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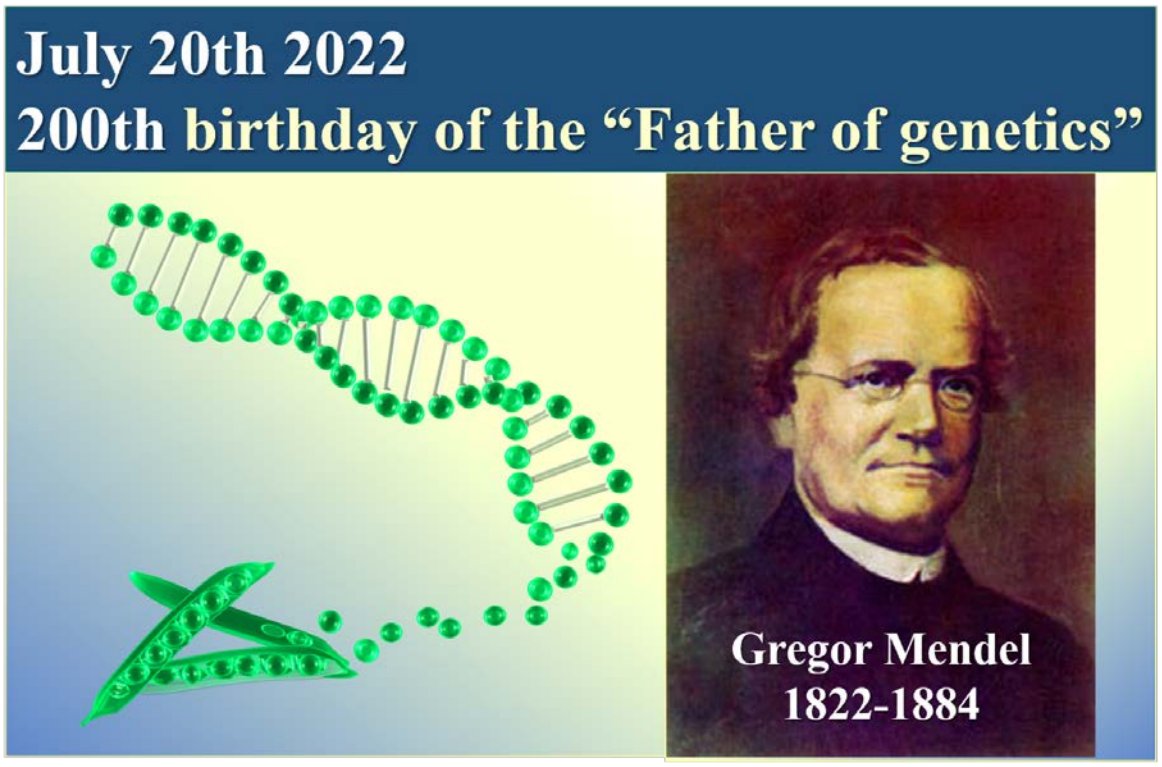

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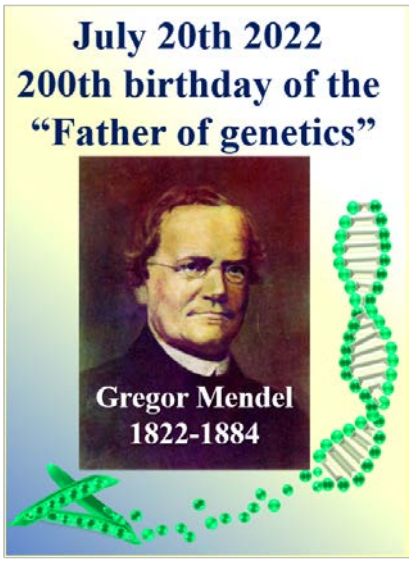

July 20th 2022 – 200th birthday of the "Father of Genetics" – Johann Gregor Mendel (1822–1884).

Mendel discovered the fundamental laws of inheritance while experimenting with garden pea (*Pisum sativum*) long before we understood the structure of DNA and genes. Although unrecognized for his scientific achievements during his life, Mendel's pioneered work shaped the field of genetics and led to advances in biology and medicine.

20. jul 2022. – 200. rodjendan "oca genetike" – Johana Gregora Mendela (1822–1884).

Mendel je otkrio fundamentalne zakone nasleđivanja zahvaljujući eksperimentisanju sa zrnima graška (*Pisum sativum*) mnogo pre nego što smo razumeli strukturu DNK i gena. Iako njegov naučni doprinos nije bio prepoznat tokom njegovog života, pionirski rad Mendela je oblikovao polje genetike i doveo do napretka u biologiji i medicini.

GENERAL REVIEW
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Radiation-induced tumors and secondary malignancies following radiotherapy

Tumori indukovani zračenjem i sekundarni maligniteti posle radioterapije

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Key words:
genetics; neoplasms metastasis; neoplasms, radiation-induced; radiotherapy; risk assessment; survival.

Ključne reči:
genetika; neoplazme, metastaze; neoplazme, radijacijom uzrokovane; radioterapija; rizik, procena; preživljavanje.

Introduction

Radiotherapy (RT) is an integral part of multidisciplinary cancer management and is indicated by evidence-based guidelines in more than 50% of all cancer patients [1]. State-of-the-art RT techniques such as intensity-modulated radiotherapy (IMRT), image-guided RT, and proton therapy have decreased the risk of cancer recurrences, improved target dose coverage with dose escalation, reduced treatment toxicities, and improved survival.

Potentially adverse effects of these treatments can reduce the quality of life and lead to morbidity and even mortality in cancer survivors. Regarding RT treatment, toxic effects are generally divided into early and late effects. Early toxic effects of radiation on healthy tissue are reversible and develop due to acute inflammation, whereas late adverse effects mostly remain permanent and are caused by chronic inflammation, microvascular damage, fibrosis, and radiation-induced genetic instability [2].

Long-term cancer survivors treated with RT treatment are at greater risk of developing late effects, including the development of radiation-induced malignancy (RIM) [2]. In 1948, Cahan et al. [3] defined a radiation-induced sarcoma, while nowadays, in practice, modified Cahan's criteria for the definition of RIM are used [4]. These criteria include that RIM must arise within the treatment field, with a significant latent period, and have different histology than primary malignancy (moreover, the origin of tissue of RIM must be metabolically and genetically normal before irradiation).

Cancer patients are at higher risk of developing a second malignancy (SM) compared to the general population. However, subsequent neoplasms may not be associated with prior cancer treatment, and RIMs make only a small proportion of SMs [5].

The risk of developing RIM after RT treatment varies on multiple factors, such as patients' age at the time of radiation, genetic susceptibility, patients' family history of cancer, lifestyle and environmental factors, the organ and tissue site receiving radiation, RT treatment modality, and dosimetric characteristics of the RT plan [2, 6].

Children are considered 10 times more sensitive to the carcinogenic effect of radiation than adults. Several studies found that pediatric cancer patients who underwent RT have a greater risk of developing RIM than adults [7, 8]. These malignancies may lead to a decrease in the overall survival after the treatment of primary cancer [2, 9].

When assessing the risk of developing RIM regarding gender, studies have shown that females are at greater risk compared to males [10]. Unfortunately, the irradiation of the breast tissue during RT treatment in Hodgkin's lymphoma is well known as a risk factor in inducing breast cancer. Previously published studies have reported that menopausal and ovarian function status in correlation with age in female patients affects the risk of developing RIM in cancer survivors treated with chest RT for Hodgkin's lymphoma. Namely, early menopause, as well as ovarian dysfunction at a younger age, may reduce the risk of breast cancer as a RIM [11].

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The dose-response relationship for radiation carcinogenesis as well as long-term effects of radiation on the development of RIM in humans are explored in Japanese atomic bomb survivors, in whom leukemia was initially diagnosed with a latent period of 5–10 years, and afterward, solid tumors with a latent period of 10–60 years (Figure 1). The latent period for developing RIM in irradiated patients is reported to be similar to that in Japanese atomic bomb survivors, and the risk continues to increase with decades gained after the exposure [7, 12].

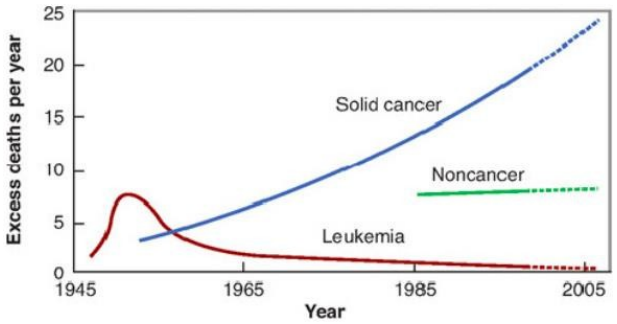

Fig. 1 – Time course of second malignant neoplasm development following A-bomb explosion in 1945. Leukemia appeared first, followed by solid cancers several years later. There was also an excess of noncancer deaths from stroke and heart disease by the late 1980s [9].

RT patients receive a high dose of radiation at a low volume and significantly lower doses at larger volumes. However, RIMs can arise from the high-dose irradiated tissues, as well as from the low-dose irradiated tissues, e.g., organs that are distant from the radiation field [13].

Cancer patients are often treated with combined treatment modalities, so it may be difficult to define the specific effect of a particular agent. Exposure to chemotherapeutic agents may be associated with an increased risk of SM neoplasms, such as anthracyclines and alkylating agents with sarcoma [14], alkylating agents with carcinomas [15], and cisplatin-based therapy with solid tumors after testicular nonseminomas [16]. Treatment-related myeloid neoplasms, including therapy-related acute myeloid leukemia and therapy-related myelodysplastic syndrome, may be linked with exposure to alkylating agents, as well as topoisomerase (TOP) II inhibitors [17].

The most frequent malignancies associated with RIMs

For the adult population, clinical data on RIM development are best reported for breast and prostate cancer due to the high rate of long-term survival. To assess the incidence of developing RIMs, an appropriate control group should be available, which is often difficult to provide; notable exceptions are prostate and cervical cancer, where patients treated with surgery provide control groups.

RT is an essential adjuvant part of breast cancer treatment, which reduces disease recurrence and improves overall survival. However, RT can also be associated with an increased risk of second cancer in exposed sites.

Radiation-induced sarcoma is a rare complication of breast irradiation with an increased risk of appearance over time after RT [18, 19]. According to Salminen et al. [18], the most common site of radiation-induced sarcoma was breast soft tissue (Figure 2), while the prevalent histological subtype was angiosarcoma.

Fig. 2 – The typical appearances of radiation-induced angiosarcoma of the breast [19].

According to the large meta-analyses conducted by Grantzau and Overgaard [20], RT treatment of breast cancer has significantly increased the risk of non-breast SMs with a relative risk (RR) of 1.22. The risk remained elevated, even 5 years after diagnosis, with a RR of 1.12. The most common SM sites were lung and esophageal cancers and soft tissue sarcoma. The estimated RRs for these sites were 1.23, 1.17, and 2.41, respectively. After a latency time of at least five years from breast cancer diagnosis, the incidence of SM gradually increased. A significant association between RT of breast cancer and second thyroid cancer was not found.

Berrington de Gonzalez et al. [21] estimated the long-term cancer risk of all solid cancers in a large cohort of patients after breast cancer RT. In the study, SM sites were divided into three dose groups (high: 1+ Gy; medium: 0.5–0.99 Gy; and low: 0.5 Gy; dose sites) according to the mean organ dose from the RT treatment. Estimated RRs were increased for the group of sites that received the highest radiation exposure (1+ Gy: lung, esophagus, pleura, bone, and soft tissue; ~1 Gy: contralateral breast cancer), while for lower dose sites, RRs were not elevated. They even found that most of the solid SMs were also related to other risk factors such as lifestyle and genetic factors.

Regarding secondary sarcomas, both studies also showed that RRs were especially highly elevated for angiosarcomas.

In the study by Mladenovic et al. [22], tumor responses and long-term outcomes were analyzed in 134 patients with

non-inflammatory locally advanced breast cancer treated with preoperative RT. Their results of pathological complete tumor response to preoperative RT were in agreement with similar previously conducted trials. The occurrence of SM was detected as breast cancer in the contralateral breast in two patients and papillary thyroid cancer in one patient.

RIMs are reported in long-term survivors of prostate cancer. Fontenot et al. [23] estimated that proton therapy reduced the risk of RIM by 26% compared to 39% with contemporary IMRT in prostate cancer patients. When comparing the risk of developing a SM treated with RT vs. surgery, Brenner et al. [24] published that RT significantly increased the risk of SMs by about 6% (p = 0.02). For patients who survived for ≥ 5 and ≥ 10 years, the increased RR was 15% and 34%, respectively. The vast majority of second solid cancer sites were bladder, rectum, and lung cancers, as well as sarcomas within the treatment field, while no significant increase in rates of leukemia was noted.

Chaturvedi et al. [25] reported that irradiated cervical cancer patients were at increased risk of SMs even after 40 years of follow-up compared to the general population. The risk of SMs was increased at sites close to the cervix, including anal, colorectal, and genitourinary sites.

Rodriguez et al. [26] analyzed the risk of developing colorectal cancer among long-term survivors of cervical cancer who received RT. Results of the study implied that RIM of the colon and rectum might occur 8 years after RT for cervical cancer. Furthermore, these patients treated with radiation at a young age should start screening for colorectal cancer earlier than the age recommended for low-risk individuals (approximately 8 years after the treatment).

Post-Operative Radiation Therapy in Endometrial Carcinoma (PORTEC)-1 trial, in which patients were randomly assigned into irradiated (postoperative external beam RT) and observational groups, has shown that after 15 years of follow-up, 22% of patients were diagnosed with second primary cancer in the RT group vs. 16% in the observational group. The most common cancer type in irradiated patients was gastrointestinal cancer [27].

Many studies have published an increased incidence of SMs in patients treated with RT for Hodgkin's and non-Hodgkin lymphoma. Factors contributing to the higher incidence of RIMs in Hodgkin's lymphoma are the following: relatively young patients, high curability, large irradiation field, the technique used in past decades, and combined therapeutic modalities, including cytotoxic drugs. The majority of SMs were thyroid, breast, lung, and stomach cancer, as well as sarcoma [28]. Moreover, RT increases the risk of developing both solid tumors and leukemia for non-Hodgkin lymphoma survivors [29].

With an increasing number of long-term cancer survivors of childhood malignancy, the occurrence of the second SMs has risen. Primary cancer treatments, including RT and chemotherapy, are associated with the risk of second malignant neoplasm (SMN) after primary childhood cancer.

The Childhood Cancer Survivor Study (CCSS) analyzed the incidence and risk factors of subsequent neoplasms after treating childhood cancer. It was reported that the cumulative incidence of all subsequent neoplasms was 20.5% thirty years after diagnosis, whereas radiation exposure was associated with an increased risk of SMs. Regarding subsequent neoplasm subtype, the 30-year cumulative incidences were 7.9%, 9.1%, and 3.1% for SMNs (excluding non-melanoma skin cancer), non-melanoma skin cancer, and meningiomas, respectively (Figure 3). The most commonly diagnosed SMs were bone, thyroid, head and neck cancer, breast cancer, central nervous system malignancies, and soft tissue sarcoma [30].

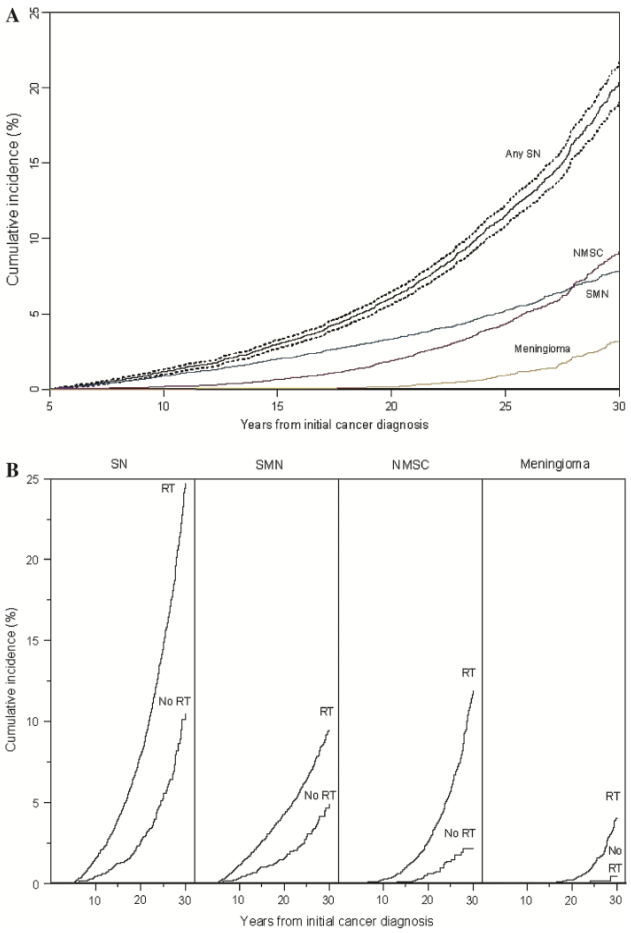

Fig. 3 – Cumulative incidence of second neoplasms (SNs) at 30 years after an initial cancer diagnosis: A) All SNs [cumulative incidence of any SN, non-melanoma skin cancer (NMSC), second malignant neoplasm (SMN), and meningioma are shown]; B) All SNs stratified by radiation therapy (RT) treatment or no RT [30].

Research of SM etiology in childhood cancer survivors is extremely important as their good prognosis enables a long period for SM occurrence. Although pediatric patients have fewer exposures to lifestyle and environmental cancer risk factors, trials of childhood cancer survivors can provide additional insight into the role of primary cancer treatments in SM etiology [31].

Genetics of radiation-induced tumors and toxicities

Radiation sensitivity is not a monogenetic trait but rather a polygenetic trait where the majority of the population

is located in the middle of the normal distribution of expression. People with mutations that could be associated with higher sensitivity to radiation, such as mutations concerning DNA repair mechanisms, might have a higher risk of developing early or late toxicities, and a possibility for deceleration of the treatment dose can be considered [32].

Nowadays, the carcinogenic effect of radiation is well-documented. Genetic susceptibility plays an important role in the pathogenesis of RIMs. Radiation can cause DNA damage to normal cells, which might lead to genomic instability and, finally, but rarely to RIM. A recent study from the University of Utah School of Medicine, published in 2017, showed that 13% of patients who received RT for breast cancer developed a SM with a median follow-up of 8.9 years, and it was estimated that only 3.4% of SMs were attributable to radiation therapy [33].

Although radiation sensitivity is largely explained as a polygenic trait encoded by numerous common variants with small individual effects, there are pathogenic mutations that cause rare conditions and have a monogenetic pattern of inheritance. Patients with these genetic syndromes are prone to developing malignancy (higher baseline risk of cancer development), and some of them have an increased risk of RIM. Some of these conditions are ataxia telangiectasia [autosomal recessive mutations of ataxia telangiectasia mutated (ATM) serine/threonine kinase gene], familiar cancers of the breast and ovarium due to breast cancer susceptibility genes (BRCA) mutations, hereditary retinoblastoma, and Li Fraumeni syndrome (both are autosomal dominant and caused by a mutation in RB1 or TP 53 gene, respectively), as well as Gorlin syndrome, neurofibromatosis type 1 and Nijmegen breakage syndrome [32, 34].

The most commonly studied heterozygous mutations are ATM and BRCA heterozygotes [34–36]. It is well known that the homology-directed repair route mediated by products of BRCA genes is activated in only 15% of DNA double-strand brakes caused by therapeutic radiation, so it should be no surprise that the clinical data consistently demonstrate no increased risk in BRCA heterozygous patients treated with standard adjuvant radiation regimens [36]. On the other hand, ATM serine/threonine kinase is directly involved in double-strand break repairs, but the clinical data on its importance in developing radiation sensitivity is contradicted. It seems that there is no increased risk of developing radiation toxicities in heterozygote carriers of pathogenic ATM mutations, but in the SEER clinical trial in breast cancer patients, an increased risk of developing contralateral breast carcinoma was observed in the irradiated group (probably due to scattering radiation) [35, 37]. Pathological ATM mutations are still not a contraindication for RT treatment. Notably, individuals with genetic syndromes with an increased risk of developing several types of cancer should be monitored for SMNs after RT treatment.

To develop a personalized medical approach in RT, with better tumor response, lower radiation toxicity, and without dose escalation, many trials explore the molecular signature concept of radiosensitivity. It would be beneficial to identify predictive biomarkers of the initial response to RT that could be helpful for predicting clinical outcomes in patients treated with RT. Tanić et al. [38] published that the MAP3K4 gene could be a potential biomarker response to RT and a potential target for radiosensitizing combination therapy.

In a genome-wide association study, Best et al. [39] identified two variants (rs4946728 and rs1040411 noncoding single nucleotide polymorphisms, located between PRDM1 and ATG1 genes) on chromosome 6q21 associated with the risk of SMNs after RT in pediatric Hodgkin's lymphoma survivors. This data indicates the significance of genetic susceptibility to SM etiology.

Testing for somatic mutations (mutations present in the tumor itself) is the most frequent scenario when cancer patients come in contact with genetic testing. Mutations in tumors occur at a higher rate than in normal tissues due to genetic instability, and their genetic information is different from the genetic information of the patient. These mutations have implications mainly for tumor response and tumor treatment decisions [32].

The somatic mutation pattern (genetic signature) could also be of diagnostic importance. Several studies have shown that RT could have its' molecular signature on the treated area, which can be detected in SMs that develop later. For instance, upregulation of MYC, RET, and FLT4 with down-regulation of CDKN2C and PRDM1 genes are frequent in radiation-induced sarcomas and other radiation-induced malignancies [40, 41].

On the other hand, somatic mutations of the key genes involved in DNA repair mentioned above can change the radiobiological behavior of the tumor. Usually, pathogenic mutations involved in DNA repairs such as ATM or BRCA 1 and 2 could be expressed at a higher rate and/or be of higher penetrance in the tumor, more than in the normal tissue due to loss of heterozygosity. In such a scenario, a full expression of recessive mutations and/or full penetrance of dominant mutations such as ATM and BRCA, respectively, can make the tumor more radiosensitive [42]. On the other hand, somatic mutations in k-RAS can make a tumor more radioresistant [43].

The effects of radiation modalities on RIM development

Nowadays, different types of ionizing radiation are used for cancer treatment that can be divided roughly into two groups: photon and particle radiation. High energy photons damage cellular molecules by producing highly reactive O_2 species, which react further with cell molecules, especially DNA. Radiation can cause single or double breaks of the DNA helix, which results in the loss of proliferation ability and cell death [44]. The biological effectiveness of ionizing radiation is quite dependent on the so-called linear energy transfer (LET), which represents the energy deposited in the targeted tissue [45]. Compared with particle radiation, photons have lower LET.

Proton beams have the highest transfer in one particular point in the body, followed by a sharp decrease of LET with the effect of sparing surrounding tissue. This point can be

manipulated, so better dose distribution and maximum dose deposition in the volume at the targeted area can be achieved while sparing the other structures along the beam way. This property gives proton therapy an advantage in treating tumors, where sparing the normal tissue is an imperative [46]. However, high LET radiation is more likely to produce cell death and mutation than low LET radiation [47, 48].

Studies that compare the incidence of SM in long-term cancer survivors following proton and photon beam therapy are limited. However, available data suggest a lower incidence of RIM in patients treated with proton beam therapy compared to photon therapy [49]. Chung et al. [50] published a comparative analysis of incidence rates of SM after radiation for cohorts of proton and photon-treated patients. After a median follow-up of 6.7 and 6.0 years in proton and photon-treated groups, the rate of SM was lower among patients treated with proton radiation compared to patients treated with photon RT (5.2% vs. 7.5%, respectively).

With the advances in RT, from conventional and three-dimensional conformal RT (3D-CRT) to IMRT and volumetric-modulated arc therapy, radiation is delivered to the targeted areas more precisely with dose escalation, and the organs at risk are better spared. As a complex radiation technique, IMRT, compared to 3D-CRT, is associated with better organ risk management and decreased frequency of acute and chronic treatment toxicities, followed by improved quality of life after treatment [51, 52]. The study by Hall and Wuu [53] showed that the move from 3D-CRT to IMRT can lead to an increase in RIMs. The rationale for this theory is that IMRT requires many fields, irradiating a larger volume of healthy tissue (so-called "low-dose bath"). Moreover, IMRT requires twofold to a threefold larger number of monitor units to deliver a preset dose compared with 3D-CRT. This larger number of monitor units leads to X-ray leakage and distant tissue irradiation. Considering that IMRT and volumetric-modulated arc therapy are fairly modern techniques and that the development of RIMs takes years and even decades, few studies compare risk in RIM development between novel techniques and 3D-CRT. However, we can assess that risk using different models. For instance, the concept of organ equivalent dose can be used to calculate the risk of RIM development in different tissues when three-dimensional dose distribution RT techniques are used [54].

Image-guided brachytherapy based on MRI, with radioactive source Ir-192, has become a standard treatment in gynecological malignancies. It provides precise information about radiation dose distribution, target volume coverage, and doses delivered to organs at risk while decreasing the toxicity [55].

Conclusion

As the number of long-term cancer survivors after RT increases, the RIMs are becoming a relevant clinical problem in long-term follow-up. RIMs are important late adverse effects of RT that can directly impact patient management and treatment decision-making. These facts could modify initial work-up, treatment, and follow-up protocols. Inclusion of genetic testing, further investigation of novel RT techniques, and additional screening and surveillance strategies should be added to the overall cancer care.

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Covid-19's impact on radiotherapy in the Republic of Srpska

Uticaj Covid-19 na radioterapiju u Republici Srpskoj

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Abstract

Background/Aim. Coronavirus disease 2019 (COVID-19) significantly affects patients with any type of chronic disease, especially the ones with neoplasm. The aim of this study was to investigate the impact of the COVID-19 pandemic on the weekly number of external beam (EB) radiotherapy (RT) (EBRT) fractions and monthly brachytherapy (BT) applications without intentional hypofractionation. We also investigated how the pandemic affected the number of EBRT patients younger or older than 70 years. **Methods.** The Affidea Radiotherapy Center, Banja Luka (BL RT Center), provides RT to the population (1.15 million) of the Republic of Srpska (Bosnia and Herzegovina). We analyzed the period of 14 months before the onset and the same period during COVID-19. **Results.** The average weekly number of EBRT fractions from January 2019 to the end of February 2020 was 680.5 [standard deviation (SD) 67.4], and from March 2020 to the end of April 2021, it was 617.1 (SD 96.4). During April 2020, the weekly number of the EBRT decreased by 67.9% compared to the same period in 2019, while in March 2021, it fell by 42.4%. Paired samples *t*-test showed that the occurrence of the COVID-19 pandemic had a statistically significant effect [$t(60) = 4.627$, $p < 0.05$] on the reduction in the number of weekly EBRT fractions in the BL RT Center. When comparing number of EBRT patients over 70 years old to those fewer than 70 years old, the decrease was 16.3% vs. 1.6%, respectively. The Wilcoxon signed-rank test revealed that the COVID-19 pandemic had a statistically significant effect ($Z = -2.42$, $p = 0.016$) on reducing the number of monthly BT applications. **Conclusion.** A statistically significant decline in EBRT and BT was observed in BL RT Center for the first fourteen months of the pandemic. The "waves" of the pandemic "closed" the medical wards needed for the diagnosis and therapy of oncology patients and converted them into COVID-19 wards. Therefore, some oncology patients who would have had an indication for RT never received it.

Key words:
age factors; brachytherapy; covid-19; epidemiology; radiotherapy; radiotherapy dosage.

Apstrakt

Uvod/Cilj. Bolest prouzrokovana koronavirusom 2019 (*coronavirus disease* 19 – COVID-19) utiče na bolesnike sa bilo kojim tipom hronične bolesti, a posebno na one sa malignitetima. Cilj studije bio je da se utvrdi uticaj pandemije COVID-19 na broj nedeljnih frakcija radioterapije (RT) sa spoljašnjim zračenjem (EBRT) i mesečne brahiterapijske (BT) aplikacije, bez namernog hipofrakcionisanja, kao i njen uticaj na broj bolesnika podvrgnutih EBRT, mlađih ili starijih od 70 godina. **Metode.** Centar za radioterapiju *Affidea* u Banjoj Luci (BL RT centar), pruža RT stanovništvu (1,15 miliona) Republike Srpske (Bosna i Hercegovina). Analizirali smo period od 14 meseci pre početka COVID-19 i isti period za vreme COVID-19. **Rezultati.** Prosečni sedmični broj EBRT frakcija u periodu od januara 2019. do kraja februara 2020. iznosio je 680,5 [standardna devijacija (SD) 67,4], a od marta 2020. do kraja aprila 2021, 617,1 (SD 96,4). Tokom aprila 2020., sedmični broj EBRT frakcija smanjio se 67,9% u odnosu na isti period 2019., dok je u martu 2021. godine pao za 42,4%; *t*-test uparenih uzoraka pokazao je da je pojava pandemije COVID-19 imala statistički značajan učinak [$t(60) = 4.627$, $p < 0,05$], na smanjenje broja sedmičnih EBRT frakcija u BL RT centru. Smanjenje broja bolesnika podvrgnutih EBRT starijih od 70 godina u odnosu na mlađe od 70 godina, iznosilo je 16,3% naspram 1,6%, redom. *Wilcoxon* rang test otkrio je da je pandemija COVID-19 imala statistički značajan učinak na smanjenje broja mesečnih BT aplikacija ($Z = -2,42$, $p = 0,016$). **Zaključak.** Za prvih 14 meseci pandemije primećen je statistički značajan pad EBRT i BT u BL RT centru. „Talasi" pandemije „zatvarali" su odeljenja potrebna za dijagnostiku i terapiju onkoloških bolesnika i pretvarali ih u COVID-19 odeljenja. Zbog toga pojedini onkološki bolesnici nikada nisu dobili RT koja im je bila indikovana.

Ključne reči:
životno doba, faktor; brahiterapija; covid-19; epidemiologija; radioterapija; radioterapija, doziranje.

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Introduction

The Coronavirus disease 2019 (COVID-19) pandemic is an entirely new experience for "modern" society that has drastically affected all aspects of life, especially the health care system. The treatment of oncology patients has become a real challenge under the influence of several "waves" of the pandemic. In addition to having infected patients, we have also had many infected health workers. Any contact with an infected person (private or at work) leads to a two-week quarantine, further reducing the number of medical staff in hospitals [1]. Radiotherapy (RT) teams are adopting new work procedures and the use of protective equipment in order to minimize the risk of infecting medical staff and patients while at the same time optimizing the treatment and care of oncology patients. Many RT centers followed the recommendation to use a hypofractionated RT (breast, prostate, palliative treatments, etc.), thus reducing the number of hospital visits [2]. Some RT centers split their staff into teams to make physical segregation and avoid the risk of infection among them. When possible, the number of medical control checks and consultations has been reduced, and telephone communication has been switched on. This has reduced the number of patients in most healthcare systems (outside the COVID-19 departments) [3].

Oncology patients are especially endangered because they need timely diagnosis and treatment therapy, primarily RT with surgery and systemic therapy [4, 5]. Pandemic "waves" have reduced the capacity of most departments in hospitals, making it harder for cancer patients to get to RT, especially during those periods. This evident impact of the COVID-19 pandemic on the health care system, including the RT services, was the subject of several studies [4, 6,] which show a quantitative decline in the number of patients per age and diagnosis.

The COVID-19 reached the Republic of Srpska (RS, Bosnia and Herzegovina) on March 5, 2020, when a patient in Banja Luka (BL), who had traveled in from Italy, tested positive. For this epidemiological retrospective study, we assume that the pandemic began on March 1, 2020.

The aim of this epidemiological study was to investigate the impact of COVID-19 on the weekly number of external beam RT (EBRT) fractions and monthly brachytherapy (BT) applications in the BL RT Center, including the age of the patients and the treatment site.

Methods

BL RT Center Affidea provides EBRT and BT services to all insured persons of the Health Insurance Fund of the RS. The Center has two Varian True Beam (Varian, Medical Systems, Palo Alto, California) linear accelerators (Linac), one Varian DHX Linac, and one Varian GammaMed plus™ iX BT high dose rate (HDR) unit. In routine clinical EBRT practice, we use 3D conformal radiotherapy (3DCRT), intensity-modulated radiotherapy (IMRT), and volumetric modulated arc therapy (VMAT) treatment techniques. In BT applications, gynecologic (GYN) cancer predominates. Patients were irradiated from Monday to Friday during one 8-h shift (260 working days per year). The estimated population and the total number of new cancer cases in the RS in 2016 were 1,157,516 and 5,786, respectively. Table 1 presents new cancer cases by leading primary sites and by sex in the RS during 2016 [7].

Table 1
New cancer cases by leading primary sites and sex, in the Republic of Srpska (RS), during 2016 [7]

Sex	Patients (%)
Male	
trachea and lungs	16.8
colon and rectum	12.6
prostate	9.5
bladder	7.0
stomach	5.2
kidney	3.1
liver	2.8
Female	
breast	16.4
colon and rectum	8.8
cervix	7.0
trachea and lungs	6.1
uterus	4.3
ovary	4.3
thyroid gland	4.0

For this epidemiological retrospective study, we assume that the pandemic began on March 1, 2020. We analyzed the number of weekly EBRT fractions and monthly BT applications in the period before (period 0: January 2019 – end of February 2020, which is the period with the largest number of EBRT fractions) and during the onset of COVID-19 in BL RT Center (period 1: March 2020 – end of April 2021). We also investigated the influence of COVID-19 on the number of EBRT patients per treatment site, younger and older than 70 years. Both periods had 14 months, i.e., 61 weeks.

Statistical analysis

Results are presented as median and arithmetic mean values with standard deviation (SD). Relative error (RE) was used to determine the percentage difference in the change in quantity. The Kolmogorov-Smirnov test was applied to assess the normality of the continuous studied data (> 50 measurements for EBRT data). The Shapiro-Wilk normality test was applied in the case of a smaller measurement sample (< 20 measurements for BT data).

The strength of the association between independent factor (presence of COVID-19) and EBRT fraction per week or BT applications (dependent factor) was determined by using parametric one-tailed paired samples *t*-test for normal distribution of data differences and nonparametric Wilcoxon signed-rank test when the data differences did not have a normal distribution [8]. All the analyses were estimated at a minimal $p < 0.05$ level of statistical significance. Complete statistical analysis of data was done with the statistical software package, SPSS Statistics 23 (IBM, Armonk NY, USA).

Results

External beam radiotherapy

Figure 1 presents the mean value of the number of EBRT fractions working per day in the period 2016–2021.

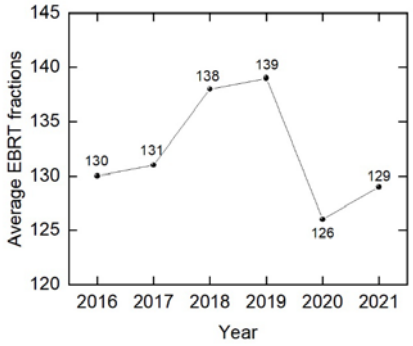

Fig. 1 – Average number of EBRT fractions working per day in the period 2016–2021.
EBRT – external beam radiotherapy.

The total weekly number of EBRT fractions in the period 2019–2021 in the BL RT Center is shown in Figure 2.

The relative difference between the weekly number of EBRT fractions for 2020 and 2021 compared to 2019 is shown in Figure 3.

