CK-MB, 0–24 U/L.

According to the PLT function, all patients were classified into four groups. Patients with ADP and ASPI tests within the RR had preserved PLT function and were classified into the control group (n = 57). Patients with ASPI test < 790 AU/min had a residual effect of ASA and were divided into two groups: mild (M) ASA (MASA) effect (MASAE) group – ASPI test in the range 410–789 AU/min (n = 37) and pronounced (P) ASA (PASA) effect (PASAE) group – ASPI test ≤ 409 AU/min (n = 47). Patients with both the ASPI test ≤ 789 AU/min and the ADP test ≤ 405 AU/min were classified into the DAPT group (n = 40), given that they had a residual effect of both ASA and PLT $P2Y_{12}R$ antagonists.

Patients from the control group and MASAE group were considered to be at low risk for hemorrhagic complications, while subjects from the PASAE group and the DAPT group were at high risk for hemorrhagic adverse events.

The investigated variables were divided into three groups: preoperative, perioperative, and postoperative data.

Preoperative data: basic demographic data (gender, age); present comorbidities: diabetes mellitus, heart failure (defined as ejection fraction < 40%), and obesity (defined as body mass index > 30 kg/m^2); characteristics of coronary disease: left main stenosis > 50%, previous PCI; APT (type of drug, discontinuation of therapy).

Perioperative characteristics were the following: number of grafts, left internal thoracic artery (also known as left internal mammary artery – LIMA) usage, aortic cross-clamp (ACC) time, extracorporeal circulation (ECC) time, autotransfusion with cell saver, and perioperative values of hsTnI and CK-MB.

Postoperative outcomes were the following: hsTnI and CK-MB values (24 hrs after surgery), postoperative chest

tube drainage, transfusion requirements, chest reexploration, PMI (defined according to the recommendations of the European Association of Cardiology [17]), postoperative inotropic therapy, heart rhythm disorders that required therapy, prolonged intubation (over 24 hrs), pneumonia, pneumothorax, pleural effusion requiring thoracentesis or drainage, cerebrovascular insult, transient ischemic attack, acute renal failure (requiring hemodialysis), chest wound infection, intensive care length of stay, total length of hospitalization (after surgery), and intrahospital mortality.

Statistical analysis

The data were described using the measures of central tendency in the form of the arithmetic mean and standard deviation or in the form of absolute and relative numbers. The normality of data distribution was tested with the Kolomogorov-Smirnov test. Comparisons of continuous variables, normally distributed between the four groups, were performed by analysis of variance (ANOVA) with the *post-hoc* Bonferroni test. If the data distribution was not normal, the comparison of values between the groups was performed using the Kruskal-Wallis test. A comparison of categorical features was performed using the Chi-squared test and Fisher's test. IBM SPSS version 16 (Chicago, Illinois) was used for the statistical analysis. The results were presented in the given Tables and Figures. Statistical significance was set at the *p*-value of less than 0.05.

Results

Data provided from 181 patients were evaluated. The patients were divided into four groups: patients with normal PLT function (n = 57); patients with MASA residual effect (n = 37); patients with PASA residual effect (n = 47); patients with ASA and $P2Y_{12}$ antagonist residual effect (n =40). The demographic and preoperative characteristics of patients are shown in Table 1.

There was no significant difference between groups regarding the demographic and preoperative clinical characteristics, except for the average time of APT cessation, which differed significantly between the studied groups. We found a statistically significant difference in the average time of ASA cessation between groups. Patients from the MASAE group had a significantly shorter average time of ASA withdrawal compared to the control group ($p < 0.001$). Patients from the PASAE group had a significantly shorter average time of ASA cessation compared to the control group ($p < 0.001$) and the MASAE group ($p < 0.001$). Patients from the DAPT group had a significantly shorter average time of ASA withdrawal compared to the control group ($p < 0.001$) and the MASAE group ($p = 0.011$). We did not find a statistically significant difference regarding ASA withdrawal average time between the PASAE and DAPT groups. Patients from the DAPT group had a significantly shorter average time of $P2Y_{12}$ antagonist withdrawal compared to the control, MASAE, and PASAE groups ($p < 0.001$ for all). There was no statistically significant difference in the average time of a $P2Y_{12}$ antagonist withdrawal between the control group and the MASAE and PASAE groups. Patients' perioperative characteristics are shown in Table 2, and the examined variables were not different between the groups. The lowest average chest tube drainage was recorded in the MASAE group (1,017.84 ± 520.11 mL), and the highest drainage was in the DAPT group (1,451.5 ± 700.5 mL). It was determined that there was a statistically significant difference in postoperative drainage among the examined groups ($p = 0.002$). Patients from the DAPT group had significantly higher drainage compared to the control ($p = 0.004$), MASAE ($p = 0.001$), and PASAE ($p = 0.006$) groups. There was no significant difference regarding postoperative drainage between the control group and the MASAE and PASAE groups.

The frequency of chest reexploration between the examined groups was significantly different ($p = 0.017$). Patients from the PASAE group had a significantly higher rate

Table 1

Demographic and preoperative characteristics of the patients

Characteristics	Groups				*p*-value
	Control	MASAE	PASAE	DAPT	
Age (years)	62.91 ± 7.98	63.19 ± 7.40	63.28 ± 7.95	63.78 ± 8.72	0.965[1]
Subjects	57 (31.5)	37 (20.4)	47 (26)	40 (22.1)	0.183[2]
female	14 (24.6)	6 (16.2)	14 (29.8)	5 (12.5)	0.179[2]
male	43 (75.4)	31 (83.8)	33 (70.2)	35 (87.5)	0.179[2]
Diabetes mellitus	22 (38.6)	16 (43.2)	19 (40.4)	10 (25.0)	0.318[2]
Obesity	14 (24.6)	10 (27.0)	17 (36.2)	7 (17.5)	0.257[2]
LM stenosis	13 (22.8)	11 (29.7)	18 (38.3)	18 (45.0)	0.107[2]
Previous PCI	7 (12.3)	4 (10.8)	7 (14.9)	4 (10.0)	0.905[2]
Ejection fraction	51.53 ± 8.88	49.32 ± 7.73	51.83 ± 7.91	51.30 ± 9.56	0.555[1]
Ejection fraction < 40%	9 (15.8)	7 (18.9)	6 (12.8)	8 (20.0)	0.797[2]
ASA withdrawal (days)	5.92 ± 1.8[a,b,c]	2.56 ± 1.7[a,b]	1.23 ± 0.7	1.88 ± 1.6	< 0.001[3]
$P2Y_{12}$ antagonist withdrawal (days)	6.06 ± 1.33[a]	6 ± 1.26[a]	5.63 ± 1.70 [a]	3.58 ± 1.4	< 0.001[3]

MASAE – mild acetylsalicylic acid effect; PASAE – pronounced acetylsalicylic acid effect; DAPT – dual antiplatelet therapy; LM – left main coronary artery; PCI – percutaneous coronary intervention. All values are expressed as numbers (percentages) or mean ± standard deviation. [1]ANOVA – analysis of variance test; [2]Chi-squared test; [3]Kruskal-Wallis test.

[a]*p* < 0.05 vs. DAPT group; [b]*p* < 0.05 vs. PASAE group; [c]*p* < 0.05 vs. MASAE group.

Table 2

Surgery-related characteristics of the patients

Parameter	Groups				*p*-value
	Control	MASAE	PASAE	DAPT	
Average graft No	2.70 ± 0.57	2.70 ± 0.57	2.77 ± 0.67	2.68 ± 0.66	0.912[1]
LIMA	53 (93.0)	35 (94.6)	39 (83)	34 (85)	0.205[2]
ACC (min)	50.19 ± 16.34	50.81 ± 22.05	52.96 ± 21.51	51.18 ± 18.09	0.907[1]
ECC (min)	96.63 ± 22	98.76 ± 29.9	99.87 ± 26.01	105.73 ± 35.5	0.467[1]
Cell saver (mL),	926.32 ± 264.09	956.76 ± 315.6	1,025.53 ± 315.69	1,152.5 ± 483.57	0.123[3]
hsTnI (ng/mL)	0.82 ± 0.78	0.84 ± 0.89	0.9 ± 1.13	0.91 ± 1.38	0.964[3]
CK-MB (U/L)	33.48 ± 11.17	31.81 ± 8.73	35.59 ± 16.19	34.03 ± 9.52	0.548[1]

LIMA – left internal thoracic artery; ACC – aortic cross-clamp; ECC – extracorporeal circulation; hsTnI – high sensitivity troponin I; CK-MB – creatine kinase-MB fraction; min – minutes. For abbreviations of other terms, see Table 1.
All results are shown as mean ± standard deviation, except the LIMA parameter which is shown as number (percentage). [1]ANOVA; [2]Chi-squared test; [3]Kruskal-Wallis test.

of chest reexploration compared to the MASAE group ($p = 0.032$). There was no statistically significant difference in the chest reexploration rate between the control group and MASAE, PASAE, and DAPT groups. Moreover, no significant difference was found regarding the chest reexploration rate between the MASAE and DAPT groups, as well as between the PASAE and DAPT groups (Table 3).

Using the Kruskal-Wallis test, we found a statistically significant difference regarding the intraoperative and postoperative PRBC transfusion requirements ($p < 0.001$). Subjects from the DAPT group required significantly more PRBC compared to the control group ($p < 0.001$) and the MASAE group ($p = 0.009$). Subjects from the PASAE group received significantly more PRBC compared to patients from the control group ($p < 0.001$) and the MASAE group ($p = 0.019$). There was no statistically significant difference regarding PRBC requirements between the control and MASAE groups, as well as between PASAE and DAPT groups. Intraoperative and postoperative PLT transfusion requirements differed significantly between the studied groups ($p < 0.001$). Patients from the DAPT group required higher amounts of PLT compared to the control ($p < 0.001$), MASAE ($p = 0.002$), and PASAE ($p < 0.001$) groups. There was no significant difference in PLT transfusion requirements between the control, MASAE, and PASAE groups. There was a statistically significant difference regarding the cryoprecipitate (Cryo) transfusion requirements ($p = 0.004$). Patients from the DAPT group received higher amounts of Cryo compared to the control group ($p < 0.002$), the MASAE group ($p = 0.009$), and the subjects from the PASAE group ($p < 0.016$). Cryo transfusion requirements did not differ significantly between the control, MASAE, and PASAE groups. No significant disparities were found regarding transfusion of fresh frozen plasma (FFP) requirements between groups ($p = 0.872$) (Table 4).

PMI was recorded in 12 subjects (6.6%). The highest rate was recorded in the control group with preserved PLT function and the lowest in the DAPT group (8.8% vs. 2.5%). Using the Chi-squared test, it was shown that there is no statistically significant difference in the frequency of PMI among the examined groups ($p = 0.541$). The hsTnI and CK-MB values 24 hrs after surgery did not differ significantly

Table 3

Adverse hemorrhagic events in the postoperative period

Parameter	Groups				*p*-value
	Control	MASAE	PASAE	DAPT	
Drainage (mL), mean ± SD	1,080.96 ± 387.02[a]	1,017.84 ± 520.11[a]	1,186.6 ± 669.54[a]	1,451.5 ± 700.5	0.002[1]
Chest reexploration, n (%)	2 (3.5)	0 (0)[b]	6 (12.8)	5 (12.5)	0.017[2]

For abbreviations, see Table 1. [1]ANOVA; [2]Chi-squared test; [a] $p < 0.05$ vs. DAPT group; [b] $p < 0.05$ vs. PASAE group (Fisher test).

Table 4

Transfusion requirements

Parameter	Groups				*p*-value
	Control	MASAE	PASAE	DAPT	
PRBC (mL)	417.54 ± 420.62[a,b]	510.81 ± 525.61[a,b]	872.34 ± 774.16	988.75 ± 834.63	< 0.001
PLT (U)	1.49 ± 3.53[a]	2.43 ± 4.95[a]	2.81 ± 7.02[a]	6.05 ± 5.34	< 0.001
Cryo (U)	1.40 ± 3.50[a]	1.57 ± 3.62[a]	1.91 ± 3.98[a]	4.50 ± 5.52	0.004
FFP (mL)	505.61 ± 284.92	508.65 ± 293.47	580.43 ± 433.80	599.50 ± 395.40	0.872

PRBC – packed red blood cells; PLT – platelets; Cryo – cryoprecipitate; FFP– fresh frozen plasma; U – unit. For abbreviations of other terms, see Table 1. [a] $p < 0.05$ vs. DAPT group; [b] $p < 0.05$ vs. PASAE group (Kruskal-Wallis test). All results are shown as mean ± standard deviation.

Table 5

Perioperative and postoperative myocardial infarction (PMI) incidence and cardiac enzyme values (24 hrs)

Parameter	Groups				*p*-value
	Control	MASAE	PASAE	DAPT	
PMI	5 (8.8)	2 (5.4)	4 (8.5)	1 (2.5)	0.541[1]
hs-TnI (ng/mL)	5.89 ± 8.87	4.1 ± 3.4	5.71 ± 9.03	4.44 ± 4.07	0.653[2]
CK-MB (U/L)	53.25 ± 31.95	54.43 ± 39.44	48.72 ± 39.4	43.48 ± 25	0.653[2]

For abbreviations, see Tables 1 and 2. All results are shown as mean ± standard deviation, except the PMI parameter which is shown as number (percentage). [1]Chi-squared test; [2]ANOVA.

Table 6

In-hospital outcomes

Parameter	Groups				*p*-value
	Control	MASAE	PASAE	DAPT	
Inotropic support	16 (28.1)	11 (29.7)	20 (42.6)	10 (25.0)	0.294[1]
Arrhythmias	16 (28.1)	9 (24.3)	16 (34.0)	13 (32.5)	0.761[1]
Intubation > 24 hrs	4 (7.0)	1 (2.7)	6 (12.8)	3 (7.5)	0.365[1]
Pneumonia	1 (1.8)	1 (2.7)	1 (2.1)	0 (0.0)	0.658[1]
Pneumothorax	0 (0.0)	2 (5.4)	3 (6.4)	4 (10.0)	0.053[1]
Pleural effusion	2 (3.5)	1 (2.7)	5 (10.6)	1 (2.5)	0.280[1]
Hospitalization (days)	7.12 ± 2.10	6.97 ± 1.17	8.66 ± 6.22	7.43 ± 1.65	0.165[2]
ICU (days)	3.00 ± 1.87	2.78 ± 1.25	4.64 ± 6.39	3.45 ± 1.81	0.268[2]

ICU – intensive care unit. For abbreviations of other terms, see Table 1. All results are shown as numbers (percentages) except hospitalization and ICU, which are shown as mean ± standard deviation. [1]Chi-squared test; [2]ANOVA.

between the examined groups ($p = 0.653$) (Table 5). Postoperative outcomes and complications are shown in Table 6. There was no significant difference between groups regarding the postoperative outcomes.

In our study, there was no intrahospital mortality, no adverse cerebrovascular events, no acute renal failure requiring hemodialysis, and no chest wound infections manifested during the early postoperative period.

Discussion

A significant number of patients who had to undergo CABG are exposed to DAPT. According to the current guidelines, preoperative withdrawal of ASA is not recommended, given that a number of studies confirm lower operative mortality and a lower rate of adverse cardiovascular events in patients who did not discontinue ASA. On the other hand, due to the increased risk of adverse hemorrhagic events, current guidelines recommend preoperative cessation of clopidogrel for at least five days and ticagrelor for three days before surgery.

However, it is not always possible to wait three to five days after cessation of DAPT to perform CABG because many patients require urgent myocardial revascularization or are at increased risk of stent thrombosis [18]. In these patients, we expect an increased rate of hemorrhagic complications in the postoperative period, as well as an increased transfusion requirement. In the majority of studies conducted so far, the risk of hemorrhagic events was assessed based on the time of APT cessation. In our opinion, the only reliable way to assess the risk of hemorrhagic complications related to the application of APT is the preoperative analysis of PLT function using impedance aggregometry. The time of discontinuing APT preoperatively is not an accurate indicator of PLT function due to individual differences in drug reactivity, as well as possible resistance to therapy.

Data related to the importance of routine application of PLT function tests in CABG patients are available in the literature [15, 19]. Furthermore, in the majority of conducted studies, all patients treated with ASA are classified in the same group. In our study, patients were divided into two groups according to the residual effect of ASA intensity. Data from the literature indicate a lower rate of PMI in patients who preoperatively received APT. In this research, we tried to determine how APT affects intraoperative and postoperative values of cardiac enzymes. There was no significant difference between the investigated variables among the examined groups, except for the average time of APT cessation, which was expected.

Although there is no significant difference, it is evident that the lower utilization of LIMA is a characteristic of the groups with an increased risk of intraoperative and postoperative bleeding (PASAE group 83.0%, DAPT group 85.0%). In the control and MASAE groups, LIMA usage corresponds to the data from the available literature (93.0%, 94.6%). A lower utilization of LIMA, without a statistically significant difference, in patients on DAPT compared to patients on ASA monotherapy was also noted in the study conducted by Qu et al. [20] (94.9% vs. 92.7%). Based on these results, it can be concluded that in patients with significantly impaired PLT function, there is a more demanding LIMA harvesting, with an increased risk of iatrogenic trauma, which is probably a consequence of difficult hemostasis during the preparation. In all subjects, we used the pedicled LIMA harvesting tech-

nique, which can affect obtained results, as well as surgeon experience. Of particular importance is the fact that in patients with a stronger residual effect of ASA, LIMA usage is 11.4% lower compared to subjects with a mild residual effect of ASA.

We did not find a statistically significant difference in the duration of ECC and ACC between the examined groups. Therefore, we can rule out the negative effect of prolonged ECC on postoperative hemostasis. The obtained results are in correlation with the study conducted by Charif et al. [21]. There was no statistically significant difference in the amount of intraoperatively saved blood among the examined groups. That correlates with the results of the study by Kapetanakis et al. [22] and Cao et al. [23]. In addition, about 200 ml more blood was saved intraoperatively in the DAPT group compared to the control group.

There was no significant difference in the intraoperative and postoperative values of myocardial necrosis markers (hsTnI/CK-MB) between the studied groups. In the available literature, there is no data regarding the influence of preoperative PLT function on intraoperative and postoperative values of myocardial necrosis markers. Based on these results, it cannot be determined with certainty whether APT has a protective effect on the myocardium in cardiac surgery patients, so additional research in this field is necessary.

Patients with a residual therapeutic effect of DAPT had the highest postoperative chest tube drainage. In this group, drainage was significantly higher compared to the group with normal PLT function and compared to both groups where the PLT function was impaired due to the residual effect of ASA. The study by Petricevic et al. [15] also confirmed a higher risk of postoperative bleeding in patients with a residual effect of PLT $P2Y_{12}$ antagonist. The difference in the amount of drained blood between patients with normal PLT function and patients from the groups with residual effect of ASA was not significant. That correlates with the study by Myles et al. [24]. On the other hand, most of the older studies conducted before the year 2000 showed significantly higher drainage in patients on ASA compared to the control group. Patients from the PASAE group had a significantly higher frequency of hemorrhage-induced chest reexploration compared to the MASAE group. The majority of the studies conducted so far did not divide patients with residual ASA effect and compare chest reexploration rate according to the residual ASA effect intensity. In our study, there was no statistically significant difference in the chest reexploration rate between the control and MASAE group, between the control and PASAE group, and between the control and DAPT group. Likewise, we did not find significant differences regarding the chest reexploration rate between the MASAE and DAPT groups, as well as between the PASAE and DAPT groups. Results from studies available in the literature are conflicting. A study by Morawski et al. [25] indicates an increased frequency of chest reexploration in patients treated with ASA preoperatively, compared to the control group, while in the study of Myles et al. [24], no such difference was found. The results of Kapetanakis et al. [22] indicate a significantly higher risk of chest reexploration in the group of patients with the preoperative effect of clopidogrel. Similar results were obtained in the meta-analysis conducted by Cao et al. [23]. Significantly greater drainage in the DAPT group compared to the other groups, without a significant difference in the frequency of chest reexploration, can be explained by the fact that during the postoperative period, in patients from the DAPT group, greater drainage was expected and tolerated, and the priority was given to pharmacological treatment, while the indication for chest reexploration was delayed.

The amount of FFP transfusions did not differ significantly among the studied groups. Patients with the DAPT effect received a significantly higher amount of PRBC compared to patients from the control and MASAE groups. PRBC transfusion requirements in subjects from the PASAE group were significantly higher compared to the subjects from the control and MASAE groups. Subjects with the DAPT effect received significantly more PLT and Cryo transfusions compared to the other three groups. Most of the studies conducted so far indicate a higher rate of transfusion requirements in patients who preoperatively received PLT $P2Y_{12}R$ inhibitors, while the results related to the influence of ASA are conflicting [16, 18, 20]. That is very important because a recent study has demonstrated that postoperative bleeding and blood transfusion are independent predictors of increased long-term mortality after CABG and have a direct negative impact on patient prognosis.

PMI was recorded in 6.6% of subjects. That correlates with the results of previous studies, where the incidence ranges from 2–12%. Although PMI occurred 3.5 times more often in patients with normal PLT function compared to the DAPT group (8.8% vs. 2.5%), no statistically significant difference was found. Furthermore, there was no significant difference between other groups. The obtained results correlated with the study by Myles et al. [24]. The results of the study by Hastings et al. [26] showed that there was a significantly lower rate of PMI in patients treated with ASA until surgery. Cao et al. [23] showed that there is a significantly lower rate of PMI in patients treated with PLT $P2Y_{12}R$ inhibitors before surgery compared to patients who did not receive this therapy. Interestingly, the frequency of this complication is almost identical in patients with normal PLT function and patients with pronounced residual effects of ASA. The unexpectedly high frequency of PIM in the PASAE group could be explained by the highest average number of grafts in this group but with very low LIMA utilization. PMI due to early venous graft occlusion is well established. Higher postoperative troponin values correlate with groups with a higher incidence of myocardial infarction. That proves the claim that postoperative troponin values are a good indicator of PMI. The frequency of postoperative arrhythmias and inotropic support did not differ significantly between the studied groups. The obtained results correlated with the study by Sadgehi et al. [27] and with the results of the study conducted by Qu et al. [20].

Prolonged intubation, pneumonia, pleural effusion, and pneumothorax are respiratory complications monitored in the postoperative period. No significant difference was found in the frequency of respiratory complications among the studied groups. That correlates with the study by Kapetanakis et al. [22],

but the results differ from the study by Cao et al. [23], where patients with residual clopidogrel effects spent more time on mechanical ventilation. The results of our study show an almost four times higher frequency of prolonged intubation and pleural effusion that required thoracentesis or drainage in the PASAE group compared to the MASAE group. The high frequency of prolonged intubation in the PASAE group could be explained by the higher incidence of hemorrhage-induced chest reexploration and peri/postoperative infarction in these patients. A large number of postoperatively detected pneumothoraces in the DAPT group could be a consequence of more frequent iatrogenic injuries of the lung parenchyma due to difficult hemostasis of the operative field.

Intensive care length of stay and total length of hospitalization did not differ significantly among the studied groups. The results of the research conducted so far are conflicting. The study by Cao et al. [23], as well as the study by Sadeghi et al. [27], show a significantly longer stay in the intensive care unit among patients with continuous administration of clopidogrel compared to subjects who were on monotherapy with ASA. The results of Kapetanakis et al. [22] correlate with the results obtained in our research. The results of the meta-analysis by Hastings et al. [26] on 2,399 patients showed no significant difference in the length of hospitalization and the length of stay in the intensive care unit between subjects who were on ASA preoperatively compared to the control group.

Conclusion

The results obtained in this study demonstrate the importance of preoperative PLT function assessment in CABG patients. Patients with a residual effect of DAPT, as well as patients with a pronounced residual effect of ASA, have a higher risk of postoperative bleeding and chest reexploration, as well as increased transfusion demands.

REFERENCES

1. *Mohr FW, Morice MC, Kappetein AP, Feldman TE, Ståhle E, Colombo A,* et al. Coronary artery bypass graft surgery versus percutaneous coronary intervention in patients with three-vessel disease and left main coronary disease: 5-year follow-up of the randomised, clinical SYNTAX trial. Lancet 2013; 381(9867): 629–38.
2. *Jawitz OK, Gulack BC, Brennan JM, Thibault DP, Wang A, O'Brien SM,* et al. Association of postoperative complications and outcomes following coronary artery bypass grafting. Am Heart J 2020; 222: 220–8.
3. *Freundlich RE, Maile MD, Hajjar MM, Habib JR, Jewell ES, Schwann T,* et al. Years of life lost after complications of coronary artery bypass operations. Ann Thorac Surg 2017; 103(6): 1893–9.
4. *Demertzis S, Tiziano C, Gabriele C.* Antiplatelet therapy before cardiac surgery. Cardiovasc Med 2016; 19(4): 110–6.
5. *Davierwala PM, Verevkin A, Leontyev S, Misfeld M, Borger MA, Mohr FW.* Impact of expeditious management of perioperative myocardial ischemia in patients undergoing isolated coronary artery bypass surgery. Circulation 2013; 128(11 Suppl 1): S226–34.
6. *Wang TK, Stewart RA, Ramanathan T, Kang N, Gamble G, White HD.* Diagnosis of MI after CABG with high-sensitivity troponin T and new ECG or echocardiogram changes: relationship with mortality and validation of the universal definition of MI. Eur Heart J Acute Cardiovasc Care 2013; 2(4): 323–33.
7. *Karthik S, Grayson AD, McCarron EE, Pullan DM, Desmond MJ.* Reexploration for bleeding after coronary artery bypass surgery: risk factors, outcomes, and the effect of time delay. Ann Thorac Surg 2004; 78(2): 527–34; discussion 534.
8. *Senage T, Gerrard C, Moorjani N, Jenkins DP, Ali JM.* Early postoperative bleeding impacts long-term survival following first-time on-pump coronary artery bypass grafting. J Thorac Dis 2021; 13(10): 5670–82.
9. *Ranucci M, Baryshnikova E, Castelvecchio S, Pelissero G; Surgical and Clinical Outcome Research (SCORE) Group.* Surgical and Clinical Outcome Research (SCORE) Group. Major bleeding, transfusions, and anemia: The deadly triad of cardiac surgery. Ann Thorac Surg 2013; 96(2): 478–85.
10. *Hudzik B, Błachut A, Lesiak M, Kubica J, Wojakowski W, Gąsior M.* Summary of the European Society of Cardiology guidelines on dual antiplatelet therapy in patients after percutaneous coronary interventions. Kardiol Pol 2022; 80(10): 974–89.
11. *Klempfner R, Barac YD, Younis A, Kopel E, Younis A, Ronen G,* et al. Early referral to coronary artery bypass grafting following acute coronary syndrome, trends and outcomes from the Acute Coronary Syndrome Israeli Survey (ACSIS) 2000–2010. Heart Lung Circ 2018; 27(2): 175–82.
12. *Hwang D, Lee JM, Rhee TM, Kim YC, Park J, Park J,* et al. The effects of preoperative aspirin on coronary artery bypass surgery: a systematic meta-analysis. Korean Circ J 2019; 49(6): 498–510.
13. *Solo K, Lavi S, Choudhury T, Martin J, Nevis IF, Kwok CS,* et al. Pre-operative use of aspirin in patients undergoing coronary artery bypass grafting: a systematic review and updated meta-analysis. J Thorac Dis 2018; 10(6): 3444–59. Erratum in: J Thorac Dis 2018; 10(12): E860.
14. *Lawton JS, Tamis-Holland JE, Bangalore S, Bates ER, Beckie TM, Bischoff JM,* et al. 2021 ACC/AHA/SCAI guideline for coronary artery revascularization: a report of the American College of Cardiology/American Heart Association Joint Committee on Clinical Practice Guidelines. Circulation 2022; 145(3): e4–17. Erratum in: Circulation 2022; 145(11): e771.
15. *Petricevic M, Knezevic J, Biocina B, Mikus M, Konosic L, Rasic M,* et al. Association among Clopidogrel cessation, platelet function, and bleeding in coronary bypass surgery: an observational trial. Thorac Cardiovasc Surg 2021; 69(7): 630–8.
16. *Gherli R, Mariscalco G, Dalén M, Onorati F, Perrotti A, Chocron S,* et al. Safety of preoperative use of ticagrelor with or without aspirin compared with aspirin alone in patients with acute coronary syndromes undergoing coronary artery bypass grafting. JAMA Cardiol 2016; 1(8): 921–8.
17. *Thielmann M, Sharma V, Al-Attar N, Bulluck H, Bisleri G, Bunge JJH,* et al. ESC Joint Working Groups on Cardiovascular Surgery and the Cellular Biology of the Heart Position Paper: Perioperative myocardial injury and infarction in patients undergoing coronary artery bypass graft surgery. Eur Heart J 2017; 38(31): 2392–407.
18. *Nardi P, Pisano C, Turturici M, Bertoldo F, Maggio VR, Bassano C,* et al. The impact of dual antiplatelet therapy administration on the risk of bleeding complications during coronary artery bypass surgery. Kardiochir Torakochirurgia Pol 2021; 18(3): 145–51.
19. *Matkovic M, Novakovic T, Bilbija I, Lazovic JM, Tutus V, Cubrilo M,* et al. The routine use of platelet function tests in elective coronary artery bypass grafting: A prospective observational trial. J Card Surg 2021; 36(2): 629–36.