Descriptive statistical analysis was performed for period 0 (n_0 = 61 weeks, when we consider that there was no influence of COVID-19 on the number of EBRT fractions) and period 1 (n_1 = 61 weeks, the period when the pandemic was present); the results are shown in Table 2.

The applied Kolmogorov-Smirnov normality test (p = 0.2) indicates the normal distribution of the values of differences (n_0-n_1) of weekly EBRT fractions (mean 63.4, SD 107). Therefore, a parametric one-tailed paired-samples t-test was performed [t (60) = 4.627, p < 0.05].

We compared the number of patients older than 70 years, younger than 70 years, and the total number of EBRT patients by the most common treatment sites (Table 3).

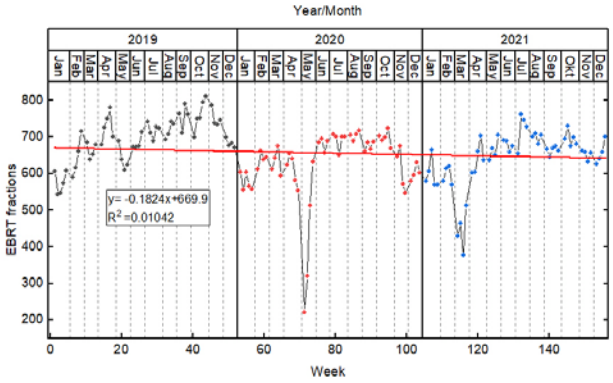

Fig. 2 – Weekly number of external beam radiation therapy (EBRT) patients in the period 2019–2021.

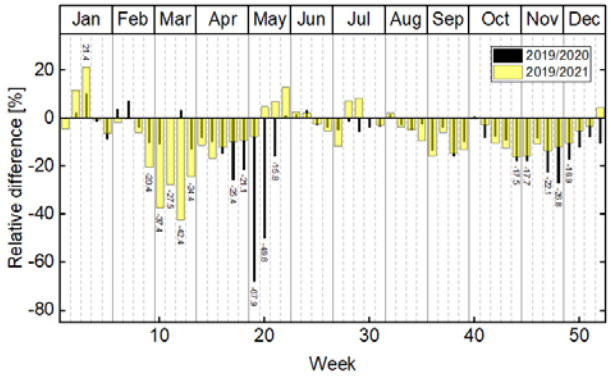

Fig. 3 – Relative difference of the weekly number of external beam radiation therapy (EBRT) fractions for 2020 and 2021 in relation to the year 2019.

Table 2

Descriptive statistics by 61 weekly external beam radiotherapy (EBRT) fractions before and during the COVID-19 pandemic

COVID -19	Number of weeks	EBRT/week	
		mean ± SD	min–max
Before	61	680.5 ± 67.4	543–811
During	61	617.1 ± 96.4	221–724

COVID-19 – corona virus disaese 2019; SD – standard deviation.

Table 3

Difference according to the treatment site for patients older than 70 years, younger than 70 years, and in total all patients (for 14 months before and during the COVID-19 pandemic)

Diagnosis sites	Number of RT	Difference (percentages)
Skin		
patients > 70 years		
before COVID-19	25	
during COVID-19	4	-84
patients < 70 years		
before COVID-19	13	
during COVID-19	5	-61.5
total		
before COVID-19	38	
during COVID-19	9	-76.3
GYN*		
patients > 70 years		
before COVID-19	101	
during COVID-19	61	-39.6
patients < 70 years		
before COVID-19	259	
during COVID-19	204	-21.2
total		
before COVID-19	360	
during COVID-19	265	-23.4
Breast		
patients > 70 years		
before COVID-19	128	
during COVID-19	90	-29.7
patients < 70 years		
before COVID-19	345	
during COVID-19	275	-20.3
total		
before COVID – 19	473	
during COVID – 19	365	-22.8
Lung		
patients > 70 years		
before COVID-19	91	
during COVID-19	77	-15.4
patients < 70 years		
before COVID-19	162	
during COVID-19	133	-17.9
total		
before COVID-19	253	
during COVID-19	210	-17
Stomach		
patients > 70 years		
before COVID-19	11	
during COVID-19	10	-9.1
patients < 70 years		
before COVID-19	30	
during COVID-19	27	-10
total		
before COVID-19	41	
during COVID-19	37	-9.8
Prostate		
patients > 70 years		
before COVID-19	130	
during COVID-19	113	-13.1
patients < 70 years		
before COVID-19	74	
during COVID-19	83	+12.2
total		
before COVID-19	204	
during COVID-19	196	-3.9

Table 3 (continued)

Diagnosis sites	Number of RT	Difference (percentages)
Bladder		
patients > 70 years		
before COVID-19	22	
during COVID-19	23	+4.5
patients < 70 years		
before COVID-19	11	
during COVID-19	14	+27.3
total		
before COVID-19	33	
during COVID-19	37	+12.1
Colo-rectum		
patients > 70 years		
before COVID-19	75	
during COVID-19	78	+4.0
patients < 70 years		
before COVID-19	128	
during COVID-19	130	+1.6
total		
before COVID-19	193	
during COVID-19	208	+7.8
Bone		
patients > 70 years		
before COVID-19	141	
during COVID-19	119	-15.6
patients < 70 years		
before COVID-19	203	
during COVID-19	237	+16.7
total		
before COVID-19	344	
during COVID-19	356	+3.5
Head and neck**		
patients > 70 years		
before COVID-19	64	
during COVID-19	69	+7.8
patients < 70 years		
before COVID-19	151	
during COVID-19	151	0
total		
before COVID-19	215	
during COVID-19	220	+2.3
Brain		
patients > 70 years		
before COVID-19	65	
during COVID-19	45	-30.8
patients < 70 years		
before COVID-19	142	
during COVID-19	165	+16.2
total		
before COVID-19	207	
during COVID-19	210	+1.4
Total		
patients > 70 years		
before COVID-19	931	
during COVID-19	779	-16.3
patients < 70 years		
before COVID-19	1,699	
during COVID-19	1,672	-1.6
total		
before COVID-19	2,630	
during COVID-19	2,451	-6.8

COVID-19 – corona virus disease 2019; Before COVID-19 – period from January 2020 to March 2020; during COVID-19 – period from March 2020 to May 2020; RT – radiotherapy; * – cervical, corpus uteri cancer, ovarian, uterine, vaginal, and vulvar cancers; ** – head and neck, hypopharynx, larynx, supraglottis, lip and oral cavity, nasal cavity and sinuses, nasopharynx, oropharynx, pharynx, salivary glands, and thyroid gland cancers.

Note: only the most common places were analyzed.

Brachytherapy applications

The total number of annual BT applications in the previous six years is shown in Figure 4.

The total monthly number of BT applications between 2019 and 2021 is shown in Figure 5.

Figure 6 shows the relative difference between the monthly numbers of BT applications for 2020 and 2021 in relation to 2019.

Descriptive statistical analysis was performed for period 0 (n_0 = 14 months, when we consider that there was no influence of COVID-19 on the number of EBRT fractions) and period 1 (n_1 = 14 months, the period when the pandemic was present); the results are shown in Table 4.

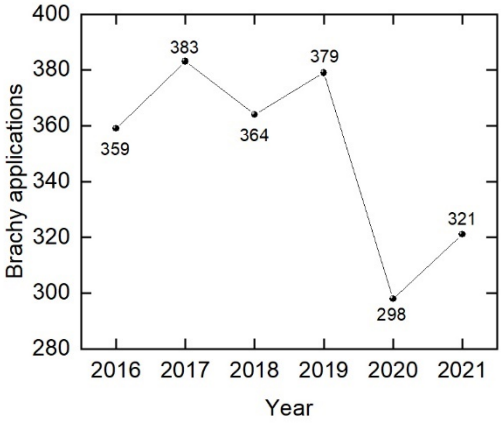

Fig. 4 – Average number of brachytherapy (Brachy) applications in the period 2016–2021.

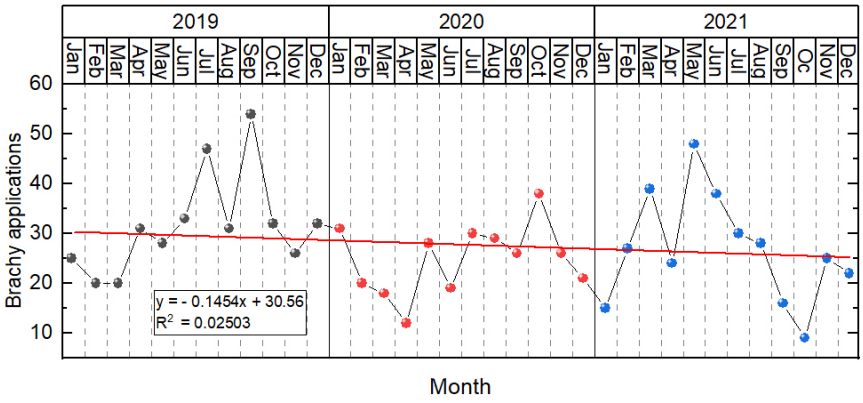

Fig. 5 – Monthly number of brachytherapy (Brachy) applications for the period January 2019 – December 2021.

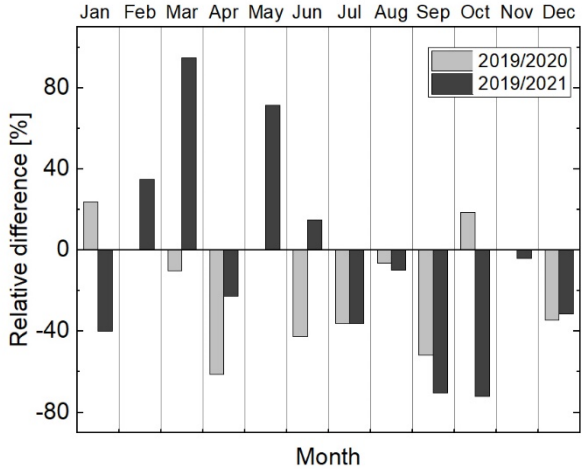

Fig. 6 – Relative difference of the monthly number of brachytherapy applications for 2020 and 2021, in relation to 2019.

Table 4

Descriptive statistics of brachytherapy (BT) applications for period before and during COVID-19

COVID -19	Number of months	BT/month	
		median	min–max
Before	14	31	20–54
During	14	26	12–39

COVID-19 – corona virus disaese 2019.

The result of the Shapiro-Wilk normality test performed for the values of the differences of the monthly BT applications (mean 5.9, SD 9.0) of the previously mentioned period (n_0-n_1) was significant (p = 0.009), which indicates that there is no normal distribution of the obtained results. Therefore, a nonparametric Wilcoxon signed-rank test was performed (for data of 28-month BT applications; Z = - 2.42, p = 0.016).

Discussion

External beam radiotherapy

The average number of daily irradiated EBRT patients in the BL RT Center grew from 2016–2019 and experienced a sharp decline in 2020. At the annual level, the total number of EBRT fractions in 2020 and 2021 decreased by 9.3% and 7.3%, respectively, compared to 2019 (Figure 1).

Figure 2 clearly shows the declining trend of the regression line obtained based on the number of weekly EBRT fractions from 2019–2021 (the direction coefficient is negative). The mean weekly number of EBRT fractions delivered in period 0 was 680.5 (SD 67.4), and in period 1 was 617.1 (SD 96.4), which is in accordance with the results of the studies by Martinez et al. [6], Chauhan et al. [9], and Nierengarten [10].

Significant declines in the number of weekly fractions in May 2020 and March 2021 are evident (Figure 2) due to the COVID-19 pandemic, i.e., the transition of other oncology services to the COVID-19 department. This is no different from a study conducted in the United States, where RT centers reported a reduction of treatments from 20% up to 39% (during April 2020) [11]. Switzerland also had a significant reduction in daily activity during April 2020 [12]. A study by Gonnelli et al. [13] for the period March end of May, shows an increase in RT treatments by 5%, which is not in accordance with our results. The Chauhan et al. [9] study agrees with our results for the period from January to the end of May 2020, which were moved two months forward (the pandemic in India started two months earlier than in RS). Amador et al. [14] reported the impact of the COVID-19 pandemic on the care of cancer patients in Spain, comparing the March–June 2019/20 periods, the number of new patients decreased by 20.8%. A study from Brazil [15] noted a 10% reduction in the number of treated patients and a 26% reduction in the number of RT sessions. Finally, the number of patients who received radiotherapy in China also declined by 31.3% during the pandemic period [16].

During the period April–May 2020 (weeks 17–21), the weekly EBRT fractions decreased by 15.8% to 67.9% compared to the same period in 2019, which is in accordance with the results of the study by Vaandering et al. [17] (decline of 45.4% in April 2020). Another significant decrease in the number of weekly patients occurred at the end of 2020 (44–49 weeks, i.e., November–December 2020), from 17.5% to 26.8%. From the 9th to the 14th week of 2021 (March), a new "wave" followed from 20.4 % to 42.4% (Figure 3).

Based on descriptive statistical analysis (Table 2), the mean values of weekly EBRT fractions before the onset of the COVID-19 virus are higher than during the pandemic, while the SD is lower. Parametric one-tailed paired samples *t*-test showed that the occurrence of the COVID-19 pandemic had a statistically significant effect [t (60) = 4.627, $p < 0.05$] on the reduction in the number of weekly EBRT fractions in BL RT Center (comparing 14 months before and during the pandemic).

A greater reduction in EBRT patients between period 0 and period 1 was seen for patients aged 70 years or older compared with those aged younger than 70 years (16.3% vs. 1.6%), which is in accordance with the results of the study by Spencer et al. [4]. Patients older than 70 years accounted for 33.7% and 31.8% of the total number of patients in periods 0 and 1, respectively, which is more than the results of the Chauhan et al. [9] study (about 5% for the first half of period 1).

The largest decrease in the number of EBRT patients older than 70 years by diagnosis is found in GYN (39.6%), breast (29.7%), brain (30.8%), lung (15.4%), prostate (13.1%), bone (15.1%), and skin (84%), which is in accordance with the results of the other study [9, 13,17].

In the group of patients younger than 70 years, we had a decrease in the number of those patients with GYN (21.2%), breast (20.3%), lung (17.9%), and skin (61.5%) cancers, while we had an increase in the number of patients diagnosed with prostate, bone, brain, and bladder cancers (Table 3). The reasons were that surgery and biopsies were stopped or reduced by 50% but also because of health care measures taken nationally and locally to limit the spread of the pandemic.

Brachytherapy

The number of BT applications in 2020 and 2021 is lower by 21.4% and 15.3%, respectively, compared to 2019 (Figure 4).

Figure 5 shows that the regression line has a negative direction coefficient, i.e., it decreased with time from 2019–2021. The total number of BT applications in period 0 (430)

and period 1 (352) indicates a decline in BT applications by 18.1%. The mean monthly number of BT applications delivered in period 0 was 30.7 (SD 9.7), and in period 1 was 25.1 (SD 7.8).

We had the most significant drop in the number of BT applications in April (61.3%) and September (51.9%) 2020 compared to the same period in 2019. Compared to the same period in 2019, there was a 40% decrease in January 2021, and a 95% increase in March 2021 (Figure 6). Based on descriptive statistical analysis (Table 4), the median values of monthly BT applications before the onset of the COVID-19 were higher than during the pandemic (31 vs. 26, respectively).

COVID-19 had a statistically significant effect on reducing the number of monthly BT applications in the BL RT Center according to the nonparametric Wilcoxon signed-rank test (for data of 28 monthly BT applications; Z = -2.42, $p = 0.016$).

Conclusion

A statistically significant decline in EBRT and BT treatments in the BL RT Center was observed in the first 14 months of the COVID-19 pandemic.

The pandemic's "waves" typically "closed" medical departments needed for oncology patient diagnosis and treatment, turning them into COVID-19 wards. Therefore, some oncology patients who would have had indications for RT never reached it.

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The relationship between insulin resistance, bone mineral density, and fracture risk in postmenopausal women

Odnos između insulinske rezistencije, mineralne gustine kostiju i rizika od frakture kod žena u postmenopauzi

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Abstract

Background/Aim. Skeletal muscles and bones are essential tissues that, in addition to supporting the body, are the primary site of postprandial glucose intake, which is significantly associated with insulin resistance. The aim of this study was to determine the effect of insulin resistance on bone mineral density (BMD) and fracture risk and re-evaluate the relationship between muscle properties and BMD and insulin resistance in postmenopausal women in Serbia. **Methods.** Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) was calculated in postmenopausal women who were divided into two groups. The "cut-off" value of insulin resistance for the group with "Low HOMA-IR" was < 2, and for "High HOMA-IR" > 2. Fat mass (FM), lean mass (LM), and BMD were measured on the hip and spine using a densitometer with dual-energy X-ray absorptiometry. **Results.** FM and LM had an evident impact on BMD. The decrease in LM and fat buildup was associated with a higher incidence of insulin resistance. A positive correlation was confirmed between HOMA-IR and BMD on the spine and hip, but there was no correlation between insulin resistance and fracture risk. **Conclusion.** LM and FM have significant effects on BMD. The association between LM, FM, BMD and the onset of insulin resistance in postmenopausal women is confirmed. However, women with higher insulin resistance levels and higher BMD do not have a lower fracture risk.

Key words:
bone density; fractures, bone; insulin resistance; postmenopause; risk assessment; serbia.

Apstrakt

Uvod/Cilj. Skeletni mišići i kosti su važna tkiva koja, osim uloge u držanju tela, predstavljaju i primarno mesto preuzimanja glukoze nakon obroka, što je značajno povezano sa rezistencijom na insulin. Cilj rada bio je da se utvrdi uticaj insulinske rezistencije na mineralnu gustinu kosti (MGK) i rizik od nastanka preloma, kao i da se ispita veza između karakteristika mišića i MGK i insulinske rezistencije kod žena u postmenopauzi u Srbiji. **Metode.** Homeostatski model – rezistencija na insulin (HOMA-IR), izračunat je kod žena u postmenopauzi koje su bile podeljene u dve grupe. Granične vrednosti (*cut-off*) insulinske rezistencije za grupu sa „niskim HOMA-IR" bila je < 2, a za grupu sa „visokim HOMA-IR" > 2. Masno tkivo (MT), bezmasno tkivo (BT) i MGK mereni su na kuku i kičmenom stubu pomoću denzitometra sa dvoenergetskom X-zračnom apsorpciometrijom. **Rezultati.** MT i BT su imali očigledan uticaj na MGK. Smanjenje BT i nakupljanje masti bilo je povezano sa višom učestalošću nastanka rezistencije na insulin. Primećena je pozitivna korelacija između HOMA-IR i MGK na kičmi i kuku, ali nije postojala korelacija između insulinske rezistencije i rizika od nastanka preloma. **Zaključak.** MT i BT imaju značajan uticaj na MGK. Potvrđena je povezanost između MT, BT, MGK i nastanka insulinske rezistencije kod žena u postmenopauzi. Međutim, žene sa višim nivoom insulinske rezistencije i većim MGK nemaju niži rizik od nastanka preloma.

Ključne reči:
kost, gustina; kost; prelomi; insulin, rezistencija; postmenopauza; rizik, procena; srbija.

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Introduction

Skeletal muscle is a vital tissue that supports body posture and is also the primary glucose uptake site after a meal. Skeletal muscle is significantly related to insulin resistance [1]. The connection between bone strength or mineral bone density (BMD) and insulin resistance is very complex [2]. According to the study, when the inflammatory response is inadequate, as in the case of aging muscles, acellular fat droplets and adipocytes tend to accumulate, so the development of insulin resistance may be the inflammatory response of the muscles [3]. This results in the secretion of different cytokines, chemokines, and adipocytes, which affects insulin resistance [1, 3].

The World Health Organization (WHO) has defined natural menopause as the least twelve consecutive months of amenorrhea, not physiological and pathological causes. According to statistics, the mean age of natural menopause is 51 years in industrialized nations, compared to 48 years in low and non-industrialized nations [1]. With the average life span extended to 70 years, most women will spend more than one-third of their life beyond the menopausal transition. Besides, the proportion of menopausal women is rising since the aging population is expanding rapidly.

A significant number of studies, on the other hand, discuss the impact of reduced muscle mass on BMD and the consequences that result from them, in the first place, a higher incidence of osteoporotic fractures [4]. It is known that muscle mass and osteoporosis, and metabolic disorders are closely related. However, data on the association of muscle properties, bone mass, and insulin resistance are lacking [5].

The aim of this study was to determine the effects of insulin resistance on bone mineral density and fracture risk and evaluate the relationship between muscle properties (muscle mass, muscle strength, and physical performance) and bone mineral density and insulin resistance in postmenopausal women in Serbia.

Methods

Ethical concerns

The protocol, as well as the study procedures, were approved by the Ethics Committee, the Clinical Center in Kragujevac (Nº 01/17-3765), and the Faculty of Medical Science, University of Kragujevac (Nº 01-15581/3-6) from November 2017 to June 2018.

Study design

The study was conducted at the Clinical Center Kragujevac, the reference healthcare institution for osteodensitometry in the region of central Serbia. The study was designed as a clinical, non-interventional, observational, cross-sectional study and included 66 women over 65 years of age who were selected through random sampling. Participants were divided into two groups and based on the Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) limit values as used in the study by Nikolić et al. [6]. The cut-off value for participants from the group "Low HOMA-IR" was < 2, and for those from the group "High HOMA-IR", the values of insulin resistance were > 2. Among the study participants were 44 women with osteoporosis (T score < 2.5) and 22 women with normal bone mineral density or osteopenia (T score ≥ -2.5, without fracture data).

Inclusion and exclusion criteria

The inclusion criteria were confirmed as a menopause of at least five years based on no menstruation. None of the participants had the diseases that affect BMD, such as hyperthyroidism, hyperparathyroidism, renal failure, malabsorption syndrome, chronic colitis, multiple myeloma, leukemia, chronic arthritis, diabetes mellitus (DM), or previous use of therapy that interfere with bone metabolism (e.g., glucocorticoids, heparin, warfarin, thyroxin, and estrogen). Moreover, the exclusion criteria were cigarette smoking, alcohol intake, body mass index (BMI) > 30 kg/m^2, and < 19 kg/m^2, respectively. Before joining the study, all participants confirmed their participation with their signatures.

The insulin resistance

Index expressed as HOMA-IR was calculated using the following equation, as described by Matthews et al. [7]: HOMA-IR = [glucose (mg/dL) × insulin (μU/mL)]/405 for each participant. Due to its simplicity and calculation, the most commonly used technique in clinical practice but also in epidemiological studies for the assessment of insulin resistance was the homeostatic test (HOMA-IR) [7].

Osteodensitometric, anthropometric, and body composition measurements

BMD (g/cm^2) was measured on the lumbar spine (LS) in the region L1-L4 and total hip in all participants. The measurement was done with a densitometer with X-ray energy absorption (DXA) (QDR 4,500, Hologic Model Discovery Inc., Waltham, MA) [8]. Participants did not wear metal items (e.g., clips, belts, brassieres, jewelry) or shoes. They were instructed to be motionless during the scan. Daily standardized quality control of DXA instruments was performed using the manufacturer's phantom spine before the start of the study. The definition of osteopenia and osteoporosis was made using WHO: -2.5 < T-score < -1 and T-score -2.5, respectively. Body weight and height were obtained from the mean of three measurements. The accurate and precise values of these body composition parameters were also estimated from the DXA scan of the total body, which included bone mineral content (BMC), lean mass (LM), and fat mass (FM).

Following the manufacturer's guidelines, all scans were obtained and analyzed by the same experienced operator [9]. Muscle strength was measured using the handgrip (HG) test

– (HGT) dynamometer and is closely related to the muscle strength of the lower extremities [10]. In our study, the Jamar dynamometer was used, which is small, portable, and easy to handle. It was considered a reduced muscle strength HGT < 16 kg [11]. To measure physical performance, we used the walk's speed test at a distance of 4 m (gait speed-GS). Physical ability was considered reduced when the gait speed was < 0.8 m/s for 4 m [12].

Fracture risk

Fracture Risk Assessment Tool (FRAX) algorithm was used to calculate the probability of major osteoporotic fractures and hip fracture (www.sheffield.ac.Uk/FRAX/) [13, 14], using data specific to our country. FRAX Index 1 represented the probability of a major osteoporotic fracture (clinical fracture of the spine, forearm, hip, or shoulder), while FRAX Index 2 represented the probability of hip fracture.

Statistical analyses

IBM SPSS version 20 (IBM Company, Armonk, NY) was used for all statistical analyses. The outcome variables used were BMDs of the whole body and at skeletal sites. The sample size was calculated using G*Power software version 3.1, and 66 subjects were required for a 90% power and 5% for the *t*-test. The cases and controls were distributed between

Table 1

Basic anthropometric characteristics of study population divided into the low-HOMA-IR (n = 44) and high-HOMA-IR (n = 18) groups

Parameters	Mean ± SD	SE	*p*-values
Age (years)			
low	70.89 ± 4.84	0.73	0.812
high	71.83 ± 4.81	1.13	
Body height (cm)			
low	160.36 ± 6.62	1.00	0.199
high	159.33 ± 7.50	1.77	
Bodyweight (kg)			
low	64.45 ± 9.77	1.47	0.071
high	72.56 ± 14.80	3.49	
Body mass index (kg/m^2)			
low	25.061 ± 3.52	0.53	0.046
high	28.133 ± 4.91	1.16	
Waist size (cm)			
low	77.64 ± 11.41	1.72	0.008
high	92.28 ± 19.57	4.61	
Lean mass (kg)			
low	3,395.06 ± 372.98	156.85	0.005
high	3,573.09 ± 583.04	137.43	
Fat mass (kg)			
low	2,411.89 ± 636.12	196.65	0.035
high	2,981.78 ± 994.23	234.87	
Total mass (g)			
low	62,335.07 ± 894.45	135.87	0.011
high	69,759.96 ± 1495.45	352.33	
Body fat (%)			
low	38,132.66 ± 587.67	189.56	0.483
high	41,600.56 ± 781.45	184.45	
Handgrip test (kg)			
low	12.40 ± 9.14	1.39	0.954
high	14.29 ± 8.31	2.01	
Gait speed (m/s)			
low	0.378 ± 0.17	0.03	0.736
high	0.360 ± 0.16	0.04	
Hip BMD (g/cm^2)			
low	0.693 ± 0.09	0.01	0.557
high	0.728 ± 0.10	0.02	
Lumbar spine BMD (g/cm^2)			
low	0.756 ± 0.10	0.01	0.179
high	0.825 ± 0.13	0.03	
BMC (g)			
low	1,592.38 ± 275.11	41.47	0.674
high	1,594.74 ± 328.94	77.53	
Lean + BMC (g)			
low	38,222.76 ± 3.99	2.02	0.004
high	39,942.17 ± 6.20	1.46	

HOMA-IR – Homeostatic model assessment – insulin resistance (the cut-off value for HOMA-IR was 2); BMD – bone mineral density; BMC – bone mineral content; SD – standard deviation; SE – standard error.
Independent *t*-test confirmed statistical differences for normally distributed data with the level of significance of 0.05.

two groups [15]. The values of all variables for the whole body and regional sites were presented as mean (M) ± standard deviation (SD). Comparison of mean values between two groups of subjects, those with osteoporosis and those with normal BMD/osteopenia, were classified according to their spine, bones, and BMD of the entire body, as well as weight, height, BMI, LM, FM, total weight and body fat. Correlation analyses of the whole body, regional sites BMD, and T-scores with the independent variables such as weight, LM, FM, and BMD were performed to obtain Pearson's correlations. We used stepwise multiple linear regression analysis to obtain determinants/predictors for the outcome variables. All *p*-values were reported significant at 0.05 or less [16].

Results

The average values and SD /standard errors (SE) of means of the examined parameters according to the level of HOMA-IR are shown in Table 1. BMI, waist size, LM, FM, total mass, and lean + BMC were significantly different in Low-HOMA-IR and High-HOMA-IR groups ($p < 0.05$). Other tested parameters were not significantly different in those groups (Table 1).

The average age of participants was 71.20 ± 4.72 years, with the range being 65 to 83. For women with normal bone mass/osteopenia, the mean (± SD) age was 70.91 ± 4.97 years, while the mean (± SD) age for women with osteoporosis was 71.34 ± 5.09 (Table 2).

In the study population, regarding their BMD values, LM was shown to have a higher degree of positive correlation with BMD on the lumbar spine ($\beta = 0.418$, $p < 0.001$) but also had a significant effect on the hip ($\beta = 0.416$, $p < 0.01$). In contrast, FM showed a high degree of positive correlation with both BMD on at the hip ($\beta = 0.473$, $p < 0.001$) and the lumbar spine ($\beta = 0.480$, $p < 0.001$).

In this study, the results showed a significant degree of a positive correlation between HGT and BMD on the hip ($\beta = 0.331$, $p < 0.01$) and spine ($\beta = 0.243$, $p < 0.05$), whereas GS was only correlated with BMD on the hip ($\beta = 0.268$, $p < 0.05$) (Table 3).

A positive correlation was confirmed between HOMA-IR and FM ($\beta = 0.322$, $p < 0.05$) and total mass ($\beta = 0.287$, $p < 0.05$). However, there was no correlation between HOMA-IR and LM ($\beta = 0.163$, $p > 0.05$) (Table 4). A significant degree of positive correlation was obtained between HOMA-IR and body mass index ($\beta = 0.381$, $p < 0.01$) and waist circumference ($\beta = 0.405$, $p = 0.001$). A high degree of positive correlation was also observed between HOMA-IR and BMD on the spine ($\beta = 0.362$, $p = 0.01$) and the T score of the spine ($\beta = 0.359$, $p = 0.01$). Besides, a correlation was also shown between HOMA-IR and hip BMD ($\beta = 0.264$, $p < 0.05$) and hip T score ($\beta = 0.305$, $p < 0.05$). In the study, no correlation was confirmed between insulin resistance and muscle strength measured by HG and physical performance measured by GS (Table 4).

Table 2

Comparison of anthropometric parameters and Dual-Energy X-Ray Absorptiometry (DXA) in women with normal BMD/osteopenia and women with osteoporosis

Parameters	Women with normal BMD/osteopenia (n = 22)	Women with osteoporosis (n = 44)	*p*-value
Age (years)	70.91 ± 4.97	71.34 ± 5.09	0.935
Weight (kg)	74.77 ± 9.47	63.41 ± 11.25	0.000
Height (cm)	161.77 ± 4.68	159.20 ± 7.61	0.096
Body mass index (kg/m^2)	28.57 ± 3.47	24.88 ± 3.99	0.000
HOMA-IR	2.684 ± 1.98	1.566 ± 1.13	0.005
Lean mass (g)	37,513.73 ± 4,128	33,168.40 ± 3979	0.000
Fat mass (g)	30,973.18 ± 6,613	23,859.95 ± 7,593	0.000
Total mass (g)	72,986.69 ± 9,162	61,197.15 ± 10,600	0.000
Body fat (%)	42.10 ± 4.98	38.14 ± 6.91	0.020
BMD LH (g/cm^2)	0.78 ± 0.06	0.66 ± 0.07	0.000
BMD spine (g/cm^2)	0.89 ± 0.08	0.72 ± 0.07	0.000
Bone mineral content (g)	1,790.69 ± 227	1,510.26 ± 272	0.000
Lean + BMC (g)	42,013.52 ± 4,282	37,337.20 ± 4,269	0.000

All values are given as mean ± standard deviation.
LH – left hip; for other abbreviations see under Table 1.

Table 3

Correlation between bone mineral density (BMD) and muscle parameters

Parameters	Hip BMD		Spine BMD	
	r	*p*-value	*r*	*p*-value
Lean mass	0.416	0.01	0.418	0.001
Fat mass	0.473	0.000	0.480	0.000
Handgrip test	0.331	0.007	0.243	0.049
Gait speed	0.268	0.031	0.232	0.061

r − **Pearson correlation coefficient.**

Table 4

Correlation between body composition parameters, bone mineral density (BMD), and Homeostasis Model Assessment of Insulin Resistance (HOMA-IR)

Parameters	*r*	*p*-value
Lean mass	0.163	0.213
Fat mass	0.322	0.012
Total mass	0.287	0.026
Body mass index	0.381	0.003
Waist circumference	0.405	0.001
Hip BMD	0.264	0.043
Spine (L1-L4) BMD	0.362	0.005
T score Hip	0.306	0.019
T score Spine	0.359	0.005
Handgrip test (kg)	0.031	0.815
Gait speed (m/s)	0.121	0.356
FRAX Index 1	-0.070	0.588
FRAX Index 2	-0.111	0.389

r − **Pearson correlation coefficient; Fracture Risk Assessment tool (FRAX) Index 1 – the probability of a major osteoporotic fracture (clinical fracture of the spine, forearm, hip, or shoulder); FRAX Index 2 – the probability of hip fracture.**

Univariate regression analysis with HOMA-IR as the dependent variable showed marginally significant associations between HOMA-IR and lumbar spine BMD ($p = 0.055$, stand. beta coefficient = 0.311), which means that an increase of HOMA-IR will lead to an increase of values of BMD of the spine (Table 5). Furthermore, univariate regression analysis confirmed the association between HOMA-IR and changes in the T-score of the spine ($p = 0.009$, stand. beta coefficient = 0.387) (Table 6). Regarding the significance of blood markers as predictors, we statistically confirmed the significance of Insulin level ($p = 0.000$, stand. beta coefficient = 0.241), glucose level ($p = 0.000$, stand. beta coefficient = 0.350) and inversed association and marginally significance of free thyroxine (fT4) levels ($p = 0.071$, stand. beta coefficient = -0.027) (Table 7).

Considering that the association between other tested parameters and HOMA-IR appears to be statistically insignificant in univariate linear regression analysis, other variables are not recognized as predictors of changing HOMA-IR values in postmenopausal women (Tables 5–7).

Table 5

Univariate linear regression analysis between HOMA-IR and hip BMD, femoral neck BMD and lumbar spine BMD

Variables	Unstandardized coefficient	SE	Standardized coefficient	*t*	Sig.	95.0% CI for B	
	(B)		β			lower bound	upper bound
Hip BMD	0.602	3.315	0.036	0.181	0.857	-6.036	7.239
Femoral neck BMD	0.544	3.128	0.032	0.174	0.863	-5.719	6.807
Lumbar spine BMD	4.334	2.211	0.311	1.960	0.055	-.093	8.762

Dependent variable: HOMA-IR; Predictors (constant): lumbar spine BMD, femoral neck BMD, hip BMD.
B – coefficient of the model; Sig. – significance level; CI – confidence interval; for other abbreviations, see under Table 1.

Table 6

Univariate linear regression analysis between HOMA-IR and T and Z score (hip and spine) and Fracture risk Assessment Tool (FRAX 1 and 2)

Variables	Unstandardized coefficients	SE	Standardized coefficients	*t*	Sig.	95.0% CI for B	
	(B)		(β)			lower bound	upper bound
T score (hip)	0.136	1.431	0.066	0.095	0.924	-2.732	3.005
Z score (hip)	0.578	1.394	0.274	0.414	0.680	-2.218	3.373
T score (spine)	0.588	1.369	0.387	0.429	0.009	-2.156	3.332
Z score (spine)	-0.234	1.385	-0.154	-0.169	0.867	-3.011	2.544
FRAX-1	0.073	0.122	0.287	0.594	0.555	-0.172	0.317
FRAX-2	-0.027	0.240	-0.062	-0.114	0.910	-0.509	0.454

Dependent variable: HOMA-IR; Predictors (Constant): T and Z score (hip and spine), Fracture risk Assessment Tool FRAX Index 1 and 2 (FRAX Index 1 – the probability of a major osteoporotic fracture (clinical fracture of the spine, forearm, hip, or shoulder); FRAX Index 2 – the probability of hip fracture).
B – coefficient of the model; SE – standard error; Sig. – significance level; CI – confidence interval; for other abbreviations, see under Table 1.

Table 7

Univariate linear regression analysis between Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) and blood markers

Variables	Unstandardized coefficient (B)	SE	Standardized coefficient (β)	*t*	Sig.	95% CI for B	
						lower bound	upper bound
Vitamin D	-0.002	0.002	-0.015	-1.021	0.313	-0.006	0.002
Somatotropin	-0.011	0.010	-0.015	-1.143	0.259	-0.031	0.009
IGF	0.000	0.001	-0.005	-0.367	0.715	-0.002	0.001
TSH	0.029	0.021	0.019	1.407	0.166	-0.013	0.071
fT4	-0.013	0.007	-0.027	-1.852	0.071	-0.028	0.001
TgAt	0.095	0.000	0.010	0.717	0.477	0.000	0.000
TPOAt	0.078	0.000	-0.007	-0.486	0.630	0.000	0.000
PTH	-0.001	0.001	-0.011	-0.767	0.447	-0.003	0.001
Insulin	0.248	0.004	0.925	65.560	0.000	0.241	0.256
Glucose	0.414	0.032	0.186	13.112	0.000	0.350	0.477

Dependent Variable: HOMA-IR; Predictors: (Constant), Glucose, Vitamin D, somatropin; TPOAt – anti-thyroid peroxidase antibodies; TSH – thyroid-stimulating hormone, fT4 – free thyroxine; TgAt – antithyroglobulin antibody; IGF – insulin growth factor; PTH – parathyroid hormone.

B – coefficient of the model; Sig. – significance level; SE – standard error; CI – confidence interval.

No significant difference was observed ($p = 0.935$) in age between women with osteoporosis (M = 69.5 years) and women in group with normal bone mass/osteopenic (M = 70 years). All participants were postmenopausal. The subjects' mean BMI was 26.11 kg/m^2 and ranged from 15.6 to 35.6 kg/m^2. The mean BMI for women with normal BMD/osteopenia was 15% higher (28.57 kg/m^2) than in women with osteoporosis (24.88 kg/m^2) ($p < 0.001$). The group of women with normal BMD/osteopenia had an 18% higher body mass ($p < 0.001$), 13% more LM ($p < 0.001$), and even 30% more FM ($p < 0.001$). This group of women had about 9% more LMI ($p = 0.003$) and about 25% more FMI ($p = 0.001$). HOMA-IR had a mean of 1.92 in the subjects and ranged from 0.2 to 6.7. HOMA-IR values were 1.56 in the subjects with osteoporosis and 2.68 in the group with normal BMD/osteopenia (Table 3).

Discussion

The association between muscle properties, BMD, and insulin resistance in this study was evaluated based on body composition parameters, muscle strength, and physical performance [17]. Based on these parameters, we provide clinical evidence that body composition changes, muscle strength, and physical performance are associated with decreased BMD. In addition, adipose tissue accumulation and an increase in total mass are closely related to insulin resistance. Finally, we confirmed the association between BMD and insulin resistance in postmenopausal women in Serbia.

FM is a significant source of proinflammatory cytokines that mediate bone metabolism, and postmenopausal women tend to accumulate visceral fat. Some authors [16, 18] reported in their studies the independent effect of FM on BMD over estrogens, insulin, and leptin. It was also observed that the relative contribution of body composition parameters to BMD depends on gender, ethnicity, and age [19].

Ho-Pam et al. [8], in their study, state that LM and FM are significant precursors to BMD. Our study results are consistent with the fact that a positive correlation was obtained between LM and FM and BMD on the hip and spine. Several studies have suggested a positive correlation between HGT and BMD in elderly people [18], whereas some studies have suggested the opposite [19]. Our study is consistent with the study that revealed a significant positive correlation between muscle strength and BMD. The results showed that HGT had a high degree of positive correlation with the BMD of the hip and a significant positive correlation with the BMD of the lumbar spine. Although multiple physiological and psychological factors influence GS, this is the most useful clinical practice test. It appeared to be a significant predictor of health events in the elderly [20]. GS had the highest degree of positive correlation with hip BMD, which confirms that maximum GS can be a useful and specific test for predicting bone status in older postmenopausal women [21].

In the present study, LM, muscle strength, and physical performance were not associated with insulin resistance. In contrast, adipose tissue and BMD on the hip, and especially on the spine, were significantly associated with insulin resistance: women with higher adipose tissue showed higher insulin resistance levels. Therefore, our study implies that the reduction of LM accompanied by its damage and the accumulation of adipose tissue contributes to insulin resistance development. Study results are in line with results reported by Park et al. [22]. These authors state in their research that LM reduction with muscle damage and fat accumulation has a close positive relationship with developed insulin resistance. The relationship between BMD and insulin resistance has been studied in different populations, and mixed results have been obtained. In a study by Kalamari M. et al. [2] involving Caucasian postmenopausal women, a statistically significant positive association between hip BMD and insulin resistance was demonstrated [2], which is consistent with our study results. The main predictors in changing the metabolic profile in postmenopausal women are lumbar spine BMD, T score of the spine, fT4, insulin, and glucose levels. Study results are in accordance with the

results of the study by Srikanthan et al. [23]. They confirmed the association between insulin resistance and strength femoral neck and suggested that obesity and hyperinsulinemia may not be bone-protective. They add just that to the growing body of evidence that points to the importance of measuring bone strength relative to load in assessing and understanding fracture risk [23]. This is consistent with our results, which indicate that although it has been found that there is a positive correlation between insulin resistance and BMD, there is no correlation between insulin resistance and fracture risk. This means that women with higher insulin resistance levels and higher BMD do not have a lower fracture risk.

Several studies have investigated the correlation between muscle parameters, fat accumulation, and insulin resistance [23]. With aging, muscle mass is lost, and muscle damage and fat accumulation occur. Specifically, the infiltration of muscle tissue by fat leads to the activation of apoptotic cells and the release of inflammatory cytokines and adipokines, leading to the development of insulin resistance [24]. On this basis, the idea that local inflammation in the muscle followed by the accumulation of fat by secretion of cytokines and adipokines instead of a decrease in muscle strength and physical performance may have a more important role in the production of insulin resistance [25]. In this regard, our results may provide clinical evidence to support the results of other studies.

Conclusion

The results suggest that LM and FM significantly affect BMD and muscle strength, and physical performance in postmenopausal women. Besides, a decrease in LM, muscle damage, and fat buildup is associated with a higher incidence of insulin resistance in these women. Finally, BMD on the hip, and especially on the spine, is associated with the onset of insulin resistance. However, there is no correlation between insulin resistance and fracture risk. These results significantly contribute to understanding the changes that occur in the body with aging in postmenopausal women.

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Red blood cell alloimmunization in pregnancy: A 10-year single-center study

Desetogodišnja studija jednog centra o aloimunizaciji eritrocitnim antigenima u trudnoći

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Abstract

Background/Aim. Pregnancy-induced red blood cell (RBC) alloimmunization is important not only because of the possible negative effects on subsequent pregnancy outcomes in case the fetus carries the antigen but also because of the optimal transfusion management in cases of obstetric hemorrhage. Timely detection of RBC antibodies is part of testing, prevention, and treatment strategy, aimed at achieving better outcomes for alloimmunized mothers with an affected fetus. The aim of the study was to determine the frequency and specificity of alloantibodies among pregnant women from the South Bačka District, Serbia, with special attention to the incidence of anti-D alloantibodies. **Methods.** A retrospective study was conducted in the Blood Transfusion Institute of Vojvodina and covered the period from January 1, 2010, to December 31, 2019. Screening and antibody identification were performed by an indirect antiglobulin test in gel-microcards (ID-Card Liss/Coombs) with two test RBC (ID-DiaCell I-II screening cells, Bio-Rad, Cressier, Switzerland) on an automated system (IH-500, Bio-Rad). **Results.** Among 25,694 tested pregnant women, 1.38% were actively immunized, while 1.12% of women acquired antibodies in the current pregnancy. Among 3,622 (14.09%) RhD-negative women, 1.77% produced anti-D antibodies during the ongoing pregnancy. Distribution of antibody specificity was: anti-D 23.34%, anti-M 11.85%, anti-E 9.41%, anti-K 9.41%, anti-C 5.92%, anti-Fy^b 5.92%, anti-c 3.13%, anti-S 3.13%, anti-Le^a 3.13%, anti-Le^b 3.13%, anti-C^w 1.75%, anti-Jk^a 1.40%, anti-P 1.05%, anti-Lu^b 0.70%, anti-Fy^a 0.35%, autoantibody of undetermined specificity 0.70%, and irregular antibodies of undetermined specificity 15.68%. **Conclusion.** Immunoglobulin prophylaxis has led to a significant reduction in the frequency of D-alloimmunization among pregnant women in the South Bačka District over the last ten years. However, the incidence of anti-D antibodies is still significantly higher than in published data for developed countries. We also identified the other, less commonly present, clinically significant antibodies. There is a need to introduce uniform recommendations for immunohematological testing in pregnancy on the territory of the Republic of Serbia in accordance with modern requirements.

Key words:
blood group antigens; blood group incompatibility; erythrocytes; immunity, humoral; prenatal diagnosis; rh-hr blood – group system.

Apstrakt

Uvod/Cilj. Aloimunizacija eritrocitnim antigenima izazvana trudnoćom nije važna samo zbog mogućih negativnih efekata na ishode sledećih trudnoća, ukoliko fetus nosi određeni antigen, već i zbog optimalnog upravljanja transfuzijom u slučajevima akušerskog krvarenja. Pravovremeno otkrivanje antieritrocitnih antitela deo je strategije ispitivanja, prevencije i lečenja, kako bi se postigao bolji ishod za aloimunizovane majke sa ugroženim fetusom. Cilj rada bio je da se utvrdi učestalost i specifičnost aloantitela među trudnicama južnobačkog okruga, Srbija, sa posebnom pažnjom na učestalost anti-D aloantitela. **Metode.** Retrospektivnom studijom, sprovedenom u Zavodu za transfuziju krvi Vojvodine, obuhvaćen je period od 1. januara 2010. do 31. decembra 2019. godine Skrining i identifikacija antitela kod trudnica vršeni su indirektnim antiglobulinskim testom u gel-mikrokarticama (*ID-Card Liss/Coombs*), dvoćelijskim test eritrocitima (*ID-DiaCell I-II screening cells, Bio-Rad, Cressier, Switzerland*), automatizovanim sistemom (IH-500, Bio-Rad). **Rezultati.** Među 25 694 testiranih trudnica ustanovljeno je da je 1,38% aktivno imunizovanih, dok su kod njih 1,12% ova antitela nastala u tekućoj trudnoći. Među 3 622 (14,09%) RhD-negativnih žena, njih 1,77% je produkovalo anti-D antitelima tokom tekuće trudnoće. Raspodela antitela prema specifičnosti bila je: anti-D 23,34%, anti-M 11,85%, anti-E 9,41%, anti-K 9,41%, anti-C 5,92%, anti-Fy^b 5,92%, anti-c 3,13%, anti-S 3,13%, anti-Le^a 3,13%, anti-Le^b 3,13%, anti-C^w 1,75%, anti-Jk^a 1,40%, anti-P 1,05%, anti-Lu^b 0,70%, anti-Fy^a 0,35%,

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autoantitela čija specifičnost se nije mogla odrediti 0,70%, i iregularna antitela čija specifičnost se nije mogla utvrditi 15,68%. **Zaključak.** Profilaksa imunoglobulinom značajno je smanjila učestalost D-aloimunizacije među trudnicama južnobačkog okruga tokom poslednjih deset godina, ali je učestalost anti-D antitela i dalje znatno viša od objavljenih podataka za razvijene zemlje. Takođe, identifikovali smo prisustvo i drugih, ređe prisutnih, ali klinički značajnih antitela. Potrebno je uvesti jedinstvene preporuke za imunohematološka ispitivanja u trudnoći na teritoriji Republike Srbije, u skladu sa savremenim zahtevima.

Ključne reči:
krvne grupe, antigeni; krvne grupe; nepodudaranje; eritrociti; imunitet, humoralni; dijagnoza, prenatalna; krvne grupe, rh-hr sistem.

Introduction

The blood group antigens recognized by the Working Party on Red Cell Immunogenetics and Blood Group Terminology (ISBT) are formally registered within 38 blood group systems [1]. The blood group systems consist of one or more antigens with varying importance to the transfusion care of patients and to the outcome of pregnancies in which the child inherits from the father an antigen that the mother does not possess [2–3].

Pregnancy-induced red blood cell (RBC) alloimmunization is important not only because of the possible negative effects on subsequent pregnancy outcomes if the fetus carries the antigen but also because of the optimal transfusion management in cases of obstetric hemorrhage. Timely detection of RBC antibodies is part of testing, prevention, and treatment strategy in order to achieve better outcomes for alloimmunized mothers with an affected fetus. The testing protocols are designed to provide recommendations for blood grouping and RBC antibody testing in pregnancy in order to protect pregnant women and their children [4].

The antigenic difference between the pregnant woman and her fetus, the patient's immune status, and the immunogenicity of the RBC antigens are some of the factors that influence the formation of antibodies. The Rhesus D antigen (RhD) has the greatest importance in pregnancy even though, in the case of maternal and infant RhD incompatibilities, there is the possibility of preventing immunization with human anti-D immunoglobulin. In the general population of Vojvodina (north part of Serbia), approximately 84% of the population is RhD-positive, while 16% is RhD-negative [5].

Once an antibody to RBC has been detected during immunohematological testing of pregnant women, their specificity must be determined in order to predict the possibility of occurrence of hemolytic disease in the fetus and newborn (HDFN) [6]. Pregnant women with antibodies (anti-D, anti-c, and anti-K) known for having the potential to cause clinically significant HDFN are monitored more frequently, according to established algorithms which define the frequency of testing, measurement of antibodies concentration, referrals to specialist examinations, monitoring after childbirth, etc. [6–8]. In the case of immunization, the child's biological father is also being tested (ABO blood group, Rh phenotype, the presence of the specific antigen). Defining the RhD fetal genotype from fetal cells and cell-free fetal DNA (cffDNA) in maternal plasma has opened up opportunities for improved noninvasive prenatal testing and allowed invasive procedures, such as amniocentesis and chorionic villus sampling, to be abandoned [9]. Cordocentesis can also be done after week 18 of pregnancy in order to perform the basic immunohematological analysis and to detect and treat fetal anemia in the pregnancies at risk (when pregnant women have clinically significant antibodies in a concentration above the critical titer). Collecting fetal blood by cordocentesis also became rare due to a high risk of complications to the child [4]. Monitoring of alloimmunized pregnancies with a risk of fetal anemia is possible using the fetal middle cerebral artery Doppler [4, 7].

Compared to other causes, immune-mediated fetal and neonatal hemolysis are accompanied by a higher bilirubin level in the early stages after birth and a longer term of hyperbilirubinemia. In the most severe cases, phototherapy and immunoglobulin therapy are not sufficient, and hyperbilirubinemia must be treated with exchange transfusion [10, 11]. For all neonates at risk, cord blood should be taken immediately after the delivery in the following cases: if the mother's group was RhD-negative, if the mother's group was O, if antibody screening for the mother was not done or if the result was positive [11]. The routine testing includes ABO grouping and RhD typing, as well as a direct antiglobulin test (DAT). These tests provide information on indications for the use of anti-D immunoglobulin in RhD-negative women [12–14]. In the DAT-positive cases, the antibodies can be obtained by removing the bound antibodies of the neonatal RBC (elution). There are several different techniques for elution based on the process used to cause dissociation of the antigen/antibody complex. The choice of elution technique depends on the type of expected antibodies. Then, that eluate is tested against screening RBC [15].

Immunohematological testing of pregnant women is undertaken to determine the presence of RhD antigens, identify RhD-negative women who need anti-D immunoglobulin prophylaxis, and identify women with clinically significant RBC alloantibodies. This testing also serves as assistance in the diagnosis and treatment of HDFN [4].

The incidence of alloimmunization in pregnant women has rarely been reported in Serbia. The aim of the study was to determine the specificity and frequency of RBC alloantibodies in pregnant women of the South Bačka District of the Autonomous Province of Vojvodina (the northern part of Serbia). Special attention was paid to the

incidence of anti-D antibodies in order to compare the obtained results with data from other published studies and to evaluate anti-D immunoglobulin protection in the District.

Methods

Study design

The retrospective study was conducted to assess the frequency and specificity of RBC alloantibodies in pregnant women from January 1, 2010, to December 31, 2019. Testing was carried out at the Blood Transfusion Institute of Vojvodina (BTIV), Novi Sad, Serbia, the secondary/tertiary health care referral provider center. BTIV is the reference transfusion institution responsible for the antenatal RBC antibody screening and monitoring of alloimmunized women as well as neonatal testing. Data gathered from transfusion registers and information system of BTIV included: the age of the pregnant women, primary and other diagnoses, transfusion or pregnancy history, ABO blood groups, and RhD antigen typing of pregnant women, antibodies screen results, and identification results. The primary focus of this study was the presence of alloantibodies, antibody specificity, and its association with other patient characteristics.

The study was approved by the Ethics Committee Number 1/2020, on February 17, 2020.

Laboratory testing

At the first antenatal visit, basic demographic characteristics and a comprehensive history of all previous pregnancies were taken (miscarriages, ectopic pregnancy, known alloimmunizations, physical intervention with associated risks to the fetus, previous fetal-maternal hemorrhage and blood transfusion, previous anemic fetus or infant, etc.) in order to detect risk factors for the woman and her fetus.

Routine laboratory testing of maternal blood samples included ABO grouping and RhD typing, as well as screening for irregular RBC antibodies by indirect antiglobulin test (IAT). Further testing included: 1. Rh phenotyping – for RhD-negative women and the presumed father; 2. monitoring of RBC alloantibody development – for pregnant RhD-negative women; 3. antibody identification and monitoring of antibody titers – for pregnant women with positive antibody screening and clinically significant antibodies.

An IAT with gel cards (ID-Card Liss/Coombs) with two RBC test cells (ID-DiaCell I-II screening cells, Bio-Rad, Cressier, Switzerland) was used to detect antibodies in the serum of women during pregnancy. Testing was performed on an automatic immunoassay analyzer (IH-500, Bio-Rad).

In case of a positive antibody screen, antibody identification was performed on LISS/Coombs gel card using commercially available eleven RBC antibody identification panels (ID-DiaPanel, Bio-Rad, Cressier, Switzerland) typed for all clinically relevant antigens (D, C, E, c, e, C^w, K, k, Kp^a, Kp^b, Js^a, Js^b, Fy^a, Fy^b, Jk^a, Jk^b, Le^a, Le^b, P, M, N, S, s, Lu^a and Lu^b). The antibody titer was determined by the test tube method [15]. In the case of anti-D antibody detection, the differentiation between prophylactic and alloimmune anti-D antibodies was done based on anamnestic data (preexisting anti-D antibodies, data of receiving routine antenatal anti-D prophylaxis, complications during pregnancy) and increase/decrease of anti-D antibody titer.

Antibody screening for all pregnant women was primarily performed in the first trimester of pregnancy. All pregnant women were advised to repeat the check of irregular RBC antibodies during the third trimester. RhD-negative and screening-positive pregnant women were controlled more often because of the risk of developing HDFN. The frequency of testing repetition (antibody screening and identification as well as antibody titration) depends on the specificity and strength of the antibody [4].

Statistical analysis

Data collected were analyzed using the statistical program Minitab 16. Descriptive statistics were conducted for all variables. Data are presented in tables and graphs. Statistical significance was set at $p < 0.05$.

Results

A total of 25,694 pregnant women were tested for ABO blood groups, RhD antigens, and antibody screening over the 10-year study period. Out of those tested, 3,622 (14.09%) were RhD-negative and 22,072 (85.91%) were RhD-positive.

A total of 761 (2.96%) antibodies were found, and out of that: 407/761 (53.48%) antibodies were passively introduced as anti-D antibodies as part of the prevention of RhD alloimmunization and 354/761 (46.52%) antibodies were actively produced. Passive anti-D antibodies were found in 128/407 (31.45%) first-time pregnant women, as well as in 279/407 (68.55%) women in second or subsequent pregnancies.

The alloimmunization rate of all pregnant women was 1.38% (354/25,694): 18.93% (67/354) of multiparous pregnant women with antibodies detected during previous pregnancies and 81.07% (287/354) of pregnant women with antibodies detected during the current pregnancy. The alloimmunization rate of pregnant women during the ongoing pregnancy was 1.12% (287/25,694). The Fisher test showed an extremely statistically significant difference between actively immunized RhD-negative pregnant women 4.53% (164/3,622) and RhD-positive pregnant women 0.56% (123/22,072) ($p < 0.0001$).

Anti-D alloantibodies were detected in 1.77% (67/3,622) of RhD-negative pregnant women during the ongoing pregnancy.

None of the pregnant women had a positive history of RBC transfusion.

The specificity of RBC antibodies detected during previous pregnancies is shown in Table 1. Only two pregnant

Table 1

Specificity of red blood cell (RBC) antibodies which are the result of previous pregnancies

Mothers' RhD status	RBC antibodies specificity					
	Anti-D	Anti-D + Anti-Fy[a]	Anti-E	Anti-K	Anti-M	Total
D-positive			9	10	8	27
D-negative	20	2	8		10	40
Total	20	2	17	10	18	67

women alloimmunized during previous pregnancies had multiple antibodies (D + Fy[a]).

The specificity of RBC antibodies detected during current pregnancies is shown in Table 2.

Trend analysis of the annual number of pregnant women with anti-D alloantibodies is given in Figure 1.

The study found 287 women with antibodies detected in current pregnancy: 1) in 141/25,694 (0.55%) women who were pregnant for the first time, antibodies were detected initially at a repeated screening during the third trimester; 2) in 146/25,694 (0.66%) women who reported previous pregnancies.

The specificity of the anti-RBC antibodies produced in the current pregnancy was as follows: anti-D 23.34% (67/287), anti-M 11.85% (34/287), anti-E 9.41% (27/287), anti-K 9.41% (27/287), anti-C 5.92% (17/287), anti-Fy[b] 5.92% (17/287), anti-c 3.13% (9/287), anti-S 3.13% (9/287), anti-Le[a] 3.13% (9/287), anti-Le[b] 3.13% (9/287), anti-C[w]

Table 2

Specificity of red blood cell (RBC) antibodies detected during current pregnancies

Antibody specificity	Number	Maternal characteristics			
		RhD status		The number of pregnancies	
		D-positive	D-negative	First	More than one
Anti-D	67		67	23	44
Anti-C	17		17	17	
Anti-c	9	9		9	
Anti-E	27	20	7	27	
Anti-C[w]	5	5			5
Anti-K	27	22	5	7	20
Anti-M	34	18	16	7	27
Anti-S	9	4	5	2	7
Anti- Fy[a]	1	1			1
Anti- Fy[b]	17		17	5	12
Anti-Le[a]	9	5	4	2	7
Anti-Le[b]	9	6	3	9	
Anti-Jk[a]	4	4		1	3
Anti-P	3	3			3
Anti-Lu[b]	2		2		2
Undetermined specificity	45	25	20	30	15
Autoantibody	2	1	1	2	
Total	287	123	164	141	146

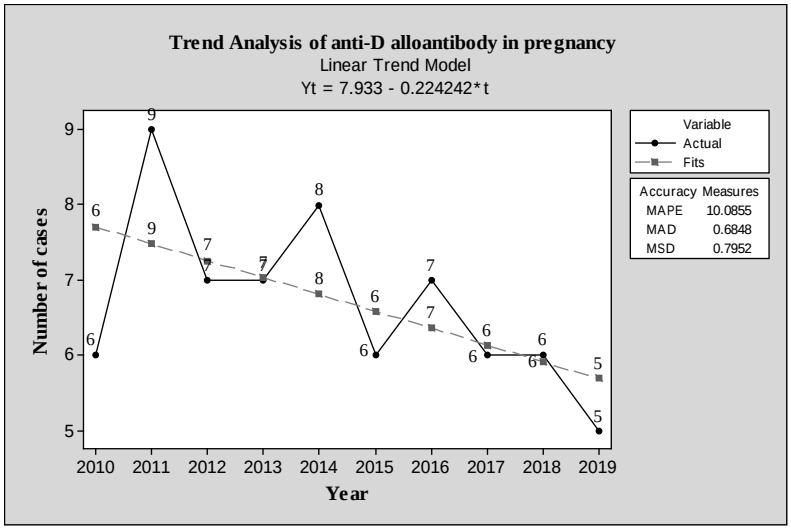

Fig. 1 – Pregnant women anti-D alloantibody, annually.

1.75% (5/287), anti-Jk^a 1.40% (4/287), anti-P 1.05% (3/287), anti-Lu^b 0.70% (2/287), anti-Fy^a 0.35% (1/287), autoantibody of undetermined specificity 0.70% (2/28,734), and irregular antibodies of undetermined specificity 15.68% (45/287).

All the pregnant women with antibodies were followed up, with an average of 3 (rank 1-6) controls for RhD-positive and 4 (rank 1-10) controls for RhD-negative pregnant women.

Discussion

The study revealed 1.12% of women who acquired anti-RBC antibodies by active immunization during an ongoing pregnancy. An extremely statistically significant difference was found between actively immunized RhD-positive pregnant women (0.56%) and RhD-negative pregnant women (4.53%). Anti-D antibodies showed the highest frequency (23.34%) among the detected antibodies. Anti-D alloantibodies were produced in 1.77% of RhD-negative pregnant women during the ongoing pregnancy.

A study conducted 20 years ago in the Republic of Serbia showed that the incidence of potentially clinically significant antibodies was 2.4%. The majority belonged to the Rh system, followed by anti-M, -Fy^a, -S, -Jk^a, and -Jk^b [16].

The 20-year retrospective epidemiological study in the West Herzegovina region found positive IAT in 1.8% of pregnant women. This study found that even though there has been significant progress in the prevention of RhD immunization, monitoring of immunization is still necessary [17]. The 15-year observational study from a tertiary care university hospital in Spain found that 76.9% of all maternal antibodies were clinically significant, and the most frequent maternal antibodies were anti-D (53%) and anti-K (19%) [18]. A five-year study conducted in West Yorkshire confirmed that the prevalence of RBC antibodies in pregnancy varies with ethnicity and geographical location. The study found RBC antibody prevalence in Yorkshire is lower compared with reports from other Caucasian populations, 1 : 242 of pregnancies (0.41%). The anti-E and anti-M antibodies were the most common [19].

Exposure to non-self RBC antigens during pregnancy may lead to the production of antibodies against RBC antigens. Antibody screening enables the detection of maternal alloimmunization early in pregnancy and a timely start of treatment. In severe cases, clinically significant maternal antibodies may lead to fetal anemia with a risk of fetal death and to severe forms of neonatal hyperbilirubinemia. Most severe HDFN cases are caused by anti-D antibodies despite antenatal – and postnatal anti-D prophylaxis in the previous pregnancy.

Although all pregnant women are exposed to fetal RBC during pregnancy and delivery, most of them do not become alloimmunized after this exposure. Exposure to immunogenic RBC antigens, such as Rhesus, Kell, Duffy, and Kidd, results in alloimmunization and leads to clinically significant HDFN. ABO incompatibility between mother and fetus plays a protective role against RBC alloimmunization [2].

In 2015, a group of authors reported the prevalence of 0.73% of unexpected anti-RBC antibodies in pregnant women in Australia, with the highest frequency of anti-E-27.6%, anti-D-10.4%, and anti-K-9.5% antibodies. The data showed that most antibodies were found in RhD-positive women and that the most commonly detected, anti-E antibodies, were present in a small number of RhD-negative women. Anti-E antibodies had a much higher detection rate compared to other studies, but the method used in this study proved both classes (IgM and IgG) of antibodies. The study showed that anti-D antibodies remain a clinically significant problem but suggested that the other antibodies can also cause severe HDFN [20]. Our study has also shown a lower frequency rate of anti-E antibodies (9.41%) than observed by the Australian study.

The Michigan Immunohematology Laboratory reported an antibody prevalence of 0.74% in pregnant women. The most common clinically significant antibodies were: anti-E (38.2%), anti-K (20.6%), and anti-M (17.6%). The most common clinically significant alloantibodies were anti-E and anti-M in RhD-positive women, and anti-K, anti-D, and anti-c in RhD-negative pregnant women [21]. The prevalence of anti-K antibodies in pregnant women was 0.15%. These antibodies lead to the suppression of erythropoiesis as a contributing factor to fetal anemia, unlike the other antibodies that cause direct erythrocyte hemolysis. In the same study, IgM class anti-M antibodies were mainly identified (naturally occurring antibodies). Compared to the US authors, we found a higher prevalence of RBC antibodies in pregnant women (1.12%), as well as a lower prevalence of anti-M (11.85%) and anti-K (9.41%) antibodies. The most frequent antibodies among South Bačka pregnant women were anti-D (23.34%), followed by anti-M, while anti-E and anti-K ranked third and fourth, respectively.

HDFN can be caused by antibodies directed against RBC antigens other than D antigen. In a study conducted in India in 2019, gynecologists' education about the necessity of antenatal antibody screening in all pregnant women was evaluated. The positive effects of the transfusion activity led to an increase in the number of RhD-positive pregnant women who came to the prenatal antibody screening (18.2% in the first phase and 72.8% in the second phase after the intervention) [22]. In order to protect pregnant women and their babies, BTIV also recommended to gynecologists a testing algorithm that included blood grouping and RBC antibody testing in pregnancy.

Studies have also shown that additional antibody screening in the third trimester of pregnancy increases the number of detected late alloimmunizations in RhD-negative pregnant women, thus enabling early treatment of HDFN [13]. Blood transfusion, multiparity, and chorionic villus sampling/amniocentesis are known risk factors for alloimmunization. A much faster secondary immune response, due to the action of these factors before the current pregnancy, explains most late alloimmunizations [12].

Administration of routine antenatal anti-D immunoglobulin prophylaxis significantly reduces the incidence of RhD immunizations. Bollason et al. [23] examined

the prevalence of anti-D antibodies in Iceland, where postnatal anti-D immunoglobulin prophylaxis was introduced in 1969 and antibody screening in 1978. Before these measures, the prevalence of alloimmunized RhD-negative pregnant women was 1.9%. In the period 1996–2015, the incidence of anti-D antibodies was significantly reduced (1.04%). Compared to their results, pregnant women in our study had a higher prevalence not only of anti-D antibodies (1.77%) but also of total antibodies (1.12%). The most frequent antibodies among Iceland pregnant women were anti-M (19.4%), anti-E (19.0%), and anti-D (12.5%). The authors of this study highlight the unusually high prevalence of anti-M antibodies, whose cause is unclear, as well as the fact that anti-E plus anti-c were the most common antibodies combination in alloimmunized pregnancies [23].

Chatziantoniou et al. [24] from London, UK, demonstrated that morbidity and mortality caused by HDFN were minimal, and reported anti-D antibodies as the most commonly encountered, followed by anti-c and anti-E antibodies.

The frequency of alloimmunization in pregnant women as well as antibody specificity varies around the world. Gavrančić et al. [25] carried out a retrospective study similar to this one from 2003 to 2010 among pregnant women from the South Bačka District. The authors found anti-D antibody specificity in 74 alloimmunized pregnant women, which represented 44% of all detected alloantibodies. Almost one decade later, our study showed a downward trend of alloimmunized pregnant women with anti-D antibodies.

In 2015 Southern Pakistan authors reported 1.6% of non-anti-D incidence and 2.9% of anti-D incidence [26]. Prevalence of RBC alloantibodies among multiparous antenatal females in North India is found to be 2% (anti-D, anti-E, anti-C, and anti-K) with anti-D antibodies most commonly encountered [27]. Ngoma et al. [28] reported incidence of RBC alloantibodies among pregnant women in Africa, ranging from 1.1% to 12.1%. Anti-D antibodies were ranked as the most common, followed by anti-K and anti-E antibodies. Al-Dughaishi et al. [29, 30] have determined an alloimmunization rate of 10% in RhD-negative Omani pregnant women and an alloimmunization rate of 2.7% in RhD-positive Omani pregnant women [29, 30]. In the Hubei province of China, among the Han population, RBC alloantibodies prevalence was 0.50%, and the most frequently identified alloantibodies were anti-E, anti-D, and anti-M [31].

Conclusion

The study found that anti-D, anti-M, anti-E, anti-K, anti-C, and anti-Fy^b were the most frequent specificities, respectively. Antenatal and postnatal anti-D immunoglobulin prophylaxis has led to a significant reduction in the frequency of D-alloimmunization over the last ten years but did not eliminate D-alloimmunization. Unfortunately, the incidence of anti-D antibodies among alloimmunized pregnant women in the South Bačka District is still significantly higher than published data for the developed countries.

The introduction of mandatory perinatal immunohematological testing of pregnant women and fetuses/newborns in a uniform manner throughout the Republic of Serbia, in all private and state healthcare institutions, would certainly lead to improved health care provision in this area.

Conflict of interest

None to declare.

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Correlation of neuropsychological indicators of child development with speech: empirical research underpinning the National Children's Health Prevention Program

Povezanost neuropsiholoških indikatora razvoja dece i govora: empirijsko istraživanje kao osnova pripreme nacionalnog preventivnog programa dečije zaštite

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Abstract

Background/Aim. Psychophysiological disorders and speech pathology is one of the priority public health problems in the Republic of Serbia. A child's growth and development proceed in a particular sequence and course, depending on individual physiological capacities, quality of neuromuscular structures, and stimuli, which can be significantly affected by the environment. The development of speech from birth to the adult age is a result of the interaction of neurocognitive factors that contribute to the gradual attainment of phonological presentation and motor control abilities. The aim of the study was to examine the connection between manipulative manual dexterity, oral praxis, and lateralization with the development and speech in children and their potential for the development of prevention programs. **Methods.** The sample included 60 children from Belgrade of both sexes, aged between 5.5 and 7 years. Of these, 30 made up the experimental group – children with deviations in speech development (articulation disorders), and 30 made up the control group – typically developing children from the general population. The instruments used were the Manipulative Manual Dexterity Test (Lafayette), Oral Praxis Test, and part of the Test for assessing lateralization (auditory, visual, and functional). **Results.** The hand grip development level was statistically significantly different between the experimental and the control group ($\chi^2 = 21.40$, df = 3, $p < 0.01$). The presence of associated involuntary movements statistically significantly differed between the experimental and the control group examinees ($\chi^2 = 10.58$, df = 1, $p < 0.01$). Oral praxis statistically significantly differed between the experimental and the control group ($t = 2.01$, $p < 0.05$). Visual laterality statistically significantly differed ($\chi^2 = 7.56$, $p < 0.05$) between the observed groups. When all predictors were taken into account, significant contribution to the explanation of the existence of speech pathology (articulation disorders) was given by the variables: Visual lateralization [odd ratio (OR) = 0.38; 95% confidence interval (CI) = 0.179–0.832; $p = 0.015$] and the level of hand grip development (OR = 0.23; 95% CI = 0.082–0.699; $p = 0.009$). **Conclusion.** The hand grip development level and visual laterality are worse in children with speech (articulation) impairment and developmental deviations. These indicators of child development should be used as a guide in designing the national programs for developmental assessment and prevention in the child healthcare system.