20. *Qu J, Zhang H, Rao C, Chen S, Zhao Y, Sun H,* et al. Dual antiplatelet therapy with clopidogrel and aspirin versus aspirin monotherapy in patients undergoing coronary artery bypass graft surgery. J Am Heart Assoc 2021; 10(11): e020413.
21. *Charif F, Hamdan R, Youness G, El Zein A, Issa M, Jassar Y,* et al. Dual antiplatelet therapy up to the time of non-elective coronary artery bypass grafting with prophylactic platelet transfusion: is it safe? J Cardiothorac Surg 2019; 14(1): 202.
22. *Kapetanakis EI, Medlam DA, Boyce SW, Haile E, Hill PC, Dullum MK,* et al. Clopidogrel administration prior to coronary artery bypass grafting surgery: the cardiologist's panacea or the surgeon's headache? Eur Heart J 2005; 26(6): 576–83.
23. *Cao L, Young N, Liu H, Silvestry S, Sun W, Zhao N,* et al. Preoperative aspirin use and outcomes in cardiac surgery patients. Ann Surg 2012; 255(2): 399–404.
24. *Myles PS, Smith JA, Forbes A, Silbert B, Jayarajah M, Painter T,* et al. Stopping vs. continuing aspirin before coronary artery surgery. N Engl J Med 2016; 374(8): 728–37.
25. *Morawski W, Sanak M, Cisowski M, Szczeklik M, Szczeklik W, Dropinski J,* et al. Prediction of the excessive perioperative bleeding in patients undergoing coronary artery bypass grafting: role of aspirin and platelet glycoprotein IIIa polymorphism. J Thorac Cardiovasc Surg 2005; 130(3): 791–6.
26. *Hastings S, Myles P, McIlroy D.* Aspirin and Coronary Artery Surgery: A Systematic Review and Meta-analysis. Br J Anaesth 2015; 115(3): 376–85.
27. *Sadeghi R, Haji Aghajani M, Miri R, Kachoueian N, Jadbabaei AN, Mahjoob MP,* et al. Dual antiplatelet therapy before coronary artery bypass grafting in patients with myocardial infarction: a prospective cohort study. BMC Surg 2021; 21(1): 449.

Received on March 6, 2023
Revised on June 7, 2023
Accepted on June 20, 2023
Online First June 2023

ORIGINAL ARTICLE
UDC: 617.7-007.681
DOI: https://doi.org/10.2298/VSP220729030M

Quality of life of patients with primary open-angle glaucoma, primary angle closure glaucoma, and pseudoexfoliation glaucoma in Central Serbia

Kvalitet života bolesnika sa primarnim glaukomom otvorenog ugla, primarnim glaukomom zatvorenog ugla i pseudoeksfolijativnim glaukomom u centralnoj Srbiji

Elizabeta Marčeta*, Tatjana Šarenac Vulović†‡, Nenad Petrović†‡, Dejan Vulović§‖, Marija Trenkić¶, Danijela Randjelović, Dušan Todorović†‡**

***Clinical Hospital Center Zvezdara, Department of Internal Medicine, Belgrade, Serbia; University Clinical Center Kragujevac, †Clinic for Ophthalmology, §Center for Plastic Surgery, Kragujevac, Serbia; University of Kragujevac, Faculty of Medical Sciences, ‡Department of Ophthalmology, ‖Department of Surgery, Kragujevac, Serbia; ¶University Clinical Center Niš, Clinic for Ophthalmology, Niš, Serbia; **Aero Medical Institute, Belgrade, Serbia**

Abstract

Background/Aim. Impaired vision resulting from glaucoma can have deleterious effects on both physical and mental health. This study aims to examine the adverse impacts of primary open-angle glaucoma (POAG), primary angle closure glaucoma (PACG), and pseudoexfoliation glaucoma (PEG) on the quality of life (QoL) in Central Serbia. **Methods.** This research, designed as a cross-sectional study, included 102 patients treated for POAG, PACG, or PEG. The patients were divided into three groups (POAG, PACG, and PEG) based on the type of glaucoma they suffered from and were monitored for six months. The data on the QoL were obtained with the National Eye Institute Visual Functioning Questionnaire 25 (NEI VFQ-25). Using the appropriate algorithm, the total NEI VFQ-25 scores were calculated for each group. **Results.** The questionnaire showed that scores for general vision were significantly lower in the PEG group than in the other two groups (PEG: 61.1 ± 12.6; POAG: 71.6 ± 17.9; PACG: 75.7 ± 11.6), $p < 0.001$. General health, eye pain, distance vision, social functioning, peripheral vision, reduced ability in daily activities, and dependence on others were also statistically significantly lower in the PEG group. The highest value of near vision was recorded for the POAG group (76.2 ± 21.2). The highest mean value in glaucomatous visual field defect was recorded in the PEG group (14.5 ± 3.6 dB). **Conclusion.** This research has demonstrated that the QoL is significantly lower in patients suffering from PEG compared to those suffering from POAG and PACG. Concerning the general health of patients, eye pain, distance vision, as well as the domain of social and everyday functioning, the lowest scores for individual areas of examination according to the NEI VFQ-25 were recorded in PEG patients. The results indicate that serious measures should be taken in order to improve the QoL of glaucoma patients.

Key words:
glaucoma, open-angle; glaucoma, angle-closure; exfoliation syndrome; quality of life; surveys and questionnaires.

Apstrakt

Uvod/Cilj. Oštećenje vida uzrokovano glaukomom ima snažan negativan uticaj na mentalno i fizičko zdravlje. Cilj studije bio je da se ispita uticaj primarnog glaukoma otvorenog ugla (PGOU), primarnog glaukoma zatvorenog ugla (PGZU) i pseudoeksfolijativnog glaukoma (PEG) na kvalitet života (KŽ) bolesnika u centralnoj Srbiji. **Metode.** U ovoj studiji preseka bila su uključena 102 bolesnika koji su lečeni zbog PGOU, PGZU ili PEG. Bolesnici su bili podeljeni u tri grupe (PGOU, PGZU i PEG) u zavisnosti od tipa glaukoma i posmatrani su šest meseci. Podaci o KŽ učesnika prikupljeni su korišćenjem upitnika *National Eye Institute Visual Functioning Questionnaire 25* (NEI VFQ-25). Korišćenjem odgovarajućeg algoritma izračunat je ukupan rezultat iz upitnika NEI VFQ-25 za svaku grupu. **Rezultati.** Parametri funkcije vida bili su statistički značajno niži u PEG grupi u poređenju sa ostalim

Correspondence to: Dušan Todorović, University Clinical Center Kragujevac, Clinic for Ophthalmology, Zmaj Jovina 30, 34 000 Kragujevac, Serbia. E-mail: drdusantodorovic@yahoo.com

ispitivanim grupama (PEG: 61,1 ± 12,6; PGOU: 71,6 ± 17,9; PGZU: 75,7 ± 11,6), $p < 0,001$. Opšte zdravlje, bol u očima, vid na daljinu, socijalno funkcionisanje, periferni vid, smanjena sposobnost u svakodnevnim aktivnostima i zavisnost od drugih, takođe su bili statistički značajno niži u PEG grupi. Najbolja oštrina vida na blizinu izmerena je u PGOU grupi (76,2 ± 21,2). Najviša srednja vrednost glaukomnog ispada vidnog polja zabeležena je u PEG grupi bolesnika (14,5 ± 3,6 dB). **Zaključak.** Istraživanje je pokazalo da su bolesnici sa PEG imali statistički značajno lošiji KŽ u poređenju sa bolesnicima koji su bolovali od PGOU i PGZU. Najniži skor po pojedinim oblastima ispitivanja po upitniku NEI VFQ-25 bio je kod bolesnika sa PEG, kada je u pitanju bilo opšte zdravlje bolesnika, osećaj bola u očima, vid na daljinu, kao i u domenu socijalnog i svakodnevnog funkcionisanja. Dobijeni rezultati ukazuju da treba preduzeti ozbiljne mere za popravljanje kvaliteta života bolesnika sa glaukomom.

Ključne reči:
glaukom, otvorenog ugla; glaukom, zatvorenog ugla; eksfolijativni sindrom; kvalitet života; ankete i upitnici.

Introduction

Glaucoma is a chronic and progressive optic neuropathy characterized by specific morphological changes in the head of the optic nerve and the retinal nerve fibers layer [1]. The defects of a visual field, which are the major clinical signs of glaucoma, result from the fiber loss in the retinal nerve. The most dominant risk factor for developing glaucoma is elevated intraocular pressure (IOP), i.e., ocular hypertension. However, there are also glaucoma patients with IOP measurements constantly lower than 21mmHg (so-called normal tension glaucoma) [2]. After cataracts, glaucoma is the second most common cause of blindness worldwide, but unlike a cataract, blindness caused by glaucoma is irreversible [3]. The global prevalence of glaucoma has been increasing. Reportedly, there were almost 80 million people with glaucoma in 2020, and 11 million of them were affected by irreversible blindness [4]. There are numerous classifications of glaucoma. Based on the mechanism of its development, glaucoma can be classified as primary, secondary, and congenital glaucoma [5]. In primary glaucoma, there is no association between glaucoma and any ophthalmic or systemic disease. Primary glaucoma is more likely to occur bilaterally [2]. On the other hand, secondary glaucoma results from another disease, and unlike primary glaucoma, more commonly manifests unilaterally [6]. The most frequent secondary glaucomas include pseudoexfoliative, pigmentary, uveitic, post-traumatic, and iatrogenic glaucoma. Congenital glaucoma is a rare condition with an incidence of 1 in 10,000 newborns. It stems from the developmental disorders of the iridocorneal angle [7]. Based on the status of the iridocorneal angle, i.e., its width, glaucoma can be classified as open-angle or angle-closure glaucoma [8]. Open-angle glaucoma is associated with abnormalities of the trabecular meshwork, which hinder the outflow of aqueous humor outflow. In angle-closure glaucoma, both primary and secondary, the closure of the iridocorneal angle occurs due to iridotrabecular contact (ITC). As a rule, in angle-closure glaucoma, the clinical symptoms are more pronounced, and IOP is more elevated [9]. Despite very dramatic clinical signs, this type of glaucoma can be pretty successfully treated with Yttrium aluminum garnet laser iridotomy, which most commonly leaves patients only with early-stage difficulties with near vision [9]. The diagnosis of glaucoma can be made with certainty only after taking IOP (tonometry), examining the width of the iridocorneal angle (gonioscopy), examining the head of the optic nerve (ophthalmoscopy), and performing a visual field testing (perimetry) [10]. Glaucoma treatments focus on lowering IOP levels, improving the circulation of the optic nerve head, and enhancing neuroprotection [1]. The reduction of elevated IOP is still the most effective method of glaucoma treatment, and it can be accomplished with anti-glaucoma drugs, laser therapy, and surgical procedures [11].

This study aims to examine the adverse impacts of primary open-angle glaucoma (POAG), primary angle closure glaucoma (PACG), and pseudoexfoliation glaucoma (PEG) on the quality of life (QoL). Countries differ in their cultural, economic, social, educational, and habituation characteristics. All these factors may be relevant for the QoL of glaucoma patients. Even within the same country, the regional differences vary significantly. To the best of our knowledge, no study comparing the QoL of patients with these three types of glaucoma has been conducted in the administrative district of Central Serbia. In addition to the general questionnaire inquiring about the demographic characteristics of the participants, this study used the National Eye Institute Visual Functioning Questionnaire 25 (NEI VFQ-25) as the main research instrument for gathering data on the QoL of the patients. NEI VFQ-25 was founded by the United States National Eye Institute (Bethesda, Maryland) in 1969. Since 1996, no further written permission has been needed for the usage of NEI VFQ-25. A final version of NEI VFQ-25 was adapted in 2000 [12]. This questionnaire is a reliable, standardized instrument for evaluating vision-dependent functions and the impacts of impaired vision on the QoL of patients suffering from a wide variety of ophthalmic diseases, including glaucoma. The initial version of the NEI VFQ-25 included 51 items. The questionnaire was later simplified and abbreviated, and its 25-item form was validated. The questionnaire uses a five-point Likert-type scale, so patients need to choose one from five provided answers. The questions cover 12 domains: general health, general vision, eye pain, near vision, distance vision, social functioning, mental health, disabilities, dependence on others, driving, color vision, and peripheral vision. The validation of the questionnaire was carried out in accordance with the cultural norms of the Serbian context in the same manner as in the study by Kovač et al. [13].

Methods

The study was conducted at the Clinic for Ophthalmology, University Clinical Center Kragujevac,

Serbia, from January to June 2022. The research was approved by the Ethics Committee of the University Clinical Center Kragujevac (No. 01/20-12323). According to the tenets of the Declaration of Helsinki, all patients gave their written consent at the beginning of the study.

The research was designed as a cross-sectional, randomized study. It included 102 participants suffering from POAG, PACG, or PEG who were treated in the outdoor unit of the Clinic. The patients visited the outdoor unit for their regular check-ups. The main inclusion criterion was the presence of one of these three types of glaucoma. They were equally divided into three groups (POAG, PACG, and PEG), depending on the type of glaucoma they suffered from. The study included only the patients treated for less than three years because, with longer treatments, it was ambiguous whether the low QoL was associated with the duration of their treatment, specific glaucoma they suffered from, or both.

The excluding criteria were: age below 18, the duration of the glaucoma treatment of more than three years, the presence of neuromuscular diseases or dementia, and pregnancy or breastfeeding. The other excluded groups included: the patients with other types of glaucoma (such as normal tension glaucoma and ocular hypertension), the patients treated for retinal diseases, optic neuropathy, ocular injuries, and the patients with a history of ocular surgeries or inflammation.

The glaucoma diagnoses were established after having determined that, in addition to the detected visual-field defects and elevated IOP levels (above 21 mmHg), the specific glaucomatous damage of the optic nerve head was recorded, i.e., the cup/disc ratio was above 0.3, there was neuroretinal rim thinning, and the asymmetry of a cup/disc ratio was larger than 0.2 between the eyes. The type of glaucoma was determined with a slit-lamp examination and gonioscopy. A wide and open iridocorneal angle was detected in the POAG patients. Angle-closure was defined based on the presence of ITC. ITC of more than 180 degrees was considered clinically relevant. Angle closure may result in raised IOP which may lead to glaucomatous optic neuropathy. In the patients with PEG, the iridocorneal angle was widely open. We also detected increased pigmentation. Finally, we detected the typical fibrillar deposits at the iris pupillary margins and the anterior lens capsules with slit lamp examination of the maximal mydriasis [14].

At the beginning of the study, the patients had to undergo a complete ophthalmological examination: visual acuity measurements, slit-lamp and funduscopic examinations, IOP measurements (taken with Goldmann applanation tonometer), gonioscopy, and standard automated perimetry (Humphrey visual field analyzer, Carl Zeiss, Oberkochen, Germany). According to Hodapp's classification, glaucoma patients were staged according to mean deviation (MD) value into early glaucomatous loss ≤ 6 decibels (dB), moderate glaucomatous loss 6 > MD ≤ 12 dB, and advanced glaucomatous loss MD > 12 dB [15]. All respondents were asked to fill in an NEI VFQ-25 questionnaire [13]. The questionnaire uses a five-point Likert-type scale; hence the patients need to choose one out of five provided answers. Each question/statement was converted into a scale ranging from 0 to 100. The appropriate algorithm was further used to calculate the total scores for the three groups. Every answer above 0 was graded with an additional 25 points; therefore, 0 was the lowest and 100 the highest possible score. The scores for each group were converted into the mean values with the standard deviation, minimum, and maximum values. The Machado et al. [16] study used a statistical program to conduct the G-power binomial test (binomial test, one sample case) aiming to examine the effects of visual acuity in the less impaired eye of the patients with POAG and their QoL. This study selected the probability of error of the first type $\alpha = 0.05$, the assumed minimum study power of 80% (0.8), and the effect size of 0.25. It identified a coefficient of determination of approximately 20% in a multiple linear regression model. We used a similar research instrument. With identical parameters, we calculated that at least 30 patients *per* group were needed to examine the impact of glaucoma type on QoL with a reasonable degree of probability. The Chi-squared (χ^2) test was used to compare differences in the frequency of categorical variables. The relationships between the dependent variable and the set of independent variables were examined with univariate and multivariate logistic regression. A value of $p < 0.05$ was considered to be statistically significant. All statistical calculations were performed in the standard SPSS software package, version 24.0. (The Statistical Package for Social Sciences software Inc, version 24.0, Chicago, IL).

Results

The mean age of the patients was 71.2 years. The highest mean value of the QoL questionnaire was recorded in the PEG group, followed by the PACG and the POAG groups (Table 1). However, the difference between the groups was not statistically significant ($p > 0.05$). Fifty-eight females and forty-four males participated in the study. The male/female ratio was not statistically significant either for the groups observed independently or in total ($p > 0.05$) (Table 1).

Table 1

Patient age and gender distribution

Characteristics	Groups			Total (n = 102)
	POAG	PACG	PEG	
Age, years				
mean ± SD	73.5 ± 6.2	68.5 ± 6.9	74 ± 5.8	71.2 ± 6.8
range, min-max	55–81 (69–76)*	52–81 (63–73)*	59–84 (72–77)*	52–84
Gender				
male/female, n (%)	15/19 (44.1/55.9)	14/20 (41.2/58.8)	15/19 (44.1/55.9)	44/58 (43.1/56.9)

POAG – primary open-angle glaucoma; PACG – primary angle closure glaucoma; PEG – pseudoexfoliation glaucoma; SD – standard deviation.

***The age range of the highest percentage of patients in the group.**

The lowest level of general health was recorded in the PEG group, followed by the POAG group. The PEG group was the most diverse in terms of general health (49.5 ± 25.1) with respect to other groups (POAG group: p <0.05; PACG group: $p < 0.001$). The average score for all three groups was 59.9 ± 22.0. The general vision of the PEG group was also significantly lower (PEG group: 61.1 ± 12.6; POAG group: 71.6 ± 17.9; PACG group: 75.7 ± 11.6), $p < 0.001$. The average score for all three groups was 69.4 ± 14.0. Eye pain was present more often in the PEG group (64.2 ± 17.6), in comparison to the groups with POAG (75.3 ± 24.5) and PACG (71.6 ± 21.0), $p < 0.05$. The average score for all three groups was 70.4 ± 21.1. Near vision was significantly better in the patients with POAG (76.2 ± 21.2), compared to the PACG (67.2 ± 18.8) and the PEG (62.1 ± 21.4) patients, $p < 0.05$. The average score for all three groups was 68.5 ± 20.5. Distance vision was significantly lower in the patients with PEG (61.2 ± 11.2) in comparison to the patients with PACG (81.4 ± 11.4) and POAG (76.4 ± 9.8), $p < 0.05$. The average score for all three groups was 73.0 ± 10.8. The difference between the latter two groups was not statistically significant, $p > 0.05$. Social functioning was hindered in the patients with PEG (82.2 ± 19.3) in comparison to the other two groups (POAG: 96.3 ± 6.8; PACG: 95.3 ±7.9), $p < 0.05$. The average score for all three groups was 91.3 ± 11.33. The patients with PEG (75.5 ±17.4) reported significantly lower levels of mental health (POAG patients: 93.5 ± 12.2; PACG patients: 92.5 ± 9.9), $p < 0.05$. The difference between the latter two groups was not statistically significant, $p > 0.05$. The average score for all three groups was 87.2 ± 13.2. The disability in daily activities was significantly lower in the PEG (63.4 ± 21.5) group than in the other two groups (POAG group: 90.8 ± 16.5; PACG group: 89.1 ± 19.3), $p < 0.001$. There were no significant differences between the latter two groups, $p > 0.05$. The average score for all three groups was 81.1 ± 19.1. The patients with PEG (82.3 ± 22.8) were more dependent on other people than the POAG (97.4 ± 13.3) and PACG patients (98.7 ±11.4), $p < 0.05$. The average score for all three groups was 92.8 ± 15.8. Lower vision abilities in the PEG patients and low general health made driving significantly more difficult for the PEG (49.4 ± 21.5) patients than for the POAG (87.3 ±17.3) and PACG (88.9 ± 14.6) patients, $p < 0.001$; there was no significant difference between the latter two groups, $p > 0.05$. The average score for all three groups was 75.2 ± 17.8. Our findings indicate that there was no statistically significant difference in color vision among the three groups (POAG: 100.0 ± 0.0; PACG: 100.0 ± 0.0; PEG: 94.2 ±16.7), while the average score for all three groups was 98.1 ± 5.6. Peripheral vision was more disturbed in the PEG (56.8 ± 21.7) group in comparison to the POAG (95.3 ± 5.2) and PACG (97.8 ±14.5) groups, $p < 0.001$. The average score for all three groups was 83.3 ± 13.8. The total score for the NEI VFQ-25 questionnaire was lower in the PEG (66.8 ± 19.1) patients (highly statistically significant) in comparison to the POAG (85.1 ± 13.7) and PACG (85.6 ± 13.5) patients, p <0.001, while the average score for all three groups was 79.2 ± 15.4 (Table 2).

Table 2

Scores of the NEIVFQ-25 questionnaire for the groups

Domains	Groups			All participants
	POAG	PACG	PEG	
General health	61.32 ± 19.34	68.82 ± 21.56	49.48 ± 25.12 $p < 0.001^a$, $p < 0.05^b$	59.87 ± 22.0
General vision	71.65 ± 17.86	75.67 ± 11.65	61.10 ± 12.57 $p < 0.001^{a,b}$	69.44 ± 14.02
Eye pain	75.35 ± 24.54	71.61 ± 21.04	64.17 ± 17.61 $p < 0.05^a$	70.37 ± 21.06
Near vision	76.24 ± 21.17	67.25 ± 18.83 $p < 0.05^a$	62.11 ± 21.43 $p < 0.05^a$	68.53 ± 20.48
Distance vision	76.45 ± 9.83	81.45 ± 11.38	61.18 ± 11.24 $p < 0.05^{a,b}$	73.02 ± 10.82
Social functioning	96.29 ± 6.79	95.34 ± 7.92	82.16 ± 19.32 $p < 0.05^{a,b}$	91.26 ± 11.34
Mental health	93.54 ± 12.22	92.48 ± 9.97	75.53 ± 17.38 $p < 0.05^{a,b}$	87.18 ± 13.19
Disability	90.82 ±16.47	89.12 ± 19.27	63.43 ± 21.48 $p < 0.001^{a,b}$	81.12 ± 19.07
Dependence on others	97.36 ±13.29	98.71 ± 11.42	82.34 ± 22.81 $p < 0.05^{a,b}$	92.8 ± 15.84
Driving	87.27 ± 17.28	88.86 ± 14.57	49.36 ± 21.52 $p < 0.001^{a,b}$	75.16 ± 17.79
Color vision	100.00 ± 0.00	100.00 ± 0.00	94.23 ±16.71 $p > 0.05$	98.07 ± 5.58
Peripheral vision	95.27 ± 5.17	97.81 ± 14.49	56.75 ± 21.72 $p < 0.001^{a,b}$	83.28 ± 13.79
Total	85.13 ± 13.66	85.59 ± 13.51	66.82 ± 19.08 $p < 0.001^{a,b}$	79.18 ± 15.42

NEI VFQ-25 – National Eye Institute Visual Functioning Questionnaire 25.
For abbreviations of other terms, see Table 1.
All results are shown as mean ± standard deviation. [a] – *p*-value vs. POAG group; [b] – *p*-value vs. PACG group.

Table 3

The influence of different glaucoma stages on the quality of life

Glaucoma stage/type	Early stage		Moderate stage		Advanced stage	
	MD (dB)	NEI VFQ-25 score	MD (dB)	NEI VFQ-25 score	MD (dB)	NEI VFQ-25 score
POAG	3.8 ± 1.0	88.1 ± 16.6	7.5 ± 2.1	84.2 ± 11.7	12.8 ± 3.2	81.3 ± 10.5
PACG	3.7 ± 1.1	89.2 ± 11.9	6.9 ± 2.3	86.4 ± 12.9	12.4 ± 2.8	83.1 ± 14.0
PEG	4.0 ± 1.2	70.2 ± 15.6	8.8 ± 1.8	68.5 ± 19.3	14.5 ± 3.6	64.7 ± 17.1
p	> 0.05	< 0.05	> 0.05	< 0.05	> 0.05	< 0.05

MD – mean deviation value; NEI VFQ-25 – National Eye Institute Visual Functioning Questionnaire 25. For abbreviations of other terms, see Table 1. All results are shown as mean ± standard deviation.

Table 3 shows the influence of different glaucoma stages on the QoL in all groups. The lowest NEI VFQ-25 score was recorded in the PEG group in all three stages. These values were significantly lower compared to POAG and PACG ($p < 0.05$). The differences between POAG and PACG were not statistically significant ($p > 0.05$). The highest mean value in glaucomatous visual field defect was recorded in the PEG group (14.5 ± 3.6 dB), while the patients with PACG had the lowest visual field defects in all three stages of glaucoma progression, but these results were not significant compared to the other two groups.

Discussion

As defined by the World Health Organization, health is "a state of complete physical, mental, and social well-being, not only the absence of a disease" [17]. Visual impairments caused by glaucoma or other ophthalmological diseases have strong adverse impacts on mental and physical health [18]. As patients' life expectancy has increased over time, the number of comorbidities has also increased, including the diseases that lead to visual impairments. Therefore, the QoL of glaucoma patients should be observed as a crucial element of any treatment. Glaucoma does not only lead to vision disturbances, but it also affects many aspects of patients' QoL [19]. Many studies suggest that advanced stages of glaucoma disease, with intensive visual field defects, are strongly associated with anxiety and depression [20, 21]. Likewise, patients' perception of the nature of the disease and the therapeutic treatments – medication, laser, or surgery – affects the QoL. In the end, glaucoma patients with impaired vision are more likely to suffer injuries while performing basic life activities, such as walking, eating, reading, and driving [22].

In this study, the patients were divided into three groups based on the glaucoma type they suffered from. Our results indicate that these three groups do not differ in age or gender. These findings are in line with the results obtained by some other studies [23, 24]. PEG is very difficult to control, regardless of the type of applied therapy. The disease, which includes high IOP levels on a daily basis, has an unpredictable course and faster progression [25]. Patients with PEG also have associated cardiovascular and cerebrovascular comorbidities more often and disturbed circulation in the optic nerve head. We recorded a significantly higher incidence of cerebrovascular diseases in the PEG group. That requires a greater number of medications to be used daily and, thus, can be associated with lower compliance rates. In addition, more comorbidities result in more financial strain because more medications must be bought. Since glaucoma patients are mainly pension users, very frequently they cannot afford all the medications they need or find them expensive. This *circulus vitiosus* can lead to decreased compliance rates and subsequent disease progressions [26, 27]. In developing countries, low income disturbs the QoL of older people significantly. There are incentives for some prescribed medicines for both systemic diseases and antiglaucomatous eye drops. However, a lot of patients choose to take only a proportion of the prescribed therapy, if any. Such patients usually live in rural areas and are not adequately informed about glaucoma and its potential complications. Since glaucoma is frequently asymptomatic, many patients believe that there is no need to take the prescribed therapy because no complications are expected. Even though it seemed less important, the financial status can have a huge influence on the QoL of patients treating glaucoma in our country. In developed countries, pensions are higher, and there are more incentives and programs available for the elderly.

Our findings indicate that there is a big discrepancy between the groups in terms of ocular pain. The patients with PEG stand out in terms of pain struggle. The ocular pain may be attributed to the number of antiglaucoma eye drops used by this group (more than one medication and more than one application per day), which is higher than for other groups. Namely, ocular pain stems from one of the side effects of benzalkonium chloride present in almost all antiglaucoma eye drops [28]. Benzalkonium chloride disturbs corneal and conjunctival epithelial cells, causing tear film instability and consequently ocular surface disease (OSD) [29]. OSD is more frequent in patients with pseudoexfoliations (PEX) because PEX affects conjunctival goblet cells [30]. These cells are responsible for producing the mucin layer of the tear film. Thus, PEX also leads to the development of dry eye that patients experience as discomfort and ocular pain [31]. Most patients subjectively described that feeling as a foreign-body sensation rather than pain. Only the patients who had acute glaucoma attacks had experienced real pain.

It is well known that vision could be highly impaired in patients with glaucoma [19]. Our findings indicate a difference in the degree of vision impairment in different glaucoma types. The PEG patients reported significantly lower general vision, as well as near and distance vision. These findings are similar to some previous studies [32–34]. In patients with PEX,

the progression of the disease is faster. It is also associated with cerebrovascular diseases and also leads more commonly to cataracts. It is also very interesting that the scores for near vision were less favorable in the PACG group than in the POAG group. The patients with PACG predominantly have hyperopic refraction anomaly; therefore, near vision is impaired on its own, but also due to the usage of myotics (if necessary).

Driving is very hard for patients with advanced glaucoma because of their poor distance vision and advanced visual-field defects [35]. Middle-aged patients with glaucoma can develop compensatory mechanisms while driving (head movement in the direction of the visual field defects). However, older patients with advanced glaucoma have much more difficulty with driving. According to our findings, PEG patients face more problems while driving than other groups. The results are not surprising, taking into consideration their poor distance vision, advanced visual-field defects, OSD symptoms, comorbidities, etc. Interestingly, a lot of participants confessed to not having a driving license due to their financial situation. The circumstances recorded for our specific context differ significantly from those recorded for developed countries. Social functioning is a very important aspect of the QoL. The ability to go out, meet friends and family, go shopping, visit the hospital, and pay bills is important for every individual [36].

The patients with PEG suffer from poor distance vision, comorbidities, ocular discomfort, etc., so they also reported the lowest levels of social functioning. The study subjects also highlighted that family gatherings and hospital visits were the most significant aspects of social functioning. Other options provided in this questionnaire, such as going out, going shopping, and going to a cinema or a theater, were not selected as crucial for our patients. These findings can be important for some future investigations, which may compare the habitual and cultural aspects between different nationalities. Daily activities, crucial elements of the QoL, are very hard for patients with PEG. Thus, patients with PEG face many obstacles in everyday activities and have to rely on other people to help them out [37].

Of all mental health disorders, anxiety and depression are predominant in glaucoma patients [38]. The available data demonstrate that anxiety is present in 13% of glaucoma patients in Japan, 14% in Turkey, 22.9% in China, and 17% in the USA [38–41]. This incidence rate is quite higher compared to the general population in these countries, where anxiety varies from 2.2 to 9.4% [36]. The incidence of depression is also statistically higher in glaucoma patients and reaches up to 22% in the USA, compared to 5.7% in the general population [41]. The high incidence rates of depression and anxiety in these patients can be attributed to their reduced visual acuity, their fear of the disease progression, as well as the difficulties they experience while driving, reading, walking, etc. Our results demonstrate that the incidence of depression and anxiety was significantly higher in our PEG patients. Many of them confessed that depression did not stem only from glaucoma. Low income and poor social opportunities for old persons were also reported as relevant factors. That is in line with the findings of other studies [42–44].

Visual field defect is proportional to QoL scores in glaucomatous patients. Higher MD values are associated with lower QoL scores [35]. Our findings show that PEG patients have the lowest QoL scores compared to the other two groups of patients. They also exhibited the lowest MD values, which can be blamed for the extremely low QoL of PEG patients. As our study suggests, patients with PEG have significantly higher incidence rates in many other domains, such as social functioning, dependence on others, driving, and peripheral vision ($p < 0.05$). However, no significant difference was recorded among the three groups in terms of matching clothing items based on their color ($p > 0.05$). The results may stem from the fact that our patients were mostly older. The elderly, especially from rural areas, do not consider matching colors of their garments an important factor in their everyday life. Similar results were obtained by Onakoya et al. [45]. Likewise, a study done by Marčeta and Todorovic [46] shows that the clinical characteristics of PEG are the least favorable, which complies with our results.

Conclusion

Our research has shown that glaucoma is much more than an eye disease because it affects many aspects of patients' lives. By comparing NEI VFQ-25 scores for three groups suffering from three different types of glaucoma, we found that the QoL of the patients with PEG was the lowest compared to the patients with POAG and PACG. The results are not surprising since the clinical characteristics of PEG are the least favorable. The findings further suggest that the QoL of glaucoma patients should receive more attention. The current state of affairs has to be improved. Medical staff can contribute by providing better insight into the nature, course, and medical prognoses of the disease, by putting more effort into increasing compliance rates, and by ameliorating the current treatments of ocular and systemic comorbidities. Psychiatric help should be also included. All these measures can greatly contribute to the more successful management of this complex disease. Studies like this can contribute to new meta-analysis studies and also in updating the literature on this topic.

Acknowledgement

We would like to thank Ph.D. Jelena Josijević for doing the language revision of the manuscript.

REFERENCES

1. *Zhou H, Shen Z.* The definition of glaucoma. In: *Zhou H, Shen Z*, editors. Intraocular pressure and Glaucoma. Wuhan, China: Hubei Science & Technology Press; 2010. p. 8–11.
2. *Liu SA, Zhao ZN, Sun NN, Han Y, Chen J, Fan ZG.* Transitions of the understanding and definition of primary glaucoma. Chin Med J (Engl) 2018; 131(23): 2852–9.

3. *Choudhari N, Pathak-Ray V, Kaushik S, Vyas P, George R.* Prevalent practice patterns in glaucoma: Poll of Indian ophthalmologists at a national conference. Indian J Ophthalmol 2016; 64(10): 715–21.
4. *Kalayci M, Cetinkaya E, Erol MK.* Prevalence of primary open-angle glaucoma in a Somalia population. Int Ophthalmol 2021; 41(2): 581–6.
5. *Lee JY, Akiyama G, Saraswathy S, Xie X, Pan X, Hong YK*, et al. Aqueous humour outflow imaging: seeing is believing. Eye (Lond) 2021; 35(1): 202–15.
6. *Camp DA, Yadav P, Dalvin LA, Shields CL.* Glaucoma secondary to intraocular tumors: mechanisms and management. Curr Opin Ophthalmol 2019; 30(2): 71–81.
7. *Mocan MC, Mehta AA, Aref AA.* Update in genetics and surgical management of primary congenital glaucoma. Turk J Ophthalmol 2019; 49(6): 347–55.
8. *Zhang N, Wang J, Chen B, Li Y, Jiang B.* Prevalence of primary angle closure glaucoma in the last 20 years: A meta-analysis and systematic review. Front Med (Lausanne) 2021; 7: 624179.
9. *Flores-Sánchez BC, Tatham AJ.* Acute angle closure glaucoma. Br J Hosp Med (Lond) 2019; 80(12): C174–9.
10. *Hagiwara Y, Koh JEW, Tan JH, Bhandary SV, Laude A, Ciaccio EJ*, et al. Computer-aided diagnosis of glaucoma using fundus images: A review. Comput Methods Programs Biomed 2018; 165: 1–12.
11. *Storgaard L, Tran TL, Freiberg JC, Hauser AS, Kolko M.* Glaucoma clinical research: Trends in treatment strategies and drug development. Front Med (Lausanne) 2021; 8: 733080.
12. *Mangione CM, Lee PP, Gutierrez PR, Spritzer K, Berry S, Hays RD*, et al. Development of the 25-item National Eye Institute Visual Function Questionnaire. Arch Ophthalmol 2001; 119(7): 1050–8.
13. *Kovač B, Vukosavljević M, Djokić Kovač J, Resan M, Trajković G, Janković J*, et al. Validation and cross-cultural adaptation of the National Eye Institute Visual Function Questionnaire (NEI VFQ-25) in Serbian patients. Health Qual Life Outcomes 2015; 13: 142.
14. *European Glaucoma Society.* Optic nerve head and retinal nerve fibre layer. In: Terminology and guidelines for glaucoma. 5th ed. Br J Ophthalmol 2021; 105(Suppl 1): 71–81.
15. *Hodapp E, Parrish RK, Anderson DR.* Clinical decisions in glaucoma. St. Louis: Mosby; 1993. 204 p.
16. *Machado LF, Kawamuro M, Portela RC, Fares NT, Bergamo V, Souza LM,* et al. Factors associated with vision-related quality of life in Brazilian patients with glaucoma. Arq Brass Oftalmol 2019; 82(6): 463–70.
17. *Ribeiro MV, Hasten-Reiter Júnior HN, Ribeiro EA, Jucá MJ, Barbosa FT, Sousa-Rodrigues CF.* Association between visual impairment and depression in the elderly: a systematic review. Arq Bras Oftalmol 2015; 78(3): 197–201.
18. *Courtney-Long EA, Carroll DD, Zhang QC, Stevens AC, Griffin-Blake S, Armour BS*, et al. Prevalence of Disability and Disability Type Among Adults--United States, 2013. MMWR Morb Mortal Wkly Rep 2015; 64(29): 777–83.
19. *Biggerstaff KS, Lin A.* Glaucoma and Quality of Life. Int Ophthalmol Clin 2018; 58(3): 11–22.
20. *Shin DY, Jung KI, Park HY, Park CK.* The effect of anxiety and depression on progression of glaucoma. Sci Rep 2021; 11(1): 1769.
21. *Tharmathurai S, Muhammad-Ikmal MK, Razak AA, Che-Hamzah J, Azhany Y, Fazilawati Q*, et al. Depression and Severity of Glaucoma Among Older Adults in Urban and Suburban Areas. J Glaucoma 2021; 30(5): e205–12.
22. *Hirneiss C, Kortüm K.* Quality of Life in Patients with Glaucoma. Klin Monbl Augenheilkd 2016; 233(2): 148–53. (German)
23. *Stein JD, Khawaja AP, Weizer JS.* Glaucoma in Adults-Screening, Diagnosis, and Management: A Review. JAMA 2021; 325(2): 164–74.
24. *Tanito M, Matsuoka Y.* Proportion of Glaucoma Types and Surgeries Among Young, Pre-Old, Old, and Oldest-Old Age Groups or Different Sex Groups. Clin Ophthalmol 2022; 16: 1815–9.
25. *Tekin K, Inanc M, Elgin U.* Monitoring and management of the patient with pseudoexfoliation syndrome: current perspectives. Clin Ophthalmol 2019; 13: 453–64.
26. *McKean-Cowdin R, Varma R, Wu J, Hays RD, Azen SP; Los Angeles Latino Eye Study Group.* Severity of visual field loss and health-related quality of life. Am J Ophthalmol 2007; 143(6): 1013–23.
27. *Wolfram C, Lorenz K, Breitscheidel L, Verboven Y, Pfeiffer N.* Health- and vision-related quality of life in patients with ocular hypertension or primary open-angle glaucoma. Ophthalmologica 2013; 229(4): 227–34.
28. *Šarenac-Vulović T, Pavlović S, Janicijević K, Todorović D, Paunović S, Petrović N,* et al. Tear film stability in patients with pseudoexfoliation. Ser J Exp Clin Res 2018; 19(3): 243–6.
29. *Zhang X, Vadoothker S, Munir WM, Saeedi O.* Ocular Surface Disease and Glaucoma Medications: A Clinical Approach. Eye Contact Lens 2019; 45(1): 11–8.
30. *Todorović D, Šarenac-Vulovic T, Jovanović S, Janičijević-Petrović M, Petrović N, Kontić M,* et al. The impact of pseudoexfoliation and artificial tear application on the tear film stability in a pseudophakic eye. Srp Arh Celok Lek 2018; 146(7-8): 422–7.
31. *Dermenoudi M, Matsou A, Keskini C, Anastasopoulos E.* Ocular Surface Disease Signs and Symptoms in Patients with Pseudoexfoliative Glaucoma: A Case-Control Study. Vision (Basel) 2022; 6(1): 11.
32. *Rao A, Raj N, Pradhan A, Senthil S, Garudadri CS, Verma PVKS,* et al. Visual impairment in pseudoexfoliation from four tertiary centres in India. PLoS One 2020; 15(5): e0233268.
33. *Singh VM, Yerramneni R, Madia T, Prashanthi S, Vaddavalli PK, Reddy JC.* Complications and visual outcomes of cataract surgery in patients with pseudoexfoliation. Int Ophthalmol 2021; 41(7): 2303–14.
34. *Cho YK, Huang W, Nishimura E.* Myopic refractive shift represents dense nuclear sclerosis and thin lens in lenticular myopia. Clin Exp Optom 2013; 96(5): 479–85.
35. *Montana CL, Bhorade AM.* Glaucoma and quality of life: fall and driving risk. Curr Opin Ophthalmol 2018; 29(2): 135–40.
36. *Jin S, Trope GE, Buys YM, Badley EM, Thavorn K, Yan P*, et al. Reduced social participation among seniors with self-reported visual impairment and glaucoma. PLoS One 2019; 14(7): e0218540.
37. *E JY, Schrack JA, Mihailovic A, Wanigatunga AA, West SK, Friedman DS*, et al. Patterns of Daily Physical Activity across the Spectrum of Visual Field Damage in Glaucoma Patients. Ophthalmology 2021; 128(1): 70–7.
38. *Mabuchi F, Yoshimura K, Kashiwagi K, Shioe K, Yamagata Z, Kanba S*, et al. High prevalence of anxiety and depression in patients with primary open-angle glaucoma. J Glaucoma 2008; 17(7): 552–7.
39. *Tastan S, Iyigun E, Bayer A, Acikel C.* Anxiety, depression, and quality of life in Turkish patients with glaucoma. Psychol Rep 2010; 106(2): 343–57.
40. *Zhou C, Qian S, Wu P, Qiu C.* Anxiety and depression in Chinese patients with glaucoma: sociodemographic, clinical, and self-reported correlates. J Psychosom Res 2013; 75(1): 72–82.
41. *Zhang X, Olson DJ, Le P, Lin FC, Fleischman D, Davis RM.* The association between glaucoma, anxiety, and depression in a large population. Am J Ophthalmol 2017; 183: 37–41.

42. *Nelson P, Aspinall P, Papasouliotis O, Worton B, O'Brien C.* Quality of life in glaucoma and its relationship with visual function. J Glaucoma 2003; 12(2): 139–50.
43. *Skalicky SE, Fenwick E, Martin KR, Crowston J, Goldberg I, McCluskey P.* Impact of age-related macular degeneration in patients with glaucoma: understanding the patients' perspective. Clin Exp Ophthalmol 2016; 44(5): 377–87.
44. *Wilson MR, Coleman AL, Yu F, Sasaki I, Bing EG, Kim MH.* Depression in patients with glaucoma as measured by self-report surveys. Ophthalmology 2002; 109(5): 1018–22.
45. *Onakoya AO, Mbadugha CA, Aribaba OT, Ibidapo OO.* Quality of life of primary open angle glaucoma patients in lagos, Nigeria: clinical and sociodemographic correlates. J Glaucoma 2012; 21(5): 287–95.
46. *Marčeta E, Todorovic D.* The effect of glaucoma on the quality of patient's life. Ser J Exp Clin Res 2021. doi: 10.2478/sjecr-2020-0046.

Received on July 29, 2022
Revised on May 8, 2023
Accepted on May 9, 2023
Online First May 2023

M E T A - A N A L Y S I S

UDC: 617.7-02-07
DOI: https://doi.org/10.2298/VSP220301024D

Prevalence of computer vision syndrome in computer users: a systematic review and meta-analysis

Prevalencija sindroma „kompjuterskog vida“ kod korisnika računara: sistematski pregled i meta-analiza

Vanja Dimitrijević*, Ivana Todorović†, Biljana Viduka‡, Igor Lavrnić§, Dejan Viduka‖

***University of Novi Sad, Faculty of Sport and Physical Education, Novi Sad, Serbia; †University of Priština/Kosovska Mitrovica, Faculty of Medicine, Kosovska Mitrovica, Serbia; ‡Educons University Sremska Kamenica, Faculty of Project and Innovation Management, Belgrade, Serbia; §Faculty of Maritime Academic Studies Belgrade, Serbia; ‖University Business Academy, Faculty of Applied Management, Economics, and Finance, Belgrade, Serbia**

Abstract

Background/Aim. Vision and health problems associated with the use of computers and other digital devices are known as computer vision syndrome (CVS). Advances in technology have led to increased use of computers, so the prevalence of these symptoms is increasing. The aim of this study was to calculate the overall prevalence of CVS and CVS symptoms using meta-analysis. **Methods.** The study was developed in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) Statement. In July 2021, a systematic search of four electronic databases with article collections was performed: PubMed, Cochrane Library, Web of Science, and Google Scholar. The key search terms were: "computer vision syndrome", "computer users", "digital eyestrain", "headache", "dry eyes", "red eyes", "eyestrain", "neck pain", "back pain", and "shoulder pain". The articles included in the study had to be original articles written in English only, and the criterion that had to be met was that the research included computer users. As a result, the prevalence of CVS or the prevalence of any of the symptoms of CVS had to be measured. **Results.** A total of 43 articles were fully reviewed, of which 20 were included in the meta-analysis. The total calculated prevalence for all studies was 74.4%, while the prevalence for individual symptoms was: headache – 43%, dry eyes – 24.4%, eyestrain – 29%, red eyes – 20.7%, and neck, back, or shoulder pain – 46.3%. **Conclusion.** The results obtained are worrying and point to the necessity of a multidisciplinary approach to solving CVS-related problems.

Key words:
asthenopia; computers; databases, bibliographic; health; meta-analysis.

Apstrakt

Uvod/Cilj. Problemi sa vidom i zdravstveni problemi povezani sa upotrebom računara i drugih digitalnih uređaja poznati su kao sindrom „kompjuterskog vida“ (SKV). Napredak u tehnologiji doveo je do povećane upotrebe računara, tako da je rasprostranjenost tih simptoma sve veća. Cilj rada bio je da se izračuna ukupna rasprostranjenost SKV-a i simptoma SKV-a primenom meta-analize. **Metode.** Studija je razvijena u skladu sa Izjavom o preferiranim stavkama izveštavanja za sistematske preglede i meta-analize (*Preferred Reporting Items for Systematic Reviews and Meta-Analyses* – PRISMA). U julu 2021. godine, izvršena je sistematska pretraga četiri elektronske baze podataka sa zbirkama članaka: *PubMed, Cochrane Library, Web of Science* i *Google Scholar*. Ključni termini za pretragu bili su: „sindrom kompjuterskog vida“, „korisnici računara“, „digitalno naprezanje očiju“, „glavobolja“, „suve oči“, „crvene oči“, „naprezanje očiju“, „bol u vratu“, „bol u leđima“ i „bol u ramenu“. Članci koji su bili uključeni u studiju morali su da budu originalni članci napisani samo na engleskom jeziku, a kriterijum je nalagao da se istraživanjem moraju obuhvatiti korisnici računara. Kao rezultat toga, morala je biti merena rasprostranjenost SKV-a ili rasprostranjenost bilo kojeg od simptoma SKV-a. **Rezultati.** U potpunosti su pregledana 43 članka, od kojih je 20 bilo uključeno u meta-analizu. Ukupna izračunata rasprostranjenost za sve studije iznosila je 74,4%, dok je

Correspondence to: Dejan Viduka, University of Business Academy, Faculty of Applied Management, Economics, and Finance, Jevrejska 24/1, 11 000 Belgrade, Serbia. E-mail: dejan@viduka.info

rasprostranjenost za pojedinačne simptome bila: glavobolja – 43%, suve oči – 24,4%, naprezanje očiju – 29%, crvene oči – 20,7% i bol u vratu, leđima ili ramenu – 46,3%. **Zaključak.** Dobijeni rezultati su zabrinjavajući i ukazuju na neophodnost multidisciplinarnog pristupa rešavanju problema vezanih za SKV.

Ključne reči:
astenopija; kompjuteri; baze podataka, bibliografske; zdravlje; meta-analiza.

Introduction

In the last few decades, advances in technology have become the focus of most countries (especially the underdeveloped ones) [1]. There is no doubt that the computer has freed us from many difficult tasks. However, its constant use leads to unforeseen vision and health problems, especially when its use is uncontrolled [2]. These vision and health problems associated with computer use are known as computer vision syndrome (CVS). Prolonged use of the screen can cause problems such as dryness of eyes, redness, eyestrain, irritation, tired eyes, blurred vision, hypersensitivity to light, headaches, and muscular problems, specifically back, shoulder, and neck pain that stem from using a computer [3, 4]. According to the American Optometric Association [5], all these symptoms of CVS are also called digital eyestrain, or in some studies, it is called asthenopia [6–10].

Research shows that as many as 90% of people who use computers for more than 2 hrs a day experience symptoms associated with vision problems [11]. A study from Japan by Iwakiri et al. [12] reported that the prevalence (PRv) of CVS among office workers was 72.1%. In Egypt, eyestrain (72.4%) and headache (64.4%) were the most commonly reported symptoms of CVS [13]. CVS is reported in 54.6% of call center operators in Sao Paulo, Brazil [14]. A study of 419 computer users in India [15] found that about 46.3% of users experienced two or more of the following symptoms during or after working on a computer: burning sensation, itchy eyes, pain, tenderness, redness, excess tears, dryness, discomfort when looking, blurred vision, and discoloration of objects. Studies among students show that the PRv of CVS among student engineers was 81.9%, while among medical students, it was 78.6% [16]. In the United States, about 54 million children use computers, 25–30% of whom have developed eye problems and have to rely on glasses for better vision [17]. CVS occurs because the eyes and brain have different reactions to characters seen on a computer screen than the characters printed on paper. Changes may occur constantly on the computer screen, but the printed characters remain stable and have clear contrast and edges [18]. When the eyes are fixed on the computer for a long time, and the distance between the eyes and the computer is small, fatigue of the ciliary muscles can occur, which can cause headaches [19]. One or more factors may be responsible for the development of CVS. These factors are infrequent blinking, prolonged viewing of digital screens, inappropriate lighting conditions, ametropia, glare, and incorrect distances between the eye and the computer [20]. Due to the consistent focus on the screen, our blinking rhythm is disturbed, which contributes to reduced tear production and reduces the natural moisture of the eyes, resulting in corneal stress and causing dry eyes, watery eyes, itching, and eye pain [21], additional cramps accommodation of the eye, the disorder of the accommodation mechanism (blurred vision, double vision, presbyopia, myopia, and slow change of focus) [4].

The computer releases electromagnetic radiation, so a great deal of energy-related stress is developed against the ciliary muscles. Poor lighting conditions, prolonged computer use, screen brightness, refractive errors, and improper workstation tuning are also risk factors for CVS [22, 23]. Improper height and angle of inclination of the visual display unit lead to pain in the back, neck, and shoulders. Twenty-two percent of computer workers report musculoskeletal disorders [24]. When the screen is at a higher level, the user turns backward, which causes muscle strain on the trapezius and neck muscles [25].

Daily use of personal computers and digital screens for 3 hrs or more leads to a high risk of developing CVS, occupational overuse syndrome, and psychosocial stress [26]. Even infrequent daily use of computers leads to various health problems [27]. Instruments used for diagnosis are usually unstructured questionnaires that focus on the frequency of symptoms occurrence [28–30], their intensity [31], or both [26, 32]. Using the previous questionnaires, Segui et al. [33] validated a questionnaire for respondents with CVS problems.

The aim of this study was to assess the overall PRv of CVS for all included studies using meta-analysis, as well as the PRv of certain symptoms that characterize CVS.

Methods

This study has been developed and reported in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [34].

Data sources and searches

A search strategy was developed to identify all relevant studies dealing with the PRv of CVS. Our systematic search included PubMed, Cochrane Library, Web of Science, and Google Scholar databases. We used combinations of the subject titles “computer vision syndrome”, “digital eyestrain”, “asthenopia”, “computer users”, “dry eyes”, “headache”, “eyestrain”, “red eyes”, “neck pain”, “back pain”, and “shoulder pain”. The diagram of the process of study selection for the meta-analysis is shown in Figure 1. We also manually searched for reference citations of identified critiques and selected original research articles to download the full text.

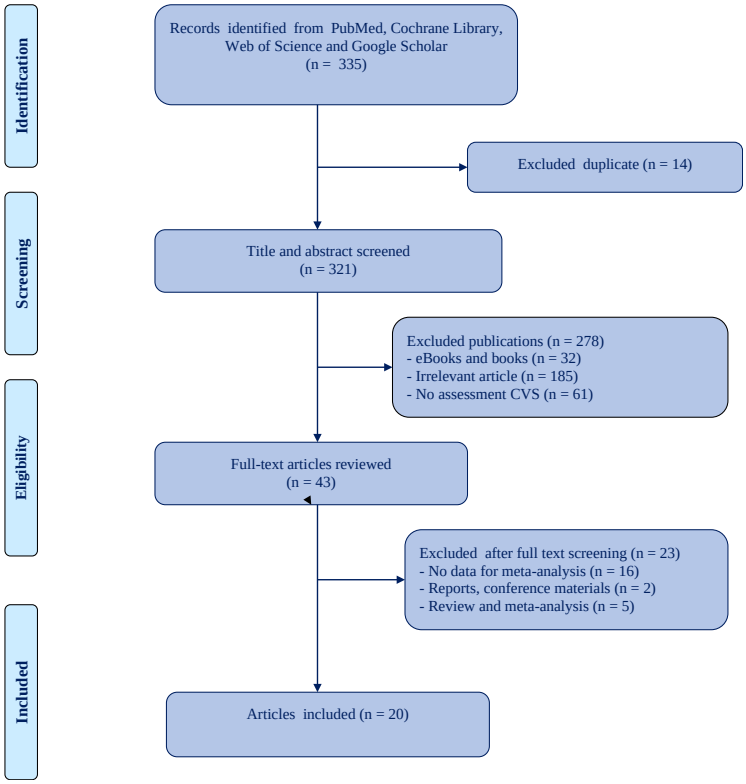


Fig. 1 – Flow diagram of the process of study selection for the meta-analysis. CVS – computer vision syndrome.

Study selection

In order to be included in our analysis, the original research article had to meet the following criteria: 1) the research had to include computer users; 2) as a result, the PRv of CVS or the PRv of any of the symptoms of CVS had to be measured. The inclusion of studies in our analysis was limited to English language only. The studies included in our analysis were not older than five years. In July 2021, a systematic search of four electronic databases was conducted. The inclusion/exclusion of studies was done by two investigators by consultation and consensus.

Data extraction and quality assessment

After selecting the studies based on the inclusion and exclusion criteria, the two investigators independently conducted data extraction. The following variables were abstracted into a pre-formatted table: authors, year of publication, number of participants, outcomes, percentage of CVS, occupation, and age of respondents. All studies included had data available.

Risk of bias was assessed for each study using Downs and Black [35] quality score for non-randomized studies and comprised of five sections: reporting (ten items) – to assess the overall quality of the study; external validity (three items) – to determine the ability to generalize the findings of the study; internal validity (seven items) – to assess bias in the intervention and outcome measures; selection bias (six items) – to determine bias from sampling or group assignment; power (one item) – to determine whether findings are due to chance. Two researchers independently assessed the quality of the studies involved and classified the studies as adequate or inadequate. As no intervention study was selected, the maximum score possible in the present review was 13 points. The minimum score of the studies included was 7 points, and below that number, the studies would not be included in the analysis.

Data synthesis and analysis

Meta-analysis and statistical analysis were performed using MetaAnalyst software version 3.2 (Brown University, 2012, USA) [36]. For six outcomes (total CVS, headache, red eyes, dry eyes, eyestrain, and neck, back, or shoulder pain), overall PRv was assessed. Freeman-Tukey Double Arcsine Proportion (PFT) Random model [DerSimonian-Laird (DL)] for dichotomous data was used in the meta-analysis to calculate the pooled PRv for all studies included. A subgroup analysis for CVS outcome was also performed, using Untransformed Proportion (PR) Random model DL for dichotomous data divided into respondents from the information technology (IT) sector and those who were not from that sector. When the *p*-value was < 0.05, the results were considered statistically significant. Since proportion estimates are usually heterogeneous [37, 38], we have not addressed the problem of increased heterogeneity. Heterogeneity between studies was assessed using the Higgins I^2 test and *p*-values. The number of degrees of freedom is presented in each analysis as *df*.

Results

Study selection and characteristics

Based on the search strategy, a total of 335 studies were selected from the initial database search. Of that number, 14 studies were excluded due to duplication, so 321 studies were selected for further analysis. Following the presentation of the abstract and title, 278 studies were excluded because they did not meet the inclusion criteria. The remaining 43 studies were fully reviewed. When the full-text articles were reviewed, 23 studies were excluded. The remaining 20 studies were included in this systematic review and meta-analysis. The flow chart of the study selection process is shown in Figure 1.

Table 1 [2–4, 19, 21, 27, 39–52] shows the main characteristics of the included studies. A total of 4,560 respondents participated in the 20 included studies; the sample size of the included studies ranged from 50 to 713. The age of the respondents ranged between 17 and 60 years. The studies included in the analysis were conducted in nine countries. Of those nine countries, only one was from Europe.

Table 1

Characteristics of the included studies

Study	Respondents n	Outcome	Country	CVS %	Computer users	Respondents age years
Agbonlahor [2]	215	CVS (eyestrain, headache, red eyes, dry eyes, neck, back, or shoulder pain)	Nigeria	65.6	working-class adults	18–35
Arjuna et al. [39]	125	CVS (headache, dry eyes, red eyes)	Indonesia	59.2	workers	no answer
Astuti et al. [40]	50	CVS	Indonesia	52	employees in Telekom	> 40 (6*) < 40 (44*)
Hadi et al. [3]	385	headache, dry eyes, neck, back, or shoulder pain	Pakistan	no answer	students	17–24
Hamdani et al. [19]	134	CVS (headache)	Indonesia	61.9	computer workers	> 40 (100*) < 40 (34*)
Iqbal et al. [41]	100	CVS (headache, dry eyes, red eyes)	Egypt	86	medical students	18–24
Kamal and Abd El-Mageed [42]	218	eyestrain, headache	Egypt	no answer	bank employees	23–59
Kausar et al. [21]	350	CVS (headache)	Pakistan	88	software engineering students	18–25
Kumar and Sharma [43]	100	CVS (eyestrain, dry eyes, headache)	India	69	computer users	20–60
Kumar [27]	60	CVS (eyestrain, headache, dry eyes, red eyes)	India	85	medical students	no answer
Noreen et al. [44]	326	CVS (eyestrain, red eyes)	Pakistan	16	medical students	17–25
Poudel and Khanal [45]	263	CVS (headache)	Nepal	82.5	IT workers	20–30 (218*) > 30 (45*)
Ranganatha and Jailkhani [46]	150	CVS (eyestrain, headache, dry eyes, red eyes, neck, back, or shoulder pain)	India	86.7	computer science students	19–22
Shahid et al. [47]	150	CVS (headache, neck, back or shoulder pain)	Pakistan	75.3	college students, employees of multinational companies	18–50
Sitaula et al. [48]	234	CVS	Nepal	76.5	computer science students	17–26
Al Tawil et al. [49]	713	dry eyes, headache, red eyes, dry eyes, neck, back, or shoulder pain	Saudi Arabia	no answer	medical and business students	no answer
Tesfa et al. [50]	217	CVS (eyestrain, headache, red eyes)	Ethiopia	75.6	secretary employees	21–48
Viduka et al. [4]	90	dry eyes, headache, red eyes, neck, back, or shoulder pain	Serbia	no answer	computer users	≤ 30 (38*) > 30 (52*)
Vikanaswari and Handayani [51]	600	CVS (headache, neck, back or shoulder pain)	Indonesia	58.8	medical students	≤ 20 (474*) > 20 (126*)
Zalat et al.[52]	80	CVS	Egypt	81.2	staff members in a medical college	47.1 ± 8.1**

CVS – computer vision syndrome; IT – information technology; n – number;
***number of respondents within the age group; **mean ± standard deviation.**

Risk of bias

Table 2 [2–4, 19, 21, 27, 39–52] presents the summary of the risk of bias for each included study. For the parameter of Reporting, eight studies had a score 6/6, eleven studies had a score 5/6, and only one study scored 4/6. For the item External validity, eleven studies had a score 2/2, six studies 1/2, and three studies 0/2. The Internal validity parameter was 2/2 in eleven studies, 1/2 in eight studies, and only one study had 0/2. For the item Selection bias, five studies had a score 2/2, eleven studies 1/2, and four studies 0/2. For item Power, 17 studies had a score 1/1, while three studies had a score 0/1. The lowest overall score was 7, while the highest was 13. The mean score in all 20 included studies was 10.1 ± 2.