Key words:
child; growth and development; motor activity; national health program; serbia; speech; speech disorders.

Apstrakt

Uvod/Cilj. Psihofiziološki poremećaji i patologija govora su jedan od prioritetnih problema javnog zdravlja u Republici Srbiji. Rast i razvoj deteta odvijaju se određenim tokom i redosledom, zavisno od individualnih fizioloških kapaciteta, kvaliteta nervno-mišićnih struktura i podsticaja, na koje sredina može značajno da utiče. Razvoj govora od rođenja do odraslog doba rezultat je interakcije neurokognitivnih faktora pomoću kojih se postepeno stiču sposobnosti fonološke prezentacije i motorne kontrole. Cilj istraživanja bio je da se ispita povezanost manipulativne manuelne spretnosti, oralne praksije i lateralizovanosti sa razvojem i govorom kod dece i njihov potencijal za izradu preventivnih programa. **Metode.** Uzorkom je obuhvaćeno 60 dece iz Beograda, oba pola, uzrasta od 5,5 do 7 godina. Njih 30 činilo je eksperimentalnu grupu – deca sa odstupanjima u razvoju govora (artikulacioni poremećaji) i

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30 kontrolnu grupu – deca urednog razvoja, iz opšte populacije. Od „instrumenata“ ispitivanja korišćeni su: test manipulativne manuelne spretnost (*Lafayette*), test oralne praksije i deo testa za procenu lateralizovanosti (auditivna, vizuelna i upotrebna). **Rezultati.** Nivo razvijenosti hvata je bio statistički značajno različit kod eksperimentalne i kontrolne grupe (χ^2 = 21,40, df = 3, p < 0,01). Prisustvo nevoljnih kretnji bilo je statistički značajno različito kod ispitanika eksperimentalne i kontrolne grupe (χ^2 = 10,58, df = 1, p < 0,01). Oralna praksija je bila statistički značajno različita kod eksperimentalne i kontrolne grupe (t = 2,01, p < 0,05). Vizuelna lateralizovanost je bila statistički značajno različita (χ^2 = 7,56, p < 0,05) među posmatranim grupama. Kada se uzmu u obzir svi prediktori, u višestrukom regresionom modelu statistički značajan doprinos u objašnjenju postojanja patologije govora (poremećaji artikulacije) dali su vizuelna lateralizovanost [*odd ratio* (OR) = 0,38; 95% *confidence interval* (CI) = 0,179–0,832; p = 0,015) i nivo razvijenosti hvata (OR = 0,23; 95% CI = 0,082–0,699; p = 0,009). **Zaključak**. Nivo razvijenosti hvata i vizuelna lateralizovanost je lošija kod dece sa oštećenjem govora (artikulacije) i odstupanjima u razvoju. Ove indikatore razvoja deteta trebalo bi iskoristiti u kreiranju nacionalnih programa procene i prevencije razvoja u sistemu zdravstvene zaštite dece.

Ključne reči:
deca; rast i razvoj; aktivnost, fiička; zdravstveni programi, nacionalni; srbija; govor; govor, poremećaji.

Introduction

Most activities in the first seven years of life are part of one process of organizing nerve impulses in the nervous system. Nerve impulses arise as a result of the direct influence of stimuli. As the child experiences stimuli during its life, learning how to organize them in the brain and discovering what each of them means, it learns how to focus its attention on a particular one, disregarding all the others. Additionally, by organizing the stimuli, the child gains control over its perceptual experiences. Nerve impulses must pass through two or more neurons to shape a sensory experience, a motor response, or an opinion. The more complex the functioning, the more neurons become involved in the message transmission. The nervous system of each human being operates in a particular, distinct manner [1].

The function of neurons is determined by the localization and a series of other circumstances throughout development. One of the basic postulates of development is that functionally higher parts develop under the influence of lower parts (e.g., development of the thalamus induces further development of the cerebral hemispheres) [2]. Neurons, by way of their intercellular connections, are organized into dynamic functional systems. During development, there occur morphological changes that form the basis of cognitive functions and various skills gained during individual maturation.

The root causes of behavioral variability and flexibility are morphological and structural changes in neurons, as well as a multitude of established synaptic connections. For neurons to develop interconnections, they must be stimulated. The development of new connections generates new possibilities for neural communication. Each new connection adds another element to the sensory perception and motor ability of a child. The more neural connections, the more capable the child is of learning [1]. Psychomotor activities with exposure to various stimuli (that stir different senses eliciting thus psychomotor response) are the stimulating factors that promote the maturation of the nervous system.

Growth and development are interrelated but not necessarily interdependent. Growth can be defined as a combination of increases in both the number and size of cells. Development is the increase in the complexity of an organism due to nervous system maturation. A child can develop normally but have a delay in growth and *vice versa*. Growth can be measured precisely but presenting the measurement of development in numbers is much more difficult and almost impossible [3]. Development means synchronized motor, intellectual, and emotional maturation. When we assess development, we can reduce this assessment to four major areas: gross motor skills (basic motor movements), fine motor skills (differentiated motor movements), speech and language, and social development.

Speech development cannot be observed solely through biological development as is, for example, the case with walking, because, once the neurological basis becomes mature, a child gradually, by itself, starts walking, which is genetically programmed, while it will never occur with speech however predisposed the child is to that function. The reason is that speech originates exclusively from the biolinguistic conjunction, that is, one of neurobiological potential and verbal social environment. Development of speech from birth to the adult age is the result of the interaction of neurocognitive factors that lead to a gradual acquisition of the abilities of phonological presentation and motor control in the presence of a range of physical and physiological changes in the morphology of the articulation system [4]. Given the dynamics of growth and development, as well as the plasticity of the nervous system, a preschool-age child is particularly susceptible to the overall influences that are, in that period, the most enduring and efficient.

It stands as an obligation of every serious society to ensure in the best possible way the timely assessment of the psychophysiological development of every child from its birth. Early detection of any, even the minimal, developmental disability should be a signal for a thorough monitoring and timely undertaking of the preventive and therapeutic stimulation of development.

Psychophysiological disorders and speech pathology pose a global problem, particularly in transition countries. Every year a significant rise in the number of children with developmental disabilities is recorded. It is estimated that, worldwide, there are about 11% of children with

psychophysiological disorders of varying types, including speech pathology [5]. Speech disorders occur both in children with developmental disabilities and in the general population [6]. Although no accurate epidemiological studies exist, it is estimated that about 20–30% of the children population in our environment suffers from some form of psychophysiological and speech disorder. If we add to this figure data on the acquired disorders that can evolve in children after the period of speech acquisition, then the proportion of the population with psychophysiological and speech disorders is considerably higher [7].

The development/review of the National Program for the Prevention and Treatment of Psychophysiological Disorders is in line with the recommendations of the World Health Organization. For this exact reason, the Republic of Serbia has advocated for decades the development of a detailed and precise National Prevention Program to decrease the number of children with psychophysiological and speech disorders. One of the preventive measures is early detection, timely diagnosis, and treatment of children with developmental disabilities. Institutional capacities in the field of public health protection need to be strengthened in order to effectively implement public policies.

Governments should have a role in maintaining and improving capacities for the benefit of populations. In health, this means being ultimately responsible for the careful management of their citizens' wellbeing. The health of the people must always be a national priority: government responsibility for it is continuous and permanent. The tasks and vision of each public policy need to be defined in accordance with public interest and priorities. Consequently, exceptional knowledge and skills – competencies have been required [8]. Representatives of public institutions have a key role in both formulating and implementing government policies. In addition to this, they should devote their entire capacities to the purpose of achieving public interest [9].

The aim of the study was to examine the connection between manipulative manual dexterity, oral praxis, and lateralization with the development and speech (articulation) in children and their potential for the development of prevention programs.

Methods

The research was organized as a quasi-experiment with two observed groups. It was conducted in Belgrade, at the Institute for Psychophysiological Disorders and Speech Pathology "Cvetko Brajović", and partly at the Children's University Clinic, as well as at the development counseling service of the Medical Center Voždovac, in 2016. The study followed the tenets of the Declaration of Helsinki and was approved by the Ethics Committee of the Medical Center Voždovac. Taking into account that the research participants were children, informed consent was obtained from the parents/guardians.

The sample included 60 children of both sexes, aged between 5.5 and 7 years. The experimental group (E) consisted of 30 children with diagnosed speech (articulation) and development disorders who were on continuous treatment. The control group (C) of 30 children comprised typically developing children. We used the technique of individual testing for both the E and C groups.

The instruments used in the research were the Manipulative Manual Dexterity Test (Lafayette), Oral Praxis Test, and part of the Test for Assessing Lateralization (Auditory, Visual, and Functional hands use). Manipulative Manual Dexterity was examined using two tests. The aspects monitored during the tests were hand dominance, manner of selecting materials, presence of involuntary movements of extremities, and facial musculature. In the first test, examinees were given a box containing balls of four different colors and a wire on which they needed to slide colored balls in a specific order. On the agreed command, the examinee started sliding the balls and stoped doing so after 2 min on the examiner's signal. The balls slid down the wire were then counted. In the second test, examinees were given a long bolt with corresponding nuts. They were required to place as many nuts as possible on the bolt within 4 min. Both tests monitor which hand is used to pick up the ball/nut and whether the same hand is used all along, then what type of hand grip is used (the pincer, the tripod, or the palmar-for fingers hand grip, or whole hand grip) and the presence of involuntary movements. The Test for Examining Oral Praxis was conducted by way of acting in imitation of the examiner, who askeds the child to repeat the same model. The test contains 22 items (motor patterns for the orofacial region). The maximum number of points is 22, and the minimum is 0 (which would mean that the respondent cannot report any movement). The examination begins with the simplest motor patterns and, if the examinee successfully repeats a given model, proceeds toward the next – more difficult ones. By using this test, we accurately identify the condition of the orofacial region and, based on the established condition, the cause of occurrence of articulation-speech disorder. The assessment of lateralization was conducted in respect of eyes, ears, and hand use. The examinees were asked to perform certain actions as instructed using the specific objects. The examiner recorded the responses by monitoring the assessed lateralization of a sense or a hand.

Results obtained from the research were statistically processed by the appropriate selection of statistical methods. Statistical data processing was performed using the software SPSS ver. 20. Of the descriptive statistics measures, we used arithmetic mean with the associated standard deviation, as well as the minimum and the maximum. We also used frequency and percent. The χ^2 test was used to examine the relationship between two categorical variables, then the *t*-test for large independent samples, as well as univariate logistic regression and multivariate regression analysis.

Univariate logistic regression was performed to determine the individual influence of indicators-predictors on the existence/non-existence of development and speech pathology. The predictive value of the following variables was examined: Hand-Use Lateralization, Visual Lateralization, Auditory Lateralization, Assessment of Oral Practice, Dominance of the Hand, the hand grip development level, and

Presence of involuntary movements. Cox & Snell R-Squared was used as a substitute for the coefficient of determination showing the percentage of explained variance.

Results

This research involved children of ages 5.5 to 7 years. There were 60 participants, 36 of which were male and 24 female, divided into two groups. As this was a prospective cross-sectional study, the structure of the sample by gender reflected the numerical representation of the groups in the population as well. In this way, a larger number of participants within the E group were boys (76.7%), while a larger number of girls (56.7%) were within the C group. The average age of sample participants in the E group was 6.07 ± 0.5 years, and in the C group, 6.34 ± 0.46.

The Manipulative Manual Dexterity Test showed that the hand dominance, the hand grip evolution level, and the presence of involuntary movements were statistically significantly different between the examinees of the E and C groups on both trials. In the hand dominance subtest in both trials, right-handers were dominant in both groups with 60% frequency. However, there were more left-handers (40%) in the C group than in the E group (23.3%).

The hand grip evolution level showed a statistically significant difference between the E and C groups (χ^2 = 21.40, df = 3, $p < 0.01$). In the C group, most were those with a group – grip with three fingers (50%) and those with a pincher grip (46.7%), while in the E group, the examinees whose hand grip was with four fingers (36.7%) and three fingers (40%) were dominant. The presence of involuntary movements was significantly different in the subjects of the E and C groups (χ^2 = 10.58, df = 1, $p < 0.01$). In the second trial, there was a statistically significant difference in the hand grip development level (χ^2 = 21.40, df = 3, $p < 0.01$). Furthermore, on the presence of involuntary movements subtest, the inverse results were identical to the first trial (χ^2 = 10.58, df = 1, $p < 0.01$). The results obtained from the tests were summarized and the analysis was conducted on the differences between the E and the C group on three items. Statistically significant differences were found on all three items: hand dominance (χ^2 = 6.31, df = 2, $p < 0.05$), hand grip development level (χ^2 = 20.25, df = 3, $p < 0.01$), and presence of involuntary movements (χ^2 = 12.00, df = 1, $p < 0.01$) (Figure 1).

The score on the Oral Praxis Test was obtained by adding the movements that were possible, i.e., where they exist. The results of the Oral Praxis Test showed a statistically significant difference between the E and the C group regarding the overall test score (t = 2.01, $p < 0.05$). The average number of proper movements of the oral region in the C group (19.6 ± 2.20) was higher than in the E group (18.1 ± 3.64).

With the lateralization assessment, a statistically significant difference between the E and C groups existed on the subtest Visual Lateralization (χ^2 = 9.32, $p < 0.01$). The largest number of subjects in the C group belonged to the group with right lateralization (83.3%), while in the E group (36.7%), subjects belonged to the group with left lateralization, and 13.3% of them to the group of ambidextrous. Figure 2 shows that both groups were predominantly right-lateralized regarding Hand use

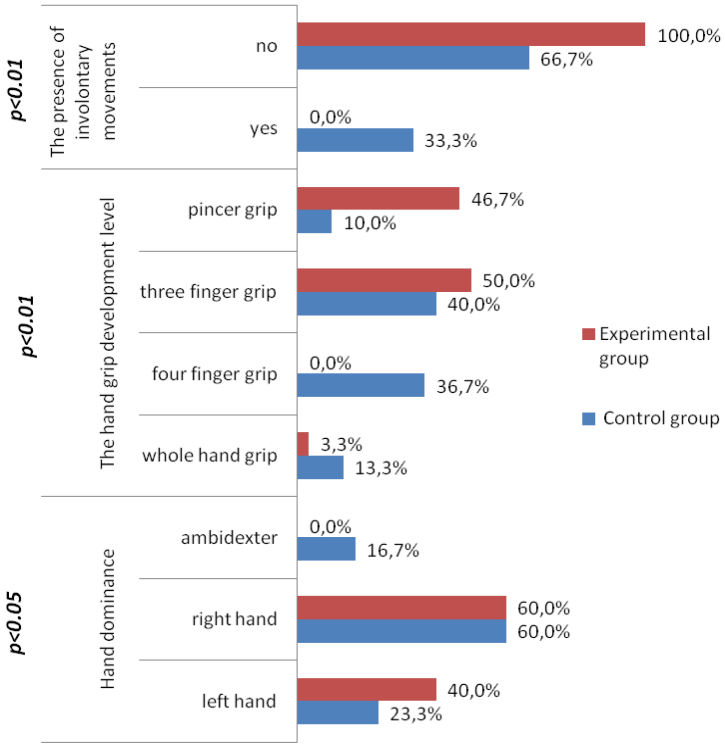

Fig. 1 – The Manipulative Manual Dexterity Test – difference between the experimental and control group.

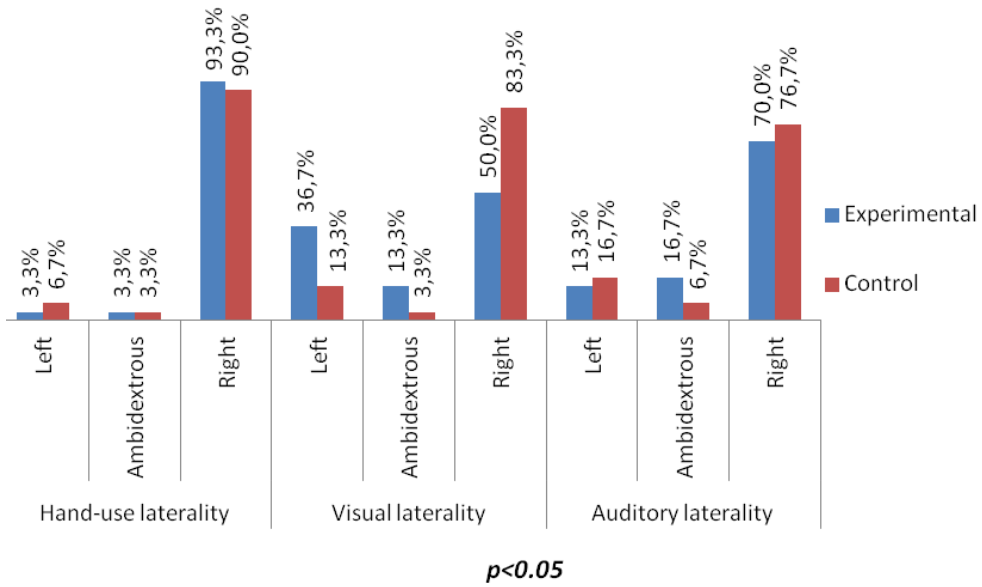

Fig. 2 – The assessment of lateralization – difference between the experimental and control group.

lateralization. By careful analysis, we can observe that the C group had more (6.7%) functionally left-handed compared to the E group (3.3%), with the number of the ambidextrous also being higher in the E group (13.3%) than in the C group (3.3%). On the subtest of Auditory lateralization, both groups are predominantly right-lateralized, but the number of ambidextrous subjects was higher in (16.7%) the E group than in the C group. After obtaining the results of the E and C groups on each subtest, we presented the results of both groups on all three subtests of lateralization (Figure 2).

Univariate logistic regression revealed that the predictors of speech pathology (articulation disorders) were: Visual Lateralization [odds ratio (OR) = 0.43; 95% confidence interval (CI) = 0.225–0.951; p = 0.015], Oral Practice (OR = 0.82; 95% CI = 0.674–1.00; p = 0.050), Hand Dominance (OR = 2.82; 95% CI = 1.08–7.36; p = 0.034), The hand grip development level (OR = 0.19; 95% CI = 0.079) -0.495; p = 0.001) (Table 1). When all predictors were taken into account, in the multiple regression model a statistically significant contribution to the explanation of the existence of speech (articulation) pathology was given by the variables: Visual lateralization (OR = 0.38; 95% CI = 0.179–0.832; p = 0.015) and The Hand Grip Development Level (OR = 0.23; 95% CI = 0.082–0.699; p = 0.009). These two variables together explain as much as 48% of the variance of the dependent variable (Table 2).

Table 1

Predictive properties of measured parameters on the existence of pathology in development and speech (articulation) – univariate logistic regression

Parameter	p	Exp(B)	95% CI for EXP(B)		Cox & Snell R-Squared	Percentage of well-classified
			lower	upper		
Hands use lateralization	0.583	1.373	0.443	4.249	0.005	51.7
Visual lateralization	0.015	0.438	0.225	0.851	0.106	66.7
Auditory lateralization	0.861	0.940	0.473	1.870	0.001	53.3
Oral Praxis Test	0.050	0.824	0.674	1.008	0.067	60.0
Hand dominance	0.034	2.822	1.081	7.368	0.080	58.3
Hand grip development level	0.001	0.198	0.079	0.495	0.272	73.3
Presence of involuntary movements	0.999	0.000	0.000		0.032	66.7

CI – confidence interval.

Table 2

Predictive properties of measured parameters on the existence of pathology in development and speech (articulation) – multivariate regression analysis

Parameter	p	Exp(B)	95% CI for EXP(B)		Cox & Snell R-Squared	Percentage of well-classified
			lower	upper		
Visual lateralization	0.015	0.386	0.179	0.832	0.480	80.0
Oral Praxis Test	0.578	0.906	0.641	1.282		
Hand dominance	0.214	2.586	0.577	11.584		
Hand grip development level	0.009	0.239	0.082	0.699		

CI – confidence interval.

Discussion

The age of examinees for purposes of this research is selected according to the opinion that, at that age, children reach a certain degree of maturity of motor, speech, and social abilities. The result shows that articulation speech disorders are more common in boys than in girls, as shown by other studies [10–12]. Timely and appropriate assessment of these abilities can point to potential deviations and deficits in the child's development. In order for a child to become fully developed, it must reach a certain degree of maturity of the nervous system, which allows it to connect with the external world [13]. Each stage of child development is characterized by certain abilities that constitute the preparatory 'elements' and a base for more complex and mature development. The psychomotor ability of hands is essential to organizing ways to exist in the social field. Its organization indicates the development degree of speech, intelligence, opinion, and feelings. The results of the Manipulative Manual Dexterity Test show that the first test records a statistically significant difference on the item "hand dominance", with the right-handers being dominant in both groups with 60% prevalence in each. However, the C group recorded more left-handers (40%) than the E group (23.3%). The E group recorded 16.7% of ambidextrous children, showing that this group had considerably more children without differentiated lateralization, which indicates slow maturation of the structures and functions that determine movement lateralization. While around 40% of children aged between 4 and 5 are weakly lateralized, this percentage drops to about 30% among those aged between 5 and 7 [14]. The hand grip development level was another item recording a statistically significant difference between the E and C groups. More dominant in the E group were those with four-finger and three-finger grip, while in the C group, those with three-finger (tripod) grip and pincer grip prevailed. The presence of involuntary movements statistically significantly differed between the E and the C group examinees. These movements were are not recorded in the C group; however, they were found in 33.3% of examinees of the E group. In the second test, there was a statistically significant difference in the hand grip development level. The results showed that hand grip was better developed among the C group examinees compared to the E group examinees. In a hierarchical development, a child first acquires simpler manipulative hand movements, such as the palmar grip, that eventually evolve into the pincer grip. In the C group, we had considerably more of those using the pincer grip, which involves the ability of proper grip (appropriate to the examined age group), characterized by thumb opposition and meaningful coordination of movements of hand segments in performing manipulative activities [15, 16]. Statistical difference was also found in the item "presence of involuntary movements". The lower prevalence of involuntary movements among the examinees of the C group relative to the E group indicates better neuromaturation of CNS within the C group. The obtained results showed that differentiated hand motor patterns are better developed in typically developing children, that is, the ones in the C group. Given that differentiated hand motor patterns hierarchically develop before oral motor patterns (responsible for oral praxis), some deficits in speech development can be expected and predicted in children without differentiated hand motor movements at a certain age [17]. The results of the Oral Praxis Test showed a statistically significant difference between the E and the C group, which shows that children with some developmental speech (articulation) deviations have a less developed oral praxis relative to typically developing children [18]. In the development and maturation of orofacial musculature, the first acts to evolve are swallowing, sucking, and chewing. Deficits in these functions at certain stages of development are the first indication of poor oral praxis [19, 20].

Lateralization assessment revealed a statistically significant difference between the E and the C group concerning visual laterality. Figure 2 shows a higher number of examinees with non-differentiated laterality in the E group and indicates the existence of disharmonic laterality, which concurrently indicates the slow maturation of certain functions among these examinees [21]. From further processing of research results, we found the following as the predictors of the existence of speech pathology (articulation disorders): hand dominance, hand grip development level, oral praxis, and visual laterality. After conducting a univariate regression analysis, the variables that proved to be statistically significant predictors in the explanation of the speech (articulation) disorders entered the multivariate regression analysis. The results showed that poorer visual lateralization increased the chance that the subject would have speech pathology by 62% and that griping with the whole hand and with four fingers increased the chance of developing speech (articulation) pathology by 77%. These two variables accurately classify 80% of participants.

These findings show that the inability to learn or immaturity of any of these functions can provide a timely indication of the delays in child development and predict future deficits in the development of speech as a more complex function. It is crucial to timely recognize disharmony and include the child in stimulating treatments to prevent potential disorders to any extent. All obtained results point to the necessity of preventive action, which should be conducted at the level of primary health care to timely prevent the occurrence of developmental disabilities. The psychophysiological growth and development of a child must be appropriate to its age; if not synchronized, whether for objective or subjective reasons, it creates disharmony in the child's development, which can particularly be observed in speech development. With a view of developing the health care system in the Republic of Serbia, special consideration is given to the protection, improvement, and promotion of health of the youngest generation. Determination of causes of disorders as well as early detection and prevention are the main aims of all health systems, including both those of our country and Europe and of the rest of the world. For this exact reason, it is necessary to develop a detailed and precise Program for preventive child protection at the national level, which

would be implemented through the level of primary health care by monitoring the child from birth. The main goals of the Program would be the promotion of and support for the health and healthy development of all children in the first years of life, particularly those with present developmental risks and disabilities. The Programs would be aimed at the primary prevention of developmental disorders by reducing and preventing the influence of risk factors and would assume the engagement of the health system and intersectoral cooperation. Another major precondition for the successful implementation of the Program is a developing partnership with families using the family orientation approach in work.

The aim of developing a National Prevention Program is to decrease the number of children with psychophysiological and speech disorders. One of the preventive measures is early detection, timely diagnosis, and treatment of children with developmental disabilities (Government of the Republic of Serbia, 2004). This National Program is one of the priority programs of the Ministry of Health, the implementation of which requires the involvement of all the defined tiers of health care delivery. The development/review of the National Program for the prevention and treatment of psychophysiological disorders is in line with the recommendations of the World Health Organization. The aims of the Program are early detection of psychophysiological disorders and speech pathology, appropriate diagnostics, and therapy aimed at decreasing the frequency of psychophysiological and speech disorders and improving the quality of life of persons with developmental disabilities and their families. The government's mission is to ensure coherence and consistency across departments and sectors by an overall reform of public administration [22].

The health care system and the organization of health services are regulated by the Health Care Act [23]. Decree on the National Program of Preventive Health Care of Children with Psychophysiological Disorders and Speech Pathology establishes the National Program for preventive health care of children with psychophysiological disorders and speech pathology and regulates the activities on early detection of children with psychophysiological and speech disorders at all tiers of health care delivery. Following the objectives of the proposed National Program, the strategy has been defined for information, education, communication, and social mobilization, along with the action plan for its implementation, time frames, and entities responsible for the implementation of the set activities in primary health care.

The key limitation of this study is the small sample size. These data can be the basis for future research in order to develop preventive and therapeutic programs.

Conclusion

The research results show that visual lateralization and the level of hand grip are worse in children with impaired speech (articulation) compared to typically developing children. Poorer visual lateralization increased the chance that the subject would have speech pathology by 62%, and that griping with the whole hand and with four fingers increased the chance of developing speech-articulation pathology by 77%. The ability of fine motor coordination, visuomotor control, and differentiated lateralization are all associated with the development of speech and higher nervous activities, which means that they can help assess the child's developmental level and maturity. This fact should be considered in creating the assessment and prevention programs in the health care system.

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The value of Gissane's angle in the population of Central Serbia

Vrednost Gissane-ovog ugla kod stanovništva centralne Srbije

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Abstract

Background/Aim. Determining the value of Gissane's angle (GA) plays a role in the diagnosis and prognosis of heel fractures, determining treatment methods, and assessing the outcome of surgical treatment. Normal values of the GA vary in relation to ethnicity, age, and gender of the examined group, which indicates its anthropometric significance. The aim of this study was to determine the range of normal GA values in the population of Central Serbia. **Methods.** GA was determined on lateral radiography of the foot of 145 subjects of both sexes (67 women and 78 men), with subjects divided into 6 age groups. The patients included in the study did not have a calcaneus fracture. The obtained results were processed in the Graph Pad program. **Results.** The average value of GA in Central Serbia was 110.8° ± 8.1°, ranging from 93° to 132.9°. The mean value of GA decreased with age, and no difference was observed between women and men, except in the youngest age group, where women had higher GA values than men. **Conclusion.** The results of the study showed a wide range of normal GA values, as well as the existence of a difference in subjects of different ages and sex.

Key words:
anthropometry; calcaneus; fractures, bone; heel; radiography; sex factors; serbia.

Apstrakt

Uvod/Cilj. Određivanje vrednosti Gissane-ovog ugla (GU) ima ulogu u dijagnostikovanju i prognozi preloma pete, određivanju metoda lečenja, i u proceni uspeha operativnog lečenja tog preloma. Normalne vrednosti GU pokazuju varijacije u odnosu na etničku pripadnost, životno doba i pol ispitivane grupe, što ukazuje na njegov antropometrijski značaj. Cilj istraživanja bio je utvrđivanje raspona normalnih vrednosti GU kod stanovništva centralne Srbije. **Metode.** GU je određivan na lateralnim radiografijama stopala 145 ispitanika, oba pola (67 žena i 78 muškaraca), a ispitanici su bili podeljeni u 6 starosnih grupa. Ispitanici koji su bili uključeni u istraživanje nisu imali prelom petne kosti. Dobijeni rezultati su obrađivani u *Graph Pad* programu. **Rezultati.** Prosečna vrednost GU u centralnoj Srbiji iznosila je 110.8° ± 8.1°, u rasponu od 93° do 132.9°. Utvrđeno je smanjenje srednje vrednosti GU sa povećanjem godina života, a nije nađena razlika između žena i muškaraca, izuzev u najmlađoj starosnoj grupi, u kojoj su žene imale veće vrednosti GU od muškaraca. **Zaključak.** Rezultati ispitivanja pokazali su veliki raspon normalnih vrednosti GU, kao i postojanje razlika kod ispitanika različite starosti i pola.

Ključne reči:
antropometrija; kalkaneus; kost; prelomi; zaceljenje; radiografija; pol, faktori; srbija.

Introduction

The calcaneus, the heel bone, is the largest tarsal bone, whose role is to provide stability and maintain a high degree of whole-body pressure [1]. All fractures of the calcaneus can be divided into extraarticular and intraarticular, totaling 2% of all bone fractures, as well as 75% of tarsal bone fractures [1, 2]. Fractures occur most often due to a fall from a height, and in most cases in the younger adult population aged 21–45 [3]. The morphometric dimensions of the calcaneus show ethnic heterogeneity and, in addition, may influence the plan of surgical treatment after injury [4]. Depending on the study, different normal values were reported: 95°–105°, 96°–152°, 100°–130°, 120°–145° [5]. The diagnosis of fractures is made based on lateral and anteroposterior radiographs of the foot [6]. Radiographs of the foot provide accurate information about calcaneus morphology and angular relationships and are, therefore, the

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method of choice in measuring the value of Gissane's angle (GA) [7]. Imaging methods play a major role in the diagnosis of calcaneus fractures, thus radiological measurement of GA values, together with Bohler angle (BA) values, is an important parameter both in the diagnosis and in the treatment and prognosis [8]. However, it should be emphasized that they cannot be used as an independent criterion when choosing a method of treatment and diagnosis and that in addition to measuring calcaneal angles, it is necessary to determine the severity and location of fractures as well as dislocation of fragments, insight to which these angles do not provide [9]. GA (also known as the critical angle, the critical angle of Gissane) is named after Dr. William Gissane (1898–1981), an Austrian professor of orthopedics [10]. GA is measured by the intersection of a line drawn from the highest point of the posterior articular facet to the highest point of the posterior tuberosity and a line from the former to the highest point on the anterior articular facet (Figure 1).

Fig. 1 – Measurement of the critical angle of Gissane.

Since it was described before the discovery of computed tomography in the 1960s, its values, along with BA values, have been the only parameter in assessing the severity of calcaneus fractures for a long time. Although nowadays the use of computed tomography is standard for assessing those injuries, GA and BA are still used in the assessment of fracture severity and decision-making about the therapy of calcaneus fractures [11].

Previous studies have shown different mean GA values measured in subjects who did not have a heel bone fracture. The aim of this study was to determine the range of normal GA values in the population of Central Serbia in order to help surgeons in decision-making. Moreover, possible differences in relation to gender, ethnicity, and the age of the respondents indicate the anthropometric significance of GA.

Methods

This retrospective study included 145 lateral radiographies of the foot, subjects of both sexes (67 women and 78 men) divided into 6 age groups (10–20; 21–30; 31–40; 41–50; 51–60; > 60 years). The research was conducted at the Military Medical Academy in Belgrade from October 1st, 2017, to June 30th, 2018. The images were taken on a Shimadzu digital X-ray machine with a Bucky grid, 40 kV, 5 mAs, and 110 cm focus film distance. Images were analyzed from radiographies taken for medical indications. The population in which the calcaneus fracture was not present was included in the study. In addition, the population under 10 years of age was not included considering that the calcaneus completes its development after the tenth year of life. The values were measured by two observers, independently of each other, and the measurement was repeated after two weeks in order to reduce the error.

Results were presented as mean ± standard deviation (SD). The obtained results were analyzed using Sigma Plot 12.0 (Systat Software) statistical program. As data had normal distribution and equal variance, as asserted by Shapiro-Wilk and equal variance tests, the appropriate parametric test was used. Data were analyzed by the two-way analysis of variance (ANOVA) followed by Holm-Sidak post hoc, with factors "sex" and "age". Significance was accepted at $p < 0.05$.

Results

GA was examined in 145 subjects of both sexes, divided into 6 age groups. The mean GA value in the whole examined group was 110.3° ± 8.4°, in the range from 93° to 132.9°. In the group of female subjects, the measured angle averaged 110.9° ± 8.9° in the range from 93° to 132.9°, while in male subjects, its value was on average 109.9° ± 8.1° while the range was from 93.8° to 132°. There was no statistically significant difference between males and females ($p = 0.0213$).

The GA value was also compared in different age groups. The youngest age group (respondents aged 10–20) had the highest mean value of the measured angle, which was expected due to the characteristics of the heel bone in the adolescent period. GA shows a tendency to decrease with age, and accordingly, the lowest values were observed in subjects older than 60 years. Differences between group mean values were shown between the 10–20 years and 21–30 years groups and all older groups (Figure 2A).

Additionally, when age groups were separated by gender, a significant difference was found within the

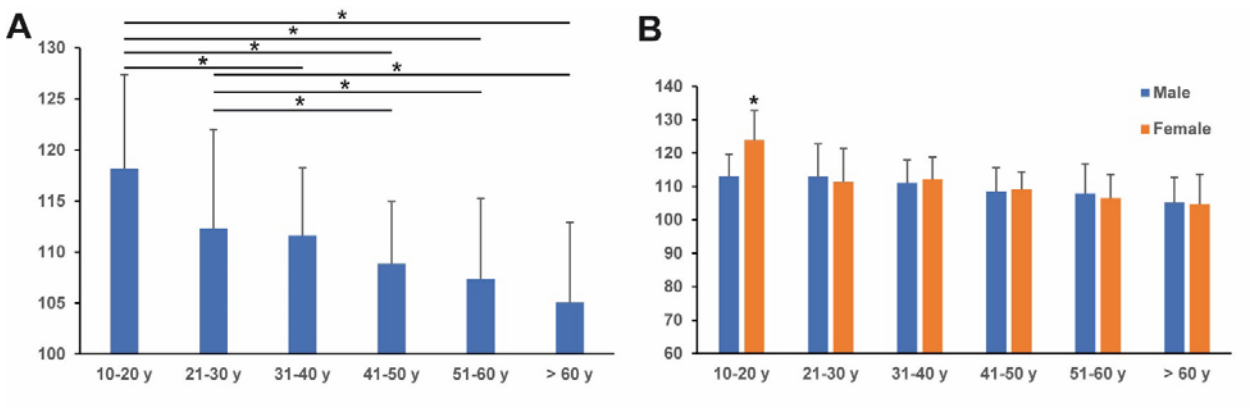

Fig. 2 – Mean values of Gissane angles per age group and gender: A) The mean ± standard deviation (SD) values for angles at various age groups. Asterisks mark significant differences in group mean values ($p < 0.05$; two-way ANOVA with Hold-Sidak post hoc); B) The mean ± SD values for angles at various age groups, separate for males and females. Asterisk marks significant differences in group mean values ($p < 0.05$; *t*-test). y – years.

youngest group (10–20 years), with females having significantly larger angles (123.9° ± 6.5°) compared with males (118.2° ± 9.2°), as shown in Figure 2B.