Table 2

Risk of bias for each study

Study	Reporting	External validity	Internal validity	Selection bias	Power	Downs and Black [35] score
Agbonlahor [2]	adequate (6/6)	adequate (2/2)	adequate (2/2)	adequate (2/2)	adequate (1/1)	13
Arjuna et al. [39]	adequate (5/6)	inadequate (0/2)	inadequate (1/2)	inadequate (1/2)	inadequate (0/1)	7
Astuti et al. [40]	adequate (4/6)	inadequate (0/2)	adequate (2/2)	inadequate (1/2)	adequate (1/1)	8
Hadi et al. [3]	adequate (5/6)	adequate (2/2)	adequate (2/2)	inadequate (1/2)	adequate (1/1)	11
Hamdani et al. [19]	adequate (6/6)	inadequate (0/2)	adequate (2/2)	inadequate (1/2)	adequate (1/1)	10
Iqbal et al. [41]	adequate (5/6)	inadequate (1/2)	inadequate (0/2)	inadequate (1/2)	inadequate (0/1)	7
Kamal and Abd El-Mageed [42]	adequate (6/6)	inadequate (1/2)	inadequate (1/2)	inadequate (1/2)	adequate (1/1)	10
Kausar et al. [21]	adequate (6/6)	adequate (2/2)	inadequate (1/2)	adequate (2/2)	adequate (1/1)	12
Kumar and Sharma [43]	adequate (5/6)	inadequate (1/2)	inadequate (1/2)	inadequate (0/2)	adequate (1/1)	8
Kumar [27]	adequate (5/6)	inadequate (1/2)	inadequate (1/2)	inadequate (0/2)	inadequate (0/1)	7
Noreen et al. [44]	adequate (5/6)	adequate (2/2)	inadequate (1/2)	inadequate (0/2)	adequate (1/1)	9
Poudel and Khanal [45]	adequate (5/6)	adequate (2/2)	adequate (2/2)	inadequate (1/2)	adequate (1/1)	11
Ranganatha and Jailkhani [46]	adequate (6/6)	adequate (2/2)	adequate (2/2)	inadequate (1/2)	adequate (1/1)	12
Shahid et al. [47]	adequate (6/6)	adequate (2/2)	adequate (2/2)	inadequate (0/2)	adequate (1/1)	11
Sitaula et al. [48]	adequate (6/6)	adequate (2/2)	adequate (2/2)	inadequate (1/2)	adequate (1/1)	12
Al Tawil et al. [49]	adequate (6/6)	adequate (2/2)	adequate (2/2)	adequate (2/2)	adequate (1/1)	13
Tesfa et al. [50]	adequate (5/6)	adequate (2/2)	adequate (2/2)	inadequate (1/2)	adequate (1/1)	11
Viduka et al. [4]	adequate (5/6)	inadequate (1/2)	inadequate (1/2)	inadequate (1/2)	adequate (1/1)	9
Vikanaswari and Handayani [51]	adequate (5/6)	adequate (2/2)	adequate (2/2)	adequate (2/2)	adequate (1/1)	12
Zalat et al. [52]	adequate (5/6)	inadequate (1/2)	inadequate (1/2)	adequate (2/2)	adequate (1/1)	9
						10.1 ± 2*

*** mean score ± standard deviation.**

Meta-analysis for computer vision syndrome

First, an analysis was performed for the overall PRv of CVS. A total of 15 studies measured the PRv of CVS. The pooled PRv for these 15 studies was: PFT = 74.4% [95% confidence interval (CI) = 68%–80.4%, $p < 0.001$]; 2,059 subjects out of 2,828 reported some of the symptoms of CVS; heterogeneity I^2 = 92.66%, $p < 0.001$; df = 14 (Figure 2). Subsequently, subgroup analysis for CVS outcome was performed using PR. Employees in the IT sector and those who were IT students had a slightly higher PRv than those who were not in the IT sector. The PRv of the IT subgroup was: PR = 78.1%, 95% CI = 71.1%–85.1%, $p < 0.001$; 986 respondents out of 1,231 reported some of the symptoms of CVS; heterogeneity I^2 = 89.89%, $p = 0.001$. The PRv of the non-IT subgroup was: PR = 71.3%, 95% CI = 63.6%–79%, $p < 0.001$;

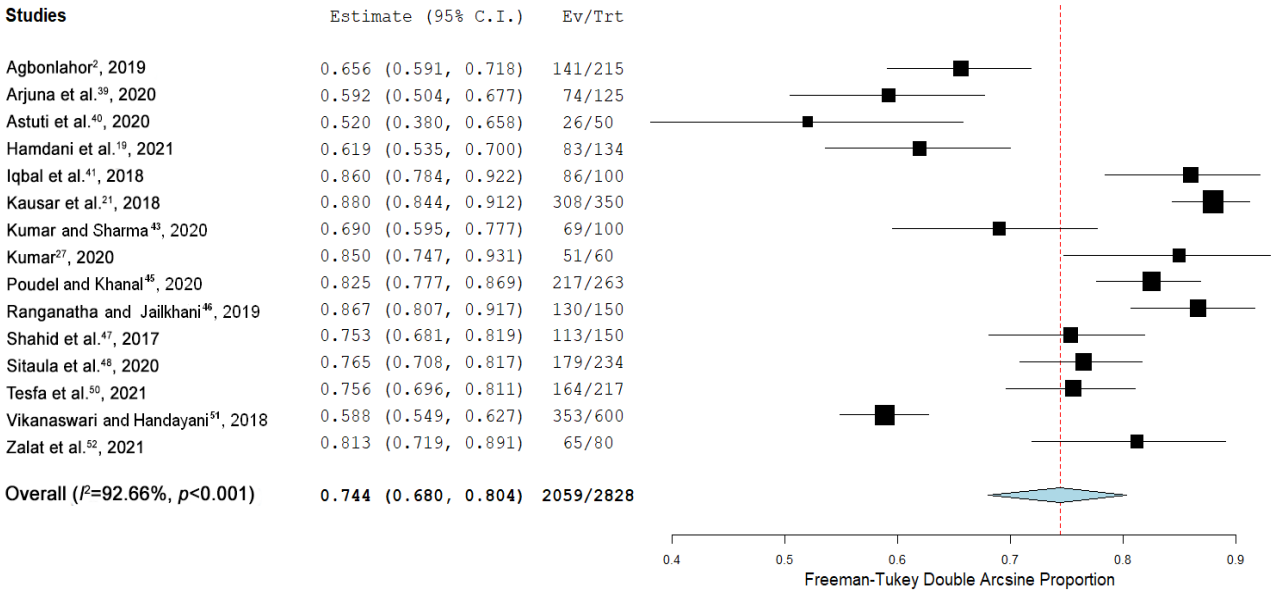


Fig. 2 – Forest plot, outcome: computer vision syndrome. C.I. – confidence interval; Ev/Trt represents the test group.

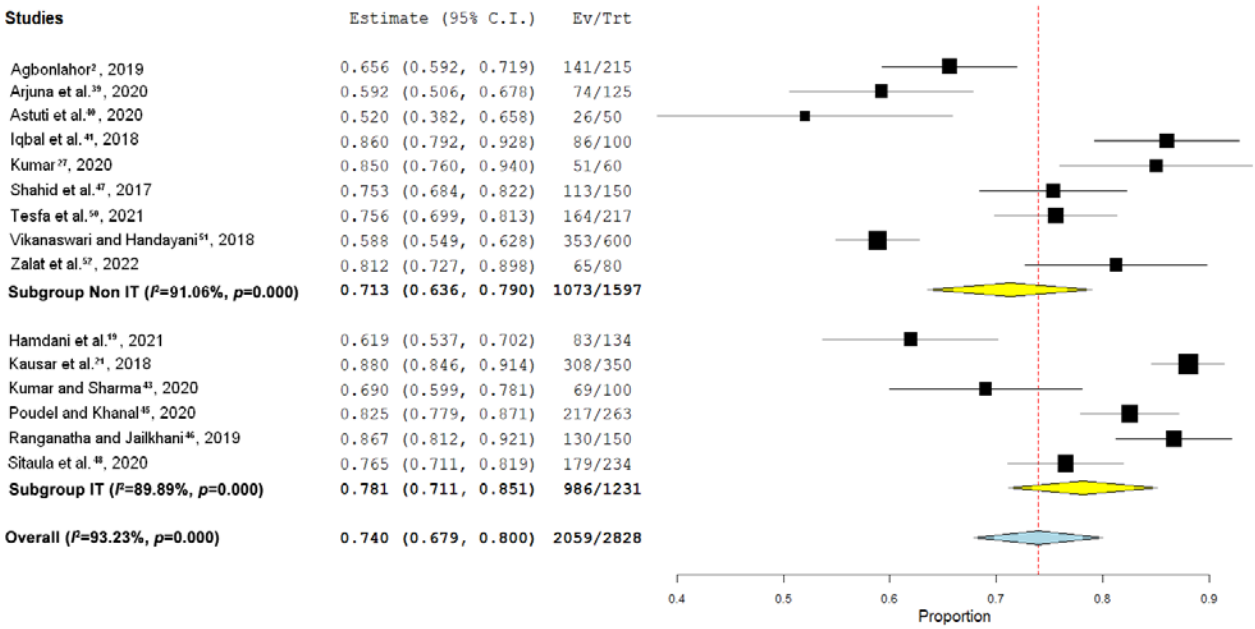


Fig. 3 – Forest plot, outcome: subgroups of computer vision syndrome – information technology (IT) and non-IT. For abbreviations, see Figure 2.

1,073 subjects out of 1,597 reported some of the symptoms of CVS; heterogeneity I^2 = 91.06%, p = 0.001 (Figure 3).

Meta-analysis for headache

Seventeen studies examined the PRv of headaches. The pooled PRv for the headache outcome was: PFT = 43%, 95% CI = 34.1%–52.1%, p < 0.001; 1,894 subjects out of 4,196 reported headache symptoms; heterogeneity I^2 = 97.1, p < 0.001; df = 16 (Figure 4).

Meta-analysis for dry eyes

Nine studies examined the PRv of dry eyes. The pooled PRv for the dry eyes outcome was: PFT = 24.4%, 95% CI = 14.4%–36.1%, p < 0.001; 632 subjects out of 1,938 reported

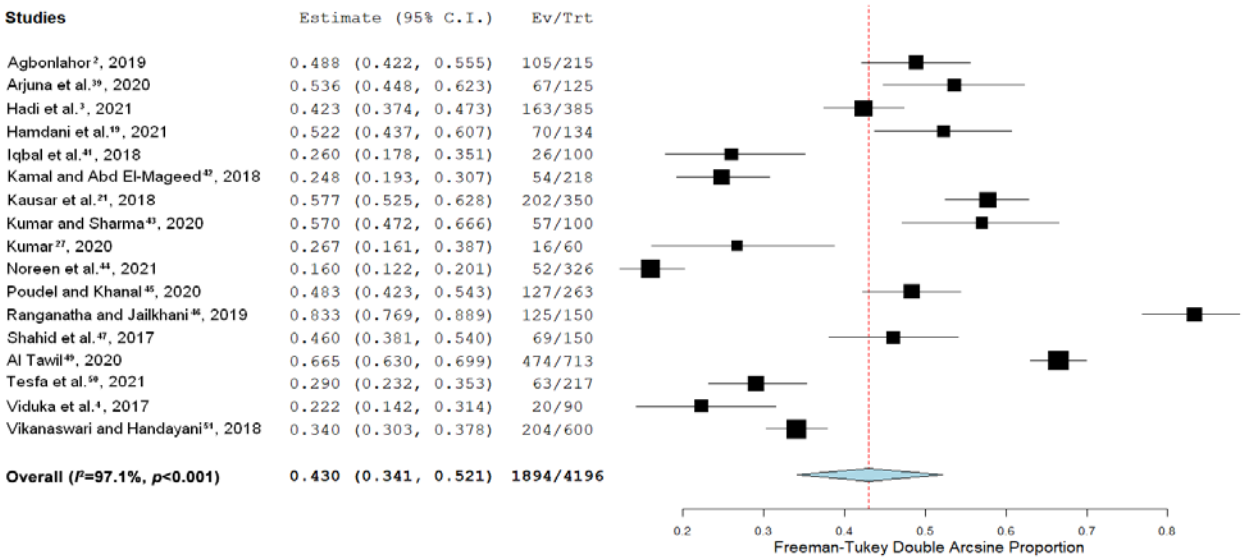


Fig. 4 – Forest plot, outcome: headache.
For abbreviations, see Figure 2.

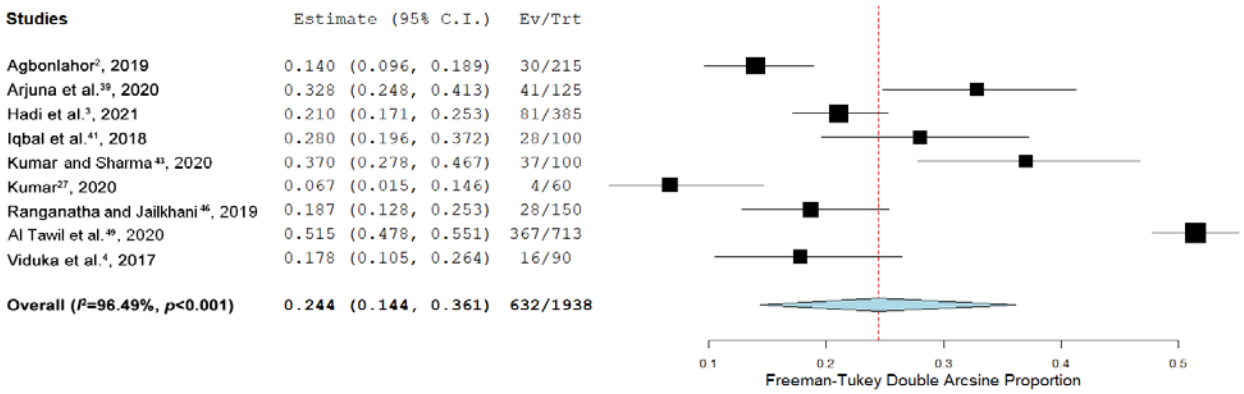


Fig. 5 – Forest plot, outcome: dry eyes.
For abbreviations, see Figure 2.

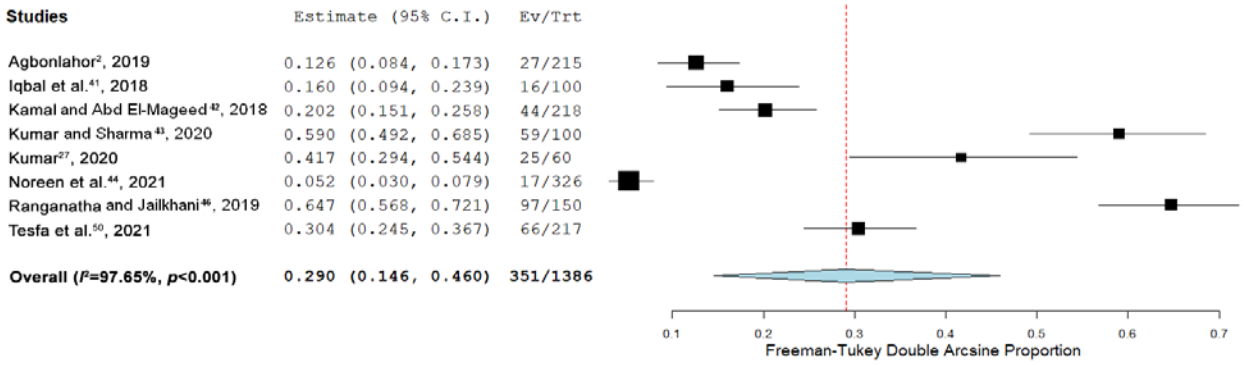


Fig. 6 – Forest plot, outcome: eyestrain.
For abbreviations, see Figure 2.

dry eye symptoms; heterogeneity I^2 = 96.49%, $p < 0.001$; df = 8 (Figure 5).

Meta-analysis for eyestrain

Eight studies examined the PRv of eyestrain. The pooled PRv for the eyestrain outcome was: PFT = 29%, 95% CI = 14.6%–46%, $p < 0.001$; 351 subjects out of 1,386 reported eyestrain symptoms; heterogeneity I^2 = 97.65%, $p < 0.001$; df = 7 (Figure 6).

Meta-analysis for red eyes

Nine studies examined the PRv of red eyes. The pooled PRv for the red eyes outcome was: PFT = 20.7%, 95% CI = 11.1%–32.2%, $p < 0.001$; 528 subjects out of 1,996 reported

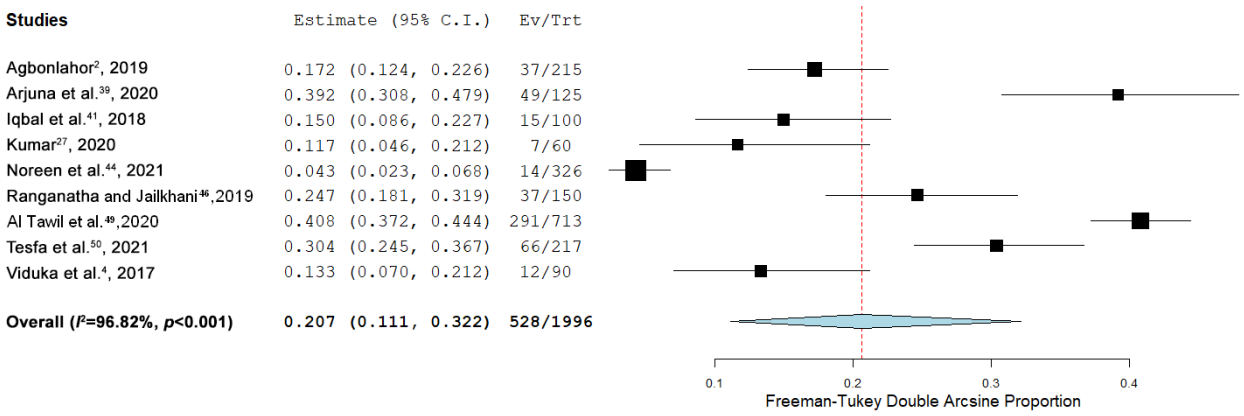


Fig. 7 – Forest plot, outcome: red eyes.
For abbreviations, see Figure 2.

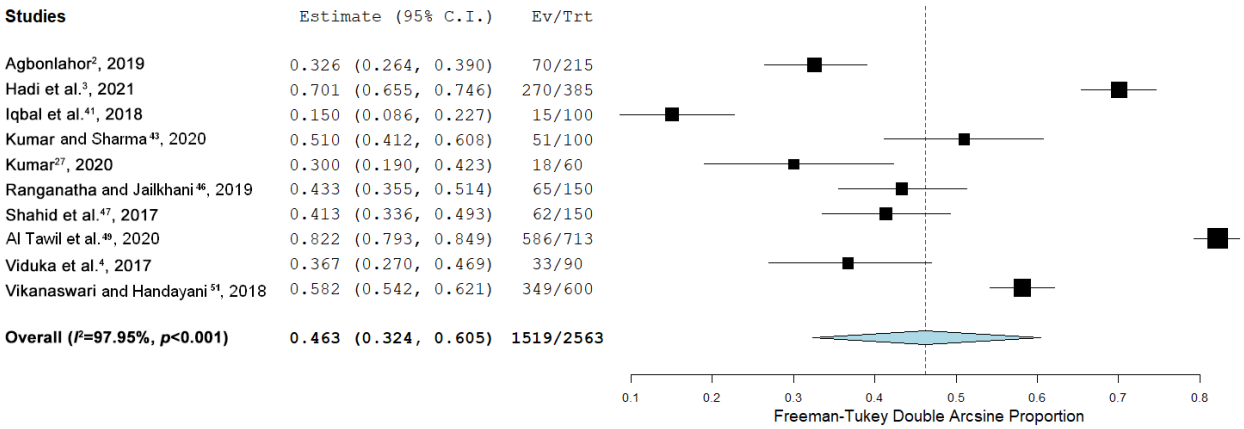


Fig. 8 – Forest plot, outcome: neck, back, or shoulder pain.
For abbreviations, see Figure 2.

red eyes symptoms; heterogeneity I^2 = 96.82%, $p < 0.001$; *df* = 8 (Figure 7).

Meta-analysis for neck, back, or shoulder pain

Ten studies examined the PRv of neck, back, or shoulder pain. The pooled PRv for the outcome of neck, back, or shoulder pain was: FTT = 46.3%, 95% CI = 32.4%–60.5, $p < 0.001$; 1,519 respondents out of 2,563 reported symptoms of neck, back, or shoulder pain; heterogeneity I^2 = 97.95%, $p < 0.001$; *df* = 9 (Figure 8).

Discussion

In this systematic review, we pooled the results of 20 studies to calculate the combined PRv of CVS and some of the symptoms of CVS. The total number of subjects in these 20 studies was 4,560. Progressive advances in technology have led to the increased use of computers and other digital devices, so the emergence of CVS is a modern problem. An increasing number of studies deal with the problems of CVS, so we also tried to make a cross-section in this paper, including in our analysis only studies that were not older than five years.

The total overall PRv in the 20 studies included in our analysis was 74.4%, which is in line with the previous statements. The subgroup analysis indicates that the PRv is slightly higher among respondents in the IT sector than among other respondents (78.1% vs. 71.3%). These results confirm that there is almost no difference in computer hrs between professional users and non-professional users. The digitalization of work has led to the fact that almost all respondents in the studies have become professional computer users. It is certain that smartphone misuse and other devices also influence these results. After this analysis, other analyses were performed to assess the overall PRv of individual CVS symptoms and those that were most evaluated by the included studies. The overall PRv for headache was 43%, for dry eyes 24.4%, for eyestrain 29%, for red eyes 20.7%, and for neck, back, or shoulder pain 46.3%. The last analysis for the symptom of the neck, back, or shoulder pain was the most difficult to do because some studies examined one of these symptoms separately, some two symptoms together, and some all three symptoms together. Due to the musculoskeletal problems caused by CVS and their importance for the normal life of computer users, we did this analysis as well. We did not deal with the amount of time users spent at the

computer because we believe a separate study should be conducted for that.

Our meta-analysis is the only one that has addressed the issues of CVS PRv and CVS symptom PRv. A meta-analysis by Vilela et al. [53] dealt with the problem of asthenopia in children aged 5–19 years. Five studies were included in the meta-analysis. Due to the large difference in the number of studies included and due to the different types of respondents, it is not possible to compare the results of our two studies. We found four reviews [18, 54–56] that addressed the problem of CVS. They detailed the problems related to CVS, but since no meta-analysis was done, we were unable to compare our results with them. Previous research [11, 12, 57–60] has shown a PRv of CVS between 64% and 90%. In our meta-analysis, this range is from 68% to 80.4%, which shows that the obtained results confirm previous research.

Certainly, these results indicate a reduced quality of work and productivity among employed computer users. CVS represents a significant health problem among computer users of different occupations: architects, accountants, flight controllers, scientists, engineers, and lecturers [23]. Ophthalmologists, doctors of various specialties, and kinesitherapists due to musculoskeletal deformities are involved in solving the problem of CVS. Some authors [61, 62] recommend different types of eye exercises or rinsing the eyes and using distilled water. Appropriate spectacle correction can resolve the visual symptoms of CVS, which include muscle asthenopia and accommodative fatigue [63]. While using the computer, the reading materials should be ideally positioned; the goal is to position the reading material in such a way that the head does not move between reading the document and reading from the computer screen [64]. It is important to position the monitor to avoid direct glare from light sources using low-voltage bulbs and fluorescent tubes [65]. Blinking is very important when working on a computer screen because it moisturizes the eyes to prevent dryness and irritation [66]. After 30 min of working on the computer, the eyes should be closed for 30 sec [67].

This study has several limitations. First, despite a comprehensive search, our study included only those written in English. Second, we did not find any study that included inclusive criteria that came from developed countries in Western Europe and America.

Conclusion

This study examined the PRv of CVS through meta-analysis. CVS is a disorder characterized by various symptoms, the most common being those we analyzed in our study: headache, dry eyes, eyestrain, red eyes, and neck, back, or shoulder pain. The results we have shown are certainly worrying and recommend better education of users for more proper use of computers and other digital devices, and require a multidisciplinary approach in eliminating the problems caused by CVS. CVS, as a modern global problem, requires the help of science and the involvement of experts from various fields in order to remedy its consequences. With our study, we have tried to make a contribution.

REFERENCES

1. *Ranasinghe P, Wathurapatha WS, Perera YS, Lamabadusuriya DA, Kulatunga S, Jayawardana N,* et al. Computer vision syndrome among computer office workers in a developing country: an evaluation of prevalence and risk factors. BMC Res Notes 2016; 9: 150.
2. *Agbonlahor O.* Prevalence and knowledge of computer vision syndrome (CVS) among the working class adults in FCT Nigeria. J Niger Optom Stud Assoc 2019; 21(1): 49–60.
3. *Hadi KN, Rehman MH, Toru HK, Orakzai AA, Khalid S, Iftikhar B.* Assessment of computer vision syndrome in University students in Peshawar; A descriptive cross-sectional study. The Stetho 2021; 2(6): 6–14.
4. *Viduka D, Dragičević M, Bašić A, Viduka B, Lavrnić I.* 21st Century engineering challenges observed through computer vision syndrome. Teh Vjesn 2017; 24(Suppl 1): 201–5.
5. *Loh KY, Redd SC.* Understanding and preventing computer vision syndrome. Malays Fam Physician 2008; 3(3): 128–30.
6. *Gowrisankaran S, Nahar NK, Hayes JR, Sheedy JE.* Asthenopia and blink rate under visual and cognitive loads. Optom Vis Sci 2012; 89(1): 97–104.
7. *Thorud HM, Helland M, Aarås A, Kvikstad TM, Lindberg LG, Horgen G.* Eye-related pain induced by visually demanding computer work. Optom Vis Sci 2012; 89(4): E452–64.
8. *Pointer JS, Gilmartin B.* Clinical characteristics of unilateral myopic anisometropia in a juvenile optometric practice population. Ophthalmic Physiol Opt 2004; 24(5): 458–63. Erratum in: Ophthalmic Physiol Opt 2004; 24(6): 607.
9. *Sheedy JE, Hayes JN, Engle J.* Is all asthenopia the same? Optom Vis Sci 2003; 80(11): 732–9.
10. *Ostrovsky A, Ribak J, Pereg A, Gaton D.* Effects of job-related stress and burnout on asthenopia among high-tech workers. Ergonomics 2012; 55(8): 854–62.
11. *Hayes JR, Sheedy JE, Stelmack JA, Heaney CA.* Computer use, symptoms, and quality of life. Optom Vis Sci 2007; 84(8): 738–44.
12. *Iwakiri K, Mori I, Sotoyama M, Horiguchi K, Ochiai T, Jonai H,* et al. Survey on visual and musculoskeletal symptoms in VDT workers. Sangyo Eiseigaku Zasshi 2004; 46(6): 201–12. (Japanese)
13. *Gabal MS, Elaziz KMA, Mostafa NS, Almadani TA.* Computer vision syndrome and musculoskeletal disorders among call center workers of a private company. Egypt J Community Med 2016; 34(4): 51–7.
14. *Sa EC, Ferreira Junior M, Rocha LE.* Risk factors for computer visual syndrome (CVS) among operators of two call centers in São Paulo, Brazil. Work 2012; 41(Suppl 1): 3568–74.
15. *Bhanderi DJ, Choudhary S, Doshi VG.* A community-based study of asthenopia in computer operators. Indian J Ophthalmol 2008; 56(1): 51–5.
16. *Logaraj M, Madhupriya V, Hegde S.* Computer vision syndrome and associated factors among medical and engineering students in Chennai. Ann Med Health Sci Res 2014; 4(2): 179–85.
17. *Bandela PK, Satgunam P, Garg P, Bharadwaj SR.* Corneal transplantation in disease affecting only one eye: does it

make a difference to habitual binocular viewing? PLoS One 2016; 11(3): e0150118.
18. *Akinbinu TR, Mashalla YJ.* Impact of computer technology on health: Computer Vision Syndrome (CVS). Med Pract Rev 2014; 5(3): 20–30.
19. *Hamdani D, Kurniawati N, Ernawati T.* Computer vision syndrome and tension type headache in computer workers. J Widya Medika Junior 2021; 3(2): 110–5.
20. *Hazarika AK, Singh PK.* Computer vision syndrome. SMU Med J 2014; 1(2): 132–8.
21. *Kausar S, Sughra U, Khan WA, Nabeel K.* Prevalence of computer vision syndrome (CVS) symptoms and its awareness among software engineering students of Twin cities. Al-Shifa J Ophthalmol 2018; 14(1): 28–33.
22. *Ihemedu CO, Omolase CO.* The level of awareness and utilization of computer shields among computer users in a Nigerian community. Asian J Med Sci 2010; 1(2): 49–52.
23. *Torrey J.* Understanding computer vision syndrome. Empl Rel Today 2003; 30(1): 45–51.
24. *Anshel J.* Diagnosing, treating CVS relies on good case history: basic eye care, ergonomics and optical correction are all part of an effective treatment plan for computer vision syndrome. Prim Care Optom News 2007; 12(9): 1081–6437.
25. *Wimalasundera S.* Computer vision syndrome. Galle Med J 2006; 11(1): 25–9.
26. *Sen A, Richardson S.* A study of computer-related upper limb discomfort and computer vision syndrome. J Hum Ergol (Tokyo) 2007; 36(2): 45–50.
27. *Kumar BS.* A study to evaluate the knowledge regarding computer vision syndrome among medical students. Biomed Pharmacol J 2020; 13(1): 469–73.
28. *Mocci F, Serra A, Corrias GA.* Psychological factors and visual fatigue in working with video display terminals. Occup Environ Med 2001; 58(4): 267–71.
29. *Woods V.* Musculoskeletal disorders and visual strain in intensive data processing workers. Occup Med (Lond) 2005; 55(2): 121–7.
30. *Rocha LE, Debert-Ribeiro M.* Working conditions, visual fatigue, and mental health among systems analysts in São Paulo, Brazil. Occup Environ Med 2004; 61(1): 24–32.
31. *Ye Z, Honda S, Abe Y, Kusano Y, Takamura N, Imamura Y,* et al. Influence of work duration or physical symptoms on mental health among Japanese visual display terminal users. Ind Health 2007; 45(2): 328–33.
32. *Carta A, Pasquini L, Lucchini R, Semeraro F, Apostoli P.* Relation of asthenopia and some ophthalmological, neuropsychological, and musculoskeletal parameters in workers assigned to video display terminals. Med Lav 2003; 94(5): 466–79. (Italian)
33. *Seguí MDM, Cabrero-García J, Crespo A, Verdú J, Ronda E.* A reliable and valid questionnaire was developed to measure computer vision syndrome at the workplace. J Clin Epidemiol 2015; 68(6): 662–73.
34. *Moher D, Liberati A, Tetzlaff J, Altman DG, PRISMA Group.* Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. Ann Intern Med 2009; 151(4): 264–9, W64.
35. *Downs SH, Black N.* The feasibility of creating a checklist for the assessment of the methodological quality both of randomised and non-randomised studies of health care interventions. J Epidemiol Community Health 1998; 52(6): 377–84.
36. *Wallace BC, Dahabreh IJ, Trikalinos TA, Lau J, Trow P, Schmid CH.* Closing the gap between methodologists and end-users: R as a computational back-end. J Stat Softw 2012; 49(5): 1–15.
37. *Lin L, Xu C.* Arcsine-based transformations for meta-analysis of proportions: Pros, cons, and alternatives. Health Sci Rep 2020; 3(3): e178.
38. *Higgins JP, Thompson SG.* Quantifying heterogeneity in a meta-analysis. Stat Med 2002; 21(11): 1539–58.
39. *Arjuna SR, Ernawati T, Djaputra EM.* Association between computer vision syndrome and attention in workers. J Widya Medika Junior 2020; 2(2): 125–9.
40. *Astuti D, Makaba S, Tingginehe RM, Ruru Y.* The determinant factors affecting the event of computer vision syndrome (CVS) on helpdesk employees at PT. Telkom Access Papua in 2020. Int J Sci Basic Appl Res 2020; 53(1): 17–34.
41. *Iqbal M, El-Massry A, Elagouz M, Elzembely H.* Computer vision syndrome survey among the medical students in Sohag University Hospital, Egypt. Ophthalmol Res Int J 2018; 8(1): 1–8.
42. *Kamal NN, Abd El-Mageed AS.* Determinants of computer vision syndrome among bank employees in Minia City, Egypt. Egypt J Community Med 2018; 36(4): 70–6.
43. *Kumar N, Sharma N.* To determine the prevalence of computer vision syndrome among computer users: a descriptive study. Eur J Mol Clin Med 2020; 7(10): 3933–8.
44. *Noreen K, Ali K, Aftab K, Umar M.* Computer vision syndrome (CVS) and its associated risk factors among undergraduate medical students in midst of COVID-19. Pak J Ophthalmol 2021; 37(1): 102–8.
45. *Poudel S, Khanal SP.* Magnitude and determinants of computer vision syndrome (CVS) among IT workers in Kathmandu, Nepal. Nepal J Ophthalmol 2020; 12(24): 245–51.
46. *Ranganatha SC, Jailkhani S.* Prevalence and associated risk factors of computer vision syndrome among the computer science students of an engineering college of Bengaluru- a cross-sectional study. Galore Int J Health Sci Res 2019; 4(3): 10–5.
47. *Shahid E, Burhany T, Siddique WA, Fasih U, Shaikh A.* Frequency of computer vision syndrome in computer users. Pak J Ophtahlmol 2017; 33(2): 108–12.
48. *Sitaula K, Kafle N, Acharya A, Mishra VP.* Prevalence and associated factors of computer vision syndrome among the computer engineering students of Pokhara University affiliated colleges of Kathmandu valley. Int J Community Med Public Health 2020; 7(6): 2027–31.
49. *Al Tawil L, Aldokhayel S, Zeitouni L, Qadoumi T, Hussein S, Ahamed SS.* Prevalence of self-reported computer vision syndrome symptoms and its associated factors among university students. Eur J Ophthalmol 2020; 30(1): 189–95.
50. *Tesfa M, Ibrahim M, Markos Y, Adere A, Temam L.* Computer vision syndrome and its predictors among secretary employees working in Jimma University, Southwest Ethiopia. Int J Sens Sensor Netw 2021; 9(1): 11–8.
51. *Vikanaswari GI, Handayani AT.* The screening of computer vision syndrome in medical students of Udayana University. Bali J Ophthalmol 2018; 2(2): 28–34.
52. *Zalat MM, Amer SM, Wassif GA, El Tarhouny SA, Mansour TM.* Computer vision syndrome, visual ergonomics and amelioration among staff members in a Saudi medical college. Int J Occup Saf Ergon 2022; 28(2): 1033–41.
53. *Vilela MA, Pellanda LC, Fassa AG, Castagno VD.* Prevalence of asthenopia in children: a systematic review with meta-analysis. J Pediatr (Rio J) 2015; 91(4): 320–5.
54. *Blehm C, Vishnu S, Khattak A, Mitra S, Yee RW.* Computer vision syndrome: a review. Surv Ophthalmol 2005; 50(3): 253–62.
55. *Gowrisankaran S, Sheedy JE.* Computer vision syndrome: A review. Work 2015; 52(2): 303–14.

56. *Rosenfield M.* Computer vision syndrome: a review of ocular causes and potential treatments. Ophthalmic Physiol Opt 2011; 31(5): 502–15.
57. *Bergqvist U, Knave B, Voss M, Wibom R.* A longitudinal study of VDT work and health. Int J Hum Comput Interact 1992; 4(2): 197–219.
58. *Thomson WD.* Eye problems and visual display terminals—the facts and the fallacies. Ophthalmic Physiol Opt 1998; 18(2): 111–9.
59. *Yan Z, Hu L, Chen H, Lu F.* Computer Vision Syndrome: A widely spreading but largely unknown epidemic among computer users. Comput Hum Behav 2008; 24(5): 2026–42.
60. *Agarwal S, Goel D, Sharma A.* Evaluation of the factors which contribute to the ocular complaints in computer users. J Clin Diagn Res 2013; 7(2): 331–5.
61. *Bhutada RS.* The effect of eye exercise, triphala kwath eyewash, and instillation of distilled water on computer vision syndrome. J Datta Meghe Inst Med Sci Univ 2019; 14(6): 78–82.
62. *Dewi MA, Rahmi FL, Saubig AN, Julianti HP.* The effect of "Permata-Ku" exercises on the improvement of computer vision syndrome score. Diponegoro Med J 2021; 10(2): 128–31.
63. *Scheiman M, Wick B.* Clinical management of binocular vision: heterophoric, accommodative, and eye movement disorders. 4th ed. Philadelphia, USA: Wolters Kluwer Health/Lippincott Williams and Wilkins; 2014. 722p.
64. *American Optometric Association*. Computer vision syndrome [Internet]. 2020 [accessed on 2023 June 30]; Available from: https://www.aoa.org/healthy-eyes/eye-and-vision-conditions/computer-vision-syndrome?sso=y
65. *Chu C, Rosenfield M, Portello JK, Benzoni JA, Collier JD.* A comparison of symptoms after viewing text on a computer screen and hardcopy. Ophthalmic Physiol Opt 2011; 31(1): 29–32.
66. *Rossignol AM, Morse EP, Summers VM, Pagnotto LD.* Video display terminal use and reported health symptoms among Massachusetts clerical workers. J Occup Med 1987; 29(2): 112–8.
67. *Anshel JR.* Visual ergonomics in the workplace. AAOHN J 2007; 55(10): 414–20.

Received on March 1, 2022
Revised on April 17, 2023
Accepted April 25, 2023
Online First April 2023

CASE REPORTS

UDC: 617.7-053.9-079.4:616.89-008.42
DOI: https://doi.org/10.2298/VSP230210022T

Complex visual hallucinations with retention of insight: four cases of Charles Bonnet syndrome

Složene optičke halucinacije kod bolesnika sa očuvanim uvidom: četiri slučaja Šarl Boneovog sindroma

Vesna Tepšić Ostojić*†, Zagorka Gojković*, Bratislav Živić*†

***Military Medical Academy, Psychiatric Clinic, Belgrade, Serbia; †University of Defence, Faculty of Medicine of the Military Medical Academy, Belgrade, Serbia**

Abstract

Introduction. Complex visual hallucinations with retention of insight due to visual impairment are key symptoms of Charles Bonnet syndrome. The syndrome is a standalone diagnosis in the International Classification of Diseases, 11th Revision. Nevertheless, in clinical praxis, it is often misdiagnosed as psychosis or early stages of dementia, and it goes underreported by patients because of the fear of being diagnosed with a mental illness. **Case report.** We presented four elderly patients, who were referred for psychiatric consultation due to visual hallucinations, with preserved insight, but with impaired vision. All four patients had complex, vivid, and colorful hallucinations consisting of realistic objects, people, animals, or scenery that tend to recur. Their emotional response and impact on quality of life differed, and psychopharmacotherapy was determined according to their psychological symptoms. Empathic explanation of the symptoms' origin and reassurance of the absence of mental illness for patients and caregivers were of vital importance in all cases. **Conclusion.** With the aging of the population, the number of patients with impaired vision also increases, and the importance of a multidisciplinary approach in the diagnostic procedures and treatment of Charles Bonnet syndrome is emphasized. Increased awareness of clinical characteristics and therapeutic approaches is required among all physicians who are in contact with elderly and/or impaired vision patients.

Key words:
charles bonnet syndrome; diagnosis, differential; hallucinations; vision disorders.

Apstrakt

Uvod. Složene optičke halucinacije sa očuvanim uvidom kod bolesnika sa oštećenjem vida predstavljaju ključni simptom Šarl Boneovog sidroma. Ovaj sindrom je uvršten u jedanaestu reviziju Međunarodne klasifikacije bolesti kao samostalna dijagnoza. Ipak, često se u kliničkoj praksi bolesnicima sa tim sindromom pogrešno dijagnostikuje psihoza ili početna faza demencije, a sindrom retko prijavljuju i bolesnici, zbog straha od postavljanja dijagnoze mentalnog oboljenja. **Prikaz bolesnika.** Prikazali smo četiri bolesnika starijeg životnog doba, koji su bili upućeni na psihijatrijsku konsultaciju zbog optičkih halucinacija, uz očuvani uvid, a sa oštećenjem vida. Sva četiri bolesnika imala su složene, žive halucinacije u boji, koje su predstavljale realne predmete, ljude, životinje i pejzaže i koje su se ponavljale. Emocionalni odgovor i uticaj na kvalitet života tih bolesnika bio je različit, a psihofarmakoterapija je bila određena u skladu sa kliničkom slikom. Pokazivanje empatije u objašnjavanju porekla simptoma i razuveravanje da se ne radi o psihijatrijskom poremećaju, bili su od velikog značaja kod svih bolesnika, kao i kod njima bliskih osoba. **Zaključak.** Starenjem populacije povećava se i broj bolesnika koji imaju oštećenje vida i naglašava se značaj multidsciplinarnog pristupa u dijagnostici i terapiji Šarl Boneovog sidroma. Potrebno je povećati svest o kliničkim karakteristikama i terapijskom pristupu kod svih lekara koji su u kontaktu sa starijim bolesnicima i/ili bolesnicima sa oštećenjem vida.

Ključne reči:
šarl boneov sindrom; dijagnoza, diferencijalna; halucinacije; vid, poremećaji.

Introduction

Charles Bonnet syndrome (CBS) is a medical condition named after a Swiss philosopher and naturalist, Charles Bonnet (1720–1792), who first documented the phenomenon of complex visual hallucinations in visually impaired patients with no mental illness in the 1760s [1]. Patognomonic CBS hallucinations are complex, vivid, and mostly colorful

Correspondence to: Vesna Tepšić Ostojić, Military Medical Academy, Psychiatric Clinic, Crnotravska 17, 11 000 Belgrade, Serbia.
E-mail: vesna.j.tepsic@gmail.com

and are comprised of realistic objects, people, animals, or scenery that tend to recur [2]. They occur involuntarily and retain insight that what is observed is not real. Hallucinations are rarely simple photopsias consisting of lights and flashes [3]. Hallucinatory content is often congruent with the emotional response and is usually not disturbing, but, in some cases, it can be anxiety-provoking and can affect the quality of life [4, 5]. They can manifest regardless of the level of the lesion of the visual system (eyes, optic nerve, or brain), but typically, there is a loss of central visual acuity [2]. Older age, social isolation, and sensory deprivation are the triads that are usually present in the CBS etiology [4, 6].

We present four cases of CBS that were referred for psychiatric consultation due to the presence of hallucinations. Permission was given from the patients, and the personal information was de-identified to protect their anonymity.

Case report

Case 1

A 64-year-old male patient diagnosed with diabetes mellitus at the age of 27 with multiple complications listed in his medical record, including diabetic neuropathy and retinopathy, was referred to psychiatric consultation after reporting visual hallucinations to his endocrinologist. Hallucinations started after a few days in the hospital and were recurring. He saw his patron saint, St. John, sitting on his bed, walking through the room from patient to patient, sometimes holding a candle or talking to the favorite female saint of the patient's wife, St. Petka. They were both smiling, with warmth in their eyes and grace in their movements. He described them as very pleasant and comforting. The patient had no previous history of psychiatric symptoms or treatment and had mild cognitive impairment. He had full insight into the quality of his visions. Even though he knew they were not real, he enjoyed the comfort they provided him in his sickness and loneliness. No psychopharmacotherapy was prescribed as he had full insight and was not afraid of them, and no follow-up was suggested.

Case 2

A 78-year-old female patient, hospitalized for hip surgery, presented with severe anxiety and sleep disturbance for the last several days and was alone in the room because of the COVID-19 preventive measures. Psychiatric evaluation showed no history of mental illness and no cognitive decline. Medical history showed she was diagnosed with hypertension and glaucoma, but no ophthalmological report was available. The patient reported disturbing images she had been seeing for the last few days. She described an ugly female person sitting on the window sill that scared her. That woman's head was shrinking and growing, sometimes looking familiar, but the patient was unable to recognize her. Sometimes the woman she saw had the head of an unpleasant insect, a wild beast, or even of a nurse that the patient did not like because she found her cruel and lazy. Occasionally, the same head would be there for several minutes, and at other times, different heads would change rapidly. The patient was terrified and angry and had a level of anxiety that interfered with all aspects of her functioning, including sleep. Since the detailed explanation of her visual experiences and reassurance showed no benefit, she was prescribed olanzapine 2.5 mg in a single evening dose during her stay in the hospital. Even though her hallucinations were still present, they were less frequent, and she did not report fear, anxiety, or sleep disturbance. She was advised to continue outpatient psychiatric follow-up.

Case 3

A 77-year-old female patient was referred by her general practitioner for outpatient psychiatric consultation accompanied by her daughter. She was a widow and had been living alone for two years; her two daughters and grandchildren visited often. The patient had severe rheumatoid arthritis with restricted mobility and hypertension. She had left eye cataract surgery six months ago and was listed for the right eye surgery as well, as explained by her daughter. She described her daily "afternoon experiences" that started a few weeks after the cataract surgery, including seeing very colorful bucolic scenery in her living room, plants with big leaves, flowers, butterflies, and small animals like rabbits, squirrels, bees, etc. It was like a magical garden with mixed colors – leaves were rarely green and instead were blue, white, etc. The flowers were of all colors, sometimes floating in the air so that animals could ride on them. Flowers and animals were often smiling or playing to entertain her. The patient understood her visual experience was not real and that there were hallucinations that did not frighten her, but she was afraid of going mad. Detailed explanations and reassurance were offered to her and her daughter, and the patient decided to calmly wait for the other eye surgery. Therefore, no psychopharmacotherapy was prescribed.

Case 4

An 84-year-old female patient came with two grandchildren, with whom she lived, to the Emergency Room after reporting seeing a little girl calmly sitting on the kitchen floor and playing with her doll. The little girl neither talked to her nor wanted anything from her. The patient had no emotional response to her vision whatsoever, but her grandchildren were afraid that this was a sign of dementia. Her grandchildren knew her eyesight was declining, and after explaining the probable nature of hallucinations, they were advised to make an appointment with an eye specialist, neurologist, and psychiatrist if necessary.

Discussion

Even though CBS was described 250 years ago, a search of the PubMed electronic database shows an upward CBS publication activity, with nearly 60% of publications

within the last ten years [4]. Unsurprisingly, the increase in the number of published papers coincides with the introduction of CBS as a standalone diagnosis by the World Health Organization in the International Classification of Diseases, 11th Revision (ICD-11). CBS is classified as a disease of the visual system as 9D56 Visual release hallucinations [7]. The CBS incidence is not easy to determine, but one can hypothesize that it is far more common than it is diagnosed. The case reports available from the literature propose that CBS is rarely recognized by clinicians and, therefore, misdiagnosed as psychosis or early stages of dementia [6, 8]. Furthermore, visual hallucinations are mostly not reported by the patients themselves because of the fear of "madness" or being diagnosed with a psychiatric disease [9]. Interestingly, the results of the Age-Related Eye Disease Study 2 Research Group [10] showed that when visual hallucinations are solicited in elderly patients with impaired sight, 11% to 15% of them reported experiencing visual hallucinations. The results of this study and by other authors also pointed out that the majority of patients did not report their symptoms to their doctor or family members [5, 10, 11]. We presented four cases that were referred to a liaison psychiatrist as a visit consultation. Two out of the four of our patients reported their hallucinations, and two were recognized by the hospital staff. CBS can be found at any age, but the the age of the vast majority of the diagnosed cases was between 70 and 85 years. Three out of the four presented patients are in this age group. CBS tends to be more frequent in the elderly, and important factors are the different conditions of visual impairment [3, 12].

Common etiologies, except for congenital blindness, include age-related macular degeneration, glaucoma, diabetic retinopathy, cataract, optic neuritis, visual cortex cerebral infarction, retinal vein, or arterial occlusion [3, 12, 13]. The etiology of presented cases is among the most common listed above. As liaison psychiatrists, we had no detailed medical data regarding the exact visual acuity and duration of eyesight impairment. A patient with no previous knowledge of eye disorder was referred to an eye specialist. The most accepted explanation for typical CBS hallucinations is that visual sensory deafferentation leads to disinhibitions of visual cortical regions [4, 14]. Hallucinations are, therefore, spontaneous activation of the disinhibited visual cortex. Neuroimaging supports that theory, showing that the visual cortex activates spontaneously during hallucinations [15]. Hallucinations can be defined as perceptions of stimuli in the absence of an external stimulus and, therefore, can be found in all sensory modalities. In this phenomenon, the hallucinations have been described as formed and vivid, of realistic objects or people, with a recurring tendency. Hallucinations described by our patients are typical for CBS. Given that our patients were from the liaison service, we had no information regarding the course of the CBS. In CBS, the patient retention of insight is present, and in most cases, they do not find these images disturbing. Over time, a decrease from 38% to 8% in emotional input described as disturbing, frightening, or horrifying was reported [14]. CBS hallucinations can affect the quality of life – 60% of patients did not experience any change, 33% had a negative impact, and 7% reported the hallucinations had a good effect [12]. Interestingly, the patient described in the first case finds his hallucinations comforting. Any patient with *de novo* hallucinations should undergo a detailed evaluation to rule out the causes. The patient should go through a detailed assessment by an ophthalmologist, neurologist, and psychiatrist. A differential diagnosis involving visual hallucinations is complex and can include neurodegenerative conditions such as Alzheimer's or Parkinson's disease, migraine aura, epileptic seizures, sensory and/or sleep deprivation, narcolepsy, hypnagogic hallucinations, metabolic encephalopathy, drugs and alcohol withdrawal, delirium, and psychosis [16]. Different common non-psychotropic medicines and over-the-counter medications (including ephedrine and synthetic cannabinoids) [16, 17], psychotropic medications and drugs of abuse must also be considered as the possible cause of hallucinations [16].

Treatment of CBS is challenging and should be planned for each individual separately. Optimizing visual functions at the maximum level possible and explaining that symptoms are not due to mental illness is crucial. The severity of symptoms is the part where therapy comes in. In most cases, clarification and reassurance with empathy are all that is needed, as was the case in three out of the four of our presented patients; only one patient required medication for intensive anxiety and sleep disturbance. In more severe cases, some kind of psychological support or treatment should be made necessary, along with medications. There is no causal medication for CBS. Atypical antipsychotics [16, 18] at low doses like olanzapine or quetiapine are the first choices because of their side effect profile, as well as cholinesterase inhibitors such as donepezil [16]. Other medications with positive effects in a modest series of patients include venlafaxine and escitalopram, as well as valproate and carbamazepine [18].

The prognosis of CBS is directly connected to the cause of vision impairment [15, 16]. The prognosis is far more promising in conditions where visual impairment can be corrected, like cataracts [15, 19], but also less favorable in those with continuous stable or progressing visual impairment [15, 16, 19].

Conclusion

We presented four cases of visual hallucinations with retained insight in elderly patients with impaired vision referred for psychiatric consultation. The aging of the population consequently increases the number of patients with impaired vision, which emphasizes the importance of the multidisciplinary approach to diagnostic procedures and treatment. CBS is a standalone diagnosis in ICD-11, but in clinical praxis, it is often misdiagnosed as psychosis or early stages of dementia and underreported by patients because of the fear of being diagnosed with a mental illness. Empathic reassurance and explanation for patients and caregivers are of vital importance.

R E F E R E N C E S

1. *Hedges TR Jr.* Charles Bonnet, his life, and his syndrome. Surv Ophthalmol 2007; 52(1): 111–4.
2. *Vukicevic M, Fitzmaurice K.* Butterflies and black lacy patterns: the prevalence and characteristics of Charles Bonnet hallucinations in an Australian population. Clin Exp Ophthalmol 2008; 36(7): 659–65.
3. *Jan T, Del Castillo J.* Visual hallucinations: Charles Bonnet syndrome. West J Emerg Med 2012; 13(6): 544–7.
4. *Jones L, Ditzel-Finn L, Enoch J, Moosajee M.* An overview of psychological and social factors in Charles Bonnet syndrome. Ther Adv Ophthalmol 2021; 13: 25158414211034715.
5. *Doeller B, Kratochwil M, Sifari L, Hirnschall N, Findl O.* Benefit of psychiatric evaluation on anxiety in patients with Charles Bonnet syndrome. BMJ Open Ophthalmol 2021; 6(1): e000463.
6. *Pang L.* Hallucinations Experienced by Visually Impaired: Charles Bonnet Syndrome. Optom Vis Sci 2016; 93(12): 1466–78.
7. *World Health Organization (WHO).* International Classification of Diseases, 11th Revision (ICD-11) [Internet]. 2022 [accessed on 2023 July 14]. Available from: https://icd.who.int/browse11/l-m/en
8. *Ffytche DH.* Visual hallucinations and the Charles Bonnet syndrome. Curr Psychiatry Rep 2005; 7(3): 168–79.
9. *Donovan NJ, Blazer D.* Social Isolation and Loneliness in Older Adults: Review and Commentary of a National Academies Report. Am J Geriatr Psychiatry 2020; 28(12): 1233–44.
10. *Le JT, Peprah D, Agrón E, Keenan TD, Clemons TE, Chew EY*, et al. Associations between Age-Related Eye Diseases and Charles Bonnet Syndrome in Participants of the Age-Related Eye Disease Study 2: Report Number 26. Ophthalmology 2022; 129(2): 233–5.
11. *Singh A, Subhi Y, Sørensen TL.* Low awareness of the Charles Bonnet syndrome in patients attending a retinal clinic. Dan Med J 2014; 61(2): A4770.
12. *Teunisse RJ, Cruysberg JR, Hoefnagels WH, Verbeek AL, Zitman FG.* Visual hallucinations in psychologically normal people: Charles Bonnet's syndrome. Lancet 1996; 347(9004): 794–7.
13. *Scott IU, Schein OD, Feuer WJ, Folstein MF.* Visual hallucinations in patients with retinal disease. Am J Ophthalmol 2001; 131(5): 590–8.
14. *Adachi N, Watanabe T, Matsuda H, Onuma T.* Hyperperfusion in the lateral temporal cortex, the striatum and the thalamus during complex visual hallucinations: single photon emission computed tomography findings in patients with Charles Bonnet syndrome. Psychiatry Clin Neurosci 2000; 54(2): 157–62.
15. *Cox TM, Ffytche DH.* Negative outcome Charles Bonnet syndrome. Br J Ophthalmol 2014; 98(9): 1236–9.
16. *Rojas LC, Gurnani B.* Charles Bonnet Syndrome. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022 [updated 2022 Dec 6; cited on 2023 Jan]. Available from: https://www.ncbi.nlm.nih.gov/books/NBK585133/
17. *Doane J, Stults B.* Visual hallucinations related to angiotensin-converting enzyme inhibitor use: case reports and review. J Clin Hypertens (Greenwich) 2013; 15(4): 230–3.
18. *Alao AO, Hanrahan B.* Charles Bonnet syndrome: visual hallucination and multiple sclerosis. Int J Psychiatry Med 2003; 33(2): 195–9.
19. *Holroyd S, Rabins PV.* A three-year follow-up study of visual hallucinations in patients with macular degeneration. J Nerv Ment Dis 1996; 184(3): 188–9.

Received on February 10, 2023
Revised on March 25, 2023
Accepted on April 4, 2023
Online First April 2023

CASE REPORT

UDC: 616.132.2-005.6-07/-08
DOI: https://doi.org/10.2298/VSP210428017D

Acute coronary syndrome in a young patient with ECG presentation of acute inferior myocardial infarction and acute thrombosis of left main stem coronary artery

Akutni koronarni sindrom kod mladog bolesnika sa EKG prezentacijom akutnog infarkta donjeg zida miokarda i akutnom trombozom glavnog stabla leve koronarne arterije

Nemanja Djenić*†, Branko Milovanović*†, Radoslav Romanović*†, Siniša Stojković‡§, Andjelko Hladiš*, Marijan Spasić*, Boris Džudović*, Dragan Dulović*†, Zoran Jović*†, Slobodan Obradović*†

***Military Medical Academy, Clinic for Emergency Internal Medicine, Belgrade, Serbia; †University of Defence, Faculty of Medicine of the Military Medical Academy, Belgrade, Serbia; ‡University Clinical Center of Serbia, Clinic for Cardiology, Belgrade, Serbia; §University of Belgrade, Faculty of Medicine, Belgrade, Serbia**

Abstract

Introduction. The left main stem (MS) coronary artery (CA) (MSCA) thrombosis is a rare but potentially lethal manifestation of acute coronary syndrome. The standard approach in treating such patients is the primary percutaneous coronary intervention (pPCI) or CA bypass graft surgery. In some cases, depending on the morphological appearance of the thrombus, findings and flow rates assessed on coronary angiography (CAn), clinical conditions, and cardiologist's experiences, another possible method of treatment can be the conservative approach using antithrombotic therapy. **Case report.** A 37-year-old male was admitted to the emergency room with symptoms of an acute myocardial infarction with an ST elevation in diaphragmal localization. Using an emergency CAn, we have visualized a thrombus at the ostial and proximal part of the left MSCA, with no complete obstruction of the blood flow. Initially, dual antithrombotic therapy (ticagrelor and acetylsalicylic acid) was applied, and in the further procedure, it was decided to introduce glycoprotein IIb/IIIa platelet receptor inhibitor (tirofiban) as an intracoronary bolus (0.3 μg/kg) and later as a continuous infusion (0.1 μg/kg/min). Four days later, a control CAn and intravascular echocardiography were performed, and it was decided to continue the treatment using conservative therapy without a pPCI procedure. The patient was discharged in good condition with no signs of illness on the eighth day after hospital admission for home recovery, with planned frequent follow-ups in the future. **Conclusion.** In the case of non-obstructive thrombotic masses without significant atherosclerotic stenotic lesions, conservative treatment modality with the use of aggressive antithrombotic therapy may be considered.

Key words:
coronary artery disease; coronary vessels; myocardial infarction; platelet aggregation inhibitors; treatment outcome.

Apstrakt

Uvod. Tromboza glavnog stabla leve koronarne arterije (GSLKA) predstavlja retku ali potencijalno smrtonosnu manifestaciju akutnog koronarnog sindroma. Standardni pristup u lečenju takvih bolesnika jeste primarna perkutana koronarna intervencija (pPKI) ili hirurška revaskularizacija miokarda (*coronary artery bypass graft surgery*). U određenim slučajevima, na osnovu morfološkog izgleda tromba, nalaza i protoka dobijenih metodom koronarne angiografije (KAn), kliničke slike, kao i iskustva kardiologa, jedan od načina lečenja može biti i konzervativni pristup intenzivnom antitrombocitnom terapijom. **Prikaz bolesnika.** Muškarac star 37 godina primljen je u jedinicu za hitne slučajeve jer je pokazivao znake akutnog infarkta miokarda sa ST elevacijom dijafragmalne lokalizacije. Urađena je hitna KAn tokom koje je pronađen tromb u proksimalnoj trećini GSLKA, bez potpune opstrukcije protoka krvi. Inicijalno, primenjena je dvojna antitrombocitna terapija (tikagrelor i acetilsalicilna kiselina), a u daljoj proceduri odlučeno je da se uvede inhibitor glikoproteinskog IIb/IIIa receptora trombocita (tirofiban) u

Correspondence to: Andjelko Hladiš, Military Medical Academy, Clinic for Emergency Internal Medicine, Crnotravska 17, 11 000 Belgrade, Serbia. E-mail: anhladis@gmail.com

vidu intrakoronarnog bolusa (0,3 μg/kg), a zatim kao kontinuirana infuzija (0,1 μg/kg/min). Četiri dana kasnije, urađeni su kontrolna KAn i intravaskularna ehokardiografija, nakon čega je odlučeno da se nastavi samo sa konzervativnom terapijom, bez procedure pPKI. Bolesnik je bez tegoba, u dobrom stanju, otpušten na kućno lečenje osmog dana nakon prijema u bolnicu, uz planirano intenzivno praćenje u daljem toku oporavka. **Zaključak**. U slučaju neopstruktivnih trombocitnih masa bez značajne aterosklerozne stenoze, može se razmotriti modalitet konzervativnog lečenja upotrebom agresivne antitrombocitne terapije.

Ključne reči:
koronarna bolest; koronarni krvni sudovi; infarkt miokarda; antiagregaciona sredstva; lečenje, ishod.

Introduction

Acute coronary syndrome is a medical condition involving acute ST-elevation myocardial infarction (MI) (STEMI), non-ST-elevation MI, sudden MI caused by myocardial ischemia and unstable angina pectoris. According to the most recent division of MIs, Type 1 acute MI is caused by rupture or erosion of unstable atherosclerotic plaque in epicardial coronary arteries (CA). Platelet adhesion and aggregation occur when the prothrombogenic material is released from plaque. That is accompanied by the activation of coagulation and the forming of a thrombus that can completely occlude the so-called infarcted artery [1].

Sometimes, a thrombus can be formed in the proximal segment of the coronary artery, and its fragments can embolize the distal segments of the artery and cause a "distant" infarction.