Discussion

On the lateral radiography of the foot, facies articularis anterior and posterior calcanei form a crucial angle or the GA. During the action of excessive axial compression forces, a calcaneus fracture occurs when the talus process acts as a 'wedge', disrupts the bone between anterior and posterior articular surface [12] of the calcaneus as well as underlying Ward's neutral triangle leading to an even bigger displacement or depression of fragments. All this leads to changes in the values of GA and BA. Since the calcaneus is the most vulnerable tarsal bone [8], knowledge of the value of GA proved important in the diagnosis of heel bone fracture, with an increase in GA values relative to the uninjured side.

We were attracted by the variations in the range of normal GA values in different populations and also by the fact that no literature suggested similar research done on the topic of average GA values in the population of Serbia.

Our study has shown a wide range of normal GA values, which coincides with previous research done on this topic [11–13]. The average value of GA in the examined population of Central Serbia was 110.3° ± 8.4°, while the range of values varied from 93° to 132.9°. The values obtained in the population of Central Serbia were lower than the values of most previously obtained results in various populations [5, 8, 9, 12, 14]. We did not observe significant differences among genders in GA values of the whole study group, similar to findings in previous studies done in Turkey, Saudi Arabia, Egypt, and Brazil [5, 11, 12]. The radiological study conducted in Brazil [11] did not show a statistically significant difference in the value of GA between the subjects of different sexes, as well as among subjects of different age groups, and the values that they reported (110.6° ± 11.9°) were similar to those in our study [11]. The mean values of the measured angle in the Egyptian population were higher (122.9° ± 6.52°) [12]. In the Serbian population, clear differences in GA values were observed between the examined age groups. Unlike our research, where the highest GA values were measured in the youngest group (10–20 years), in the Egyptian study, the highest value was found in the 41–50 years age group [12]. In the population of Egypt, the lowest values were measured in people aged 51–60 [12], while we observed a linear decline in GA values among the age groups, with the lowest values measured in people older than 60.

We also analyzed data from two studies performed in India. In the first study, the value of GA was measured in the population of South India, and it was on average lower (108.5°) than in the Serbian population [8]. In another study, which involved subjects from all regions in India, average GA values were measured at 126.72°, higher than those in our study [3]. Neither of these two studies found an association between the value of the measured angle and age or sex [3, 8].

A similar retrospective study was also conducted in South Korea using cadaveric calcaneus and a digital goniometer for the estimation of calcaneal angles. The mean value of GA was 114° ± 8.2°, and their values were higher than in our study. In this study, a statistically significant difference was shown between the values obtained in the female population (112.5° ± 8.1°) and the male population (117.7° ± 7.4°) [14]. In our study, the difference between males and females was present only in the youngest age group, and it was the opposite – females had larger angles than males. The GA of the population of Serbia is higher compared to the populations of Japan and Thailand, where the mean GA value was 105.1° ± 7.5° [15]. Similar mean GA values were obtained in a study conducted in Nigeria (116.29° ± 8.29°), Turkey (115° ± 6.5°), and Saudi Arabia (116.16°) [5, 13, 16]. None of these studies demonstrated a significant difference between the results obtained in males and females. In studies conducted in Turkey and Saudi Arabia, the value of GA was measured on both lower extremities, and no statistically significant difference between the left and the right side was shown [5, 13].

Table 1

The review of various studies reporting regional Gissane's angle (GA) values

Country	GA value	Age difference	Sex difference	Reference
Serbia	110.3	young > old	F > M	Our study
Brazil	110.6	no	no	11
Egypt	122.9	yes	N/D	12
South India	108.5	no	no	8
India	126.7	no	no	3
South Korea	114.8	N/D	M > F	14
Japan, Thailand	105.1	N/D	N/D	15
Nigeria	116.3	no	no	16
Turkey	115	no	no	13
Saudi Arabia	116.2	no	no	5
Kwara	64	N/D	no	17

F – female; M – male; N/D – not done.

The lowest GA values of all previously published research on this topic were reported among the population of Kwara, where a significant difference was found in the values of the measured angle on the right and left foot (right foot: 63.94° ± 20.58°; left foot: 80.23° ± 26.68°) [17]. In this study, there was also no difference between the sexes [17].

Variations in normal GA values concerning the ethnicity of the subjects were shown in different studies. The highest values obtained in the works published so far were observed in the population of India (126.79°) and Egypt (122.9° ± 6.952°), among Caucasians [3, 12]. In a study conducted in Nigeria, the mean GA value was 116.29° ± 8.29°, while in the Kwara population, it was 63.94° ± 20.58°, indicating a wide range of normal values of the negroid race [16, 17]. Within the Mongoloid race, the study was conducted on the population of the Korean people and Thai and Japanese people, where the mean GA value was 114° ± 8.2° and 105.1° ± 7.5° [14, 15], respectively. The mean values in a Portuguese group of subjects older than 18 years were 112.8° ± 7.4° [18]. The summary of the aforementioned studies in relation to our data is shown in Table 1.

When diagnosing a calcaneus fracture, it is important to know that GA values may be enlarged due to this injury [19], whereby maintaining normal GA values is a good prognostic sign. Therefore, such patients are considered to have a better long-term outcome [20]. Conservative treatment is usually recommended for extraarticular fractures, while intraarticular fractures should be treated surgically, where one of the goals is to return GA to the normal, pre-injury range [19].

One of the study limitations is that the measurements were performed on one foot only. In available studies among Caucasians, no significant difference was published comparing left and right foot calcaneal angle values. However, some authors recommend the use of lateral radiographies of the contralateral foot while planning operative procedures [3].

Conclusion

The range of normal GA values varies in different populations, which was confirmed in this study as well. A statistically significant difference in GA in the population of Central Serbia in relation to the age of the respondents was also shown.

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The effect of computer use on the occurrence of migraine

Uticaj upotrebe računara na pojavu migrene

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Abstract

Background/Aim. Risk factors concerning computer use-related migraine onsets are still unknown. The aim of this study was to determine the prevalence of headaches in computer users as well as the effects of computer use and behavior on the prediction of migraine presence. **Methods.** A cross-sectional study included 1,500 subjects from the general population who were given a questionnaire to assess the presence and type of headache and questions regarding computer-assisted behavior. All examinees were divided into two groups: the first group consisted of respondents who had a headache, and the second group consisted of subjects without a headache. **Results.** A total of 67.9% of the subjects had a headache, of which 23.9% had a migraine. Results of multinomial regression analysis showed that significant predictors of the migraine group, compared to the group without headache, were gender (female) and family anamnesis positive for migraine, as well as spending more time on the computer, making shorter and infrequent breaks in which physical activities were less included. Furthermore, members of the migraine group, compared with the group with other types of headaches, were younger and had family anamnesis, rarely made pauses during computer use, and their pauses were shorter. **Conclusion.** Improper and excessive computer use could be considered a risk factor for migraine occurrence, particularly in young people who have positive family anamnesis for migraine.

Key words:
computers; headache; migraine without aura; risk assessment; risk factors; sex factors; work; workplace.

Apstrakt

Uvod/Cilj. Faktori rizika od nastanka migrene usled rada na računaru još uvek su nepoznati. Cilj rada bio je da se utvrdi učestalost glavobolje kod korisnika računara i efekat načina upotrebe i ponašanja pri radu na računaru u predviđanju prisustva migrene. **Metode.** U studiju preseka je bilo uključeno 1 500 ispitanika iz opšte populacije kojima je zadat upitnik za procenu prisustva i tipa glavobolje, kao i pitanja u vezi sa ponašanjem prilikom rada na računaru. Svi ispitanici su bili podeljeni u dve grupe: prvu grupu su činili ispitanici sa glavoboljom, a drugu grupu ispitanici bez glavoblje. **Rezultati.** Glavobolju je imalo 67,9% ispitanika, od kojih je 23,9% imalo migrenu. Rezultati multinomalne regresione analize pokazali su da su pol (ženski), porodična anamneza, kao i više vremena provedenog za računarom, ređe i kraće pauze u toku kojih su često fizički neaktivni, bili značajni prediktori u grupi ispitanika sa migrenom, u odnosu na grupu bez glavobolje. Štaviše, pripadnici grupe sa migrenom u poređenju sa grupom ispitanika koja je patila od drugih vrsta glavobolja, bili su mlađi i imali pozitivnu porodičnu anamnezu za migrenu, ređe pravili pauze u toku rada na računaru, a te pauze su bile kraće. **Zaključak.** Nepravilna i prekomerna upotreba računara može se smatrati faktorom rizika od pojave migrene, posebno kod mladih osoba sa pozitivnom porodičnom anamnezom za migrenu.

Ključne reči:
kompjuteri; glavobolja; migrena bez aure; rizik, procena, faktori rizika; pol, faktor; rad; radno mesto.

Introduction

The number of people suffering from frequent headaches is increasing every day [1]. Headache is the most common neurological symptom today, which can have a significant impact on reducing the quality of life [2, 3]. This also affects work life, as headaches can reduce productivity at work [3], and pupils and students may experience learning disabilities [4].

Nowadays, working on a computer is becoming more and more common within the professional as well as in the private sphere of life [5, 6]. In addition to the many benefits that

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the use of a computer can bring, it can also lead to numerous damages to the health of users, of which, in the past research, besides spinal pain, shoulder pain, visual impairment [7], fatigue, depression, and obesity, headache has become the most mentioned [6, 8–10]. Some studies show that the use of computers can be considered a significant risk factor for migraines [6, 8, 11]. Although the use of computers has recently been increasingly associated with the onset of headache attacks, not all the risk factors for working on a computer, that contribute to its occurrence, are known yet [8].

Consequently, all strategies for preventing computer harm are not yet well known [8, 12], and further research is needed [8]. The aim of the study was to determine the prevalence of headaches in computer users as well as to determine the effect and the pattern of using the computer in a migraine occurrence.

Methods

This cross-sectional study was approved by the Ethics Committee of the Faculty of Medicine in Novi Sad (No 01-39/81/1). The study was conducted over one year in primary and secondary schools, preschools, colleges, and enterprises in the municipalities of Sombor, Apatin, Novi Sad, and Mali Idjos, with prior approval of the management of these institutions.

The minimum number of subjects was calculated based on G-power software, with an a priori set test power of 0.95. The baseline for initial values were the headache prevalence results from a previous study [13], as this is the only study in these areas that presents headache prevalence results. Consistent with the prevalence of headaches in the aforementioned study, it was calculated that the minimum sample size should be 1,040 subjects to control the type II error. A total of 1,506 subjects that use a computer were included in the survey. The respondents in the study were adult pupils, students, teaching and non-teaching staff in schools and colleges, and workers in enterprises. The exclusion criteria were as follows: persons with poor mental development and dementia, severe and life-threatening acute and chronic illnesses, which excluded 6 subjects. Data collection was performed using a set of questions that was modeled after other studies [2, 6, 8, 13–15]. Immediately before the questionnaire was distributed, the study participants were introduced to the method of filling in correctly, the meaning of certain terms, and were informed about the objectives of the test after receiving their written consent for voluntary participation in the study. The survey lasted about 45 min. The questionnaire consisted of a total of three parts. The first part contained questions about general demographic data (gender, age, place of residence, educational level, occupation, and employment), data on the health status of the respondents (about the presence of headaches in the previous year, about the presence of chronic diseases, about the existence of neck pain and so on) and information about the presence of headaches in the family. The second part contained questions about computer use and behavior while working on the computer (time spent on the computer during the day, whether pauses were made while working on the computer, and if so, how long, and of what content, as well as if the subject occupied the correct position while operating the computer). The third part was filled in exclusively by the respondents who answered that they had at least one headache attack in the last year. It contained questions about the characteristics of headaches and was partly taken from previous research [13]. This set of questions follows the criteria of the International Classification of Headaches [16]. All participants initially were divided into two groups: subjects with headaches and subjects without headaches. Furthermore, the first group was divided into two subgroups: the ones with migraine and the ones with other types of headaches.

Data analysis

Multinomial regression analysis was used in order to test the prediction of belonging to the migraine group, compared to the controls and other headache (non-migraine) groups. Category predictors were gender, employment, family anamnesis, and whether participants make a pause during the computer use, and continuous predictors were age, pause frequency (on a scale from 1 = after 30 min to 5 = never), pause duration (on a scale from 1 = never to 5 = 2 hrs and more), correct position during computer use (on a scale from 1 = never to 4 = always), and total hrs of computer use per day. Due to the missing data on some questions, the total number of answers was not the same across variables. Using the χ^2 test, the difference in the prevalence of migraine and other types of headaches between school children and adults was determined. Analysis was performed in SPSS v.23 for Windows.

Results

The total sample was first divided into two groups. The first group consisted of 1,019 (67.9%) respondents who had a headache. The second group consisted of subjects who did not have a headache (control group), which consisted of 481 (32.1%) respondents. The headache group was divided into two subgroups. The first subgroup consisted of 243 (23.9%) subjects with migraine, and the second group consisted of 776 (76.1%) subjects with other types of primary and secondary headaches.

Table 1 shows the demographic characteristics, usage patterns, and behavior of respondents while working on a computer.

Using the χ^2 test, it was determined that there were statistically significant differences in the prevalence of migraine and other types of headaches between school children and adults [χ^2 (4) = 10.55, p = 0.032]. The prevalence of migraine and other types of primary and secondary headaches was significantly higher in school children than in adults. Results of multinomial regression analysis showed that model was significant [χ^2 (20) = 1,044.93, p < 0.001], with R^2 ranging from 0.59 (Cox & Snell) to 0.68 (Nagelkerke) and 77.3% of overall correct classification. Significant prediction of membership to the migraine group

Table 1

Demographic characteristics, usage patterns, and behavior of examinees while working on a computer

Parameter	Migraine n = 243	Other headaches n = 776	Control n = 481
Demographics characteristics			
gender, n (%)			
male	47 (19.3)	228 (29.4)	226 (47.0)
female	196 (80.7)	547 (70.6)	255 (53.0)
employment, n (%)	88 (36.2)	311 (40.1)	234 (48.6)
yes			
no	155 (63.8)	465 (59.9)	247 (51.4)
age (years)			
mean ± SD	26.66 ± 10.82	28.69 ± 12.7	31.38 ± 14.46
school children, n (%)	110 (45.3)	347 (44.7)	184 (38.3)
adults, n (%)	133 (54.7)	429 (55.3)	297 (61.7)
family anamnesis, n (%)			
yes	114 (46.9)	228 (29.4)	48 (10.0)
no	129 (53.1)	548 (70.6)	433 (90.0)
Characteristics of computer use			
total hours per day, mean ± SD	6.85 ± 3.43	6.23 ± 2.88	2.91 ± 2.06
pause (break), n (%)			
yes	185 (76.1)	620 (79.5)	390 (81.1)
no	55 (22.6)	156 (20.5)	49 (10.2)
pause frequency, n (%)			
after 30 min	15 (6.3)	121 (15.9)	261 (59.5)
after 1 h	18 (7.5)	246 (32.3)	94 (21.4)
after 2 hrs	47 (19.7)	181 (23.8)	27 (6.2)
after 3 or more	104 (43.5)	58 (7.6)	8 (1.8)
never	55 (23.0)	156 (20.5)	49 (11.2)
pause duration, n (%)			
up to 10 min	103 (56.3)	252 (41.7)	52 (13.3)
between 15 and 30 min	62 (33.9)	232 (38.3)	147 (37.6)
between 31 min and 1 h	14 (7.7)	85 (14.0)	98 (25.1)
about 2 hrs and more	4 (2.2)	36 (6.0)	94 (24.0)
activity during the pause, n (%)			
mobile/tablet	47 (25.5)	158 (26.1)	72 (18.4)
relaxing	77 (41.8)	262 (43.2)	109 (27.9)
physical activity, other	60 (32.6)	186 (30.7)	210 (53.7)
correct position, n (%)			
never	32 (13.3)	421 (55.1)	62 (14.2)
sometimes	129 (53.8)	277 (36.3)	199 (45.6)
often	68 (28.3)	51 (6.7)	141 (32.3)
always	11 (4.6)	15 (2.0)	34 (7.8)

n – number of subjects; SD – standard deviation.

compared to controls showed gender (with more females in the migraine group) and family anamnesis (with more participants with family anamnesis in the migraine group) from the demographics characteristics (Table 2). Regarding variables about computer use, results showed that the migraine group compared to controls, spent more total hrs per day on the computer, rarely made pauses during computer use (after 3 hrs or never), made shorter pauses (up to 10 min), and were more relaxing in pauses than engaging in physical activity. Compared to participants with other headaches, participants from the migraine group were younger and had family anamnesis more often, while there were no significant gender differences nor differences in employment (Table 2). Furthermore, although there were no differences in total spent hrs per day, participants with migraine rarely made pauses during computer use and made shorter pauses. However, they reported that they sat more correctly during computer use compared to the participants with other headaches. There were no differences in the type of activity during the pause between migraine and other headache groups.

Most subjects with both migraine and other types of headaches reported having a headache after 2 to 6 hrs of computer work (Table 3).

Table 2

Prediction in the migraine group based on demographics characteristics and characteristics of computer use

Parameter	Migraine vs. controls				Migraine vs. other headaches			
	Exp(B)	*p*	CI		Exp(B)	*p*	CI	
			lower	upper			lower	upper
Demographics characteristics								
gender (male)	2.45	0.003	1.35	4.45	1.59	0.075	0.96	2.64
age	1.01	0.537	0.97	1.05	1.05	0.003	1.02	1.09
employment (no)	1.36	0.521	0.53	3.49	0.75	0.492	0.34	1.69
family anamnesis (no)	0.13	0.000	0.07	0.24	0.47	0.000	0.30	0.71
Characteristics of computer use								
total hours per day	0.69	0.000	0.62	0.77	1.06	0.145	0.98	1.13
pause frequency	0.15	0.000	0.11	0.21	0.32	0.000	0.25	0.41
pause duration	3.54	0.000	2.56	4.91	1.77	0.000	1.33	2.36
correct position	0.90	0.544	0.65	1.25	0.29	0.000	0.22	0.38
activity during pause (mobile/tablet)	0.53	0.076	0.26	1.07	0.80	0.454	0.45	1.42
activity during pause (relaxing)	0.38	0.002	0.21	0.70	0.75	0.266	0.46	1.24

CI – confidence interval.

Table 3

Presence of computer use as a headache trigger

Parameter	Migraine	Other primary and secondary headaches
Computer as a headache trigger (yes), n (%)	207 (85.2)	560 (72.2)
Time onset of the headache after the beginning of computer use (hours), n (%)		
1–2	31 (15.1)	81 (14.5)
2–6	116 (56.6)	307 (54.8)
> 6	58 (28.3)	172 (30.7)

n – number of subjects.

Discussion

Due to the increasing number of people suffering from headaches, many studies conducted so far have focused on discovering significant triggers for headache attacks [17–19]. One of the triggers analyzed, which has recently become increasingly significant, is the use of computers [4–6, 8, 11, 14, 15, 17–20]. As in many other studies [6, 9, 11, 21], our study also found a high prevalence of headaches among computer users. In our study, as many as 67.9% of respondents who are computer users experienced a headache in the previous year. A similar prevalence of headaches among computer users (64.5%) was observed in a study conducted in Sweden [11]. Moreover, in a survey conducted in Iceland, 65.2% of computer users had a headache [11]. A slightly higher prevalence of headaches (74.9%) among computer users was observed in a study conducted in Finland [11] and in a study conducted in Brazil, where 80.6% had a headache [6]. A significantly lower prevalence of headache (26%) in computer users has been observed in a study conducted in Australia [8]. In our study, 23.9% of the subjects were affected by migraine. A slightly lower prevalence of migraine (19.3%) is observed in a study on the prevalence of headaches in adolescents and their association with the use of computers and video games [6]. The prevalence of migraine (30.2%) is observed in the study by Saueressig et al. [9]. These differences in the prevalence of headaches among computer users between the different surveys may be primarily due to different demographic characteristics of the respondents (due to differences in gender and age structure) and different methodology since it is observed that the inclusion criteria for determining the presence of headaches differ from study to study (from three months to one year). Given that, headache prevalence is expected to be higher in studies where the inclusion criterion for headache was the presence of headache for at least one year prior to the survey.

This study, in addition to determining the prevalence of migraine among computer users, was also conducted to identify behaviors during computer use to determine risk factors contributing to the onset of migraine attacks.

In addition to the already known fact that migraines are more common in women and those with a positive family history of headaches, the results of our study also indicate the importance of the length of work and certain computer behaviors as risk factors for the presence of migraines. Specifically, the subjects with migraine, in comparison with the control group without headaches, spend significantly more time during the day working on the computer, rarely taking a break, and when they do, they are of shorter duration and more often physically inactive during the break. In comparison with the group of patients with other types of primary and secondary headaches, the subjects with migraine belong to the younger age category and have a positive family history. Although there are no significant differences in daily computer exposure, subjects with migraine compared with subjects with other types of primary and secondary head-

aches are significantly less likely to take a break, and when they do, those breaks have a significantly shorter duration. However, it is noted that migraine sufferers are more likely to occupy a proper position while working on a computer than those suffering from other types of headaches. The length of computer exposure during the day is the most studied factor to date, which has proven to be significant in the onset of migraine attacks [6, 9, 14, 15]. Specifically, Saueressing et al. [9] point out that the chance of a migraine is even 2.54 times higher for computer users who use the computer for more than 3 hrs during the day. In their research, Xavier et al. [6] point out that computer users who used a computer for more than 4 hrs a day were more likely to experience primary headaches, especially migraines. Milde-Busch et al. [15] in their research warn that even shorter exposure times to computers/the Internet (as little as 30 min) may result in an increased risk of migraine attacks. Additionally, confirmation of the importance of the length of work on a computer during the day for the onset of migraine is found in the results of the research conducted by Montagni et al. [14]. They cite two potential "scenarios" that could explain the effects of computer screens on migraine. The first is the brightness and frequency of the screen that can directly trigger the attack, and the second is the screen exposure time, which can reduce the threshold for headache, which is then induced by other factors. In contrast to our and the results of the aforementioned studies, different results, namely that the length of computer exposure does not play a significant role in the onset of headache attacks, were obtained by Smith et al. [8] in the study of the prevalence of neck pain and headache in computer users. However, the results of their study show the importance of the length of work on the computer for the onset of pain in the cervical spine. It is well known that ergonomic recommendations aimed at preventing the harmful effects of a computer on the health of users require the proper positioning of the body while operating the computer [6, 18, 22, 23]. Prolonged irregular position of the body when working on a computer in an environment that is not designed according to ergonomic rules is thought to be stressful for the trapezius muscle, which in addition to pain in the neck and shoulder can lead to headaches [24, 25]. In our study, taking the proper position when working on a computer proved to be a significant predictor between migraines and other types of headaches.

In order to determine how much and whether computer users who were suffering from headaches were aware of the computer as a trigger in our study, the respondents were asked if the occurrence of a headache attack could be affected by computer work. As a result, 85.2% of migraine and 72.2% of other primary and secondary headache sufferers reported that the computer could be a trigger. More than half of migraine and other primary and secondary headache sufferers reported that the headache usually occurs after 2–6 hrs of computer work.

As in other studies [4, 8], the results of our study show that computer users with headaches generally do not adhere to existing ergonomic recommendations to prevent the harmful effects of computers on health. Given that the number of headache sufferers is increasing every day, there is a need to develop and implement measures to prevent the onset of headache attacks, especially migraines. It is recommended that computer users receive adequate ergonomic training to prevent headaches [26].

In addition to confirming the results of previous research that the length of work on the computer is probably a risk factor for the presence of different types of headaches, this study found that the main difference between the types of headaches is the dynamics of work at the computer, i.e., the key is to take a break.

Limitations of the study

This research has several limitations. One of them refers to the way data was collected, which is a survey, and, therefore, not the best way to obtain reliable data. The limited time of 45 min provided for completing the survey questionnaire conditioned the limited number of questions. Therefore, questions about the diagnosis, the use of drugs in case of a headache, whether drugs are used on the recommendation of a doctor or independently, what is the effect of these drugs, and the like, would have given additional weight to the study and indicated the complexity of this problem.

Conclusion

The results of our study indicate a high prevalence of headaches in computer users. In addition to being female and having a positive family history of headaches, length of computer work and individual computer behaviors were significant predictors of belonging to the migraine group compared to the headache-free group. Respondents with migraine compared to the group without headaches significantly spend more time during the day working on the computer, taking a break less often, and when they do, they are more often physically inactive during the break which is most often of shorter duration. Respondents with migraine compared to subjects with other types of headaches are younger and have a positive family history of headaches. Although there are no significant differences in the length of work on the computer during the day, subjects with migraine compared to those with other types of headaches are much less likely to take a break, and when they do, those breaks are of a significantly shorter duration. Therefore, the length of time you work on your computer is probably a risk factor for the presence of different types of headaches, but the main difference between the types of headaches is in the dynamics of working on the computer, that is, taking breaks. Computer users with headaches generally do not adhere to existing ergonomic recommendations for the prevention of the harmful effects of computers on health, and there is a need to develop and implement preventative measures, which can be achieved by training users on ergonomic principles for proper and adequate use of computers.

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Awareness of patients' orthodontic problems and referral practices among general dental practitioners and non-orthodontic specialists

Svest o ortodontskim problemima pacijenata i praksi njihovog upućivanja među stomatolozima opšte prakse i specijalistima koji nisu ortodonti

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Abstract

Background/Aim. Dentists and particularly general dental practitioners (GDPs) should be able to identify problems such as malocclusions and refer patients with such problems to orthodontics. The aim of the study was to evaluate the awareness of orthodontic problems and referral practices among GDPs and non-orthodontic specialists practicing in the Kingdom of Saudi Arabia. **Methods.** A cross-sectional prospective study included GDP and specialists/consultants of both genders, aged between 22–60 years, practicing general dentistry and specialists other than orthodontics who had worked in their field for two or more years. Non-practicing dentists and nonregistered dentists were excluded. Data was analyzed on SPSS version 21.00. **Results.** Among the respondents, 55.5% were men, and 44% were GDPs. Out of the total number of respondents, 107 (53.5%) GDPs and 66 (33%) non-orthodontic specialists practiced orthodontic referral in their practices. By using the χ^2 test, statistical analysis of different variables depending on the participants' gender and specialty was performed. It revealed that variables with significant *p*-value were: orthodontist referral, the recommendation of orthodontic treatment only after the eruption of all permanent teeth, awareness of tooth extraction for alignment of irregular teeth, and awareness regarding worsening of temporomandibular joint problems by orthodontic treatment. **Conclusion.** The referral practices of dentists practicing in the Kingdom of Saudi Arabia concerning orthodontic patients were satisfactory, although awareness regarding orthodontic problems requires continuous learning and considerate positive progression toward basic orthodontics.

Key words:
dentists; knowledge; orthodontics; referral and consultation; surveys and questionnaires.

Apstrakt

Uvod/Cilj. Stomatolozi, uopšteno, i posebno stomatolozi opšte prakse (SOP) trebalo bi da budu edukovani da mogu da prepoznaju probleme kao što je malokluzija i upute pacijente sa takvim problemima specijalisti ortodoncije. Cilj rada bio je da se proceni svest o ortodontskim problemima i praksi u vezi upućivanja među SOP i neortodontskim specijalistima koji rade u Kraljevini Saudijskoj Arabiji. **Metode.** Prospektivnom studijom preseka bili su obuhvaćeni SOP i specijalisti/konsultanti starosti 22–60 godina koji su se bavili opštom stomatologijom, kao i specijalisti svih grana sem ortodoncije, zaposleni dve ili više godina. Svi podaci su analizirani korišćenjem SPSS programa verzije 21.00. **Rezultati.** Među ispitanicima bilo je 55,5% muškaraca, a SOP 44%. Ukupno, 107 (53,5%) SOP i 66 (33%) neortodontskih specijalista je u svojim ordinacijama praktikovalo upućivanje ortodontima. Pomoću χ^2 testa izvršena je statistička analiza različitih varijabli u zavisnosti od pola i specijalnosti ispitanika. Pokazano je da sledeće varijable imaju statistički značajnu *p*-vrednost: upućivanje ortodontu, preporuke za ortodontsko lečenje tek nakon nicanja svih stalnih zuba, svest o vađenju zuba radi poravnanja nepravilnih zuba i svest o pogoršanju problema temporomandibularnog zgloba usled ortodontskog lečenja. **Zaključak.** Upućivanje ortodontskih pacijenata od strane stomatologa koji rade u Kraljevini Saudijskoj Arabiji je zadovoljavajuće, mada svest o ortodontskom lečenju zahteva neprekidno učenje i pažljiv napredak ka bazičnoj ortodonciji.

Ključne reči:
stomatolozi; znanje; ortodoncija; upućivanje i konsultacija; ankete i upitnici.

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Introduction

Orthodontics deals with facial and dental aesthetics [1], and the correct association between dental and skeletal tissues provides the most pleasing aesthetics and functions to the patient [2].

Awareness of a dentist about the existence of orthodontic problems in their patients is of paramount importance. A dentist should be able to identify dental and skeletal incorrect relations such as malocclusion. Numerous factors influence orthodontic practices, which include the personal and practice characteristics of dentists. A study published in 2019 reported that there was a significant difference between the orthodontic care providers and non-orthodontic care providers – general dental practitioners (GDPs) – in terms of knowledge, attitude, and orthodontic needs among two groups of practitioners [3]. Their results pointed out and highlighted that continuous dental education programs and the use of information resources play a valuable role in GDP and non-orthodontic specialists' education.

GDPs and even pedodontists are the primary sources of identification and referral of patients to orthodontists. Various studies reported that many GDPs provide basic orthodontic treatment and referral to an orthodontist in the majority of patients [4–6]. Another study conducted in India and published in 2016, determining the practices of pedodontists for orthodontic referral, revealed that the knowledge and practices of pediatricians are not sufficient [7]. They should be aware of the common understanding of orthodontic problems and develop a habit of referral to an orthodontist. A study conducted to evaluate the basic knowledge and practices of GDPs and non-orthodontist practitioners revealed reduced knowledge and practices [8, 9]. Unfortunately, fewer studies determining patient referral to orthodontists among Saudi dental practitioners were available [10, 11].

Therefore, the aim of this study was to evaluate the knowledge, awareness, attitude, and referral practices of dentists and non-orthodontic specialists in the Kingdom of Saudi Arabia.

Methods

This cross-sectional prospective study was conducted from a predesigned questionnaire, which consisted of 22 questions addressing dentists and non-orthodontic specialists. Samples were recruited through nonprobability convenience sampling.

Inclusion criteria for the sample population were general dentists and specialists/consultants of both genders, aged between 22–60 years, practicing general dentistry or other dental specialties for at least 2 or more years.

Exclusion criteria were non-practicing dentists, nonregistered dentists, as well as fresh dental graduates.

The duration of the study was 6 months, i.e., from April 2019 to September 2019. Raosoft software was used to calculate the sample size, which was 200, with a margin of error of 5%, a population size of 100, a confidence level of 95%, and a response distribution of 50%.

The participants were asked for verbal informed consent to be included in the study. All the responses were recorded on a predesigned form by the researchers. The study was carried out on a previously used [11] structured questionnaire containing 22 questions, which were validated after a pilot study. The questionnaires were formulated to study the knowledge and attitude of GDPs and non-orthodontic specialties toward the practice of orthodontic treatment. The first part of the questionnaire was designed to collect demographic and other practice-related information, such as specialty and years of experience. The second part consisted of 13 questions framed to study the knowledge of general dental surgeons and non-orthodontic specialties. The questions were Yes/No type, questions indicating their knowledge regarding starting age of orthodontic treatment, mixed dentition stage treatment, facial appearance, functional therapy, inclined teeth, extraction of teeth for orthodontic purposes, etc. The last part, which consisted of 9 questions (Yes/No type), was prepared to study the attitude toward orthodontic treatment, like diagnostic orthodontic procedures, opinion of the orthodontist, giving information to the patient about malocclusion when the patient comes for the other dental treatment, orthodontic treatment in patients with periodontal problems, orthognathic surgeries, etc.

Statistical analysis was done with the SPSS version 21.00. Frequencies and percentages were calculated for qualitative variables. Cross tabulation was performed using descriptive analysis. A χ^2 test was done to evaluate the association of different qualitative variables with gender and specialty. The p-value of ≤ 0.05 was considered significant.

Results

The results revealed that among questioned dentists and non-orthodontic specialists, females made up 45.5% and males 55.5%. Among specialties, GDPs constituted the highest percentage (44%), followed by others (Figure 1).

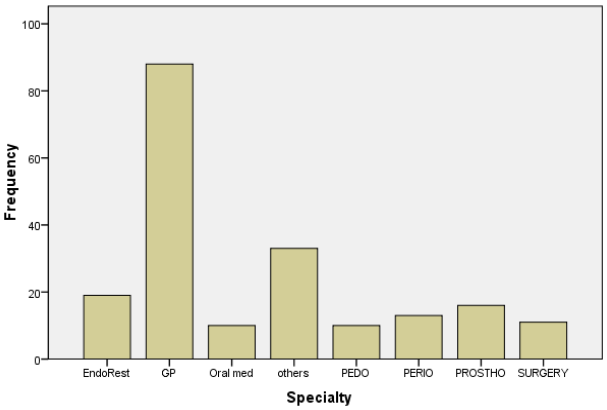

Fig. 1 – Specialty distribution among questioned dentists. GP – general practitioners; PEDO – pedodontists; PERIO – periodontists; PROSTHO – prosthodontists.

Figure 2 shows the percentage of orthodontic referrals among GDPs and non-orthodontic specialists. In their practices, 107 (53.5%) GDPs and 66 (33%) non-orthodontic spe-

cialists practiced orthodontic referral. The comparison revealed a high significance (*p*-value of 0.000).

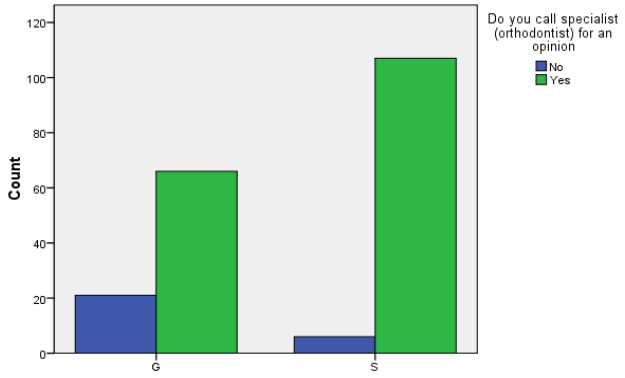

Fig. 2 – Percentage of orthodontic referrals among general dental practitioners (G) and non-orthodontic specialists (S).