Plaque rupture and occlusive thrombosis in the left main stem CA (MSCA) are usually associated with sudden death or major shock MI. Occlusive thrombi and thrombi that cause critical myocardial ischemia must be resolved urgently either by percutaneous coronary angioplasty or bypass surgery [2]. If the mentioned procedures cannot be done urgently, one of the remaining options would be fibrinolytic therapy. However, this therapy has poor results in patients who are in a state of shock, which is often the case here. If percutaneous or surgical reperfusion is performed, the treatment is continued with dual antiplatelet therapy (DAPT) in order to ensure the permeability of stents or grafts. Rarely, a non-occlusive thrombus in which thrombogenic material has been embolized can be seen in the left MSCA, as a result of which a minor MI is registered [3]. The aim of this case report was to present a patient with a thrombus in the left MSCA and embolization of the distal segment of the left anterior descending (LAD) CA caused by thrombus migration, which also resulted in a smaller occluded posterior descending artery. Therefore, according to its localization, STEMI is manifested as an MI with ST-elevation in the inferior leads on the electrocardiogram (ECG). That raises the question of whether a heart attack lesion is actually a thrombus in the left MSCA or an occluded distal segment of the LAD CA. Questions are also raised regarding the treatment of non-occlusive thrombus mass in the MSCA that does not lead to myocardial ischemia itself. Dual anti-aggregation therapy in combination with glycoprotein (GP) inhibitors, which inhibit the aggregation by blocking the IIb/IIIa receptors on platelets, may be one of the more successful approaches in treating these patients [4, 5].

Case report

The patient was a 37-year-old male admitted to the coronary intensive care unit with chest pain lasting for two hours, clinically and hemodynamically stable, with a blood pressure of 120/80 mmHg and a pulse rate of 60 beats/min. ECG on arrival showed ST-segment elevation in leads II, III, and augmented vector foot (AVF) and tall T waves on the anterior wall (Figure 1). The patient was diagnosed with STEMI of the inferior myocardial wall. He received DAPT (180 mg of ticagrelor and 300 mg of acetylsalicylic acid) and

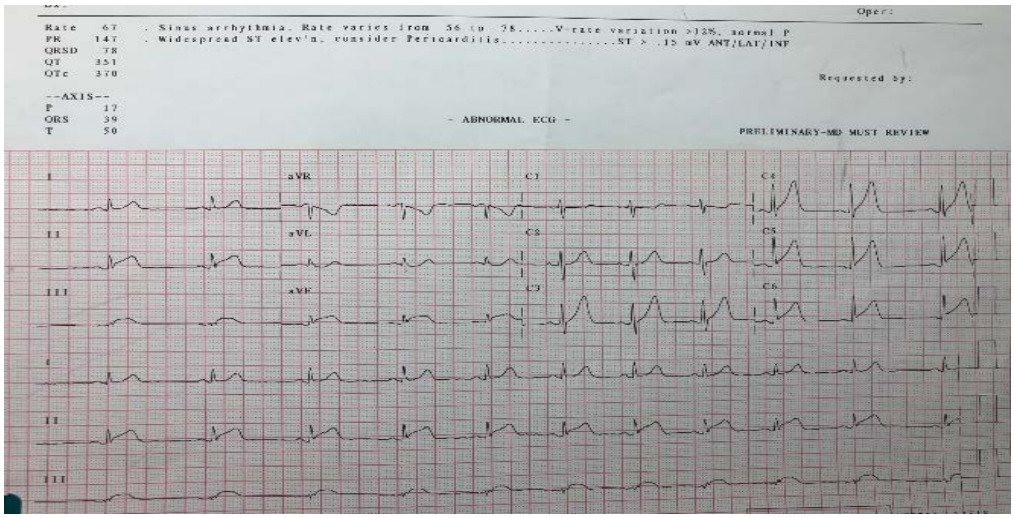


Fig. 1 – Electrocardiogram on admission to the coronary unit. ST-segment elevation in leads II, III, and augmented vector foot and tall T waves on the anterior wall.

was immediately admitted to the cardiac catheterization room for emergency coronary angiography (CAn). CAn was performed using a radial approach. Prior to the review of the coronarographic findings, the patient intravenously received 7,000 units of unfractionated heparin in a bolus. CAn showed a thrombus on the ostial and proximal part of the left MSCA without flow obstruction (Figure 2A). The right CA was without significant changes (Figure 2B). Occlusion of the distal LAD artery in front of the apex of the heart has also been observed, where the artery was less than 2 mm in diameter (Figure 2C). Due to the risk of distal embolization and further iatrogenic microcirculatory injury associated with the use of thrombus aspiration, the clinical decision of the cardiologist performing the intervention was to introduce GP IIb/IIIa platelet receptor inhibitor (tirofiban) as an intracoronary bolus (0.3 μg/kg). The patient was transferred to the coronary intensive care unit where the treatment was continued with tirofiban for 24 hrs as an intravenous infusion (0.1 μg/kg/min) and after that with low-molecular-weight heparin in addition to acetylsalicylic acid, ticagrelor, and atorvastatin. Bedside transthoracic echocardiogram showed normal left ventricular size with an ejection fraction of 60%, with hypokinesis of the distal anterior wall and apex and no pericardial effusion.

In the following days, the patient received DAPT and anticoagulant therapy with enoxaparin subcutaneously in therapeutic doses twice a day, and he was absolutely clinically stable.

Intravascular ultrasound (IVUS) was performed seven days after admission, with atherosclerotic plaque found, narrowing the ostium and proximal segment of the left MSCA by 48%. The minimum lumen area of the left MSCA was 8.12 mm (Figure 3).

There was also an opening of the distal, occluded LAD CA segment (Figure 4) and resolution of ST-segment

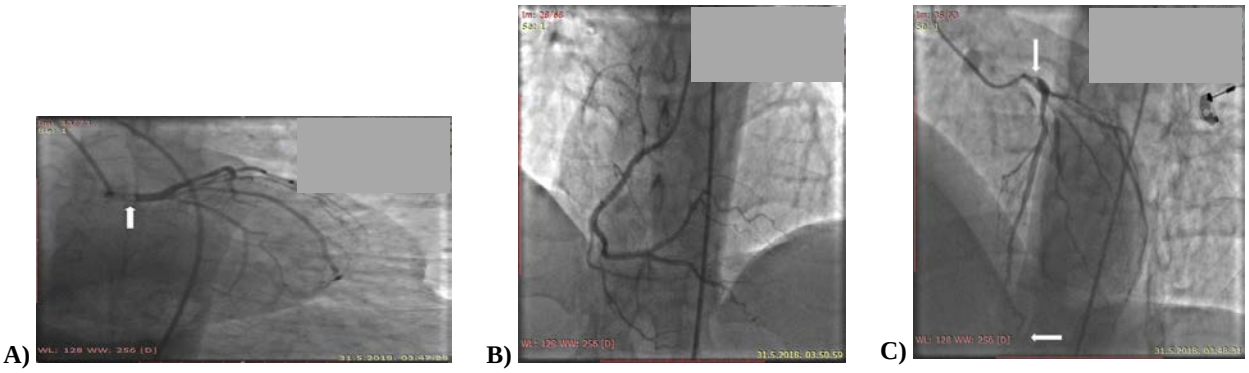


Fig. 2 – Coronary angiography image of: the fluctuating thrombus in the left main stem coronary artery (arrow) (A); the right coronary artery with no obstructions (B); the left coronary artery with distal embolization (arrows)(C).

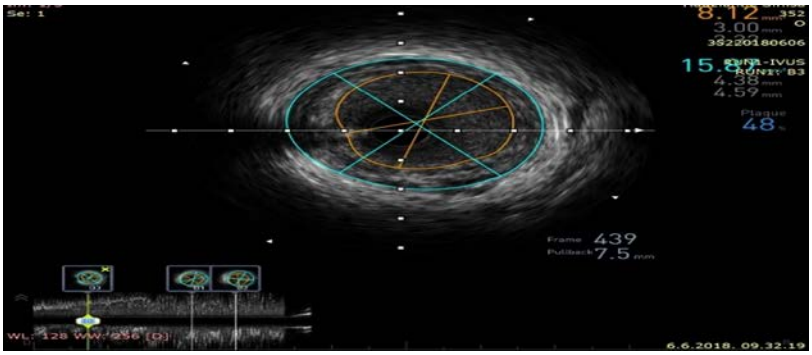


Fig. 3 – Intravascular ultrasound shows 48% stenosis in the left main stem coronary artery.

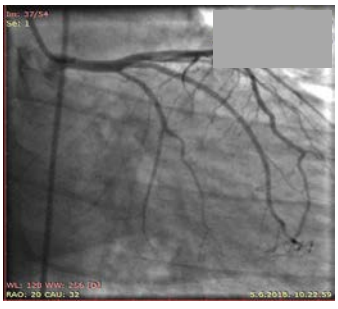


Fig. 4 – Coronary angiography shows an opening of the distal occluded left anterior descending coronary artery segment.

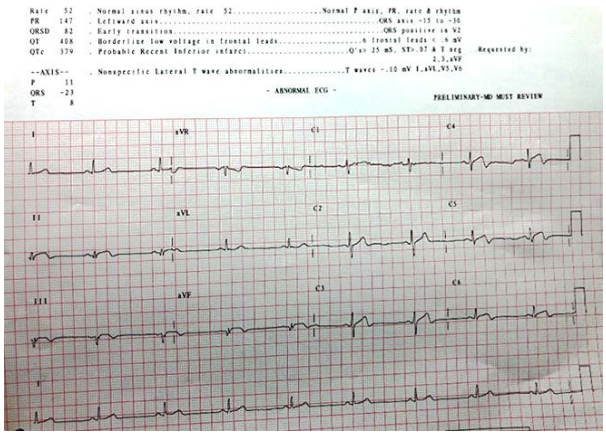


Fig. 5 – Electrocardiogram shows resolution of ST-segment elevation in inferior leads.

elevation in inferior leads (Figure 5). The patient was discharged after eight days and treated with dual antithrombotic therapy for up to 12 months. A follow-up exercise stress test was performed one month later, and the test result was normal. No adverse events occurred during the 12-month follow-up period.

Discussion

As mentioned above, the thrombosis of the left MSCA may result in extensive heart muscle necrosis, cardiogenic shock, or sudden cardiac death [6]. Considering this, the challenge for every cardiologist is an immediate diagnosis and the problem solution. In addition to the acute thrombosis of the MSCA, it is also necessary to think about other potential causes of the clinical condition and ECG changes by the type of necrosis that accompanies MSCA thrombosis, such as aortic dissection, embolic events, CA dissection, vasculitis, or spasm [7–10]. Etiological disorders may include other causes that lead to increased thrombogenic potential, such as taking cocaine (cocaine-induced spasm or rupture of plaque), as well as other reasons [11–17].

In this case report, we describe a patient with acute coronary syndrome but with an ECG presentation of STEMI at a diaphragmal location. Unexpectedly, a thrombosis of the left MSCA was found using CAn, yet no obstructions of the right system were present. An interesting finding was the CA visualization of ostial stenosis and thrombus in the proximal 1/3 of the left MSCA but without complete left MSCA luminal obstruction, which explains the hemodynamic stability of the patient. The infarction in our patient was obviously the consequence of a thrombus fragment causing embolization of the distal segment of the LAD, which passes to the diaphragmal side into the small posterior descendent artery that supplies blood to one part of the lower myocardial wall. We had several treatment options available. The first option was primary percutaneous intervention (pPCI) on the left MSCA, with stent implantation, with or without thrombus aspiration. Another option was the tirofiban therapy for 2–3 days, which would be followed by a bypass surgery. However, as no symptoms were shown during CAn, and the ECG showed a minor lower wall infarction with preserved R wave in inferior standard leads with thrombolysis in myocardial infarction (TIMI)3 flow through all branches of the left CA (LCA) except the distal LAD, which was about 2 mm in diameter, we opted for the third solution. The patient was young and had a low risk of hemorrhagic complications. He had already received a loading dose of ticagrelor and acetylsalicylic acid, and he also received an intracoronary bolus of the GP IIb/IIIa inhibitor, followed by the intravenous infusion of the GP IIb/IIIa inhibitor in the following 24 hrs. Selective CAn was repeated in IVUS after a few days when the atherosclerotic plaque was detected in the proximal segment of the left MSCA with a slight reduction in the circulating lumen of the MSCA, but with no thrombus observed there and with the distal LAD that was passable. We considered that pPCI carried a high risk of infarction extension and thrombus re-embolization. Emergency bypass surgery carried a high risk of hemorrhagic complications because the patient had already received 180 mg of ticagrelor and 300 mg of acetylsalicylic acid. In addition, apart from the thrombus on the ostium of the LCA, no other hemodynamically significant atherosclerotic changes were observed in the branches of the LCA. The triple antiplatelet therapy with therapeutic doses of low molecular weight heparin significantly reduced the chance of growth of thrombus mass and embolization. On the other hand, there was a dethrombosis effect observed with GP inhibitors. Namely, in fresh arterial thrombi, there were a lot of incompletely activated platelets completely inhibited by GP inhibitor, which led to a weakening of the structure and disintegration of the thrombus because activated platelets are thrombus nodes.

Among existing recommendations, there are no particular ones when it comes to the thrombus of the left MSCA. Anyway, it is recommended that the blood flow is restituted to the embolized coronary circulation segregation as soon as possible [18, 19]. The standard for treating such patients is CA bypass graft surgery or pPCI intervention [19]. Nevertheless, in the light of a better understanding of these methods of treatment and greater use of antithrombotic therapy, we have defined a group of patients that have fully recovered after only being treated with the conservative method – the antithrom-

botic therapy [20–23]. As in the above-mentioned case, hemodynamically stable patients are evaluated clinically, using CAn (for non-obstructive thrombus), as well as using additional morphological methods recommended for the left MSCA, such as IVUS, and, after that, a decision can be made to apply the method of treatment with antithrombotic therapy [24].

Conclusion

This case is interesting from two aspects. First of all, thrombus embolization of the left MSCA led to the development of STEMI of inferior myocardial wall localization as a consequence of occlusion of the distal LAD. In addition, and more importantly, the patient was clinically stable with TIMI3 flow through all LCA branches, except the distal LAD artery, with the development of low ischemia of the lower myocardial wall. Due to the high risk of infarction extension by thrombus migration during pPCI and the high risk of bleeding in the case of bypass surgery, we successfully applied triple antiplatelet therapy with anticoagulant therapy while doing subsequent angiographic control using IVUS. In this case, conservative treatment led to a complete lysis of the thrombus mass, and the patient was discharged to home treatment with minimal heart tissue necrosis. In the case of non-obstructive thrombotic masses without the presence of a significant atherosclerotic stenotic lesion, the use of aggressive antithrombotic therapy as a treatment modality may be considered.

REFERENCES

1. *Bentzon JF, Otsuka F, Virmani R, Falk E*. Mechanisms of plaque formation and rupture. Circ Res 2014; 114(12): 1852–66.
2. *Lee MS, Dahodwala MQ*. Percutaneous coronary intervention for acute myocardial infarction due to unprotected left main coronary artery occlusion: status update 2014. Catheter Cardiovasc Interv 2015; 85(3): 416–20.
3. *Arora NP, Joumaa M, Rosman H, Mehta R*. Left Main Coronary Artery Thrombosis with Acute Myocardial Infarction: A Management Dilemma. Am J Med Sci 2017; 353(6): 597–602.
4. *Mahmoudi M, Delhaye C, Wakabayashi K, Torguson R, Xue Z, Suddath WO*, et al. Integrilin in patients undergoing primary percutaneous coronary intervention for ST-elevation myocardial infarction. J Interv Cardiol 2011; 24(4): 351–6.
5. *Shah I, Khan SO, Malhotra S, Fischell T*. Eptifibatide: the evidence for its role in the management of acute coronary syndromes. Core Evid 2010; 4: 49–65. Erratum in: Core Evid 2014; 9: 49.
6. *Akcay M*. Evaluation of thrombotic left main coronary artery occlusions; old problem, different treatment approaches. Indian Heart J 2018; 70(4): 573–4.
7. *Patel M, Bhangoo M, Prasad A*. Successful percutaneous treatment of suspected embolic left main thrombosis in a patient with a mechanical aortic valve. J Invasive Cardiol 2011; 23(11): E263–6.
8. *Grion DDS, Grion DC, Silverio IV, Oliveira LS, Larini IF, Martins AV*, et al. Percutaneous Coronary Intervention in Unprotected Left Main Coronary Artery Lesions. Arq Bras Cardiol 2021; 116(6): 1101–8. (English, Portuguese)
9. *Ali M, Becker RC*. Bridging Anticoagulation with Mechanical Heart Valves: Current Guidelines and Clinical Decisions. Curr Cardiol Rep 2020; 22(11): 130.
10. *Hudec S, Hutyra M, Precek J, Latal J, Nykl R, Spacek M*, et al. Acute myocardial infarction, intraventricular thrombus and risk of systemic embolism. Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub 2020; 164(1): 34–42.
11. *Kappetein AP, Head SJ, Osnabrugge RL, van Mieghem NM*. Role of percutaneous coronary intervention in the treatment of left main coronary artery disease. Semin Thorac Cardiovasc Surg 2014; 26(3): 187–91.
12. *Apostolakis E, Tsigkas G, Baikoussis NG, Koniari I, Alexopoulos D*. Acute left main coronary artery thrombosis due to cocaine use. J Cardiothorac Surg 2010; 5: 65.
13. *Olcay A*. Concomitant left main coronary artery and prosthetic mitral valve thrombosis treatment. Anatol J Cardiol 2018; 20(6): 365–7.
14. *Vinod VC, Yousif ZE, Salim NO, Majwal T*. Proximal Left Main Coronary Artery Aneurysm Presenting as ST-Elevation Myocardial Infarction Treated by Stenting. Case Rep Cardiol 2020; 2020: 8833917.
15. *Dunn S, Dave N, Rodriguez-Blanco YF, Aljure O*. Incidental finding of a left atrial thrombus during surgical management of a massive pulmonary embolism. Ann Card Anaesth 2020; 23(1): 87–9.
16. *Thomopoulou S, Sfirakis P, Spargias K*. Angioplasty, stenting and thrombectomy to correct left main coronary stem obstruction by a bioprosthetic aortic valve. J Invasive Cardiol 2008; 20(4): E124–5.
17. *Ayari J, Mourali MS, Farhati A, Mechmeche R*. Left main coronary artery thrombosis revealing angio-Behçet syndrome. Egypt J Intern Med 2014; 26(2): 88–90.
18. *Sanchez-Recalde A, Calvo Orbe L, Galeote G*. Cardiogenic shock due to complete thrombotic occlusion of the left main coronary ostium in a young female. J Invasive Cardiol 2006; 18(6): E188–90.
19. *Fajadet J, Chieffo A*. Current management of left main coronary artery disease. Eur Heart J 2012; 33(1): 36–50b.
20. *Choi J, Kim IS, Cho S, Kim JS, Hong SJ, Shin DH,* et al. Optimal Duration for Dual Antiplatelet Therapy After Left Main Coronary Artery Stenting. Circ J 2020; 85(1): 59–68.
21. *Chandrasekhar J, Baber U, Sartori S, Aquino M, Tomey M, Kruckoff M*, et al. Patterns and associations between DAPT cessation and 2-year clinical outcomes in left main/proximal LAD versus other PCI: Results from the Patterns of Non-Adherence to Dual Antiplatelet Therapy in Stented Patients (PARIS) registry. Int J Cardiol 2017; 243: 132–9.
22. *Lee HJ, Yu CW, Hwang HK, Choi RK, Park JS, Li H*, et al. Long-term effectiveness and safety of triple versus dual antiplatelet therapy after percutaneous coronary intervention for unprotected left main coronary artery disease. Coron Artery Dis 2013; 24(7): 542–8.
23. *Sarma J, Laan CA, Alam S, Jha A, Fox KA*, *Dransfield I*. Increased platelet binding to circulating monocytes in acute coronary syndromes. Circulation 2002; 105(18): 2166–71.
24. *Collet JP, Thiele H, Barbato E, Barthélémy O, Bauersachs J, Bhatt DL,* et al. 2020 ESC Guidelines for the management of acute coronary syndromes in patients presenting without persistent ST-segment elevation. Eur Heart J 2021; 42(14): 1289–367.

Received on April 28, 2021
Revised on March 1, 2022
Accepted on March 7, 2023
Online First April 2023

C A S E R E P O R T

UDC: 616.447:616.716.4-006
DOI: https://doi.org/10.2298/VSP220809004M

Brown tumor of the mandible – a possible clinical manifestation of primary hyperparathyroidism

Smeđi tumor mandibule – moguća klinička manifestacija primarnog hiperparatireoidizma

Andrijana Milankov*†, Milena Mitrović*†, Tijana Icin*†, Branislav Bajkin†‡, Vukadin Milankov†§

***University Clinical Center of Vojvodina, Clinic for Endocrinology Diabetes and Metabolic Diseases, Novi Sad, Serbia; †University of Novi Sad, Faculty of Medicine, Novi Sad, Serbia; ‡Dentistry Clinic of Vojvodina, Novi Sad, Serbia; §Institute for Child and Youth Healthcare of Vojvodina, Novi Sad, Serbia**

Abstract

Introduction. One of the possible manifestations of primary hyperparathyroidism (PHPT) is the appearance of a benign bone tumor. We hereby present a case of a young woman whose first clinical manifestation of PHPT was a brown tumor of the mandible. **Case report.** A 27-year-old female patient was hospitalized at the Clinic for Endocrinology, Diabetes, and Metabolic Diseases due to problems occurring in the form of nausea, exhaustion, the feeling of suffocation, dysphagia, pain in the right ear and the right half of the cheeks, with suspected PHPT. Initial laboratory findings pointed out the high levels of parathyroid (PT) hormone (PTH) and calcium (Ca^{2+}) ions, low levels of vitamin D, and increased parameters of bone metabolism with signs of osteopenia. Cone beam computed tomography revealed the presence of bilateral radiolucent lesions of the mandible. Scintigraphy verified a retrosternal hot focus consistent with PT adenoma. After parathyroidectomy was performed, there was a normalization of PTH values, bone metabolism parameters, and the Ca^{2+} values. Four months after parathyroidectomy, a significant regression of the mandibular tumefaction was confirmed, clinically and radiologically. **Conclusion.** Brown tumors are rare first clinical manifestations of PHPT. Owing to their histological similarities with other giant-cell lesions (GCLs), definitive diagnosis is sometimes difficult and is based on a correlation of pathohistological, radiological, and laboratory findings. Due to the spontaneous regression of bone lesions after treatment of the basic cause of PHPT, brown tumors should be considered in the differential diagnosis of any GCLs in order to avoid unnecessary surgical procedures.

Key words:
bone diseases, endocrine; diagnosis; giant cell tumors; hyperparathyroidism, primary; mandible.

Apstrakt

Uvod. Jedna od mogućih manifestacija primarnog hiperparatireoidizma (PHPT) jeste pojava benignog tumora koštanog tkiva. Prikazana je mlada žena kod koje je prva klinička manifestacija PHPT bila smeđi tumor mandibule. **Prikaz bolesnika.** Bolesnica stara 27 godina bila je hospitalizovana na Klinici za endokrinologiju, dijabetes i metaboličke bolesti zbog tegoba u vidu mučnine, iscrpljenosti, osećaja gušenja, otežanog gutanja, bola u desnom uhu i desnoj polovini obraza, sa sumnjom na PHPT. Početni laboratorijski nalazi ukazivali su na visoke nivoe paratireoidnog (PT) hormona (PTH) i jona kalcijuma (Ca^{2+}), nizak nivo vitamina D, kao i povišene vrednosti parametara koštanog metabolizma, sa znacima osteopenije. Kompjuterizovanom tomografijom konusnog zraka utvrđeno je bilateralno rasvetljenje u donjoj vilici. Scintigrafijom je potvrđeno prisustvo promene, retrosternalno, koja je mogla odgovarati adenomu PT žlezde. Nakon sprovedene paratireoidektomije, došlo je do normalizacije vrednosti PTH, parametara koštanog metabolizma i vrednosti Ca^{2+}. Četiri meseca nakon paratireoidektomije, potvrđena je, klinički i radiološki, značajna regresija tumefakcije donje vilice. **Zaključak.** Smeđi tumori su retka prva klinička manifestacija PHPT. Zbog njihove patohistološke sličnosti sa drugim gigantocelularnim tumorima, postavljanje definitivne dijagnoze je nekada teško i zasniva se na korelaciji patohistološkog, radiološkog i laboratorijskog nalaza. S obzirom na to da nakon izlečenja PHPT dolazi do sponatne regresije lezija u kosti, smeđi tumori bi trebalo da budu razmotreni u diferencijalnoj dijagnozi svih gigantocelularnih lezija, kako bi se izbegao nepotreban hirurški tretman.

Ključne reči:
kosti, endokrine bolesti; dijagnoza; tumor gigantskih ćelija; hiperparatireoidizam; mandibula.

Correspondence to: Andrijana Milankov, University Clinical Center of Vojvodina, Clinic for Endocrinology, Diabetes, and Metabolic Diseases, Hajduk Veljkova 1–9, 21 000 Novi Sad, Serbia. E-mail: andrijana.milankov@mf.uns.ac.rs

Introduction

The incidence of diagnosis of primary hyperparathyroidism (PHPT) has been substantially growing since the early 70s when a routine biochemical analysis began to determine the level of serum calcium (Ca) and diagnose asymptomatic patients (catch-up effect). Epidemiological studies suggest that the majority of patients (80%) have asymptomatic disease, while the classical clinical presentation, such as hypercalcemic syndrome, nephrolithiasis, osteoporosis, and gastrointestinal and mental disorders, is rarely seen [1, 2]. Due to its effect on bone metabolism, PHPT can also manifest itself as a type of pseudo-tumor – a brown tumor (BT) of bone – which appears in the advanced stages of the disease. We hereby present a case of a young woman whose first clinical manifestation of PHPT was BT of the mandible.

Case report

A 27-year-old female patient was hospitalized at the Clinic for Endocrinology, Diabetes, and Metabolic Diseases, University Clinical Center of Vojvodina, Serbia due to nausea, exhaustion, feeling of suffocation, dysphagia, and pain in the right ear and the right half of the cheeks, which appeared a month before. Five years earlier, the patient was followed up for a tumor on the left side of the mandible. Biopsy and excision of tumefaction were performed, and pathohistological findings determined that it was a giant-cell lesion (GCL). After the operation, hormonal testing was planned, but the patient did not show up for the follow-up exams. The family history was positive for nephrolithiasis.

Initial clinical examination found lower right facial deformity, with approximate dimensions 40 × 30 mm, without changes of the skin above. From the intraoral side, in the region of the right mandible, there was a tumefaction, with necrosis and granulation tissue, about 30 mm in diameter (Figure 1). In the diagnostic algorithm, X-ray and cone beam computed tomography were performed, and the presence of bilateral radiolucency in lateral segments of the mandible was observed. There was evidence of severe osteolysis of the right mandible, with root resorption of the first molar. In addition, there was a partial recurrence of the previous tumor in the left mandible, in the region beneath the lower left incisors and canines (Figures 2 and 3).

Laboratory findings pointed to PHPT with significantly increased bone turnover (Table 1). The performed bone densitometry discovered osteopenia (Z-score: L1–L4 -2.2,

Fig. 1 – The image on the left depicts the initial intraoral clinical finding. The image on the right shows the intraoral finding after the tumor biopsy.

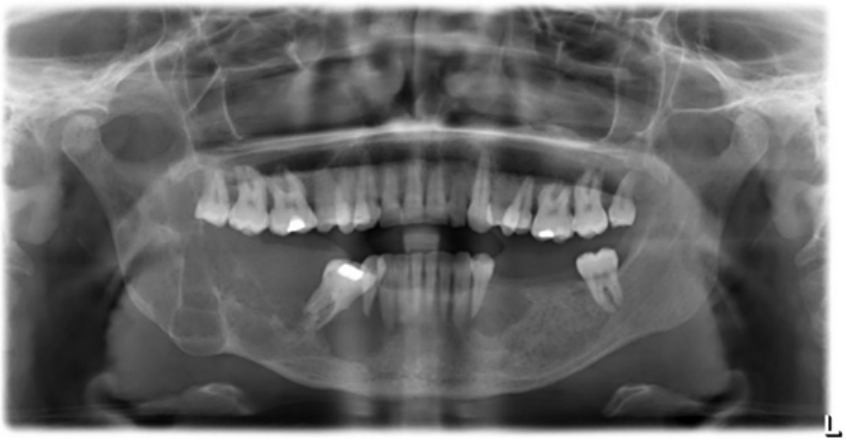


Fig. 2 – Initial state X-ray (orthopantomogram). On the right side of the mandible, tumefaction is poorly delineated, with a breach of cortical bone. There is no periosteal reaction, and there is dislocation of the right lower molar tooth with partial root resorption. On the left side, the osteolytic lesion is confined to the region beneath the lower incisors and canine. There is good delineation from the rest of the mandible. Laterally, there is evidence of previous cystic formation that has healed.

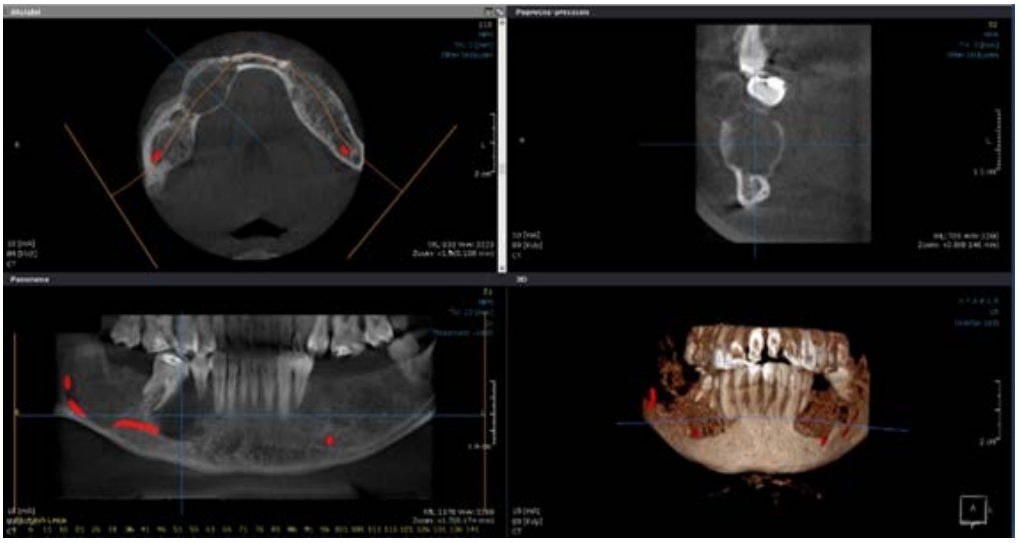


Fig. 3 – The initial appearance of the lesion on cone beam computed tomography in the region of the right side of the mandible.