Statistical analysis through the χ^2 test was performed between different variables compared with participants' gender, and it showed that variables with significant *p*-value were orthodontist referral, recommendation of orthodontic treatment only after the eruption of all permanent teeth, consideration of skeletal malocclusions in patients with incompetent lips and inclined teeth, awareness of tooth extraction for alignment of irregular teeth, and awareness regarding worsening of temporomandibular joint (TMJ) problems by orthodontic treatment (Table 1).

The χ^2 test results among different variables depending on the specialty showed a significant *p*-value of the following variables: recommendation of orthodontic treatment for patients with periodontal problems, orthodontist referral, the conducting of diagnostic orthodontic procedures, counseling of patients for orthodontic treatment, and awareness regarding worsening of TMJ problems by orthodontic treatment (Table 2).

Table 1

Relationship between gender of GDPs and non-orthodontics specialists with orthodontic treatment and other features

Variable	Male	Female
First orthodontic consultation	0.763	0.376
Possibility of treating orthodontic problems during the mixed dentition stage	0.234	0.333
Assessment of orthodontic problems on clinical examination	0.567	0.111
Importance of well-aligned teeth for facial aesthetics	0.209	0.161
Awareness regarding functional orthodontic therapy	0.173	0.440
Recommendation of orthodontic treatment for patients with periodontal problems	0.532	0.492
Relationship of caries in primary teeth and orthodontic problems	0.981	0.846
Effect of a functional appliance with treatment advised during pre-pubertal growth spurt period	0.240	0.151
Orthodontist referral	0.036	0.005
Conducting diagnostic orthodontic procedures	0.389	0.158
Counseling of patients for orthodontic treatment	0.583	0.169
Recommendation of orthodontic treatment only after the eruption of all permanent teeth	0.041	0.025
Consideration of skeletal malocclusions in patients with incompetent lips and inclined teeth	0.046	0.030
Awareness of tooth extraction for alignment of irregular teeth	0.007	0.009
Awareness of mouth breathing or thumb-sucking leading to improper skeletal form	0.529	0.419
Importance of awareness of teeth eruption and exfoliation timing	0.728	0.948
Awareness regarding the treatment of TMJ problems by orthodontic treatment	0.167	0.378
Awareness regarding worsening of TMJ problems by orthodontic treatment	0.028	0.017

GDPs – general dental practitioners; TMJ – temporomandibular joint.

Table 2

Impact of GDPs and specialists or consultants on orthodontic treatment characteristics

Variable	GDPs	Specialists/ consultants
First orthodontic consultation required at which stage of dentition	0.009	0.040
Possibility of treating orthodontic problems during the mixed dentition stage	0.385	0.487
Assessment of orthodontic problems on clinical examination	0.050	0.041
Importance of well-aligned teeth for facial aesthetics	0.972	0.346
Awareness regarding functional orthodontic therapy	0.001	0.041
Recommendation of orthodontic treatment for patients with periodontal problems	0.595	0.055
Relationship of caries in primary teeth and orthodontic problems	0.115	0.089
Effect of a functional appliance with treatment advised during pre-pubertal growth spurt period	0.078	0.047
Orthodontist referral	0.000	0.023
Conducting diagnostic orthodontic procedures	0.049	0.567
Counseling of patients for orthodontic treatment	0.009	0.237
Recommendation of orthodontic treatment only after the eruption of all permanent teeth	0.000	0.012
Consideration of skeletal malocclusions in patients with incompetent lips and inclined teeth	0.412	0.381
Awareness of tooth extraction for alignment of irregular teeth	0.003	0.045
Awareness of mouth breathing or thumb-sucking leading to improper skeletal form	0.617	0.891
Importance of awareness of teeth eruption and exfoliation timing	0.005	0.057
Awareness regarding the treatment of TMJ problems by orthodontic treatment	0.192	0.018
Awareness regarding worsening of TMJ problems by orthodontic treatment	0.000	0.055

GDPs – general dental practitioners.

Discussion

This study was carried out to evaluate the awareness of patients' orthodontic problems and referral practices among GDPs and non-orthodontic specialists practicing in the Kingdom of Saudi Arabia.

In this study, GDPs constituted the highest percentage, i.e., 44%, followed by other specialists. This is inconsistent with the study conducted by AlBaker et al. [12], who reported that the total number of dentists working in Saudi Arabia was 16,887 in December 2016, with the majority of them being general dentists (70.27%), followed by prosthodontists and orthodontists.

Considering different variables connected with orthodontist referral, the most significant were the recommendation of orthodontic treatment only after the eruption of all permanent teeth, consideration of skeletal malocclusions in patients with incompetent lips and inclined teeth, awareness of tooth extraction for alignment of irregular teeth, and awareness regarding worsening of TMJ problems by orthodontic treatment.

Orthodontics is a specific specialty of dentistry that requires deep knowledge of dentofacial mechanics and thorough treatment planning. According to Reddy et al. [13], GDPs have poor referral practices due to an ineffective referral pathway. However, in our study, orthodontic referral appeared to be a significant variable, and awareness regarding orthodontist referral was well established among GDPs and specialists. Another study published in 2017 by Alnusayri et al. [11] revealed a significant difference between knowledge and attitude of GDPs and non-orthodontic specialties regarding orthodontic referral. However, no difference was observed in a study published by Acharya et al. [10] among GDPs and non-orthodontic specialists in terms of knowledge and orthodontic referral.

The recommendation of orthodontic treatment only after the eruption of all permanent teeth is another significant factor in both cross-tabulations. Orthodontic treatment depends upon the age at which the patient reports and seeks orthodontic treatment. Treatment after the age of eruption of all teeth has the advantage of patient cooperation and compliance but limited treatment outcome. It also depends upon the emergence and eruption of teeth. A study published in 2016 revealed that the age of the mixed dentition period is increased in children born in the period between 1999–2000 compared to the children born between 1976–1985. Girls showed more advanced eruption compared to boys [14]. Therefore, the patient should be thoroughly examined and treatment outcomes discussed after considering all factors for the orthodontic referral.

The consideration of skeletal malocclusions in patients with incompetent lips and inclined teeth is another factor that showed a significant influence on patient referral [15]. It has been considered that patients with inclined teeth and incompetent lips are more likely to have skeletal malocclusion, and the treatment of these patients along with orthodontic fixed appliance therapy in combination with orthognathic surgery will dramatically modify the treatment outcomes and save overall treatment time [16]. Patients with incompetent lips and inclined anterior teeth should be thoroughly investigated and referred to the orthodontist for further treatment [17].

A study of Hassan et al. [18], published in the Saudi Medical Journal in 2014, evaluated different factors of lip incompetency among Saudi children. Their study revealed that lip incompetence was not related only to bimaxillary protrusion, pointing to other skeletal, dental, and soft tissue factors, which also play an important role in lip competency.

GDPs are aware of tooth extraction significance for the alignment of irregularly positioned teeth. However, the extraction of permanent teeth performed to create space and relieve crowding could be a factor that demotivates patients to seek orthodontic treatment. A study conducted on Yemeni children revealed that only 2% of children had undergone orthodontic consultation and treatment, the extraction of teeth being one of the factors that deferred orthodontic treatment [19]. Another study revealed that almost 100% of non-orthodontic specialists and 87.5% of GDPs are aware of the significance that the extraction of teeth has for orthodontic treatment [13].

Another important factor is awareness of the possible worsening of TMJ problems by orthodontic treatment. It is considered that orthodontic treatment could worsen a pre-existing TMJ problem. In our study, this factor appeared significant, showing a general belief that GDPs and non-orthodontic specialists consider worsening of TMJ problem after orthodontic treatment [20]. However, a study published in 2016, conducted at Oulu University Hospital, revealed that treatment of severe malocclusion leads to improvement in the overall Visual Analogue Scale (VAS) score of facial and TMJ-related pain [21].

In our study, we compared and determined the awareness and referral practices of dentists for orthodontic patients reported to dental clinics and compared different variables connected with referral practice. The variables which showed significant *p*-value were the recommendation of orthodontic treatment for patients with periodontal problems, the conducting of diagnostic orthodontic procedures, counseling of patients for orthodontic treatment, and awareness regarding worsening of TMJ problems by orthodontic treatment. The limitations of the study were the small sample size and a cross-sectional design. Other factors should also be considered for evaluating referral for orthodontic treatment, i.e., socioeconomic status, patient expectations, practitioner's expertise, etc.

It is recommended that awareness programs, talks, and seminars be arranged for GDPs and non-orthodontist specialists to enhance their understanding of orthodontics. The use of social media should be made effective so that patients seek early orthodontic treatment and consultation.

Conclusion

Referral practices for orthodontic patients of dentists practicing in the Kingdom of Saudi Arabia were satisfactory, although awareness regarding orthodontic problems requires continuous learning and considerate positive progression towards basic orthodontics.

Acknowledgement

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Informed consent in cardiac surgery – current institutional practice and legislation

Pristanak informisanog pacijenta u kardiohirurgiji – aktuelna institucionalna praksa i zakonodavstvo

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Abstract

Background/Aim. Growing waiting lists for cardiac surgery have become a real problem in the Republic of Serbia, imposing serious difficulties in patient-surgeon communication. The aim of the study was to determine the current state of the institutional informed consent policy before elective cardiac surgical procedures in light of actual national legislation. **Methods.** An anonymous, voluntary survey was conducted among 200 consecutive patients at the Clinic for Cardiac Surgery, University Clinical Centre of Serbia, from September to December 2019, after signing an official institutional consent form. A targeted questionnaire was created to determine the quantity and quality of patients' information about general and the most important aspects of cardiac surgical care. **Results.** The mean age of respondents was 66.2 years, with male predominance (68.0%), homogenous ethnicity, and low-to-middle (84.0%) education levels. A significant percentage had no information on the type of surgery (16.0%), extracorporeal circulation (46.0%), anaesthesia (56.0%) and transfusion (51.5%). Of those having some information, 7.0–20.0% graded them sufficient. The worst situation was recorded concerning risks of disease and surgical treatment, where 88.0% of patients had no information and almost 90.0% had some information and graded them as non-sufficient. Surprisingly, 81.5% of patients signed the consent form without any prior discussion with the operating surgeon. For 56.0% of patients, the information in the actual consent form was clear and sufficient. While 85.5% of patients claimed the importance, the others (14.5%) were not interested to know the most relevant information about their disease and surgery. **Conclusion.** The results unambiguously indicate an unacceptably low level of our patients' information about the cardiac surgical procedure, extracorporeal circulation, anesthesia, transfusion, and estimated risk. The majority of them (85.5%) comprehends the importance and expects timely and adequate information. An extremely high percentage (81.5%) of patients had no chance to discuss the procedure with the operating surgeon. Both surgical indifference and insufficient knowledge of professional, ethical, and legal importance are the most important reasons for the actual informed consent policy in cardiac surgery.

Key words:
ethic, medical; informed consent; legislation; physician-patient relation; risk assessment; therapeutics; thoracic surgery; serbia.

Apstrakt

Uvod/Ciljevi. Sve duže liste čekanja za kardiohirurgiju u Republici Srbiji postale su problem i ozbiljno otežavaju komunikaciju između lekara i bolesnika. Cilj rada bio je da se u svetlu aktuelnog nacionalnog zakonodavstva utvrdi trenutno stanje institucionalne prakse pristanka informisanog pacijenta pre elektivnih kardiohirurških operacija. **Metode.** Istraživanje je sprovedeno na 200 uzastopnih bolesnika, koji su potpisali zvanični obrazac o pristanku inforimsanog pacijenta u Klinici za kardiohirurgiju Univerzitetskog Kliničkog centra Srbije, od septembra do decembra 2019. godine. Za tu potrebu, kreiran je ciljani upitnik za utvrđivanje kvaliteta i kvantiteta informacija o opštim i najvažnijim aspektima kardiohirurške nege. **Rezultati.** Srednja starost ispitanika bila je 66,2 godine, sa predominacijom bolesnika muškog pola (68,0%), homogenom etničkom pripadnošću i niskim ili srednjim (84,0%) nivoom obrazovanja. Značajan procenat bolesnika nije bio informisan o vrsti hirurgije (16,0%), vantelesnom krvotoku (46,0%), anesteziji (56,0%) i transfuziji (51,5%). Od onih bolesnika koji su bili delimično informisani, 7,0–20,0% je navedene informacije ocenilo dovoljnim. Najniža informisanost zabeležena je u pogledu informisanosti o rizicima od bolesti i hirurškog lečenja, gde

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88,0% bolesnika nije imalo nikakve informacije, dok je skoro 90,0% delimično informisanih ocenilo te informacije kao nedovoljne. Čak 81,5% bolesnika potpisalo je saglasnost za operaciju bez ikakve prethodne konsultacije sa svojim hirurgom. Za 56,0% bolesnika informacije u aktuelnom obrascu pristanka informisanog pacijenta okarakterisane su kao jasne i dovoljne. Dok je 85,5% bolesnika tvrdilo da je to važno, ostali bolesnici (14,5%) nisu uopšte bili zainteresovani za dobijanje najvažnih informacija o njihovoj bolesti i operaciji. **Zaključak.** Rezultati ispitivanja ukazuju na neprihvatljivo nizak nivo informisanosti naših bolesnika o kardiohirurškom zahvatu, vantelesnoj cirkulaciji, anesteziji, transfuziji i procenjenim rizicima. Većina ispitanih bolesnika (85,5%) shvatila je važnost obaveštenosti i očekuje pravovremene i adekvatne informacije. Izuzetno visok procenat bolesnika (81,5%) bili su oni koji nisu imali priliku da razgovaraju o proceduri sa odgovornim hirurgom. Nezainteresovanost hirurga i nedovoljno poznavanje profesionalnog, etičkog i pravnog značaja pristanka informisanog pacijenta najvažniji su razlozi za trenutno stanje institucionalne prakse u vezi sa ovim pitanjem u kardiohirurgiji.

Ključne reči:
etika, medicinska; pristanak informisanog pacijenta; zakonodavstvo; lekar-bolesnik odnos; rizik, procena; lečenje; hirurgija, torakalna; srbija.

Introduction

Patient-centered care is widely recognized as a core dimension of quality and one of the most pressing priorities in modern healthcare systems. Accordingly, during the last century, informed consent (IC) became a professional obligation, legal necessity, and ethical imperative [1–3]. Essentially, IC is not simply about getting a patient's signature on the consent form as a liability waiver but "…the process of providing patients with sufficient information that allows them to make a voluntary and informed decision whether to undergo or forego a procedure, provided that the information given is capable of being understood by the patient" [3]. Moreover, as a specific kind of "emotional inoculation" to the patients overwhelmed by anxiety, IC helps them develop a "psychological immunity" to the stressful effects of surgery, resulting in a better outcome [4].

Yet, in cardiac surgery and some other highly specialized areas of modern medicine, it is almost impossible to convey all the knowledge required for IC in the limited time available [5, 6].

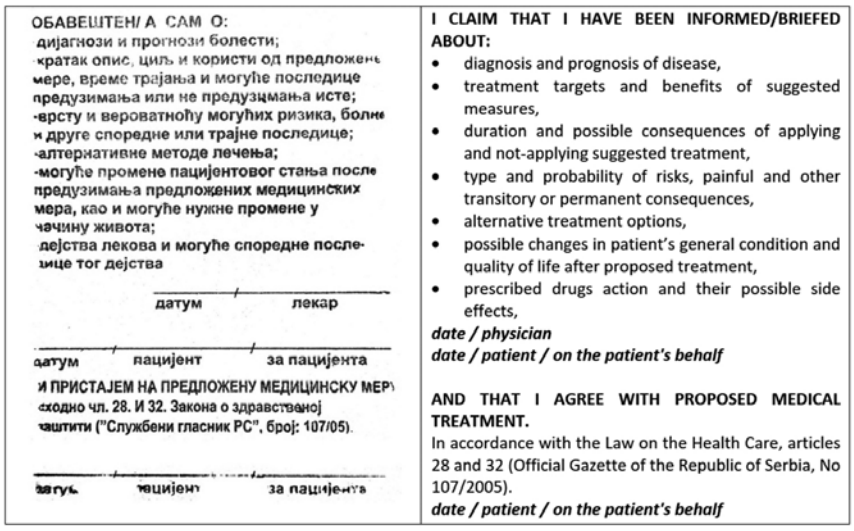
Growing waiting lists for cardiac surgery became a real and top priority problem in the Republic of Serbia (ROS), imposing serious difficulties in patient-surgeon communication. The aim of this study was to determine the current state of the IC concept before elective cardiac surgical procedures and analyze it considering actual legislation in the ROS.

Methods

The cross-sectional study, approved by the Institutional Review Board, used a targeted survey questionnaire (Supplement 1) and 200 consecutive patients admitted to the Clinic for Cardiac Surgery, Emergency Center of the University Clinical Center of Serbia, from September to December 2019, who voluntarily consented to complete it anonymously after having signed an actual consent form (Figure 1).

The first part of the questionnaire, containing 10 questions, was targeted to determine the quantity and quality of patients' information about general and the most important aspects of clinical cardiac surgical care (surgical procedure, extracorporeal circulation, anesthesia, transfusion, estimated risk). The second part of the questionnaire with three additional questions was aimed to depict a patient's opinion on the clarity and importance of information gained before surgery.

In addition, we created two completely new guides for patients bearing in mind what they should be aware of before signing the consent for cardiac surgery (Supplements 2 and 3).



ОБАВЕШТЕН/ А САМ О:
-дијагнози и прогнози болести;
-кратак опис, циљ и користи од предложен
мере, време трајања и могуће последице
предузимања или не предузимања исте;
-врсту и вероватноћу могућих ризика, болн
и друге споредне или трајне последице;
-алтернативне методе лечења;
-могуће промене пацијентовог стања после
предузимања предложених медицинских
мера, као и могуће нужне промене у
начину живота;
-дејства лекова и могуће споредне после-
дице тог дејства

датум лекар

датум пацијент за пацијента

И ПРИСТАЈЕМ НА ПРЕДЛОЖЕНУ МЕДИЦИНСКУ МЕР
сходно чл. 28. И 32. Закона о здравственој
заштити ("Службени гласник РС", број: 107/05).

датум пацијент за пацијента

I CLAIM THAT I HAVE BEEN INFORMED/BRIEFED ABOUT:

- diagnosis and prognosis of disease,
- treatment targets and benefits of suggested measures,
- duration and possible consequences of applying and not-applying suggested treatment,
- type and probability of risks, painful and other transitory or permanent consequences,
- alternative treatment options,
- possible changes in patient's general condition and quality of life after proposed treatment,
- prescribed drugs action and their possible side effects,

date / physician
date / patient / on the patient's behalf

AND THAT I AGREE WITH PROPOSED MEDICAL TREATMENT.
In accordance with the Law on the Health Care, articles 28 and 32 (Official Gazette of the Republic of Serbia, No 107/2005).
date / patient / on the patient's behalf

Fig. 1 – Left: Original consent form (stamp) currently in use in the Republic of Serbia in Serbian (Cyrillic) language; Right: The same consent form translated into the English language.

Results

The general characteristics of 200 surveyed patients are summarized in Table 1.

Table 1

Patients' general characteristics

Characteristics	Patients
Age (years)	66.2 (21–92)
Gender	
male	136 (68.0)
female	64 (32.0)
Ethnicity	
Serbian	190 (95.0)
other	10 (5.0)
Education	
low (primary, incomplete primary)	67 (33.5)
middle (secondary)	101 (50.5)
high (high, higher)	32 (16.0)
Indication for surgery	
coronary	88 (44.0)
valvular	52 (26.0)
combined	37 (18.5)
other	23 (11.5)

Results are shown as number (%) of patients except age which is shown as mean (range).

The mean age of 66.2 years and male predominance (68%) depicted the reality of the population in the ROS submitted to cardiac surgical procedures. Concerning ethnicity, the group was almost homogenous. The patients were of different educational levels, with the majority (84.0%) being with low (incomplete primary or primary) and middle (secondary) education. Indications for the cardiac surgical procedure were different, and this fact was a salutary fact for this survey.

Despite the already signed consent form (Figure 1), the results in the first part of the questionnaire (Table 2) generally indicated a low level of patient information about the upcoming cardiac surgery procedure on the type of cardiac surgery procedure they expect, extracorporeal circulation, anesthesia, and transfusion. Of those having information about the upcoming surgery, only 7.0–20.0% graded them as sufficient and satisfactory. Surprisingly, the worst situation was recorded in questions about risks of disease and upcoming surgical treatment, where 88.0% of patients had no information about it, and almost 90.0% of those with some information graded them insufficient.

The second part of the questionnaire (Table 3), where patients were asked to express their opinions on the clarity

Table 2

Quantity and quality of patient's information about general and the most important aspects of clinical cardiac surgical care

Questions	Answers, n (%)
1. Do you know which type of cardiac surgical procedure you will be submitted to (e.g., coronary, valvular, aortic, etc.)?	
yes	168 (84)
no	32 (16)
2. How do you grade your information about upcoming cardiac surgical operation?	
good (+)	33 (19.5)
average (±)	97 (58.0)
poor (−)	38 (22.5)
3. Do you know what extracorporeal circulation or heart-lung machine is?	
yes	108 (54)
no	92 (46)
4. How do you grade your information about extracorporeal circulation?	
good (+)	10 (9.5)
average (±)	36 (33.5)
poor (−)	62 (57.0)
5. Do you know which type of anaesthesia you will be submitted to (e.g., local, regional, general)?	
yes	88 (44)
no	112 (56)
6. How do you grade your information about cardiac surgical anaesthesia?	
good (+)	10 (11.0)
average (±)	30 (34.5)
poor (−)	48 (54.5)
7. Do you know that you will most likely receive blood, plasma, or derivatives during and/or after upcoming surgery?	
yes	97 (48.5)
no	103 (51.5)
8. How do you grade your information about perioperative transfusion?	
good (+)	7 (7.0)
average (±)	24 (25.0)
poor (−)	66 (68)
9. Do you know what the estimated individual risks of your disease(s) and upcoming surgical treatment are?	
yes	24 (12)
no	176 (88)
10. How do you grade your information about perioperative risks?	
good (+)	3 (12.0)
average (±)	11 (45.5)
poor (−)	10 (42.5)

Table 3

Patient's opinion on the clarity and importance of information gained before surgery

Questions	Answers, n (%)	
	yes	no
1. Did you talk with your operating surgeon about the upcoming operation?	37 (18.5)	163 (81.5)
2. Was the text of the consent form you have signed completely clear?	112 (56.0)	88 (44.0)
3. Do you consider that all relevant information related to your upcoming surgery should be explained to you?	171 (85.5)	29 (14.5)

and importance of information gained before surgery, revealed rather confusing and contradictory facts. It was surprising that 81.5% of patients signed the actual consent form (Figure 1) without any discussion about relevant issues with the operating surgeon. For slightly more than half of them (56.0%), the information offered in the consent form was clear and sufficient. Yet, 85.5% think that all relevant information about upcoming surgery should be explained to them. At the same time, 14.5% of patients are not interested at all to know even the most relevant information about their disease and surgery.

Discussion

Historically, during the last century, the importance of the IC concept was strengthened mainly in the courts. In January 1908, Mary Schloendorff was submitted to surgical intervention and suffered severe hand complications, even though she had adamantly declined it beforehand. After that, Schloendorff sued the hospital and won a 50,000 USD suit [7]. Justice Benjamin Cardozo famously wrote in the Court's opinion (Schloendorff vs. Society of New York Hospitals trial): "Every human being of adult years and sound mind has a right to determine what shall be done with his own body; and a surgeon who performs an operation without his patient's consent commits an assault, for which he is liable in damages." [3].

Paul G. Gebhard was an American attorney credited with creating the phrase "informed consent" in a 1957 medical malpractice case, in which a patient contended that a physician at a Stanford University hospital (i.e., Frank A. Gerbode 1907–1984, a famous surgeon and former president of American Association for Thoracic Surgery) had not fully disclosed the risks in a recommended treatment (i.e., translumbar aortography) [3].

Patients' rights are given great importance at the international, regional, and national levels through different agreements, declarations and charters, special laws or provisions of various laws that are fulfilled, protected, and respected by these rights [3, 8–10].

By adopting the Law on Patients' Rights (LPR) in 2013 and the Law on Health Care (LHC) in 2019, to harmonize national with European Union legislative, the Government of the ROS defined 19 distinctive rights, three of which (i.e., the right to information, the right to notice, and the right to consent) directly define IC, while several others indirectly support this issue [1, 2]. Quality of legislation is a formal and necessary framework, but the quality of the actual practice is more important, an essential component of any efficient healthcare system [11]. The same is true for patient rights and IC as a part of them.

Cardiovascular diseases remain the major burden for the healthcare system in the ROS [12]. In the absence of accurate data, it is a rough estimation that out of six million inhabitants, up to 10,000 patients annually need some kind of cardiac surgical intervention. The actual annual capacity of the five national cardiac surgical centers is 50–60% of that number [13]. Attempts to solve the problem of growing waiting lists by increasing the number of patients operated within existing capacities decreased "time per patient", negatively affecting the quality of care in many aspects.

Effective LPR does not oblige medical doctors (i.e., operating surgeons) to participate in the IC process but uses the term "authorized health care professional" instead [10]. Thus, in practice, a cardiac surgical patient may be informed by nurses or less experienced doctors (e.g., residents, fellows). Our study has shown that 81.5% of patients have not even seen their operating surgeon before the operation (Table 2). On the one hand, this fact is not strictly against LPR, but on the other hand, it definitively precludes the patient from being appropriately informed before signing the consent, as evident from Table 3. In a cross-sectional study at nine surgical hospitals, Agozzino et al. [14] showed much better results than ours, and still, they find their IC practice insufficient. Results of our survey also identify 85.5% of patients claiming their need to be properly informed (Table 2). This fact, along with the previous one, indicates that both legislation and practice should be more precise and efficient. The consent form (i.e., "stamp"), used in our institutional cardiac surgical practice (Figure 1), is a copy of the list of generic information the patient should have before surgery, taken from LPR [10]. Essentially, it is not IC but rather a liability waiver document. Moreover, LPR applied to cardiac surgical patients (i.e., expecting invasive medical treatment), in different articles [7, 10, 14, 15] insists that effective oral communication, even without the patient's explicit request, obligatory precede their voluntary consent and signature [2]. Law does not limit cardiac surgical centers to establish appropriate IC practice, so the actual practice is predominantly professional deficiency, depicting sluggishness and indifference among cardiac surgeons in ROS.

This kind of performing "the modern clinical ritual of trust" on a global level was nicely caricatured by Glyn Elwyn, who also raised the question of whether the nowadays patient consents were a "decision or assumption" [15]. Similarly, many others wondered if ICs for cardiac surgical

patients were "achievable" and "truly informed" [5, 16]. Although both the meaning and performance of IC are still debatable, illustrating rather social (i.e., population, legislative, health care systems) than medical and ethical differences, the efforts to adjust and improve IC practice are globally evident [8–10, 14, 17, 18]. In a comprehensive review by Hall et al. [9], finding no universally accepted standards, they nicely concluded that IC should be pragmatical: "…aimed to facilitate and document a good-faith effort to involve patients in medical decisions to whatever degree they are interested and able". Salzburg's statement on shared decision-making recognized that the IC concept has emerged from multiple disciplines and accordingly launched suggestions for its improvements on different "addresses" (i.e., clinicians, patients, researchers, editors, journalists, policymakers) [8].

It was not only the result of this study and available literature but rather our professional and ethical compulsion that motivated us to propose and initiate a completely new approach to the cardiac surgical IC practice. Educating the patient, like Brenner et al. [4] and many others [6, 19, 20] described, may help overcome different weak points of the IC process, allowing the patient and surgeon to develop an alliance rather than rivalry. Accordingly, we designed completely new consent forms (generic and disease-specific) covering all important issues that patients should be aware of before signing the consent for cardiac surgery (Supplements 2 and 3). These forms, written in non purely medical language empirically recognized to be understandable by our average patient (age and education level), are distributed during the patient's first visit when admission for the elective cardiac surgical procedure is scheduled. We had in mind that the majority (84%) of our examinees had an education level in accordance with the population of the ROS [21]. Thus, they have enough time to read and understand their content and (alone or with the help of family members and others) be well prepared to ask additional questions before they sign the consent form. Moreover, as a part of our initiative, we prepared the second edition of our patient-dedicated monograph, "Life after heart surgery: initial recovery", to create an integrated system of preoperative and postoperative notification for timely and more complete patient informing [22]. For any further enhancements (e.g., internet portal, audio-visual material, telemedical support, etc.), it is necessary to launch an initiative for the amendment of the LPR [10] specifying other alternative ways to inform patients. In more developed healthcare systems and cardiac surgical hospitals, a case manager is responsible for coordinating patient care throughout the cardiac surgical care continuum and ensuring the effective coordination and continuation of patient care from presurgery to postsurgery treatments [23]. Our healthcare system still does not recognize this professional profile, so we will have to propose it to our healthcare authorities. Meanwhile, we intend to start (weekly) with inpatient "guided virtual cardiac surgical tours", a form of interactive education where newly admitted patients would be able to see video material with all clinical wards and technologies explained by surgeons and nurses. With all these activities towards the improvement of our traditional IC policy, we hope to achieve a new level of confidence which would, in turn, result in better surgical outcomes.

Conclusion

The results of this study unambiguously indicate an unacceptably low level of our patients' information about the forthcoming cardiac surgical procedure, extracorporeal circulation, anesthesia, transfusion, and estimated risk, despite the already signed consent form. The majority of them (85.5%) comprehend the importance and expect timely and adequate information. It is not the patients' attitude or legislative restrictions to blame for such inadequate IC policy. An extremely high percentage (81.5%) of patients who had no chance to discuss the procedure with the operating surgeon indicates that both surgical indifference and insufficient knowledge of professional, ethical, and legal importance are the most important reasons for actual IC policy in cardiac surgery. The measures we took to change and improve established IC practice in national cardiac surgery should be validated with similar, more extended studies in the future.

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Conflict of interest

None declared.

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Supplement 1

SURVEY QUESTIONNAIRE

THE LEVEL OF YOUR INFORMATION ABOUT THE UPCOMING OPERATION

Please read the questions carefully and answer them. The way to fill out the survey is simple - circle the number in front of one of the answers provided. The purpose of this survey is to find out to what extent you are informed about your upcoming surgery. Analysis of the results of the survey will help us improve this segment of our activity.

The survey is anonymous and should not be signed.

1) Do you know what type of surgery/procedure will be performed on you?
1. Yes, I know
2. No, I don't know

2) To what extent are you informed about the upcoming surgery/procedure?
1. I am fully informed
2. I am partially informed
3. I am a little or not at all informed

3) To what extent are you informed about the possible following complications of the operation/procedure?

1. I am fully informed
2. I am partially informed
3. I am a little or not at all informed

4) Do you know that most open-heart surgeries are done using the technology of extracorporeal blood flow (during operation, your heart and lungs do not work)?
1. Yes, I know
2. No, I don't know

5) To what extent are you informed about the possible risks of using the technology of extracorporeal blood flow?
1. I am fully informed
2. I am partially informed
3. I am a little or not at all informed

6) Do you know which type of anaesthesia will be applied to you during surgery (general, regional, local, etc.)?
1. Yes, I know
2. No, I don't know

7) To what extent are you informed about the possible risks and complications of applying anesthesia?
1. I am fully informed
2. I am partially informed
3. I am a little or not at all informed

8) Do you know that you will most likely receive blood, plasma or its derivatives during and after surgery?
1. Yes, I know
2. No, I don't know

9) To what extent are you informed about possible accompanying phenomena and complications of the use of blood, plasma and its derivatives?
1. I am fully informed
2. I am partially informed
3. I am a little or not at all informed

10) Do you know how much your operational risk (Euro Score, STS) is in percentages?
1. Yes, I know
2. No, I don't know

11) Is the text of the consent form that you sign for the operation completely clear?
1. Yes, I know what I signed, and everything is clear to me
2. I am not sure, or I do not know

12) Do you consider that all the above important information related to the upcoming operation should be available to you?
1. Yes, I am interested in getting to know all the information before surgery
2. No, I am not interested

Thank you for your participation in this survey.

Supplement 2

01. GENERIC CONSENT FORM FOR CARDIAC SURGERY PROCEDURE

I confirm that I have read in written form and that the selected physician has explained the following issues to me:

1. My general health and proposed procedure, including additional procedures and proceedings. If intraoperative findings are not in line with preoperative diagnostics, I approve additional procedures. I understood the risks, including those specific to me.
2. That this procedure requires the use of anesthesia. I realize the risks of administering anesthesia, including those specific to me.
3. Possibilities of alternative procedures and the risks associated with them.
4. The prognosis of my disease and the risks if the proposed surgery is not done.
5. That there is no guarantee that my condition after the surgical procedure will be significantly better despite the professional efforts of the doctors and staff.
6. That the procedure may require the use of blood transfusions and/or blood products.
7. That a sample of my blood and/or tissues may be taken and used to make a definitive diagnosis and apply an adequate treatment, following the current state of health.
8. If suddenly a life-threatening situation arises during or after a procedure, proceedings and procedures may be applied in accordance with generally accepted principles to save my life.
9. That doctors who do not have the title of specialist can participate in my surgical treatment.

I was allowed to read and ask for more information on the following:

1. The procedure that will be performed.
2. Use and risks of extracorporeal blood flow.
3. Applying sedation and anesthesia.
4. Applying blood transfusions and blood products.
5. The overall individual risk of cardiac surgery treatment calculated and objectified using the Euro Score II and/or STS calculator.

I had the opportunity to ask my chosen physician questions about general health, the proposed procedure and the risks and the application of other therapeutic options. I was pleased with the answers to the questions asked.

I consent to the proposed cardiac surgery treatment on my own will and in a state of full and clear awareness, fully informed of the forthcoming cardiac surgery procedure and its risks, as well as possible complications.

I realized that it was my right to change my mind at any time before the procedure, even after signing this consent.

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Doctor signature Patient signature Authorized person signature

Supplement 3

2. CORONARY ARTERY BYPASS SURGERY - INFORMATION FOR PATIENT

In most cases, these surgical procedures are performed using extracorporeal blood flow. Procedures include the use of general anesthesia and the frequent use of blood transfusions and/or blood products. These types of operations may include the use of veins from your hands and/or feet and/or arteries located inside the chest (internal thoracic artery), less often the radial arteries of the hands, and extremely rarely the gastroepiploic arteries (the artery that runs along with the gastric band), which includes the opening of the abdominal cavity. The purpose of surgery is to establish a bypass flow of blood (from the aorta or subclavian artery) to the coronary artery (cardiac artery) below the site of narrowing or obstruction. The newly established bloodstream will deliver an adequate amount of blood and oxygen to the compromised part of the heart muscle and allow it to be functional to the extent necessary.

After admission to the hospital and placement in the hospital room, preoperative protocol preparation procedures will be performed. The day before the surgery, your hygiene preparation (bathing, shaving) is performed. On the day of surgery, you will be taken to a cardiac surgery room and taken into general anesthesia (immediately before and during surgery, you are in a controlled sleep state, unconscious). The surgery opens the thorax, most commonly by a longitudinal surgical incision in the middle of the sternum, and surgery is performed on the heart itself. When you wake up after completion of the surgery, you will be in the Intensive Care Unit connected to an artificial respiration machine. After clinical examination and laboratory findings confirm that you are capable of breathing independently, separation from the apparatus and removal of the endotracheal tube (plastic tube) from your mouth will be performed. Postoperative stay in the Intensive Care Unit in the absence of complications usually lasts 24-48 hours. After removal of the chest drains (plastic tubes inserted through the skin into the chest during surgery) and proper preparation, you will be transferred to the semi-Intensive Care Unit or the General Care Unit, where further postoperative hospital care continues. It involves getting out of bed and moving, doing physical exercises, administering appropriate medication, taking blood samples for laboratory tests, postoperative ultrasound examination of the heart, etc. If necessary, transfusions of blood and/or blood products may be used for therapeutic purposes. Discharge from the Cardiac Surgery Clinic is usually planned for the seventh postoperative day. Depending on your general condition, you may be discharged for home treatment or referred for further treatment to a regional healthcare facility.