Table 1

Laboratory findings before and after surgery

Parameters	Reference range	Before surgery	After surgery		
			5 days	2 months	6 months
Ca (mmol/L)	2.10–2.60	3.4	2.15	2.11	2.45
Ca^{2+} (mmol/L)	0.95–1.35	1.75	1.07	1.07	1.29
P (mmol/L)	0.74–1.52	0.78	1	1.24	0.97
ALP (U/L)	30–115	141	100	91	89
PTH (pg/mL)	14–72	1,290	126.3	43	83.2
Cross Laps (pg/mL)	162–436	2,911	707	692	399
P1NP (g/mL)	10–55.7	233.8	594.2	140.7	31.8
25(OH)-D total (nmol/L)	30–150	29	36	22.45	69

Ca – total calcium; Ca^{2+} – ionized calcium; P – phosphorus; ALP – alkaline phosphatase; PTH – parathyroid hormone; Cross Laps – cross-linked C-telopeptide of type I collagen; P1NP – procollagen type 1 N propeptide; 25(OH)-D – 25-hydroxy vitamin D.

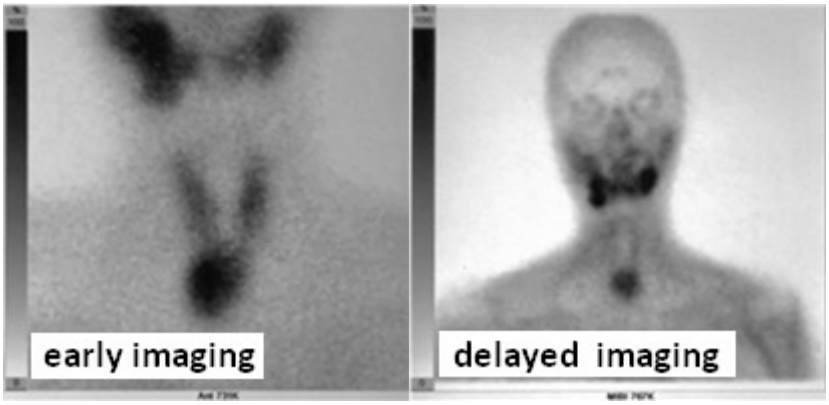


Fig. 4 – Scintigraphy of parathyroid glands (99mTc-MIBI). The early imaging shows the radioactive concentration at the site of the anterior mediastinum. The delayed imaging shows that it did not degrade.

femur: neck -2.0, total -2.2). Ultrasound examination of the abdomen verified nephrolithiasis in the right kidney. Moreover, the ultrasound examination of the thyroid and parathyroid (PT) glands discovered a focal, oval hypo-echogenic nodule near the inferior pole of the right thyroid lobe, at the superior entrance of the anterior mediastinum, measuring 32 × 13 mm (antero-posterior × latero-lateral diameter). Scintigraphy of the PT glands revealed more focal changes in the projection of the anterior mediastinum, which could match the localized ectopic adenoma/hyperplasia of the PT gland (Figure 4). PHPT was treated surgically by removing the upper and lower right PT glands. Laboratory findings

Fig. 5 – Clinical finding two months after parathyroidectomy. Significant regression of the tumor was noticed.

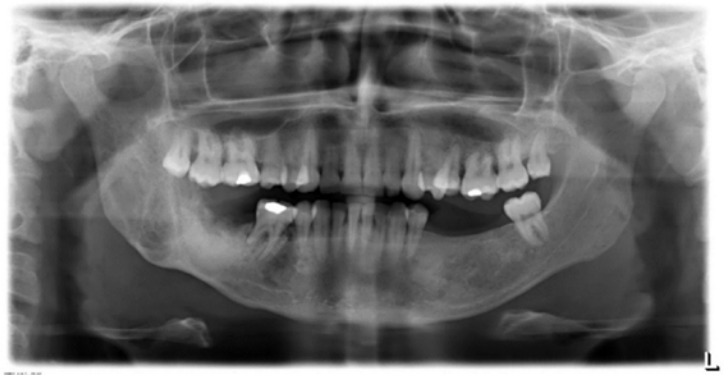


Fig. 6 – Control X-ray (orthopantomogram) four months after parathyroidectomy. There are signs of new bone formation and almost complete resolution of cystic tumefaction on both sides of the mandible.

were taken in different time periods – a couple of days, two months, and six months postoperatively, and they are shown in Table 1. Due to low levels of vitamin D, replacement therapy was introduced, followed by normalization of bone turnover. Two months after parathyroidectomy, there was significant regression of the tumor (Figure 5). Despite the significant reduction in the tumor masses, six months following parathyroidectomy, the oral surgeon decided to remove part of the tumor tissue that protruded beyond the borders of the alveolar ridge. Pathohistological findings reaffirmed that it was a GCL. Even though the lesion was large at the beginning, after treating the cause of PHPT, there was almost complete resolution of the lower jaw lesion with slight residual deformity of the alveolar ridge (Figure 6) that did not require any further operative corrections. The patient was absolutely satisfied with the functional and aesthetic outcomes.

Discussion

PHPT is a common endocrine disorder that affects one out of 500 women in the third and after the sixth decade of life. In 85% of cases, it is caused by a PT adenoma; in 15% of cases, it is caused by multiple gland disease (multiple adenomas or hyperplasia), while it is rarely a result of PT carcinoma. Familial cases may occur as a part of multiple endocrine neoplasia (MEN) 1, MEN 2a, hyper-PT jaw tumor syndrome, or familial isolated hyperparathyroidism [1–3]. Due to the rare occurrence of bone manifestations of PHPT, especially as the first sign [4], it is hard to distinguish PHPT bone changes from other osteolytic lesions. We describe a case of PHPT in which the first clinical manifestation of the disease was BT of the mandible. GCL in the jaws includes several types of lesions whose common microscopic characteristic is the presence of gigantic multinucleate cells in the vascular stroma. These lesions exhibit different behaviors, from benign to malignant and locally aggressive behavior, and sometimes, it is quite difficult to classify them only based on the microscopic findings. We are familiar with the pathohistological description of GCLs since the 1940s, and nowadays, they include peripheral giant-cell granuloma, central giant-cell granuloma, giant-cell tumor, BT in hyperparathyroidism, aneurysmal bone cysts, and cherubism [5]. Histological features of a BT are characterized by the replacement of bone marrow with loose, richly vascularized connective tissue. In the remaining cancellous bone, there is increased osteoclast activity. Due to the weakening of the bone, there are on-site microfractures, with consecutive hemorrhage and abundant hemosiderin pigment deposits that give the tumor its characteristic brown color [6]. Purely based on the pathohistological examination, the distinction between BT and giant cell tumor

is extremely difficult, and the key issue for a correct diagnosis is the correlation of clinical, laboratory, and radiological findings, as was in our case.

As a result of hyperparathyroidism, BT occurs in the incidence rate range from 1.5 to 4.5%, three times more frequently in females, especially after 50 years of age [7, 8], and in less than 2% of patients with PHPT [9]. According to the literature, common structures affected by BT are facial bones, long bones, ribs, clavicle, and pelvis. BT can develop in any bone and any stage of PHPT; however, it rarely appears as the initial sign of the disease [10]. In our case, BT was the first sign of PHPT at the moment when other extra-skeletal manifestations were not verified.

The treatment of choice for GCL depends on its etiology. GCL that is not associated with PHPT requires surgical excision of the lesion itself. On the other hand, for the giant cell tumor associated with PHPT, primary treatment is focused on the diagnosis and treatment of the basic cause of PHPT. In most cases, adenoma of PT glands is found as a source of PHPT and is treated by parathyroidectomy [11]. After normalization of hormonal changes, complete resolution of bone lesions can be expected. Since the regression rate of tumor changes depends on the scope of the change, which is proportional to the length of PHPT, it is necessary to diagnose PHPT in the shortest period of time [8, 12].

Based on our knowledge, this is a rare case of a young person having a large tumor of the mandible as a first clinical manifestation of PHPT, especially nowadays, when serum Ca^{2+} levels are routinely measured and PHPT has evolved into a typically asymptomatic disease.

Conclusion

GCLs, such as BTs, associated with PHPT are a non-typical clinical manifestation of the disease. Because of their microscopic similarities, histological differentiation between BTs and GCLs is very difficult, and definitive diagnosis is based on radiological findings of typical localization of the bone lesion, as well as laboratory findings of elevated Ca and PTH levels. Due to their spontaneous regression after treatment of the basic cause of PHPT, BTs should be considered in the differential diagnosis of any GCLs to avoid unnecessary surgical procedures in the mandible.

REFERENCES

1. *Kim LT*. Hyperparathyroidism [Internet]. 2020. Available from: https://emedicine.medscape.com/article/127351-overview#a3 [accessed 2022 July 22].
2. *Rai S, Rattan V, Bhadada SK*. Giant Cell Lesions Associated with Primary Hyperparathyroidism. J Maxillofac Oral Surg 2015; 14(4): 930–4.
3. *Bojković G, Caparević Z, Stojanović D, Lalosević D, Stojanović M*. Case report: parathyroid gland adenoma. Med Pregl 2003; 56(7–8): 377–80. (Serbian)
4. *Mahfoudhi M, Khamassi K, Battikh AG, Lahiani R, Sami T, Salah MB*. Brown tumor of the maxilla revealing primary hyperparathyroidism. Int J Clin Med 2015; 6(4): 252.
5. *Vera L, Dolcino M, Mora M, Oddo S, Gualco M, Minuto F,* et al. Primary hyperparathyroidism diagnosed after surgical ablation of a costal mass mistaken for giant-cell bone tumor: a case report. J Med Case Rep 2011; 5(1): 1–6.
6. *Ohi LG, da Cunha IW, Santini-Araujo E, Kalil RK.* "Brown Tumor" of Hyperparathyroidism. In: *Santini-Araujo E, Kalil RK, Bertoni F, Park YK,* editors. Tumors and Tumor-Like Lesions of Bone. London: Springer; 2015. p. 815–25.
7. *Thomas S, Nair P, Hegde K, Neelakantan S*. A bilaterally recurring exophytic mass on the lower jaw. BMJ Case Rep 2011; 2011: bcr0920103362.
8. *Zhong Y, Huang Y, Luo J, Ye Y*. Misdiagnosis of brown tumour caused by primary hyperparathyroidism: a case report with literature review. BMC Endocr Disord 2022; 22(1): 66.
9. *Walker MD, Bilezikian JP*. Primary Hyperparathyroidism. 2021. In: *Feingold KR, Anawalt B, Blackman MR, Boyce A, Chrousos G, Corpas E,* et al, editors. Endotext [Internet]. South Dartmouth (MA): MDText.com Inc; 2000.
10. *Xu B, Yu J, Lu Y, Han B*. Primary hyperparathyroidism presenting as a brown tumor in the mandible: a case report. BMC Endocr Disord 2020; 20(1): 6.
11. *Stefanović D, Petrović M, Dugonjić S*. The accuracy of ultrasonography for detection of enlarged parathyroid glands in patients with different forms of hyperparathyroidism. Vojnosanit Pregl 2020; 77(12): 1277–88.
12. *Mittal S, Gupta D, Sekhri S, Goyal S*. Oral manifestations of parathyroid disorders and its dental management. J Dent Allied Sci 2014; 3(1): 34–8.

Received on August 9, 2022
Revised on December 21, 2022
Accepted on January 10, 2023
Online First January 2023

CASE REPORT

UDC: 575:616-053.2-056.7-07
DOI: https://doi.org/10.2298/VSP220803003J

Clinical manifestations of Johanson-Blizzard syndrome in a patient with nucleotide variants in the *UBR1* gene

Kliničke manifestacije Johanson-Blizzard-ovog sindroma kod bolesnika sa nukleotidnim varijantama *UBR1* gena

Danijela Jojkić-Pavkov*†, Jela Tošić*†, Ivana Kavečan*†, Milica Plazačić†

***University of Novi Sad, Faculty of Medicine, Novi Sad, Serbia; †Institute for Child and Youth Health Care of Vojvodina, Department of Gastroenterology, Hepatology, and Nutrition, Novi Sad, Serbia**

Abstract

Introduction. Johanson-Blizzard syndrome (JBS) is a very rare genetic disorder caused by a mutation of the ubiquitin protein ligase E3 component N-recognin 1 (*UBR1*) gene. Clinical diagnosis is based on the pathognomonic combination of congenital exocrine pancreatic insufficiency and characteristic signs of facial dysmorphology (nasal wing hypoplasia/aplasia and oligodontia of permanent teeth). Diagnosis is confirmed by genetic screening of the *UBR1* gene. The aim of this case report was to emphasize that nucleotide variants in the *UBR1* gene, described as benign or unclassified, should still be considered a genetic cause of the clinical characteristics in patients with JBS. **Case report.** We present an 8-month-old child, with clinical features of JBS, who was admitted to our hospital due to poor weight gain and loose stools. Upon admission, signs of protein-energy malnutrition, facial dysmorphology, and other anomalies were observed. The child had hypotonia and convergent strabismus. A laboratory examination confirmed exocrine pancreatic insufficiency and hypothyroidism. Genetic testing confirmed two single nucleotide variants in the *UBR1* gene – chromosome 15q15.2: NM_174916.3:c.4700+12A>G (intron 42) and NM_174916.3 *UBR1*:c.862-18C>T (intron 07). A pancreatic enzyme replacement therapy with liposoluble vitamin supplementation and adequate nutrition was conducted. **Conclusion.** Recognizing the clinical features of JBS and confirming it with genetic analysis is essential, especially in patients with idiopathic pancreatic insufficiency. Even when genetic confirmation is not possible, adequate treatment is necessary for normal growth and development of the child.

Key words:
exocrine pancreatic insufficiency; genes; hypothyroidism; johanson-blizzard syndrome; mutation; ubr1 protein, human.

Apstrakt

Uvod. Johanson-Blizzard-ov sindrom (JBS) je veoma redak genetički poremećaj uzrokovan mutacijom gena *ubiquitin protein ligase E3 component N-recognin 1* (*UBR1*). Klinička dijagnoza se zasniva na prepoznavanju patognomonične kombinacije kongenitalne egzokrine insuficijencije pankreasa i karakterističnih znakova facijalne dismorfologije (hipoplazija/aplazija nosnih školjki i oligodoncija stalnih zuba). Dijagnoza se potvrđuje genetičkim skriningom *UBR1* gena. Cilj rada bio je da se ukaže na to da nukleotidne varijante *UBR1* gena, koje se opisuju kao benigne ili neklasifikovane, ipak treba razmotriti kao genetički uzrok kod bolesnika sa kliničkim karakteristikama JBS-a. **Prikaz bolesnika.** Prikazano je dete uzrasta osam meseci, sa kliničkim karakteristikama JBS-a, koje je primljeno u bolnicu zbog nenapredovanja u telesnoj masi i neformiranih stolica. Na prijemu su bili prisutni znakovi proteinsko-energetske malnutricije, facijalne dismorfologije i druge anomalije. Dete je imalo hipotoniju i konvergentni strabizam. Laboratorijskim ispitivanjem potvrđena je egzokrina insuficijencija pankreasa i hipotireoidizam. Genetičkom analizom potvrđene su dve nukleotidne varijante *UBR1* gena – hromozom 15q15.2: NM_174916.3:c.4700+12A>G (intron 42) i NM_174916.3 *UBR1*:c.862-18C>T (intron 07). Sprovedena je supstituciona terapija pankreasnim enzimom uz suplementaciju liposolubilnim vitaminima i uz adekvatnu ishranu. **Zaključak.** Prepoznavanje kliničkih karakteristika JBS-a i potvrda sindroma primenom genetičkih analiza, posebno je važna kod bolesnika sa idiopatskom insuficijencijom pankreasa. Čak i kada genetička potvrda nije moguća, za normalan rast i razvoj deteta neophodna je adekvatna terapija.

Ključne reči:
pankreas, egzokrini, insuficijencija; geni; hipotireoidizam; johanson-blizzardov sindrom; mutacija; ubr1 protein, humani.

Correspondence to: Danijela Jojkić-Pavkov, Institute for Child and Youth Health Care of Vojvodina, Department of Gastroenterology, Hepatology, and Nutrition, Hajduk Veljkova 10, 21 000 Novi Sad, Serbia. E-mail: danijela.jojkic-pavkov@mf.uns.ac.rs

Introduction

Johanson-Blizzard syndrome (JBS) is a very rare genetic multisystem disorder with an autosomal recessive inheritance pattern. The incidence of JBS in Europe is approximately 1 in 250,000 live births. JBS is caused by mutations in the *UBR1* gene [1, 2]. Typical clinical features predominantly affect skeletal muscles and pancreatic acinar cells. It is considered that the *UBR1* gene plays a critical role in the development and maintenance of acinar cells of the pancreas [1–5].

Destruction of acinar tissue, which may begin *in utero* in patients who suffer from JBS, results in the development of exocrine pancreatic insufficiency and fatty infiltration of the pancreas [3–7]. Clinical diagnosis is suspected according to the identification of the pathognomonic combination of congenital or infantile exocrine pancreatic insufficiency with facial dysmorphology (nasal wing hypoplasia/aplasia and oligodontia of permanent teeth) and additional features such as intrauterine growth restriction, short stature, microcephaly, scalp defects, hearing impairment, cognitive impairment of variable degree, hypothyroidism, congenital heart defects, urogenital and anorectal malformations, renal anomalies, and diabetes with onset during adolescence. The diagnosis is confirmed by the detection of a *UBR1* gene mutation [1–7]. Early diagnosis, as well as symptomatic and supportive therapy, contribute to the survival of these patients until adulthood; otherwise, pancreatic insufficiency and complications of severe malnutrition can lead to death in infancy or early childhood [4–8].

Case report

In the report, we present a boy, born as a second child from a second pregnancy (the first child from the first pregnancy was healthy) at 37 weeks of gestation by Cesarean section. The boy's birth weight was 3,180 g (57th percentile), his birth length was 52 cm (77th percentile), and his head circumference was 34 cm (55th percentile). The family history was unremarkable. When the child was four months old, his mother noticed poor weight gain and loose stools. When the boy was eight months old, he was hospitalized for diagnostic evaluation at the Department of Pediatric Gastroenterology, Hepatology, and Nutrition at the Institute for Child and Youth Health Care of Vojvodina, Novi Sad, Serbia. Upon admission to our hospital, the signs of protein-energy malnutrition were observed: body weight 5,500 g (< 3rd percentile); body length 63 cm (< 3rd percentile); head circumference 42 cm (< 3rd percentile). Furthermore, facial dysmorphological signs (prominent forehead, long eyelashes, otapostasis, malformed ears) and anomalies (micropenis, bell-shaped thorax, umbilical hernia, irregular dentition, and poor tooth quality) were present. The child had hypotonia and convergent strabismus. The major clinical hallmarks in the case of our patient include severe pancreatic insufficiency, irregular dentition, and poor dental quality (the primary dentition), with additional features of the JBS but without nasal wing anomaly. The varying frequencies of features in JBS [1] are shown together with the features of our patient in Table 1.

Table 1

Typical clinical characteristics of Johanson-Blizzard syndrome (JBS) [1] and the presence of features in the case of our patient

Clinical characteristics of JBS (frequency)	Presence / Type of presence
Pancreatic exocrine insufficiency (100%)	yes
Hypoplasia/aplasia *alae nasi* (> 95%)	no
Dental anomalies, oligodontia/hypodontia of permanent teeth (> 90%)	irregular dentition, poor tooth quality
Sensorineural hearing loss (75%)	no
Scalp defects/aplasia cutis congenital (65%)	no
Short stature, developmental and intellectual delays, hypotonia (60%)	yes
Hypothyroidism (40%)	yes
Microcephaly (35%)	yes
Anorectal malformations (20%)	no
Eye anomalies (lacrimal duct anomalies, coloboma, congenital cataract) (#)	no
Other minor signs* (#)	long eyelashes, eyes slanting downwards, otapostasis, malformed ears, prominent forehead, bell-shaped thorax, liver disease
Impaired glucagon secretion, abnormal response of insulin ** (#)	yes
Diabetes mellitus ** (#)	no
Congenital heart defects (25%)	no
Intrauterine growth restriction (30%)	no
Genitourinary malformations (30%)	micropenis

 # – the frequency varies widely; * Other minor signs described in JBS: abnormal frontal hair pattern (upsweep), severe facial clefting (cleft lip/palate), natal teeth, poly-/syndactyly, prostate aplasia, gastroesophageal reflux, cholestatic liver disease, café au-lait spots, growth hormone deficiency, hypopituitarism, brain and osseous malformations; ** – older children are at high risk, suggesting the progressive nature of pancreatic disease.

A laboratory examination was conducted, and exocrine pancreatic insufficiency was confirmed with elevated values of transaminases and gamma-glutamyl transferase (all laboratory results are shown in Table 2). Abdominal ultrasound showed normal pancreatic structure, but magnetic resonance cholangiopancreatography revealed severe pancreatic atrophy with fatty infiltration. Genetic testing confirmed two single nucleotide variants in the *UBR1* gene, chromosome 15q15.2 – NM_174916.3:c.4700+12A>G (intron 42) and NM_174916.3 *UBR1*:c.862-18C>T (intron 07). As the examination showed exocrine pancreatic insufficiency, pancreatic enzyme replacement therapy (pancrelipase) was started with liposoluble vitamin (A, D, E, K) supplementation. Adequate nutrition with high-fat content was introduced. The child had normal levels of free triiodothyronine (T3) [2 nmol/L; reference range (RR) 1.34–2.73 nmol/L] and thyroxine (T4) (100 nmol/L; RR 78.38–157.4 nmol/L) and an increased thyroid-stimulating hormone (TSH) level (9 mU/L; RR 0.34–5.60 mU/L), after which levothyroxine was introduced to treatment. The level of postprandial insulinemia was 3 pmol/L (RR 3.5–41 pmol/L), and it revealed a subnormal insulin response. Other analyses showed adrenocorticotropic hormone, cortisol, and prolactin levels in the RR, prepubertal levels of testosterone, luteinizing, and the follicle-stimulating hormone (2 U/L, RR 0–5 U/L), and a low normal insulin-like growth factor 1 level. An endocrinologist achieved normal genital size after three turns of testosterone depot. The electrocardiogram and echocardiogram were normal. Ophthalmic examination and hearing test were normal.

Table 2

Patient's laboratory test results

Parameter	Result	Reference range
Leukocytes ($x10^9$/L)	8.9	4–10
Red blood cells ($x10^{12}$/L)	3.8	4.1–6.0
Platelets ($x10^9$/L)	307	150–450
AST (U/L)	194.4	16.2–52.2
ALT (U/L)	193.2	12–58.8
GGT (U/L)	42	1.2–39
Amylase (U/L)	< 12	27.6–99.6
Lipase (U/L)	< 3	4.2–39
Albumin (mmol/L)	0.54	0.57–0.81
Components of complement (C) system		
C3 (g/L)	0.35	0.8–1.6
C4 (g/L)	1.3	0.15–0.45
Ammonia (μmol/L)	16	10–30
Serum lactate (mmol/L)	0.8	0.5–1
Fecal calprotectin (μg/g)	< 100	< 100
Pancreatic elastase (μg/g)	< 15	> 200
Antiviral antibodies, ratio		
HCV	< 1 s/c	< 1 s/c (negative); ≥ 1 s/c (positive)*
HBsAg	< 1 s/c	< 1 s/c (negative); ≥ 1 s/c (positive)*
CMV (IgM and IgG)	< 0.8	< 0.8 (negative); ≥ 0.8 to < 1.1 (cut-off); ≥ 1.1 (positive)**
HSV (IgM and IgG)	< 0.8	< 0.8 (negative); ≥ 0.8 to < 1.1 (cut-off); ≥ 1.1 (positive)**
Adenovirus (IgM and IgG)	< 0.8	< 0.8 (negative); ≥ 0.8 to < 1.1 (cut-off); ≥ 1.1 (positive)**
EBV (IgM and IgG)	< 0.8	< 0.8 (negative); ≥ 0.8 to < 1.1 (cut-off); ≥ 1.1 (positive)**
Coxsackie B (IgM and IgG)	< 0.8	< 0.8 (negative); ≥ 0.8 to < 1.1 (cut-off); ≥ 1.1 (positive)**
Toxoplasma gondii (IgM and IgG)	< 0.8	< 0.8 (negative); ≥ 0.8 to < 1.1 (cut-off); ≥ 1.1 (positive)**
Parvovirus B19 (IgM and IgG)	< 0.8	< 0.8 (negative); ≥ 0.8 to < 1.1 (cut-off); ≥ 1.1 (positive)**
Autoantibodies, titers		
ANA (on HEp-2 cells/primate liver tissue)	< 1:100	< 1:100 (negative); ≥ 1:320 (positive)¤
APA	< 1:100	< 1:100 (negative); > 1:100 (positive)¤
AMA	< 1:100	< 1:100 (negative); > 1:100 (positive)¤
ANCA	< 1:10	< 1:10 (negative); > 1:10 (positive)¤
LKM1	< 1:100	< 1:100 (negative); > 1:100 (positive)¤
ASMA	< 1:100	< 1:100 (negative); > 1:100 (positive)¤
Iron (μmol/L)	6.2	7.2–17.9
Transglutaminase antibody IgA (U/mL)	< 2	< 20
IgA (g/L)	0.33	0.19–2.2
IgM (g/L)	0.55	0.4–1.4
IgG (g/L)	6.79	3.5–10.0

AST – aspartate aminotransferase; ALT – alanine aminotransferase; GGT – gamma-glutamyl transferase; HCV – Hepatitis C virus; HbsAg – Hepatitis B surface antigen; CMV – cytomegalovirus; HSV – Herpes Simplex virus; EBV – Epstein Barr virus; Ig – immunoglobulin; ANA – anti-nuclear antibodies; APA – anti-parietal antibodies; AMA – anti-mitochondrial antibodies; ANCA – anti-neutrophil cytoplasmic antibodies; LKM1 – liver kidney microsome antibodies type 1; ASMA – anti-smooth muscle antibodies; ratio – extinction of the control or patient sample/extinction of calibrator; s/c – signal detected on sample/cut-off value ratio; *chemoluminescent immunoassay; **enzyme-linked immunosorbent assay; HEp – human epithelial; ¤ indirect immunofluorescence.

The electroencephalogram was orderly. The child had a mild developmental delay (Brunet Lezine scale). The global development quotient range (QR) was 85. The main characteristics of the psychological aspect of the child were hyperactivity, short-term attention, and short-term interests.

Discussion

JBS is a very rare autosomal recessive disorder that affects many organ systems. The molecular basis of JBS has been mapped to chromosome 15q15-q21 with identified mutations in the *UBR1* gene. The *UBR1* gene contains 47 exons that encode one of several E3 ubiquitin ligases of the N-end rule pathway (ubiquitin-dependent proteolytic pathway) [1–5]. The spectrum of dysmorphological and clinical manifestations in JBS is variable and heterogeneous, but nasal wing hypoplasia/aplasia and exocrine pancreatic insufficiency are considered the most consistent manifestations [3–8]. Severe pancreatic insufficiency, irregular dentition, and poor dental quality (the primary dentition), with some additional features of the syndrome, were present in our patient (nasal wing hypoplasia/aplasia absent).

Exocrine pancreatic insufficiency could be a manifestation of many childhood diseases, so in the differential diagnosis, apart from JBS, the following conditions should be considered: cystic fibrosis, Shwachman-Diamond syndrome, Pearson syndrome, Jeune syndrome, pancreatic aplasia and hypoplasia, isolated enzyme deficiencies, and chronic pancreatitis (hereditary and autoimmune) [9, 10]. In patients with cystic fibrosis, the pancreatic juice is abnormally thick, causing its retention in the pancreatic canalicular system. The primary disorder in the Shwachman-Diamond syndrome is hypoplasia of the exocrine tissue of the pancreas (similar to JBS). Pearson syndrome is a very rare mitochondrial cytopathy characterized by poor fluid and electrolyte secretion in addition to reduced acinar function. Although Jeune syndrome is characterized by skeletal abnormalities of the thorax and extremities, its association with pancreatic fibrosis has been confirmed. Chronic pancreatitis is a progressive inflammatory disorder that leads to irreversible destruction of pancreas tissue. The causes of chronic pancreatitis, except ductal obstruction by stones or cystic fibrosis, are hereditary pancreatitis and autoimmune pancreatitis. In hereditary pancreatitis, gene mutations such as *PRSS1*, *SPINK1*, *CASR*, and *CTRC* increase the risk of developing pancreatitis. These mutations could impair trypsin autolysis and promote the auto-activation of trypsinogen. Autoimmune mechanisms contributing to pancreatitis can lead to multifocal fibrosclerosis. In type I autoimmune pancreatitis (IgG_4-related disease), other organs can be affected besides the pancreas, such as the intrahepatic bile ducts, salivary glands, kidneys, and lymph nodes. Recent studies have found that the hypothyroidism detected in patients with this type of pancreatitis was mild and infrequent; therefore, further studies are necessary to clarify whether hypothyroidism is another manifestation of IgG_4-related disease. In autoimmune pancreatitis type II, only the pancreas is affected, but inflammatory bowel disease can develop [9, 10]. In the case of our patient, anti-nuclear antibodies (ANA), anti-parietal antibodies (APA), anti-mitochondrial antibodies (AMA), anti-neutrophil cytoplasmic antibodies (ANCA), anti-smooth muscle antibodies (ASMA) and anti liver kidney microsome antibodies type 1 (LKM1) autoantibodies were negative. IgG_4 analysis was not performed for technical reasons.

Recent research indicates that new *UBR1* gene mutations are being discovered at an increasing rate, as well as the impact of gene mutations on phenotypic characteristics in children with JBS. On the other hand, it is very difficult to identify all *UBR1* gene mutations and their variants [8, 11, 12]. In the case of our patient, genetic testing confirmed two single nucleotide variants in the *UBR1* gene, chromosome 15q15.2: NM_174916.3:c.4700+12A>G (intron 42) and NM_174916.3 *UBR1*:c.862-18C>T (intron 07). The *UBR1* was covered 100% in the whole exome sequencing (BioExome). Because those two unclassified intronic variants of the *UBR1* gene in a patient are considered benign, we assume that this connection is not a coincidence; because of that, we present this case. Further investigations are needed to confirm this connection. Early treatment with pancreatic enzyme and nutrition, as was done in the case of our patient, improves the patient's normal growth and development and has a normal global development quotient [7, 8, 11–13].