During surgery as well as postoperatively, unwanted complications can occur.

THE MOST COMMON COMPLICATIONS (in total, more than 5% of patients)

1. Bleeding from a surgical incision or the chest. This condition may require transfusion of blood, plasma, or derivatives thereof and, in the cranial line, reopening of the thorax.
2. Heart rhythm disorders, which are usually transient and usually require medical treatment.
3. Cognitive impairment, short-term memory loss, difficulty concentrating and reading, and blurred vision, which can take several weeks after surgery.
4. Chest pain and/or insensitivity of the chest wall (a consequence of surgical access and/or use of the internal thoracic artery for graft) may be transient, last for months, or occasionally occur.
5. Feeling of "shortness of breath" and easy fatigue for less effort, which may be transient or permanent, depending on the functional state of the heart after surgery.
6. Inadequate healing of a surgical incision wound. After 1-2 months, there may be thickening and scarring accompanied by pain.
7. Deep vein thrombosis is characterized by painful swelling of the leg, which in rare cases causes embolism of the lungs, which can be life-threatening.

RARE COMPLICATIONS (in total 1-5%)

1. Lung collapse as a result of compression by air and/or fluid, which may require placement of the chest drainage, to facilitate respiratory function. It is more common in smokers or if there is a previous lung disease (emphysema, chronic bronchitis).
2. Renal insufficiency (renal impairment). It is more common in patients with preoperatively impaired renal function. The occurrence of this complication may require hemodialysis.
3. Infection of the mediastinum (midgut), chest, surgical cuts on the arm or leg, rarely followed by septic conditions. This will require the administration of antibiotics and, possibly, additional surgery (debridement, stabilization of the sternum).
4. Perioperative myocardial infarction, in extent from mild to severe.
5. Acute thrombosis of the graft may require an additional procedure.
6. Pulmonary function impairment, which, in addition to medication administration, may require the use of artificial respiration and/or tracheostomy devices.
7. Disorders of cardiac conduction or rhythm. This complication may require the implantation of a permanent pacemaker.
8. Problems in the functioning of the abdominal organs. An acute bleeding ulcer of the stomach or duodenum occurs, which may require the use of blood transfusion and/or blood products, gastroscopic intervention, and surgical treatment.
9. Stroke whose effects can be long-lasting.

EXTREMELY RARE COMPLICATIONS

1. Decubitus formation as a consequence of postoperative severe general weakness or for other reasons when the mobilization of the patient from the bed is not possible. This complication requires frequent dressings and, if necessary, additional surgical procedures.
2. Intraoperative burn formation when using the necessary electrical devices (thermocouple). Despite the implementation of all available prevention measures, an accidental generation of anomalous circuits can occur. The burns are treated by dressing and possibly applying antibiotics and additional surgical procedures.
3. Transmission of blood transmissible diseases by transfusions of blood and/or blood products.

Information on your operational risk calculated by the Euro Score II and/or STS calculator and expressed as a percentage will be available to you on the day of admission to the Cardiac Surgery Clinic.
You will be informed about the risks of using extracorporeal blood flow technology, general anesthesia, and transfusions of blood and/or blood products.
The fatal outcome may be due to the procedure proposed and undertaken.
If the proposed surgical procedure is not accepted as a solution, the natural course of the disease, despite the use of medication, will lead to exacerbation of the problems and the eventual appearance of emergencies. Acute exacerbations of the disease (acute heart attack, acute impairment of global heart function, malignant heart rhythm disorders) can be life-threatening and can cause sudden cardiac death. Life expectancy will be shorter and quality of life in progressive deterioration.

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Doctor signature Patient signature Authorized person signature

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Influence of the health status and other relevant factors on the quality of life of elderly people

Uticaj zdravstvenog statusa i drugih relevantnih faktora na kvalitet života starijih ljudi

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Abstract

Background/Aim. The world population is aging rapidly. It has become a challenge to meet the vital needs of the elderly in big cities. The aim of the study was to investigate the influence of the health status as well as other relevant factors on the perceived quality of life in aged people in Belgrade, Serbia. **Method.** The survey was conducted in October 2019 on a representative sample of 764 people (39.9% male and 60.1% female) aged between 65 and 79 years (mean ± standard deviation = 72.68 ± 7.11 years) living in Belgrade. In the research, a questionnaire developed on the basis of the World Health Organization (WHO) methodology for age-friendly cities was used, aimed to explore 11 factors important for the elderly. **Results.** Factor of physical accessibility of community and socioeconomic factors were highly significant [χ^2 (df = 3, n = 764) = 238.905; $p < 0.001$ and χ^2 (df = 3, n = 764) = 207.571; $p < 0.001$, respectively] and explained 39.4% and 35% of variance of perceived quality of life, respectively. Social environment explained 24.6% of variance of perceived quality of life [χ^2 (df = 4, n = 764) = 140.242; $p < 0.001$]. Health status had greatest explanatory power regarding perceived quality of life and explained as much as 46.7% of variance [χ^2 (df = 8, n = 764) = 292.083; $p < 0.001$]. When the unique impact of health status on quality of life was analyzed, when other variables were controlled, health status explained 21.6% of the variance in addition to the variance explained by physical accessibility, socioeconomic status, and social environment. **Conclusion.** Although health status has the greatest impact on the perceived quality of life of older people, interventions on other life important domains such as physical accessibility, socioeconomic status, and social environment could have a positive impact on the perceived quality of life where health status alone could not be improved.

Key words:
aged; health services accessibility; health status; social environment; socioeconomic factors; surveys and questionnaires; quality of life.

Apstrakt

Uvod/Cilj. Svetska populacija ubrzano stari. Pravi izazov je izaći u susret najvažnijim potrebama starih ljudi u velikim gradovima. Cilj rada bio je da se istraži uticaj zdravstvenog stanja kao i drugih bitnih faktora koji doprinose kvalitetu života starijih ljudi u Beogradu, Srbija. **Metode.** Istraživanje je sprovedeno u oktobru 2019. godine na reprezentativnom uzorku koji su činile 764 osobe starosti između 65 do 79 godina života (srednja vrednost ± standardna devijacija = 72,68 ± 7,11 godina), (39,9% muškog i 60,1% ženskog pola) koje žive na teritoriji Beograda. U istraživanju je korišćen upitnik formiran na osnovu metodologije Svetske zdravstvene organizacije (SZO) za gradove prilagođene starijim osobama (*age-friendly cities*), a cilj je bio da se istraži 11 faktora koji su važni za pomenutu populaciju. **Rezultati.** Faktor fizičke dostupnosti zajednice i socio-ekonomski faktori bili su izuzetno značajni [χ^2 (df = 3, n = 764) = 238,905; $p < 0,001$, odnosno χ^2 (df = 3, n = 764) = 207,571; $p < 0,001$] i objasnili su 39,4%, odnosno 35% varijanse perceptivnog kvaliteta života. Socijalno okruženje je objasnilo 24,6% varijanse perceptivnog kvaliteta života [χ^2 (df = 4, n = 764) = 140,242; $p < 0,001$]. Zdravstveni status je imao najznačajniji efekat u odnosu na percepciju kvaliteta života i objasnio je čak 46,7% varijanse [χ^2 (df = 8, n = 764) = 292,083; $p < 0,001$]. Kada se analizirao jedinstveni uticaj zdravstvenog stanja na kvalitet života, uz istovremenu kontrolu drugih varijabli, zdravstveni status je objasnio

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21,6% varijanse, uz onu koja se objašnjava fizičkom dostupnošću, socijalno-ekonomskim statusom i socijalnim okruženjem. **Zaključak.** Iako je zdravstveni status imao najveći uticaj na percepciju kvaliteta života starijih ljudi, intervencije na drugim životno važnim poljima kao što su fizička dostupnost, socijalno-ekonomski status i socijalno okruženje mogu imati pozitivan uticaj na percipirani kvalitet života tamo gde sam zdravstveni status starijih ljudi ne može biti poboljšan.

Ključne reči:
stare osobe; zdravstvene službe, dostupnost; zdravstveno stanje; socijalno okruženje; socijalno-ekonomski faktori; ankete i upitnici; kvalitet života.

Introduction

Acceleration of global population aging is one of the most important challenges the world is facing [1].

Meeting the increased need for health care services for the aging population has a significant economic impact on society [2]. Healthy aging and preserving the quality of life of the older population is one of the most important means of keeping rising costs bearable. World Health Organization (WHO) promotes healthy aging through maintaining the functional ability of the older people [3]. It depends mostly on health status but also on other domains which influence older people's quality of life as proposed in WHO methodology for assessing age-friendliness of the cities [4]. In the global WHO network for age-friendly cities, there are currently 760 cities, local communities, and other initiatives in 39 countries with more than 213 million older people living in it [5]. Ensuring mobility through different domains is of crucial importance for older people. Besides, older people can decide whether they want to stay in their homes as long as possible or be placed in the institutions such as elderly homes. Given the chance to decide, majority of them choose to stay in their own environment [6]. Living conditions, often, could be worse for the elderly, because of the necessity for adjustment of living space, and these interventions could be costly [7]. However, recent studies showed that there were no significant systematic differences in the WHO aging-friendly city domains in developed and developing countries, while physical accessibility, service proximity, affordability, and inclusiveness were the most important features related to healthy aging and quality of life [8].

The quality of life could be best described as a statistical index that is based on various parameters, such as economic-related, health-related, and environmental-related [9]. Poor social networks [10] and additional contributing features such as poor living conditions, poverty, and poor social relations [11] underwrite deterioration of quality of life. There is a well-established positive correlation between the social participation of the elderly and active healthy aging [12]. Older people actively involved in community events are less likely to ask for health services because of health problems or depression. Maintaining or even increasing social participation in the community could be seen as a protective factor against the harmful effect in different life situations such as functional impairment, disability, or even lack of family support. Therefore, social participation has a significant impact together with physical and mental health and the overall quality of life of older people [13]. In real-life circumstances through, participation in social networks decreases with aging, especially among low-income elderly people and members of minority groups. In fact, the rate of social exclusion of the elderly is constantly growing. At the level of public policies, the problem of loneliness of the elderly is increasingly opening up.

Nevertheless, health status is considered the most important factor that influences older persons' quality of life. The more health problems older people face, the lower the subjective estimation of quality of life and social inclusion is [14]. Health status impact on quality of life could be considered not only by a direct impact but also by consequences poor health has on older people's mobility and physical accessibility, as well as socioeconomic and social environment. Thus, improving the health status of each individual contributes not only to the quality of life but also to other life domains of the elderly.

The aim of the study was to investigate the influence of the health status as well as other relevant factors on the perceived quality of life in aged people in Belgrade, Serbia.

Methods

A study was conducted on a representative sample of 764 elderly living in Belgrade, Serbia. Data were collected in October 2019. The questionnaire was filled in during interviews with the elderly in their homes which ensured the participation of the respondents with reduced functional efficacy. Interviews were conducted by trained professionals, and all participants gave formal consent to participate in the study. The questionnaire was developed specifically for the study, based on the methodology developed by WHO for assessing the age-friendliness of the cities. The questionnaire consisted of 63 items covering further areas: physical accessibility of community, accessibility of public transport, accessibility of public spaces and buildings, accessibility and quality of housing, availability of information, safety, participation in community, accessibility and quality of health and social services, socioeconomic status, health status, and perceived quality of life. In fact, the connection between the quality of life on the one and the community design recommended on the other side is of extreme importance for planning for the aging population [15].

Socioeconomic status was measured through three indicators, ability to pay communal expenses, afford heating when cold, and provide an adequate diet. Perceived quality of life was measured by the standard question: "How would you rate your quality of life?". Health status was measured through the subjective perception of health, functional efficacy, and presence of specific health problems. The validity

of the questionnaire was assessed, and it was adjusted through cognitive interviews with 20 elderly with different functional efficacy and tested on a sample of 100 elderly.

Statistical analysis was performed using Statistical Package for Social Science version 23. All variables are present using frequency (n) and percentage (%).The binary logistic regression method with all variables in the model was used to analyze the relationship between physical accessibility of community, socioeconomic status, social environment, and health status with quality of life. For each group of factors, a separate model was created. A *p*-value of 0.05 and less was considered to be statistically significant. The effect size was estimated using Nagelkerke R-Squared, while the goodness-of-fit model was assessed based on Hosmer-Lemeshow (H-L) test, although the H-L test is found biased except for small samples, as small departures from the proposed model are identified as significant. In this sense, R-Squared was used primarily when discussing results. Based on previous work, a sample larger than 500 provided adequate power of logistic regression [16] (differences within ± 0.5 for coefficients and differences within ± 0.02 for Nagelkerke R-Squared). In order to assess the unique contribution of health status to quality of life, hierarchical binary logistic regression was used where variables representing physical accessibility, socioeconomic status, and social environment were entered in the first block, and in the second step, health variables were entered.

Results

The study sample consisted of 764 elderly, among which 39.9% were men (n = 305) and 60.1% were woman (n = 459). The age of participants was in the range from 65 to 79 years, with an average (mean ± standard deviation) age of 72.68 ± 7.11 years. The educational structure showed that majority of the participants had unfinished or finished primary education (44.1%). A smaller number (39.6%) completed secondary education, while 16.3% had a university degree. More than half of the participants (53%) lived with a spouse, 12% were divorced or did not live with a spouse, and 35% were widowed. The pension was the main source of income for the majority of the participants; for 75.5%, it was an old-age pension, while 15.6% received family pension. A total of 7.4% of participants had some other kind of income, 0.64% still worked earning a salary, and 0.64% had social assistance as the main source of income, while 0.13% had no income at all. Range of monthly income between 30 and 50 thousand dinars received 32.2% of respondents and 12.2% had an income higher than 50 thousand dinars. Range from 20 to 30 thousand dinars is the amount that 30.64% of respondents received monthly, while 19.7% of participants got less than 20 thousand dinars. More than half of the participants (54.03%) could not meet monthly needs with their personal income. When it comes to poverty indicators, a quarter of participants (26%) were unable to cover communal expenses, while 11.9% could not provide heating, 13.4% adequate diet, and 27.4% could not buy the medication they needed. About one-quarter of the elderly faced inaccessible physical surroundings – 24.5% reported that the neighborhood is inaccessible, 20.9% that public transport is inaccessible, and 18.6% that public spaces and buildings are inaccessible. Elderly face significant barriers concerning participation in the social environment. Although the elderly participate in family events at least once every three to four months (71.6%), they are excluded from the cultural life of the community, where just 17.9% of the elderly reported that they have participated in a cultural event. Moreover, one-third of the elderly (36.1%) did not feel they belong to the community. Most of the elderly (89.9%) felt safe in the communities they live in. Regarding health status, 15.3% of elderly saw their health as very bad or bad, 54.1% as satisfactory, while 30.6% assessed their health as very good or good. In line with this, 11.1% reported that their health condition severely restricts their ability to perform daily activities, 48.4% reported that their ability to function is slightly impaired, while 40.4% reported that their health status does not restrict them in daily functioning. Types of impairments were measured by asking participants if they had total or partial impairment (Table 1). Regarding the quality of life, 74.2% of the elderly assessed their quality of life as good or very good and 25.8% as bad or very bad.

Table 1

Frequency of health difficulties

Health difficulties	Participants n (%)
Impairment	
vision	97 (12.7)
hearing	101 (13.2)
physical	253 (33.1)
Problems with memory and concentration	88 (11.5)
Psychological and emotional problems	54 (7.1)
Chronic pain	308 (40.3)

Model representing factor of physical accessibility of community was highly significant [χ^2 (df = 3, n = 764) = 238.905; $p < 0.001$] and explained 39.4% of variance of perceived quality of life, with significant H-L test [$\chi^2 = 92.022$; $p < 0.001$]. All factors had significant contribution to the model, where accessibility of public transport and objects and spaces was more significant than accessibility of neighborhood. Socioeconomic factors explained approximately the same amount of variance as physical accessibility of community, 35% [χ^2 (df = 3, n = 764) = 207.571; $p < 0.001$], with H-L test being not significant [$\chi^2 = 0.245$; $p = 0.885$]. The greatest negative influence on perceived quality of life had the inability to meet living expenses such as to pay bills. Social environment had somewhat less explanatory power than physical accessibility which contributed with 39.45% and socioeconomic status with 35% in explanation of variance, although it explained 24.6% of variance of perceived quality of life [χ^2 (df = 4, n = 764) = 140.242; $p < 0.001$], with significant H-L test [$\chi^2 = 67.804$; $p < 0.001$]. The two variables that were significant predictors of perceived quality of life were the sense of belonging to a community and inclusion in family life, while inclusion in cultural events and perceived safety were not connected to perceived quality of

Table 2

Analysis by groups of variables

Model	OR	95% CI (lower – upper)	*p*-value
Physical accessibility			
neighborhood	1.776	1.142–2.761	0.011
public transport	6.728	4.295–10.540	0.000
public objects and spaces	5.972	3.740–9.538	0.000
Socioeconomic			
communal expenses	5.076	3.318–7.765	0.000
heating	4.304	2.144–8.639	0.000
food	2.257	1.195–4.263	0.012
Social environment			
belonging to community	3.813	2.647–5.490	0.000
inclusion in cultural events	1.524	0.871–2.667	0.140
inclusion in family life	4.105	2.816–5.983	0.000
safety	0.872	0.478–1.590	0.655
Health			
self-perceived health status (bad: satisfactory)	6.820	3.647–12.754	0.000
self-perceived health status (bad: good)	6.951	4.294–37.243	0.000
vision impairment	12.645	3.270–14.775	0.000
hearing impairment	0.689	0.303–1.569	0.375
physical impairment	2.388	1.375–4.147	0.002
problems with memory and concentration	0.236	0.111–0.500	0.000
psychological and emotional problems	0.048	0.018–0.132	0.000
chronic pain	1.598	1.010–2.529	0.045
functional efficacy (significant constraints: mild constraints)	10.322	4.218–25.257	0.000
functional efficacy (significant constraints: no constraints)	70.141	24.656–199.532	0.000

OR – odds ratio; CI – confidence interval.

Table 3

Results of hierarchical binary logistic regression

Factors	χ^2	df	*p*-value	Nagelkerke R-Squared
Block 1 (physical accessibility, socioeconomic, social environment)	370.057	10	0.000	0.564
Block 2 (health status)	208.185	10	0.000	0.216
Block 3 (public objects and spaces)	578.242	20	0.000	0.780

df – degree of freedom.

life. Health status had greatest explanatory power regarding perceived quality of life and explained as much as 46.7% of variance [χ^2 (df = 8, n = 764) = 292.083; $p < 0.001$], with H-L test being significant [χ^2 = 52.900; $p < 0.001$]. The most significant predictors of quality of life were functional efficacy and psychological and emotional problems. It was unexpected that vision impairment and physical impairment had a positive relation to the quality of life (Table 2).

If we analyze the unique impact of health status on quality of life, when other variables are controlled, it can be concluded that health status explained 21.6% of the variance in addition to the variance explained by physical accessibility, socioeconomic status, and social environment (Table 3).

Discussion

Population aging remains one of the biggest challenges we are facing, and it will remain the same in the decades to come. The global share of the population over the age of 65 in the total world population in 2019 was 9%, while it is estimated that it will be 23% in 2050. If the number of people older than 80 is considered, the acceleration of aging is even greater. While it is estimated that there were 54 million people over 80 years in 1990, in 2019, their number was 143 million, and it is expected that in 2100 there will be 881 million people older than 80 [17]. In Serbia, in 2019, 23.06% of the population was older than 65 years. The average age of the population has steadily increased over the last 70 and more years, and in 2019, it was 43.3 years. The aging index of the population, whose value does not exceed 20 index points, indicates a distinctly young population and over 40 to the old population. While the aging index of the population was 22.42, in 1950 in Serbia, in 2019, it was 144.05 [18].

Maintaining functional ability in older age and quality of life remains a strong starting point for aging in place in an older person's environment. Different factors influence the quality of life of older adults. Our study shows that 25.8% of the older population in Belgrade evaluates their quality of life as bad or very bad. Physical inaccessibility was reported by 24.5% of respondents when asked to evaluate their immediate neighborhood, 20.9% reported public transport, and 18.6% evaluated public spaces and buildings as inaccessible. However, accessibility is not commonly considered an obstacle in obtaining health and social care services, but it is an

important factor for maintaining physical and social activities aimed at preserving physical and mental health [19]. It is worrying that almost one-third of the older population (29.9%) finds its own socioeconomic status bad or very bad, with a significant number of elderly unable to cover basic needs like heating, living costs, or diet. Although such status has an undoubted impact on quality of life [20], there is no explicit proof of whether greater longevity has amplified the functional incapacity of older people and the number of dependents [21]. The social environment stays one of the key components of healthy aging and good quality of life in older age. In this area elderly faces significant barriers. Although 71.6% of them meet with their family at least once a month, they do not participate in cultural events (82.1%) and more than one-third of them (36.1%) feel rejected by the community. Participation in social events significantly reduces the risk of functional decline, just like frequent participation in family events [22]. Finally, among older people in Belgrade, the majority (84.7%) find their health status good, very good, or at least satisfactory, but even 15.3% find their health status bad or very bad. Even 11.1% report that they are severely restricted from performing daily activities important for their functional ability, and almost half of the older population (48.4%) reports some form of restriction, at least a slight one.

Studies show that health status is the most important factor that influences the quality of life of the elderly [23]. Results from our study are in line with these findings and clearly show that health status has the greatest explanatory power concerning the perceived quality of life and explains as much as 46.7% of the variance. Functional efficacy and psychological and emotional problems remain the greatest significant predictors of quality of life. Quite unexpectedly, our results show that vision impairment and physical impairment have a positive relation with the quality of life, which could be explained by the greater support these people acquire from their families. Other studies showed different findings pointing that visual and hearing impairment had a negative influence on the quality of life [24, 25].

However, when the unique impact of health status on quality of life is analyzed, when other variables are controlled, health status explains 21.6% of the variance in addition to the variance explained by physical accessibility, socioeconomic status, and social environment. These three domains are very important for their capacity to reduce negative health status impact on the quality of life. Physical accessibility of community explains 39.4% of the variance of perceived quality of life, socioeconomic factors describe 35%, while the social environment has less explanatory power with 24.6% of the variance of perceived quality of life.

Conclusion

While health status remains the key factor for good quality of life in older age, contributing features, such as physical accessibility, socioeconomic status, and social environment, could have a significant potential for reducing its negative influence on the quality of life. Health status also remains an important field for different types of interventions when health status solely could not be improved.

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Relationship between plasma high-sensitivity C-reactive protein and traditional cardiovascular risk factors among active-duty military personnel in the Republic of Serbia

Veza između koncentracije C-reaktivnog proteina visoke osetljivosti u plazmi i tradicionalnih kardiovaskularnih faktora rizika kod aktivnih vojnih lica u Republici Srbiji

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Abstract

Background/Aim. Approximately one-third of individuals with only one cardiovascular (CV) risk factor or without any, as well as 40% of individuals with a concentration of cholesterol less than average, die from CV diseases (CVD). Recent studies underlined the significant role of inflammation in atherosclerosis and its complications. The aim of this study was to analyze the association of high-sensitivity C-reactive protein (hs-CRP) with traditional risk factors for coronary heart disease. This is the first such study in Serbia. **Methods.** This is an observational cross-sectional study, which included active-duty military personnel of similar socio-epidemiological and economic characteristics. Plasma hs-CRP and traditional CV risk factors were evaluated. The relative CV risk was staged as low (hs-CRP < 1 mg/L), intermediate (hs-CRP between 1 and 3 mg/L), and high (hs-CRP > 3 mg/L). The Systematic Coronary Risk Evaluation (SCORE) system was used for absolute CV risk assessment and total risk (fatal and non-fatal events). **Results.** The study included 205 participants**,** average age of 39 (35–43) years, with median and interquartile range values of hs-CRP 0.80 mg/L (0.43–1.75 mg/L), with average hs-CRP values of 0.71 mg/L in participants younger than 40 years of age and 1.2 mg/L in the older than 40 years. The difference in hs-CRP values between these two groups was statistically significant ($p = 0.006$). There was a significant positive correlation between hs-CRP and age ($r = 0.266$, $p < 0.001$), weight ($r = 0.223$, $p = 0.001$), body mass index (BMI) ($r = 0.344$, $p < 0.001$), diastolic hypertension ($r = 0.190$, $p = 0.007$), LDL cholesterol ($r = 0.152$, $p = 0.032$), triglycerides ($r = 0.144$, $p = 0.039$), number of risk factors ($r = 0.210$, $p < 0.003$), as well as negative correlation with HDL cholesterol concentration ($r = -0.148$, $p < 0.035$). There was no significant correlation between hs-CRP concentration and total cholesterol ($r = 0.131$, $p = 0.062$). According to hs-CRP values, high CVD risk was found in 17.7% of participants older than 40 years of age, and based on SCORE system staging, 90% of participants have intermediate CVD risk. The results of stepwise multiple regression analyses showed that BMI was independently associated with an hs-CRP concentration in the group younger than 40 years of age. Among the older participants, age was found to be associated with concentration of fibrinogen. **Conclusion.** In the population of active military personnel in the Republic of Serbia, hs-CRP is correlated with some of the risk factors for CVD, and only BMI is independently correlated with hs-CRP in those under 40 years of age. Levels of plasma hs-CRP are increased with aging, implying that hs-CRP measurement may provide a more accurate assessment of the individual overall risk profile for CVD in the Serbian military personnel population.

Key words:
cardiovascular disease; high sensitivity c-reactive protein; military personnel; risk factor.

Apstrakt

Uvod/Cilj. Skoro trećina osoba bez ili sa jednim faktorom kardiovaskularnog (KV) rizika, kao i 40% osoba sa koncentracijom holesterola manjom od prosečne, umire od KV bolesti (KVB). Nedavna istraživanja su potvrdila značajnu ulogu upale u aterosklerozi i njenim komplikacijama. Cilj rada bio je da se utvrdi povezanost C-

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reaktivnog proteina visoke osetljivosti (hs-CRP) sa tradicionalnim faktorima rizika za koronarnu bolest kod profesionalnih vojnika. Ovo je prvo takvo istraživanje u Srbiji. **Metode.** Studija preseka obuhvatila je aktivna vojna lica sličnih socio-epidemioloških osobina i ekonomskog statusa. Ispitani su hs-CRP u plazmi i tradicionalni faktori KV rizika. Relativni KV rizik definisan je kao nizak (hs-CRP < 1 mg /L), srednji (hs-CRP između 1 i 3 mg/L) i visok (hs-CRP > 3 mg/L). Skala sistemske procene koronarnog rizika (SCORE) je korišćena za procenu apsolutnog i ukupnog KV rizika (fatalni i nefatalni događaji). **Rezultati.** Studija je obuhvatila 205 ispitanika prosečne starosti 39 (35–43) godina, sa srednjom vrednošću i vrednostima interkvartilnog raspona hs-CRP 0,80 mg/L (0,43–1,75 mg/L), sa srednjom vrednošću hs-CRP 0,71 mg/L kod mlađih od 40 godina, odnosno 1,2 mg/L kod starijih od 40 godina. Razlika u vrednosti hs-CRP između ove dve grupe bila je statistički značajna ($p = 0,006$). Zabeležena je značajna pozitivna korelacija između hs-CRP i životnog doba ($r = 0,266$, $p < 0,001$), telesne mase ($r = 0,223$, $p = 0,001$), indeksa telesne mase (ITM) ($r = 0,344$, $p < 0,001$), vrednosti dijastolnog pritiska ($r = 0,190$, $p = 0,007$), LDL holesterola ($r = 0,152$, $p = 0,032$), triglicerida ($r = 0,144$, $p = 0,039$), i broja faktora rizika ($r = 0,210$, $p < 0,003$), kao i negativna korelacija sa koncentracijom HDL holesterola ($r = -0,148$ $p < 0,035$). Nije bilo značajne korelacije između koncentracije hs-CRP i ukupnog holesterola ($r = 0,131$, $p = 0,062$). Prema vrednosti hs-CRP, visok rizik od KVB ustanovljen je kod 17,7% ispitanika starijih od 40 godina, a na osnovu vrednosti SCORE sistema, njih 90% je imalo srednji rizik od razvoja KVB. Rezulati multiple regresione analize pokazali su da je ITM bio nezavisno udružen sa koncentracijom hs-CRP-a u grupi ispitanika mlađih od 40 godina. Kod starijih ispitanika nađena je povezanost godina života i koncentracije fibrinogena. **Zaključak.** U populaciji aktivnih vojnih lica u Republici Srbiji, hs-CRP je u korelaciji sa nekim od faktora rizika od KVB, a samo je BMI u nezavisnoj korelaciji sa hs-CRP i to kod mlađih od 40 godina. Koncentracija hs-CRP u plazmi raste sa starenjem, što ukazuje na to da bi određivanje hs-CRP moglo biti značajno za precizniju procenu profila individualnog rizika od KVB u populaciji vojnog osoblja Srbije.

Ključne reči:
kardiovaskularne bolesti; c-reaktivni protein, visoko osetljiv; kadar, vojni; faktori rizika.

Introduction

Recent studies considering innate and acquired immunity pointed out their role in the initiation and progression of atherosclerosis and its complications [1, 2]. Numerous pathophysiological pathways link chronic inflammation, presented as a low-grade systemic inflammatory response (LGI), to the process of atherogenesis and the development of atheroma, unstable plaque, and/or acute coronary syndrome (ACS) [2, 3]. The importance of LGI in the identification of individuals at increased risk for the occurrence of adverse cardiovascular (CV) events has been confirmed in more than 50 prospective epidemiological and clinical studies worldwide [4–6]. These results are very suggestive of the clear link between markers of inflammation and short- and long-term CV outcomes. Inflammation markers (and markers of LGI) also include various acute phase reactants, such as C-reactive protein (CRP) [7].

CRP is produced predominantly in hepatocytes, especially after stimulation with the cytokines IL-6 and IL-1, as a consequence of infection, chronic inflammatory diseases, cancer, and tissue trauma, but the increase of CRP is also related to aging [8, 9]. Serum CRP levels can be determined by both standard and high-sensitivity CRP (hs-CRP) assays in clinical practice. Measurement of hs-CRP levels can accurately detect LGI state [9]. The hs-CRP is also one of the independent parameters to use in a situation where the clinical decision to initiate statin therapy is uncertain. The hs-CRP may also be used in primary prevention as a risk factor for CV disease (CVD) and a well-validated marker for the risk of future atherothrombotic events and CV mortality [6, 10].

As the inflammatory biomarker, hs-CRP adds prognostic information on CV risk comparable to arterial blood pressure (BP) or cholesterol. The prospective cohort studies have supported the view that relatively high levels of hs-CRP in otherwise healthy individuals are linked to an increased risk of sudden cardiac death, future heart attack, stroke, and/or peripheral arterial disease, as well as cardiac events in CVD patients with obesity and complications of diabetes [4, 5, 7, 11]. The values less than 1 mg/L, between 1 mg/L and 3 mg/L, and more than 3 mg/L indicate low, intermediate, or high relative CV risk, respectively [4]. The value of 2 mg/L is often used as CV cut-off in larger clinical studies that dealt with the relationship between inflammation and risk factors (RFs), but it also may be used as a modifier of CV risk and an indicator that may be helpful in the decision about the application of drug therapy [12, 13]. The American College of Cardiology and American Heart Association (ACC/AHA) primary prevention recommendations from 2019 categorize patients at intermediate risk as class IIa, and those with borderline risk as class IIb. They recommend hs-CRP as more predictive in the assessment of CV risk than the traditional CV RFs such as high-density lipoprotein (HDL) or low-density lipoprotein LDL cholesterol [12]. This could be very important if we keep in mind that more than 40% of people with lower-than-average cholesterol values and about one-third of those with no or only one traditional RF die from CVD [14–16].

The Republic of Serbia (RS) belongs to the group of countries with a high risk of developing CVD [17]. RS is a middle-income country, which has undergone significant economic changes and crises in the last 30 years, and, in this context, there are some population groups that have been particularly exposed to a higher risk of developing CVD [18–19]. The recruitment of professional soldiers (PS) should ensure that part of the population that is "healthier" than the general population is being engaged according to the principle of "a

kind of healthy worker effect" or "healthy warrior effect" [20, 21]. The specificity of professional military service due to exposure to stress as preparation for special tasks, i.e., the nature of work, duties, and lifestyle, can further burden the PS in terms of predisposition for the development of CVD [22, 23].

Our study is the first one in RS that has the aim to analyze the association of hs-CRP with traditional RFs for coronary heart disease.

Methods

This is a prospective cross-sectional study (conducted from September 2018 to July 2019) that included 205 active male military personnel (MP), more than 20 years of age, members of the same military unit, and similar socio-epidemiological and economic characteristics. The study is a part of a scientific project of the Ministry of Defence of the RS named "Primary prevention of ischemic heart disease of professional military personnel and military officers in the Republic of Serbia", which aims to implement modern principles of CVD prevention in the part of the MP that is subject of the regular systematic examinations and, possibly, suggest new ones. That part of the population is, practically, under systemic control, so there is a possibility of daily insight into the health condition of individuals.

According to the current recommendations on the safety and health at the workplace that define periodic examinations of persons at high-risk workplaces, professional members of the Serbian Army under the age of 40 years undergo a regular systematic examination every two years, while those over 40 years have a regular annual systematic examination [24].

Recommendations for primary CVD prevention and systematic assessment of CV risk are applied in men over 40 years and women over 50 years in the postmenopausal period without known CV RFs [17]. Therefore, we divided the MP into two groups, the younger and the older than 40 years.

Anthropometric measurements and calculations included body height and weight as well as body mass index (BMI). The BMI was derived from body weight expressed in kilograms divided by squared body height (kg/m^2). Recommended criteria were used for the assessment of overweight and obesity versus normal BMI. Cut-off value for overweight and obesity was BMI $\geq$ 25 kg/m^2. By using a flexible inch tape, the waist circumference (WC) was measured at the midpoint between the lower border of the rib cage and the iliac crest. The following cut-off value of the WC was used to assess the abdominal obesity for men: normal < 94 cm, moderate 94–101 cm, and large $\geq$ 102 cm [25].

The systolic BP (SBP) and the diastolic BP (DBP) were assessed by the sphygmomanometer with the participant in a sitting position and recorded as the arithmetic mean of three repeated measurements. All BP measurements were always taken by the same researcher and with the same-sized cuff for adults [17, 26].

According to the classification of the 2018 European Society of Cardiology (ESC) and European Society of Hypertension (ESH) Guidelines, hypertension (HTN) is defined as SBP $\geq$ 140 mmHg and/or the DBP $\geq$ 90 mmHg. All the participants who used antihypertensive therapy over the last 4 weeks were considered to have arterial HTN [26].