Prenatal diagnosis of JBS is possible. The ultrasound-verified dysmorphological signs such as hypoplasia or aplasia of the *alae nasi*, dilated sigmoid colon, or imperforated anus can be seen at 21 weeks of gestation. The molecular testing of gene *UBR1* and searching for the same mutation in a fetus as the proband using invasive procedures such as chorionic villi sampling, amniocentesis, or cordocentesis could confirm the diagnosis of JBS [1, 8, 11–13].

Conclusion

Recognition of features of JBS and genetic confirmation is very important, especially in patients with idiopathic pancreatic insufficiency. Even when genetic confirmation of the diagnosis of JBS is not possible, adequate treatment is necessary for normal growth and development of the child.

Conflict of interest

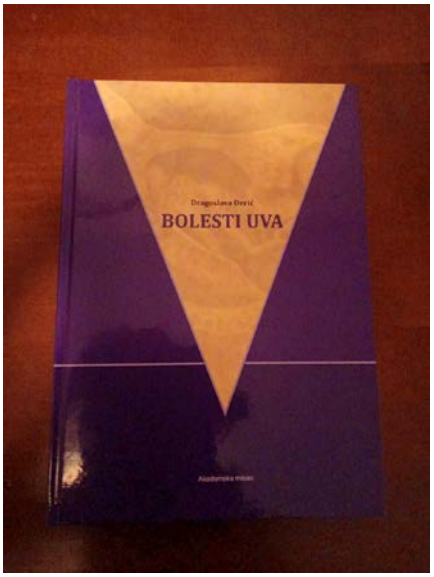
The authors declare no conflict of interest.

R E F E R E N C E S

1. *Orphanet*. Johanson-Blizzard syndrome [Internet]. Available from: https://www.orpha.net/consor/cgi-bin/OC_Exp.php?Lng=GB&Expert=2315 [accessed on: 2023 October 19]
2. *Sukalo M, Fiedler A, Guzmán C, Spranger S, Addor MC, McHeik JN,* et al. Mutations in the human UBR1 gene and the associated phenotypic spectrum. Hum Mutat 2014; 35(5): 521–31.
3. *Almashraki N, Abdulnabee MZ, Sukalo M, Alrajoudi A, Sharafadeen I, Zenker M*. Johanson-Blizzard syndrome. World J Gastroenterol 2011; 17(37): 4247–50.
4. *Atik T, Karakoyun M, Sukalo M, Zenker M, Ozkinay F, Aydoğdu S*. Two novel UBR1 gene mutations ın a patient with Johanson Blizzard Syndrome: A mild phenotype without mental retardation. Gene 2015; 570(1): 153–5.
5. *Alkhouri N, Kaplan B, Kay M, Shealy A, Crowe C, Bauhuber S,* et al. Johanson-Blizzard syndrome with mild phenotypic features confirmed by UBR1 gene testing. World J Gastroenterol 2008; 14(44): 6863–6.
6. *Rezaei N, Sabbaghian M, Liu Z, Zenker M*. Eponym: Johanson-Blizzard syndrome. Eur J Pediatr 2011; 170(2): 179–83.
7. *Demir D, Kendir Demirkol Y, Gerenli N, Aktaş Karabay E*. Johanson-Blizzard's Syndrome with a Novel UBR1 Mutation. J Pediatr Genet 2020; 11(2): 147–50.
8. *Liu S, Wang Z, Jiang J, Luo X, Hong Q, Zhang Y*, et al. Severe forms of Johanson-Blizzard syndrome caused by two novel compound heterozygous variants in UBR1: Clinical manifestations, imaging findings and molecular genetics. Pancreatology 2020; 20(3): 562–8.
9. *Lee JK, Enns R*. Review of idiopathic pancreatitis. World J Gastroenterol 2007; 13(47): 6296–313.
10. *Kunovský L, Dítě P, Jabandžiev P, Eid M, Poredská K, Vaculová J,* et al. Causes of Exocrine Pancreatic Insufficiency Other Than Chronic Pancreatitis. J Clin Med 2021; 10(24): 5779.
11. *Zenker M, Mayerle J, Lerch MM, Tagariello A, Zerres K, Durie PR,* et al. Deficiency of UBR1, a ubiquitin ligase of the N-end rule pathway, causes pancreatic dysfunction, malformations and mental retardation (Johanson-Blizzard syndrome). Nat Genet 2005; 37(12): 1345–50.
12. *Ellery KM, Erdman SH*. Johanson-Blizzard syndrome: expanding the phenotype of exocrine pancreatic insufficiency. JOP 2014; 15(4): 388–90.
13. *Sukalo M, Schäflein E, Schanze I, Everman DB, Rezaei N, Argente J,* et al. Expanding the mutational spectrum in Johanson-Blizzard syndrome: identification of whole exon deletions and duplications in the UBR1 gene by multiplex ligation-dependent probe amplification analysis. Mol Genet Genomic Med 2017; 5(6): 774–80.

Received on August 3, 2022
Revised on January 8, 2022
Accepted January 10, 2023
Online First January 2023

BOOK REVIEW/PRIKAZ KNJIGE



Diseases of the Ear

Title: Diseases of the Ear
Original title: Bolesti uva (Serbian)
Author/Autor: Dragoslava Đerić
Publisher/Izdavač: Akademska misao, Beograd
Year/Godina izdanja: 2022.
ISBN: 978-86-7466-933-4

The book *Diseases of the Ear* is written by Professor Dragoslava Đerić, otorhinolaryngologist, full professor at the Department of Otorhinolaryngology, as part of regular and post-graduate studies at the University of Belgrade Faculty of Medicine.

Diseases of the ear are common problems in daily clinical practice and are still the cause of significant complications in the structure of mortality in our environment. The field of otology is connected with many other branches of medicine in different aspects (etiological, clinical, diagnostic, and therapeutic); hence, this book represents a significant contribution to the education of physicians of various specialties. The textbook deals with the problems of the most common otological diseases, covered in 14 chapters.

The first chapter refers to the morphology of the ear and is based on the original research work of Professor Đerić, who published her results in many prestigious peer-reviewed international and domestic journals. The results of that work were a useful basis within the ear microsurgery school in Serbia, elaborated through the temporal bone dissection courses, with an emphasis on the anatomical aspect of the applications of otosurgical methods.

The second chapter refers to the most common pathological conditions of the external ear, and the following chapters include problems of middle and inner ear diseases, such as congenital malformations, injuries, inflammatory diseases and their complications, cholesteatoma, otosclerosis, and benign and malignant tumors.

Special attention is paid to new findings in the field of etiopathogenesis, clinical manifestations, modern diagnostics, and new concepts in ear microsurgery. Novelties in the classification of diseases and terminology in the notification of certain entities are presented. Particularly significant otological problems are highlighted by presenting cases from clinical practice.

Knjigu „Bolesti uva“ napisala je prof. dr Dragoslava Đerić, otorinolaringolog, redovni profesor na Katedri za otorinolaringologiju u okviru redovne i poslediplomske nastave na Medicinskom fakultetu Univerziteta u Beogradu.

Bolesti uva su čest problem u svakodnevnoj kliničkoj praksi i još uvek su uzrok komplikacija koje zauzimaju značajno mesto u strukturi mortaliteta u našoj sredini. Oblast otologije povezana je sa mnogim drugim granama medicine u različitim aspektima (etiološki, klinički, dijagnostički i terapijski), tako da ova knjiga predstavlja značajan doprinos u edukaciji lekara različitih specijalnosti. Knjiga se bavi problemima najčešćih otoloških oboljenja, koji su obrađeni u 14 poglavlja.

Prvo poglavlje odnosi se na morfologiju uva, a bazirano je na orginalnom istraživačkom radu profesorke Đerić, koja je svoje rezultate objavila u brojnim prestižim recenziranim međunarodnim i domaćim časopisima. Rezultati tog rada bili su korisna osnova u okviru škole mikrohirurgije uva u Srbiji, razrađene kroz kurseve disekcije temporalne kosti, sa akcentom na anatomsku osnovu primene otohirurških metoda.

Drugo poglavlje odnosi se na najčešća patološka stanja spoljašnjeg uva, a sledeća poglavlja obuhvataju probleme bolesti srednjeg i unutrašnjeg uva poput kongenitalnih malformacija, povreda, zapaljenjskih procesa i njihovih komplikacija, holesteatoma, otoskleroze, kao i benignih i malignih tumora.

Posebna pažnja posvećena je novim saznanjima na polju etiopatogeneze, kliničkih manifestacija, savremene dijagnostike i novog koncepta u mikrohirurgiji uva. Prikazane su novine u klasifikaciji oboljenja i terminologiji pri označavanju određenih entiteta. Posebno značajni otološki problemi istaknuti su prikazom slučajeva iz kliničke prakse.

In the chapter dedicated to facial nerve palsy, all the details related to intratemporal nerve disorders, from multifactorial etiopathogenesis to therapy, are listed.

The chapter “Otological Manifestations of Systemic Diseases” contains an overview of the connection between systemic disorders and pathological processes in the ear region. This topic, based on new knowledge related to the etiology and pathogenesis of systemic diseases, as well as the characteristics of their otological manifestations, gives special importance to technological achievements in the field of diagnostics and treatment of these diseases and indicates the importance of a multidisciplinary approach.

The author’s style is recognizable, and the content of the textbook is the result of decades of work by Professor Đerić, who was guided by contemporary knowledge and trends in otology and medicine in general during the preparation of the manuscript. A significant source of information is the extensively cited literature with over 600 bibliographic items.

The book “Diseases of the Ear", according to the topic, is intended for specialists in otorhinolaryngology, primarily focused on the field of otology and otosurgery, but it can also be of great use to doctors of other specialties. I recommend this book as a very valuable contribution to the medical literature published in the Serbian language, in line with other highly valued works in this field.

U poglavlju posvećenom paralizama facijalnog živca navedeni su svi detalji vezani za intratemporalne poremećaje živca, od multifaktorijalne etiopatogeneze do terapije.

Poglavlje „Otološke manifestacije sistemskih oboljenja“ sadrži pregledan prikaz povezanosti sistemskih poremećaja i patoloških procesa u regionu uva. Ova tema, bazirana na novim saznanjima vezanim za etiologiju i patogenezu sistemskih oboljenja, kao i karakteristikama njihovih otoloških manifestacija daje poseban značaj tehnološkim dostignućima u oblasti dijagnostike i lečenja ovih bolesti i ukazuje na značaj multidisciplinarnog pristupa.

Stil autora je prepoznatljiv, a sadržaj knjige je rezultat višedecenijskog rada profesorke Đerić, koja se tokom pripremanja rukopisa rukovodila savremenim saznanjima i trendovima u otologiji i medicini uopšte. Značajan izvor informacija čini obimno citirana literatura sa preko 600 bibliografskih jedinica.

Knjiga „Bolesti uva“, prema tematici, namenjena je specijalistima otorinolaringologije, prevashodno usmerenim na oblast otologije i otohirurgije, ali od velike koristi može biti i lekarima drugih specijalnosti. Ovu knjigu preporučujem kao veoma dragocen doprinos medicinskoj literaturi objavljenoj na srpskom jeziku, u ravni sa drugim visoko vrednovanim delima iz ove oblasti.

Professor Aleksandar Perić, MD, PhD
University of Defence, Faculty of Medicine of the Military Medical Academy, Belgrade, Serbia
Military Medical Academy, Department of Otorhinolaryngology, Belgrade, Serbia
E-mail: aleksandarperic1971@gmail.com

C O R R I G E N D U M

DOI: https://doi.org/10.2298/VSP2310892E

In the original article titled “Prescription patterns of diclofenac in patients with cardiovascular diseases or at high risk for cardiovascular diseases at primary health care level in Montenegro: retrospective, national, drug utilization study” published in the September 2023 issue of the Vojnosanitetski Pregled [1], the heading for the third, right-hand column of data in Table 1 should have been given as “Patients with CVD/risk for CVD on diclofenac therapy, n (%)” instead of “Patients with CVD on diclofenac therapy, n (%)”.

1. *Stanković M, Turković N, Dobrić S, Rančić N.* Prescription patterns of diclofenac in patients with cardiovascular diseases or at high risk for cardiovascular diseases at primary health care level in Montenegro: retrospective, national, drug utilization study. Vojnosanit Pregl 2023; 80(9): 778–88.
(DOI: https://doi.org/10.2298/VSP221229021S)

INSTRUCTIONS TO THE AUTHORS

The VSP publishes only papers not published before, nor submitted to any other journals, in the order determined by the Editorial Board. Any attempted plagiarism or self-plagiarism will be punished. When submitting a paper to the VSP electronic editing system **(http://aseestant.ceon.rs/index.php)**, the following should be enclosed: a statement on meeting any technical requirements, a statement signed by all the authors that the paper on the whole and/or partly has not been submitted nor accepted for publication elsewhere, a statement specifying the actual contribution of each author, no conflict of interest statement that make them responsible for meeting any requirements set. What follows subsequently is the acceptance of a paper for further editing procedure. The manuscripts submitted to the VSP pass in-house and external peer review. All authors pay "Article Processing Charge" for coverage all editing and publishing expenses. Domestic authors pay 5,000 RSD, and those from aboard 150 euros. The editing and publishing fee is required for substantive editing, facts and references validations, copy editing, and publishing online and in print by editorial staff of the Journal. No additional fees, other than stated above, are required even if an author who already paid the fee would have more articles accepted for publishing in the year when fee was paid. All authors who pay this fee may, if want, receive printed version of the Journal in year when fee is payed. Please note that the payment of this charge does not guarantee acceptance of the manuscript for publication and does not influence the outcome of the review procedure. The requirement about paying "Article Processing Charge" does not apply to reviewers, members of the Editorial Board and the Publisher's Council of the Journal, young researchers and students, as well as any of the subscribers of the Journal.

The VSP publishes: **editorials, original articles**, **short communications**, **reviews**/**meta-analyses, case reports**, **medical history** (general or military), personal views, invited comments, letters to the editor, reports from scientific meetings, book reviews, and other. Original articles, short communications, meta-analyses and case reports are published with abstracts in both English and Serbian.

General review papers will be accepted by the Editorial Board only if the authors prove themselves as the experts in the fields they write on by citing not less than 5 self-citations.

Papers should be written on IBM-compatible PC, using 12 pt font, and double spacing, with at least 4 cm left margin. **Bold** and *italic* letters should be avoided as reserved for subtitles. Original articles, reviews, meta-analyses and articles from medical history should not exceed 16 pages; current topics 10; case reports 6; short communications 5; letters to the editor and comments 3, and reports on scientific meetings and book reviews 2.

All measurements should be reported in the metric system of the International System of Units (SI), and the standard internationally accepted terms (except for mmHg and °C).

MS Word for Windows (97, 2000, XP, 2003) is recommended for word processing; other programs are to be used only exceptionally. Illustrations should be made using standard **Windows** programs, **Microsoft Office** (**Excel**, **Word Graph**)**.** The use of colors and shading in graphs should be avoided.

Papers should be prepared in accordance with the **Vancouver Convention**.

Papers are reviewed anonymously by at least two editors and/or invited reviewers. Remarks and suggestions are sent to the author for final composition. Galley proofs are sent to the corresponding author for final agreement.

Preparation of manuscript

Parts of the manuscript are: **Title page; Abstract with Key words; Text; Acknowledgements** (to the authors' desire), **References**, **Enclosures**.

1. ***Title page***

a) The title should be concise but informative, while subheadings should be avoided;

b) Full names of the authors signed as follows: ***, †, ‡, §, ‖, ¶, **, ††, ...** .

c) Exact names and places of department(s) and institution(s) of affiliation where the studies were performed, city and the state for any authors, clearly marked by standard footnote signs;

d) Conclusion could be a separate chapter or the last paragraph of the discussion;

e) Data on the corresponding author.

2. ***Abstract and key words***

The second page should carry a structured abstract (250-300 words for original articles and meta-analyses) with the title of the article. In short, clear sentences the authors should write the **Background**/**Aim**, major procedures – **Methods** (choice of subjects or laboratory animals; methods for observation and analysis), the obtained findings – **Results** (concrete data and their statistical significance), and the **Conclusion**. It should emphasize new and important aspects of the study or observations. A structured abstract for case reports (up to 250 words) should contain subtitles **Introduction, Case report, Conclusion**). Below the abstract **Key words** should provide 3–10 key words or short phrases that indicate the topic of the article.

3. ***Text***

The text of the articles includes: **Introduction**, **Methods**, **Results**, and **Discussion**. Long articles may need subheadings within some sections to clarify their content.

Introduction. After the introductory notes, the aim of the article should be stated in brief (the reasons for the study or observation), only significant data from the literature, but not extensive, detailed consideratuion of the subject, nor data or conclusions from the work being reported.

Methods. The selection of study or experimental subjects (patients or experimental animals, including controls) should be clearly described. The methods, apparatus (manufacturer's name and address in parentheses), and procedures should be identified in sufficient detail to allow other workers to reproduce the results. Also, give references to established methods, including statistical methods. Identify precisely all drugs and chemicals used, with generic name(s), dose(s), and route(s) of administration. State the approval of the Ethnics Committee for the tests in humans and animals.

Results should be presented in logical sequence in the text, tables and illustrations. Emphasize or summarize only important observations.

Discussion is to emphasize the new and significant aspects of the study and the conclusions that result from them. Relate the observations to other relevant studies. Link the conclusions with the goals of the study, but avoid unqualified statements and conclusions not completely supported by your data.

References

References should be superscripted and numerated consecutively in the order of their first mentioning within the text. All the authors should be listed, but if there are more than 6 authors, give the first 6 followed by *et al*. Do not use abstracts, secondary publications, oral communications, unpublished papers, official and classified documents. References to papers accepted but not yet published should be cited as "in press". Information from manuscripts not yet accepted should be cited as "unpublished data". Data from the Internet are cited with the date of citation.

Examples of references:

Jurhar-Pavlova M, Petlichkovski A, TrajkovD, Efinska-Mladenovska O, Arsov T, Strezova A, et al. Influence of the elevated ambient temperature on immunoglobulin G and immunoglobulin G subclasses in sera of Wistar rats. Vojnosanit Pregl 2003; 60(6): 657–612.

DiMaio VJ. Forensic Pathology. 2nd ed. Boca Raton: CRC Press; 2001.

Blinder MA. Anemia and Transfusion Therapy. In: Ahya NS, Flood K, Paranjothi S, editors. The Washington Manual of Medical Therapeutics, 30th edition. Boston: Lippincot, Williams and Wilkins; 2001. p. 413-28.

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.

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

Tables

Each table should be typed double-spaced 1,5 on a separate sheet, numbered in the order of their first citation in the text in the upper left corner and supplied with a brief title each. Explanatory notes are printed under a table. Each table should be mentioned in the text. If data from another source are used, acknowledge fully.

Illustrations

Any forms of graphic enclosures are considered to bi figures and should be submitted as additional databases in the System of Assistent. Letters, numbers, and symbols should be clear and uniform, of sufficient size that when reduced for publication, each item will still be legible. Each figure should have a label on its back indicating the number of the figure, author's name, and top of the figure (**Figure 1**, **Figure 2** and so on). If a figure has been published, state the original source.

Legends for illustrations are typed on a separate page, with Arabic numbers corresponding to the illustrations. If used to identify parts of the illustrations, the symbols, arrows, numbers, or letters should be identified and explained clearly in the legend. Explain the method of staining in photomicrographs.

Abbreviations and acronyms

Authors are encouraged to use abbreviations and acronyms in the manuscript in the following manner: abbreviations and acronyms must be defined the first time they are used in the text consistently throughout the whole manuscript, tables, and graphics; abbreviations should be used only for terms that appear more than three times in text; abbreviations should be sparingly used.

An alphabetical list of all abbreviations used in the paper, followed by their full definitions, should be provided on submission.

Detailed Instructions are available at the web site:

www.vma.mod.gov.rs/vsp

UPUTSTVO AUTORIMA

VSP objavljuje radove koji nisu ranije nigde objavljivani, niti predati za objavljivanje redosledom koji određuje uređivački odbor. Svaki pokušaj plagijarizma ili autoplagijarizma kažnjava se. Prilikom prijave rada u sistem elektronskog uređivanja „Vojnosanitetskog pregleda“**(http://aseestant.ceon.rs/index.php)** neophodno je priložiti izjavu da su ispunjeni svi postavljeni tehnički zahtevi uključujući i izjavu koju potpisuju svi autori da rad nije ranije ni u celini, niti delimično objavljen niti prihvaćen za štampanje u drugom časopisu. Izjavu o pojedinačnom doprinosu svakog od autora rada potpisanu od svih autora, treba skenirati i poslati uz rad kao dopunsku datoteku. Takođe, autori su obavezni da dostave i potpisanu izjavu o nepostojanju sukoba interesa čime postaju odgovorni za ispunjavanje svih postavljenih uslova. Ovome sledi odluka o prihvatanju za dalji uređivački postupak. Rukopisi pristigli u Redakciju časopisa podležu internoj i eksternoj recenziji. Svi autori dužni su da plate “Article Processing Charge” za pokriće troškova jezičke, stručne i tehničke obrade rukopisa, kao i njegovog objavljivanja. Domaći autori plaćaju iznos od 5 000 dinara, a inostrani 150 eura. Dodatna plaćanja nisu predviđena čak i u slučaju da autor koji je već prethodno platio traženi iznos, ima više prihvaćenih radova za objavljivanje u godini u kojoj je izvršio uplatu. Svi autori koji su platili “Article Processing Charge” mogu, ukoliko žele, dobijati štampanu verziju časopisa tokom godine u kojoj je izvršena uplata. Plaćanje ovog iznosa ne garantuje prihvatanje rukopisa za objavljivanje i ne utiče na ishod recenzije. Od obaveze plaćanja pokrića navedenih troškova oslobođeni su recenzenti, članovi Uređivačkog odbora i Izdavačkog saveta VSP, studenti i mladi istraživači, kao i pretplatnici časopisa.

U VSP-u se objavljuju **uvodnici**, **originalni članci, prethodna** ili **kratka saopštenja**, revijski radovi tipa **opšteg pregleda** (uz uslov da autori navođenjem najmanje 5 autocitata potvrde da su eksperti u oblasti o kojoj pišu), **aktuelne teme, metaanalize**, **kazuistika, seminar praktičnog lekara,** članci iz **istorije medicine**, lični stavovi, naručeni komentari, pisma uredništvu, izveštaji sa naučnih i stručnih skupova, prikazi knjiga i drugi prilozi. Radovi tipa originalnih članaka, prethodnih ili kratkih saopštenja, metaanalize i kazuistike **objavljuju se uz apstrakte na srpskom i engleskom jeziku**.

Rukopis se piše sa proredom 1,5 sa levom marginom od **4 cm**. Koristiti font veličine 12, a načelno izbegavati upotrebu **bold** i *italic* slova, koja su rezervisana za podnaslove. Originalni članci, opšti pregledi i metaanalize i članci iz istorije medicine ne smeju prelaziti 16 stranica (bez priloga); aktuelne teme – deset, seminar praktičnog lekara – osam, kazuistika – šest, prethodna saopštenja – pet, a komentari i pisma uredniku – tri, izveštaji sa skupova i prikazi knjiga – dve stranice.

U celom radu obavezno je korišćenje međunarodnog sistema mera (SI) i standardnih međunarodno prihvaćenih termina (sem mm Hg i °C).

Za obradu teksta koristiti program **Word for Windows** verzije 97, 2000, XP ili 2003. Za izradu grafičkih priloga koristiti standardne grafičke programe za **Windows**, poželjno iz programskog paketa **Microsoft Office** (**Excel, Word Graph**). Kod kompjuterske izrade grafika izbegavati upotrebu boja i senčenja pozadine.

Radovi se pripremaju u skladu sa **Vankuverskim dogovorom**.

Prispeli radovi kao anonimni podležu uređivačkoj obradi i recenziji najmanje dva urednika/recenzenta. Primedbe i sugestije urednika/recenzenata dostavljaju se autoru radi konačnog oblikovanja. Pre objave, rad se upućuje autoru određenom za korespodenciju na konačnu saglasnost.

Priprema rada

Delovi rada su: **naslovna strana, apstrakt sa ključnim rečima, tekst** rada, zahvalnost (po želji), literatura, prilozi.

1. ***Naslovna strana***

a) Poželjno je da naslov bude kratak, jasan i informativan i da odgovara sadržaju, podnaslove izbegavati.

b) Ispisuju se puna imena i prezimena autora sa oznakama redom: *, †, ‡, §, ‖, ¶, **, ††, ...

c) Navode se puni nazivi ustanove i organizacijske jedinice u kojima je rad obavljen mesta i države za svakog autora, koristeći standardne znake za fusnote.

d) Zaključak može da bude posebno poglavlje ili se iznosi u poslednjem pasusu diskusije.

e) Podaci o autoru za korespodenciju.

2. ***Apstrakt i ključne reči***

Na drugoj stranici nalazi se strukturisani apstrakt (250-300 reči za originalne članke i meta-analize) sa naslovom rada. Kratkim rečenicama na srpskom i engleskom jeziku iznosi se **Uvod/Cilj** rada, osnovne procedure **– Metode** (izbor ispitanika ili laboratorijskih životinja; metode posmatranja i analize), glavni nalazi **– Rezultati** (konkretni podaci i njihova statistička značajnost) i glavni **Zaključak**. Naglasiti nove i značajne aspekte studije ili zapažanja. Strukturisani apstrakt za kazuistiku (do 250 reči), sadrži podnaslove **Uvod, Prikaz bolesnika** i **Zaključak**). Ispod apstrakta, „Ključne reči“ sadrže 3–10 ključnih reči ili kratkih izraza koje ukazuju na sadržinu članka.

3. ***Tekst članka***

Tekst sadrži sledeća poglavlja: **uvod**, **metode**, **rezultate** i **diskusiju**. **Uvod.** Posle uvodnih napomena, navesti cilj rada. Ukratko izneti razloge za studiju ili posmatranje. Navesti samo važne podatke iz literature a ne opširna razmatranja o predmetu rada, kao ni podatke ili zaključke iz rada o kome se izveštava.

Metode. Jasno opisati izbor metoda posmatranja ili eksperimentnih metoda (ispitanici ili eksperimentne životinje, uključujući kontrolne). Identifikovati metode, aparaturu (ime i adresa proizvođača u zagradi) i proceduru, dovoljno detaljno da se drugim autorima omogući reprodukcija rezultata. Navesti podatke iz literature za uhodane metode, uključujući i statističke. Tačno identifikovati sve primenjene lekove i hemikalije, uključujući generičko ime, doze i načine davanja. Za ispitivanja na ljudima i životinjama navesti saglasnost nadležnog etičkog komiteta.

Rezultate prikazati logičkim redosledom u tekstu, tabelama i ilustracijama. U tekstu naglasiti ili sumirati samo značajna zapažanja.

U **diskusiji** naglasiti nove i značajne aspekte studije i izvedene zaključke. Posmatranja dovesti u vezu sa drugim relevantnim studijama, u načelu iz poslednje tri godine, a samo izuzetno i starijim. Povezati zaključke sa ciljevima rada, ali izbegavati nesumnjive tvrdnje i one zaključke koje podaci iz rada ne podržavaju u potpunosti.

Literatura

U radu literatura se citira kao superskript, a popisuje rednim brojevima pod kojima se citat pojavljuje u tekstu. Navode se svi autori, ali ako broj prelazi šest, navodi se prvih šest i *et al*. Svi podaci o citiranoj literaturi moraju biti tačni. Literatura se u celini citira na engleskom jeziku, a iza naslova se navodi jezik članka u zagradi. Ne prihvata se citiranje apstrakata, sekundarnih publikacija, usmenih saopštenja, neobjavljenih radova, službenih i poverljivih dokumenata. Radovi koji su prihvaćeni za štampu, ali još nisu objavljeni, navode se uz dodatak „u štampi“. Rukopisi koji su predati, ali još nisu prihvaćeni za štampu, u tekstu se citiraju kao „neobjavljeni podaci“ (u zagradi). Podaci sa interneta citiraju se uz navođenje datuma pristupa tim podacima.

Primeri referenci:

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

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

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

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.

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

Tabele

Sve tabele pripremaju se sa proredom 1,5 na posebnom listu. Obeležavaju se arapskim brojevima, redosledom pojavljivanja, u levom uglu (**Tabela 1**), a svakoj se daje kratak naslov. Objašnjenja se daju u fus-noti, ne u zaglavlju. Svaka tabela mora da se pomene u tekstu. Ako se koriste tuđi podaci, obavezno ih navesti kao i svaki drugi podatak iz literature.

Ilustracije

Slikama se zovu svi oblici grafičkih priloga i predaju se kao dopunske datoteke u sistemu **aseestant**. Slova, brojevi i simboli treba da su jasni i ujednačeni, a dovoljne veličine da prilikom umanjivanja budu čitljivi. Slike treba da budu jasne i obeležene brojevima, onim redom kojim se navode u tekstu (**Sl. 1; Sl. 2** itd.). Ukoliko je slika već negde objavljena, obavezno citirati izvor.

Legende za ilustracije pisati na posebnom listu, koristeći arapske brojeve. Ukoliko se koriste simboli, strelice, brojevi ili slova za objašnjavanje pojedinog dela ilustracije, svaki pojedinačno treba objasniti u legendi. Za fotomikrografije navesti metod bojenja i podatak o uvećanju.

Skraćenice i akronimi

Skraćenice i akronimi u rukopisu treba da budu korišćeni na sledeći način: definisati skraćenice i akronime pri njihovom prvom pojavljivanju u tekstu i koristiti ih konzistentno kroz čitav tekst, tabele i slike; koristiti ih samo za termine koji se pominju više od tri puta u tekstu; da bi se olakšalo čitaocu, skraćenice i aktinome treba štedljivo koristiti.

Abecedni popis svih skraćenica i akronima sa objašnjenjima treba dostaviti pri predaji rukopisa.

Detaljno uputstvo može se dobiti u redakciji ili na sajtu: www.vma.mod.gov.rs/vsp