Plasma levels of total cholesterol (TC) and LDL-cholesterol, HDL-cholesterol, triglyceride (TG), and hs-CRP were determined by spectrophotometry using the ADVIA 1800 biochemistry analyzer (Siemens Healthcare Diagnostics, Tarrytown, NY, USA). Dyslipidemia is defined as TC > 5.2 mmol/L, TG > 1.7 mmol/L and HDL cholesterol < 1.0 mmol/L, as well as LDL cholesterol $\geq$ 3.4 mmol/L. In individuals with TG levels of 400 mg/dL and more, LDL-cholesterol was assessed by using the Friedewald formula [27].

The hs-CRP values less than 1 mg/L, between 1 mg/L and 3 mg/L, and more than 3 mg/L indicate low, intermediate, or high relative CV, respectively [4]. Smokers were defined as the individuals that have used more than one cigarette a day for at least 1 year or at least 20 packets of cigarettes in their lifetime [27].

The family history of premature CVD is when the occurrence of CVD is in the first generation, before the age of 55 years in men and 65 years in women [17]. The ten-year risk of the first fatal event caused by atherosclerosis, based on clinical features and laboratory tests, was calculated based on the Systematic Coronary Risk Evaluation (SCORE) system [17].

Statistical analysis

Categorical variables, presented as frequencies, were analyzed using the χ^2 test. All continuous variables, presented as mean value (± standard deviation) or median (interquartile range: 25–75 percentile) for normally or non-normally distributed data, were analyzed using the non-parametric Mann-Whitney test and Kruskal-Wallis test. The relationship between variables was tested with Spearman's Rank Order Correlation. The Shapiro-Wilk test was used to test the normality of data distribution. Multiple regression analysis was used for the assessment of each independent variable significance in predicting or influencing hs-CRP (dependent variable). Statistical significance was defined as $p < 0.05$ for all comparisons. All data were analyzed using the Statistical Package IBM-SPSS, version 26.0.

The principles of ICH Good Clinical Practice were strictly followed, and ethical approval No 151/2019 (05/11/2019) from the Ethics Committee of the Military Medical Academy was obtained for the study protocol. Written informed consent was obtained from each participant.

Results

The basic characteristics of MP are shown in Table 1.

The 10-year risk of the first fatal event caused by atherosclerosis (SCORE risk) was identified in 10% of low-risk subjects (SCORE < 1%), while 90% of subjects were at moderate risk (SCORE $\geq$ 1% and < 5%) (Table 2).

All respondents were divided according to age into two subgroups: those under 40 and those over 40 years of age. In

Table 1

Basic demographic, biochemical, and clinical characteristics of subjects (n = 205)

Parameters	Values
Demographic	
smoking	61 (29.7)
family anamnesis for CVD	43 (21.0)
alcohol	18 (8.8)
body height, cm	181.00 (177.00–186.00)
body weight, kg	88 (78.75–96.05)
waist circumference, cm	107.00 (100.00–113.50)
waist circumference ≥ 102 cm	144 (70.2)
body mass index, kg/m^2	26.80 (24.80–28.70)
Biochemical	
cholesterol, mmol/L	5.30 (4.56–6.13)
cholesterol ≥ 5.2 mmol/L	113 (55.1)
triglycerides, mmol/L	1.17 (0.78–1.74)
triglycerides ≥ 1.7 mmol/L	54 (26.3)
HDL cholesterol, mmol/L	1.26 (1.11–1.50)
HDL cholesterol ≥ 3.4 mmol/L	26 (12.7)
glycaemia, mmol/L	5.40 (5.10–5.80)
sedimentation, %	7.00 (5.00–10.00)
creatinine, μmol/L	81 (74.50–87.00)
fibrinogen, g/L	2.90 (2.40–3.40)
hs-CRP, mg/L	0.80 0.43–1.75)
low risk (< 1 mg/L)	120 (58.5)
intermediate risk (1–3 mg/L)	58 (28.3)
high risk (≥ 3 mg/L)	27 (13.2)
Clinical	
overweight (BMI: ≥ 25 kg/m^2)	149 (72.7)
normal (BMI: < 24.9 kg/m^2)	56 (27.3)
systolic blood pressure, mmHg	120 (120–130)
diastolic blood pressure, mmHg	80 (80–80)

Values are presented as number (%) or median (interquartile range: 25–75th percentile).
CVD − cardiovascular diseases; HDL − high-density lipoprotein; hs-CRP − high-sensitive C-reactive protein; BMI – body mass index.

Table 2

The Systematic Coronary Risk Evaluation (SCORE) for individuals older than 40 years of age

Score (%)	Examinees, n (%)
< 1	8 (10.1)
1	45 (57.0)
2	16 (20.2)
3	6 (7.6)
4	3 (3.8)
5	1 (1.3)

the group of subjects younger than 40, the median age was 36 (34–38) years, while in the group over 40, the median age was 44 (42–47) years ($p < 0.001$) (Table 3).

In the group younger than 40 years, there were significantly lower values of body mass and WC, BP, TC, LDL cholesterol, TG, as well as fibrinogen and hs-CRP (the average value 0.71 mg/L vs. 1.20 mg/L, $p = 0.006$). There were no statistically significant differences between the groups in body height, glycemic value, and HbA1C.

Distribution of hs-CRP and correlation with risk factors

The average value of hs-CRP was 0.8 mg/L. Using widely available high-sensitivity assays, hs-CRP levels of < 1 mg/L, 1 mg/L to 3 mg/L, and > 3 mg/ L corresponded to low-, moderate-, and high-risk groups for future CV events; low risk was detected in 58.5% of participants, moderate in 28.3%, and high in 13.2% of participants (Table 1).

Table 3

Biochemical characteristics of participants

Parameters	Age of participants (years)		*p*-value
	< 40	≥ 40	
Age, years	36 (34–38)	44 (42–47)	< 0.001*
Total cholesterol (C), mmol/L	5.11 (4.37–5.76)	5.57 (4.94–6.36)	< 0.001*
< 5.2	65 (51.6)	27 (34.2)	0.022**
≥ 5.2	61 (48.4)	52 (65.8)	
Triglycerides, mmol/L	1.08 (0.75–1.43)	1.40 (0.92–2.17)	0.005*
< 1.7	101 (80.8)	49 (62.0)	0.005**
≥ 1.7	24 (19.2)	30 (38.0)	
HDL-C, mmol/L	1.25 (1.10–1.46)	1.32 (1.12–1.56)	0.198*
≥ 1.0	17 (13.5)	9 (11.4)	0.823**
< 1.0	109 (86.5)	70 (88.6)	
LDL-C, mmol/L	3.21 (2.65–3.94)	3.45 (3.06–4.23)	0.030*
< 3.4	72 (58.5)	36 (46.2)	0.116**
≥ 3.4	51 (41.5)	42 (53.8)	
Glycemia, mmol/L	5.40 (5.10–5.80)	5.50 (5.20–5.90)	0.154*
HbA1c, (%)	5.50 (5.30–5.60)	5.75 (5.45–5.97)	0.138*
Fibrinogen, g/L	2.60 (2.30–3.00)	3.20 (2.80–3.50)	< 0.001*
hs-CRP, mg/L	0.71 (0.40–1.39)	1.20 (0.56–2.26)	0.006*
Arterial hypertension	16 (12.69)	23 (29.11)	0.005**
Normal arterial tension	110 (87.3)	56 (70.9)	
Systolic blood pressure, mmHg	120.00 (120.00–125.00)	130.00 (120.00–135.00)	< 0.001*
Diastolic blood pressure, mmHg	80.00 (80.00–80.00)	80.00 (80.00–86.25)	0.002*
Body mass, kg	87.50 (80.00–96.00)	91.15 (83.32–97.80)	0.001*
Body height, cm	181.00 (177.00–186.00)	181.00 (177.00–185.87)	0.443*
Waist circumference, cm	105.00 (95.00–112.10)	110.20 (105.00–116.00)	< 0.001*
Body mass index, kg/m^2	26.40 (24.70–28.60)	27.41 (25.72–29.77)	< 0.001*
normal (< 24.9 kg/m^2)	41 (32.5)	15 (19.0)	0.050**
overweight (≥ 25 kg/m^2)	85 (67.5)	64 (81.0)	

Values are presented as number (%) or median (interquartile range: 25–75th percentile).
HDL – high-density lipoprotein; LDL – low-density lipoprotein; hs-CRP – high-sensitive C-reactive protein.
*** – Mann-Whitney U test; ** – Chi-squared (χ^2) test.**

In 13% or 10.3% of respondents younger than 40 years, the value of hs-CRP was in the high-risk category, and in those older than 40 years, it was 17.7%. There was a statistically significant difference in the categorization of risk by hs-CRP values in the groups of the younger/older than 40 years (Table 4).

Table 4

Category of relative risk for cardiovascular diseases (CVD) based on high-sensitive C-reactive protein (hs-CRP) levels in participants younger and older than 40 years

hs-CRP level (mg/L)	< 40 years	≥ 40 years	*p*-value
< 1 (low risk)	83 (65.9)	37 (46.8)	
1–3 (moderate risk)	30 (23.8)	28 (35.4)	0.025*
≥ 3 (high risk)	13(10.3)	14 (17.7)	

Values were presented as number (%).
*** – Chi-squared (χ^2) test**

The significant correlations between some RFs and hs-CRP values were recorded. Those correlations were registered among the hs-CRP values and the age, body weight, BMI, BP and DBP, LDL cholesterol, and TG, respectively. There was a negative correlation between the hs-CRP values and HDL cholesterol, while there was no statistically significant correlation of hs-CRP with the values of TC (Table 5).

Table 5

Correlation of risk factors with high-sensitive C-reactive protein (hs-CRP) values

Risk factor	Correlation coefficient*	*p*-value
Age	0.266	< 0.001
Total cholesterol (C)	0.131	0.062
LDL-C	0.152	0.032
HDL-C	-0.148	0.035
Triglycerides	0.144	0.039
Arterial hypertension	0.135	0.050
Systolic blood pressure	0.137	0.050
Diastolic blood pressure	0.190	0.007
Body mass	0.223	0.001
Body mass index	0.344	< 0.001

HDL – high-density lipoprotein; LDL – low-density lipoprotein.
*** – Spearman's rank correlation coefficient.**

The correlation between hs-CRP and the number of RF was also statistically significant (r = 0.206, p = 0.003). The value of hs-CRP in subjects without RFs was 0.68 mg/L (0.40–1.15 mg/L), with 1 RFs 0.79 mg/L (0.41–1.54 mg/L),

Table 6

Correlation of the individual number of risk factors with high-sensitive C-reactive protein (hs-CRP) values

Individual number of risk factors	Number (%) of participants	hs-CRP, mg/L median (IQR)	*p*-value*	Correlation coefficient**
0	54 (26.3)	0.68 (0.40–1.15)		
1	84 (41.0)	0.79 (0.41–1.54)		
2	50 (24.4)	1.16 (0.55–2.92)	0.037	r = 0.206, *p* = 0.003
3	16 (7.8)	1.22 (0.59–2.65)		
4	1 (0.5)	3.77 (-)		

IQR – interquartile range: 25–75th percentile.
*** – Kruskal-Wallis test; ** – Spearman's rank correlation coefficient.**

Table 7

Clinical and biochemical characteristics of the subjects according to high-sensitivity C-reactive protein (hs-CRP) value

Characteristics	hs-CRP (mg/L)		*p*-value
	< 3 n = 178 (86.2%)	≥ 3 n = 27 (13.2%)	
Cholesterol, mmol/L	5.28 (4.51–5.99)	5.44 (4.63–6.26)	0.240*
Triglycerides, mmol/L	1.08 (0.78–1.55)	1.30 (0.97–2.21)	0.031*
HDL cholesterol, mmol/L	1.29 (1.12–1.54)	1.16 (1.06–1.37)	0.008*
LDL cholesterol, mmol/L	3.31 (2.72–3.96)	3.50 (2.81–4.37)	0.155*
Glycaemia, mmol/L	5.40 (5.10–5.80)	5.60 (5.20–6.00)	0.139*
HbA1c, %	5.50 (5.40–5.60)	5.80 (5.10-6.30)	0.633*
Fibrinogen, g/L	2.80 (2.30–3.30)	3.40 (2.80–3.70)	< 0.001*
Systolic blood pressure, mmHg	120.00 (120.00–130.00)	120.00 (120.00–130.00)	0.220*
Diastolic blood pressure, mmHg	80.00 (80.00–80.00)	80.00 (80.00–85.00)	0.131*
Arterial hypertension ≥ 140/90 mmHg	28 (15.7)	11 (25.0)	0.279**
Body mass, kg	87.00 (78.65–95.20)	90.50 (80.77–103.35)	0.077*
Body height, cm	181.00 (176.00–185.00)	180.00 (173.50–184.50)	0.449*
Waist circumference, cm	105.00 (95.00–112.10)	110.20 (105.00–116.00)	< 0.001*
<102	54 (33.5)	7 (15.9)	0.030**
≥102	107 (66.5)	37(84.1)	
Body mass index, kg/m^2	26.50 (24.55–28.34)	27.63 (26.00–31.10)	0.004*
normal (< 24.9 kg/m^2)	53 (29.8)	3 (11.1)	0.043**
overweight (≥ 25) kg/m^2)	125 (70.2)	24 (88.9)	

Values were presented as number (%) or median (interquartile range: 25–75th percentile).
HDL – high-density lipoprotein; LDL – low-density lipoprotein; HbA1c – glycated hemoglobin.
*** – Mann-Whitney U test; ** – Chi-squared (χ^2) test.**

Table 8

Univariate and multivariate linear regression analysis of assessment the importance of each independent variable in predicting or influencing high-sensitivity C-reactive protein (hs-CRP) in participants younger and older than 40 years

Independent variables	< 40 years				≥ 40 years			
	univariate analysis		multivariate analysis		univariate analysis		multivariate analysis	
	Beta	*p*-value	Beta	*p*-value	Beta	*p*-value	Beta	*p*-value
Age	0.143	0.110	0.050	0.602	0.264	0.019	0.190	0.030
Cholesterol (C)	0.081	0.367			-0.041	0.717		
Triglycerides	0.019	0.832			0.198	0.080		
HDL-C	-0.094	0.298	-0.075	0.414	-0.240	0.033	-0.169	0.145
LDL-C	0.127	0.161			-0.044	0.705		
Fibrinogen	0.216	0.021	0.183	0.057	0.069	0.561	0.269	0.026
BMI	0.274	0.002	0.240	0.012	0.132	0.248	-0.093	0.453
Systolic BP	0.035	0.698			0.086	0.453		
Diastolic BP	0.055	0.547			0.145	0.206		
AH	-0.032	0.722	-0.065	0.485	0.225	0.048	0.199	0.093

HDL − high-density lipoprotein; LDL − low-density lipoprotein; BMI − body mass index; BP – blood pressure; AH – arterial hypertension.
hs-CRP is a dependent variable (Multivariate analysis in the group < 40 years: F = 2.917, *p* = 0.017, R^2 – R-squared 12.0%; Multivariate analysis in the group ≥ 40 years: F = 3.678, *p* = 0.005, R-squared 21.8%).

with 2 RFs 1.16 mg/L (0.55–2.92 mg/L), and in subjects with 3 RFs was 1.22 mg/L (0.59–2.65 mg/L) (Table 6).

Our results single out a group of 27 (13%) subjects with hs-CRP values > 3 mg/L (Table 7). In this group, compared to group with hs-CRP values 3 mg/L and less, TG, fibrinogen, WC and BMI values were significantly higher, but the HDL cholesterol concentration was lower ($p = 0.031$, $p < 0.001$, $p < 0.001$, $p = 0.004$, $p = 0.008$, respectively).

Multiple regression analysis pointed out that age and fibrinogen had a significant effect on hs-CRP in the group of individuals older than 40 ($p = 0.030$ and $p = 0.026$, respectively). Among the younger, only BMI had an effect on hs-CRP ($p = 0.012$) (Table 8).

Discussion

This is the first study in Serbia to investigate the association of hs-CRP with RF in a healthy male population. The results of our study showed that hs-CRP as a measure of LGI identifies 27 (13%; 13 younger than 40 years, 14 older than 40 years) subjects with a high relative risk for coronary occlusive disease, in contrast to the SCORE system that identified only intermediate risk subjects. The hs-CRP values correlate with individual RFs (age, BMI, body weight, TG values, negative correlation with HDL cholesterol values) but not with TC values. The correlation is the most significant between age and BMI. Moreover, the research showed that hs-CRP is significantly correlated with the number of RFs of individual participants. Furthermore, stepwise multiple regression analysis points out that age and fibrinogen significantly affect the value of hs-CRP in the participants older than 40 years and BMI in the younger group.

According to the European Association of Cardiologists and a European study that involved 7 countries, there are growing trends in mortality in RS, which is part of the phenomenon observed in Eastern European countries during the second half of the last century [18]. It is also stated that reliable and complete official data on mortality are not available for RS due to war events and reforms, which prevented the systematic collection of data [18].

The average value of hs-CRP among our study participants was 0.8 mg/L. Data on hs-CRP values in “apparently healthy” in RS are unavailable to us. In healthy young adults (both sexes, age 18–63 years) volunteer blood donors, the median concentration of hs-CRP is 0.8–10 mg/L (90th – 99th percentile) [8]. In the United States, 56% of the male population has hs-CRP values up to 1.9 mg/L. The age group of 30–49 years (51% of all) of both sexes has the same value [28]. The HUNT study [29] pointed out that the average value of hs-CRP in the male aged 49.7 ± 16.2 is 1.0 mg/L (IQR 1.8 mg/L), which is comparable to our results, with the notice that the average age in our group is significantly lower, i.e., 39 (35–43) years. It is well known that hs-CRP values “increase” with age, and in this context, the hs-CRP values of our group are equivalent to the significantly older population Norwegian study population [30, 31]. In a study population of 507 healthy people of different ages, the CRP value in individuals under the age of 40 years was 0.95 (± 0.37) mg/L, and in the age between 40 and 44 years was 1.17 (± 0.59) mg/L [32]. In Chinese middle-aged males, the value of hs-CRP is 1.24 (0.65–2.57) mg/L [33].

It should be emphasized that the values of hs-CRP are different in the Asian and European populations, so they are variable depending on the ethnic origin [34, 35]. In the Whitehall II study, a long-term prospective cohort of 7,636 British civil servants with an average age of 50.7 ± 6.6 years, hs-CRP values are 0.84 mg/L (IQR, 1.30 mg/L), and according to this and the previously cited Norwegian study, our study group hs-CRP values are comparable to the values for the elderly persons [36]. An explanation for these values in our group may be the fact that almost 73% of our participants are overweight with BMI ≥ 25 kg/m^2, which strictly correlates with hs-CRP values. Adipose tissue has a great inflammatory potential, especially the central type of obesity recorded in 70% of our study participants, also in one-third of respondents with at least 2 RFs, affecting LGL [4, 6, 37, 38].

According to ESC recommendations, SCORE risk is calculated in individuals over 40 years of age. According to the SCORE risk, 90% of respondents belong to the group of medium (moderate risk), and 10% are low risk (Table 2). The lifestyle change is the therapy of choice in those patients. In case of inefficiency of this strategy, in low-risk persons, it is recommended to consider the use of drugs in individuals with LDL cholesterol values of 3.0–4.9 mmol/L, and the use of drugs is recommended when LDL cholesterol values are > 4.9 mmol/L; in case of SCORE risk 1–5% (moderate risk) with LDL cholesterol values of 2.6–4.9 mmol/L, the use of drugs may be considered, and in case of LDL cholesterol values over 4.9 mmol/L, drug therapy should be considered [39]. We did not have subjects with LDL cholesterol values > 4.9 mmol/L.

According to the Centers for Disease Control and Prevention and the American Heart Association criteria, hs-CRP values > 3 mg/L are qualified as "high risk", and, therefore, 27 (13.2%) individuals in our study are at “high risk", which would imply the use of drug therapy [4, 13]. That also means that 14 (17.7%) participants older than 40 years should have drug therapy. Particularly important is the fact that 13 (10.3%) respondents under the age of 40 (the group that is not included in the recommendations of primary prevention based on SCORE) should have drug therapy [17].

We wittingly did not use the Reynolds Risk Score for men to have a comparison of only two scores for total CV risk, which are based on different models [40].

Our research showed that hs-CRP positively correlates with some RFs, especially with age, body weight, BMI, DBP values, LDL cholesterol, and TG values, and has negative correlation with HDL cholesterol values (Table 5). The correlation between hs-CRP and traditional RFs has already been proven, but the relationships are not completely clear [4, 6, 41, 42]. Inflammation is present in all stages of atherosclerosis, and it is the basic

pathophysiological process of CVD [1, 2]. On the other side, according to generally accepted views, the risk of developing CVD depends on the influence and number of traditional RFs [13, 17, 40]. The complexity of the pathophysiological process in inflammation and its links with RFs was underlined in well-documented studies (JUPITER and CANTOS) [43, 44]. Those studies referred to CV mortality decrement as the result of inflammation reduction [43, 44]. It seems to be very important to have in mind that 40% of people with lower-than-average cholesterol values and about one-third of those with no or one traditional RF die from CVD [13–15].

The results of the JUPITER, and later the CANTOS study, could shift principles and focuses on primary and secondary prevention of CVD. The JUPITER study with prospective follow-up showed that statin therapy improved CV outcomes in individuals with hs-CRP values > 2 mg/L and LDL cholesterol values < 3.4 mmol/L without a previous history of coronary heart disease [43]. The CANTOS study practically confirmed the inflammatory hypothesis in the development of atherosclerosis [44]. The CANTOS study showed that reducing inflammation by inhibiting IL-1β significantly reduced vascular risk beyond the level that can be achieved by lowering lipid concentrations. CANTOS further showed a 31% reduction in CV mortality and all-cause mortality among canakinumab-treated patients who achieved the greatest reduction in hs-CRP, as well as therapy efficacy in high-risk patients with chronic kidney disease and diabetes [44]. Such results impose a special role of LGI inflammation in the prevention of CVD.

Both ESC recommendations in the treatment of dyslipidemias as well as ESC recommendations for primary prevention neglect the importance of hs-CRP, while ACC/AHA recommendations are significantly more liberal in this context. Bearing in mind that MPs belong to a particularly "sensitive category" due to the specificity of the workplace, any data that would indicate an increase in both relative and absolute risk among this population is more than welcome. In this context, our results single out a group of 27 (13%) subjects with hs-CRP values > 3 mg/L. These are subjects with an increased inflammatory response expressed through hs-CRP that should not only be linked with CVD but may also be used in the prediction of certain malignant diseases [8, 9].

The incidence of premature CVD has not decreased, while the prevalence of obesity, arterial HTN, and diabetes increased in the younger population, which poses a specific and growing therapeutic challenge [45]. Furthermore, the results of the INTERHEART study (Effect of Potentially Modifiable Risk Factors Associated with Myocardial Infarction in 52 Countries) showed that 9 traditional RFs pose 90% of the population's risk for myocardial infarction, particularly influencing young people that are less aware of their RFs, and would, therefore, participate less in primary prevention programs [46, 47].

A particular problem is the identification of the total CV risk in the part of the population younger than 40 years, for which there is no validated and generally accepted algorithm, except recently published individual standardizations adapted to particular regions [48]. There are over 250 similar logarithms, and another CV calculator probably will not be needed [49]. However, MPs belong to the specific part of the population, which are, according to their professional obligations, to a great degree exposed to the risk of developing CVD [22, 23]. They are mostly younger than 50 years, and it is of primary significance that prevention of CVD and preclinical atherosclerosis in this population be implemented into their regular systematic examinations [45, 50].

Last but not least, multiple regression analysis showed that age and fibrinogen have a significant influence on the increase in hs-CRP values in the group older than 40 years, while in younger people, only BMI has such influence on hs-CRP. These results are in accordance with recently published study data considering the relationship between inflammation and aging [30, 31, 33]. Interestingly, age has no significant influence on hs-CRP in younger people. This is due to the fact that the older group has been exposed to RFs for a long time, as well as that the number of RFs increases with age [30, 42, 48].

Taking these facts into account and incorporating them into the context of professional military service, we tried to adapt this knowledge to our circumstances in order to early detect high-risk individuals for the development of CVD followed by adequate primary prevention measures implementation.

Based on the previously presented facts, it may be advised, in addition to ESC recommendation standards for primary prevention, to evaluate hs-CRP as an affordable biomarker that might help identify individuals who should be under more frequent medical supervision. It provides reliable information for early clinical diagnosis and treatment of incident CVD, as well as monitoring the therapeutic effect of the anti-inflammatory therapy.

Limitations of the study

Bearing in mind that recommendations for primary CVD prevention and systematic assessment of CV risk are applied in men over the age of 40, we divided the MP into two groups – younger and the older than 40 years of age – who are members of the same military unit, with similar socio-epidemiological and economic characteristics. Our study is the first one in RS with the aim to analyze the association of hs-CRP with traditional RFs for coronary heart disease but has some limitations considering study population size and diversity (no females) that may influence the generalization of the obtained conclusions.

Conclusion

In the population of active military personnel in the RS, hs-CRP is correlated with some of the risk factors for CVD, but only BMI is independently correlated with hs-CRP in those under 40 years of age. Levels of plasma hs-CRP are increased with aging, implying that hs-CRP

measurement may provide a more accurate assessment of the individual overall risk profile for CVD in the Serbian MP population.

Conflict of interest

Not applicable.

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Anatomical variations of the superficial palmar arch and its clinical relevance

Anatomske varijacije površinskog luka dlana i njihov klinički značaj

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Abstract

Background/Aim. In-depth knowledge of the vascular network of the hand is of great importance in modern medicine. The main vessel of the hand is the superficial palmar arch (SPA). As typically described in anatomical textbooks, it arises as a terminal branch of the ulnar artery, which then anastomoses with the superficial palmar branch of the radial artery. However, the SPA is characterized by remarkable variability, which has been the area of interest of many researchers so far. The aim of this study was to exert a comprehensive examination of the anatomy of the SPA. **Methods.** The research was conducted at the Institute of Anatomy "Niko Miljanić" on a total number of 14 cadavers. After careful dissection, variations of the modality of formation of the SPA and its distance of the SPA from Kaplan's cardinal line were observed on the right hands. Collected data were then analyzed statistically in SPSS 11.0 using the Mann-Whitney *U* test, with the accepted level of statistical significance of $p < 0.05$. **Results.** According to Coleman and Anson's classification, the higher incidence of the incomplete type (57.14%) of the SPA was observed compared to the complete type (42.86%). In addition to this, a statistically significant difference was discovered in the distance of the SPA from Kaplan's cardinal line between two groups with complete and incomplete types, respectively. The parameter examined in the group with the complete type took the value of 2.13 ± 0.32 cm, while in the group with the incomplete type measured 3.33 ± 0.87 cm. **Conclusion.** The present study showed a very important complexity in the domain of anatomy of the SPA with numerous clinical implications. For that reason, a thorough evaluation of the hand circulatory system should be considered while planning surgical procedures in order to avoid operative and postoperative complications.

Key words:
anatomic variation; ulnar artery; hand.

Apstrakt

Uvod/Cilj. Detaljno poznavanje vaskularne mreže šake od velikog je značaja u savremenoj medicini. Glavni krvni sud šake jeste površinski arterijski luk dlana (PLD), koji klasično nastaje anastomoziranjem lakatne arterije sa površinskom granom žbične arterije dlana. PLD odlikuje izuzetna varijabilnost koja se manifestuje različitim načinom nastanka i položajem ovog krvnog suda. Cilj rada bio je da se ispita anatomija PLD. **Metode.** Istraživanje je sprovedeno na Institutu za anatomiju „Niko Miljanić" na ukupno 14 kadavera. Nakon pažljive disekcije, na svim desnim šakama analizirane su varijacije u načinu nastanka kao i udaljenost PLD od Kaplanove kardinalne linije. Dobijeni podaci statistički su obrađeni u programu SPSS 11.0 primenom *Mann-Whitney* testa, pri čemu je prihvaćeni nivo statističke značajnosti bio $p < 0,05$. **Rezultati.** Prema *Coleman* i *Anson* klasifikaciji uočena je veća učestalost nekompletnog (57,14%) u odnosu na kompletni tip (42,86%) PLD. Takođe, utvrđena je statistički značajna razlika u rastojanju PLD od Kaplanove linije između kompletnog i nekompletnog tipa. Rastojanje kod kompletnog tipa bilo je 2,13 ± 0,32 cm, dok je kod nekompletnog tipa iznosilo 3,33 ± 0,87 cm. **Zaključak.** Sadašnja studija pokazala je veoma značajnu kompleksnost anatomije PLD, koja povlači za sobom veliki broj kliničkih implikacija. Da bi se izbegle operativne i postoperativne komplikacije neophodna je procena cirkulatornog sistema šake u toku planiranja hirurških procedura.

Ključne reči:
anatomija, varijacije; a. ulnaris; šaka.

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Introduction

From the evolutionary point of view, the human hand has become highly specialized in performing quite delicate and finely coordinated movements that have enabled us to skillfully use the objects from our surroundings. The hand, furthermore, contains numerous, densely packed tactile receptors and, therefore, plays an important role as a sensory apparatus that helps us perceive the environment [1]. Such a complex function of the hand is nothing else but a reflection of its complex anatomical structure. It is in this regard that we can analyze the vascular network of the hand, which is complicated and extremely variable. In-depth knowledge of hand vasculature is of great importance in modern medicine.

In broad terms, the two main blood vessels of the hand are the ulnar artery (UA) and the radial artery (RA). These two arteries anastomose on the anterior side of the hand and form two arterial arches – superficial and deep palmar arch [2]. The superficial palmar arch (SPA) undoubtedly occupies a central place in the hand vascular system [3]. Since the SPA presents the primary focus of our study, its origin, course, and side branches are described in more detail in the text that follows.

As standardly described in anatomical textbooks, the SPA arises from the UA, just distal to the pisiform. The vessel passes first medially around the hook of the hamate, then swings laterally, forming an arch that is convex downward [4]. The terminal part of the SPA communicates typically with the superficial palmar branch of the RA in the first web space [5]. The SPA lies in the loose connective tissue just beneath the palmar aponeurosis and above the terminal branches of the ulnar and median nerves, the long flexor tendons, and the lumbrical muscles [6].

The SPA gives rise to three or four common digital palmar arteries. At the base of the fingers, each of these arteries split into two proper digital palmar arteries. The vascular territory of proper digital palmar arteries includes the anterior surfaces of the second to the fifth finger and the posterior surfaces of these fingers at the level of the intermediate and distal phalanges. The medial side of the little finger is supplied by a separate branch that arises from the UA or represents the first side branch of the SPA [7].

Many anatomical variations of the SPA have been described by now in the literature. These variations manifest in the unusual formation and localization of the vessel and the atypical number of its side branches [8]. Variability of the SPA was the object of interest of many researchers back in the 19th century [9]. In the past century, studies were done to classify the SPA according to its formation. Important investigations in that period were conducted by Coleman and Anson [10] and Ikeda et al. [11].

In addition to having introduced detailed systematization of the SPA, scientists also tried to explain the causes of this remarkable variability. The most accepted theory is based on the ontogenetic development of the hand. This concept says that primitive vascular patterns of the hand during embryogenesis undergo many changes in terms of regression of already existing blood vessels and appearance of new ones [11]. Identification of the most common variations of SPA is very important for surgical techniques such as the use of vascularized skin flaps, synovial flaps, cardiac catheterization and bypass grafting, and wrist joint surgery [2, 12, 13].

Besides the origin of the SPA, two significant aspects in the analysis of this blood vessel are the diameters of the vessels involved in its formation and the localization of the SPA with respect to the topographic landmarks of the hand, such as Kaplan's cardinal line. Unlike the anatomical variations of the SPA, this topic has not been discussed in a huge number of research papers, so in this area, there is a need for further investigation.

The aim of this study was a comprehensive examination of the anatomy of the SPA, which included two parameters: the formation of the SPA and its localization related to the topographic landmarks of the hand.

Methods

Our research was conducted on cadaver material of the Institute of Anatomy "Niko Miljanić", Faculty of Medicine, University of Belgrade, with the approval of the Ethics Committee for the use of human and laboratory animal material, Faculty of Medicine, University of Belgrade, Serbia (Nº 325-07-01245/2014-05/2). A total number of 14 cadavers were examined in the study, of which 7 were male and 7 were female cadavers aged 67 to 79 years. At the very beginning, a visual inspection of every hand was performed to exclude the specimens with deformities and traces of trauma or surgical interventions. After fixation in 10% formalin solution, the right hands were carefully dissected, following standard protocols from dissection manuals.

In the first part of our research, the variations in the formation of the SPA were examined. Several classifications of the SPA were made in the previous period based on its formation. One of the most practical and most used is the classification into a complete and incomplete type [14]. The complete type of the SPA is defined when there is an anastomosis between the vessels involved in its formation or when the UA reaches the first web space. On the other hand, if there is no such anastomosis, the SPA is considered incomplete [15].

In the second part of our research, the focus was put on the localization of the SPA in relation to the topographic landmarks of the hand. While choosing the landmark that will be used in the study, we took into consideration two criteria – simplicity of determination and practical use in surgical procedures on the hand. Keeping that in mind, Kaplan's cardinal line was selected. According to the original definition, Kaplan's cardinal line connects the apex of the first web space with the ulnar side of the hand and runs parallel to the middle palmar crease [16]. For this study, two groups were formed – one with the complete and one with the incomplete type of the SPA, respectively. The parameter that was examined and then compared between the two groups was the vertical distance from Kaplan's cardinal line to the SPA at the level of the fourth common digital palmar artery. Measurements were taken twice on each hand, and the average of two values was recorded. The measuring instruments used in the study were the ruler and electronic digital caliper (range of 0–500 mm, resolution 0.01 mm).

The methodology of the parameters examined is shown schematically (Figure 1), while the parameters are shown in Figure 2.

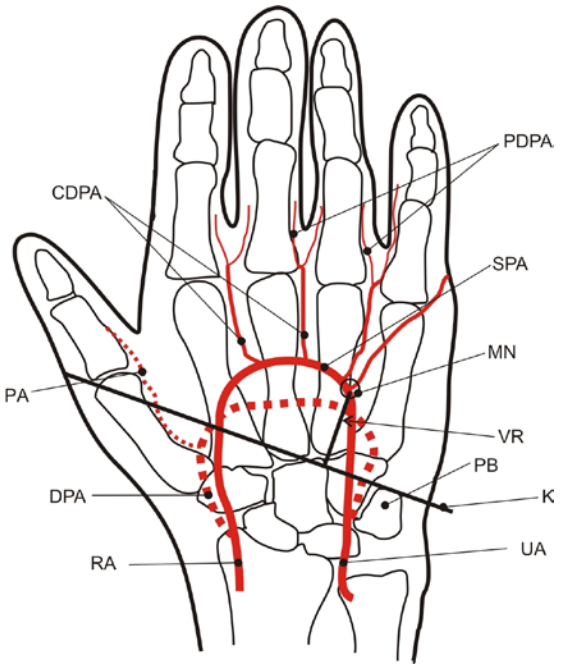

Fig. 1 – Vascular network of the hand and methodology of measurement of the parameters examined.
UA – ulnar artery; RA – radial artery; DPA – deep palmar arch; SPA – superficial palmar arch; PA – principal artery of the thumb; CDPA – common digital palmar arteries; PDPA – proper digital palmar arteries; PB – pisiform bone; K – Kaplan's cardinal line; MN – origin of the fourth common digital palmar artery in the fourth web space; VR – vertical distance between the superficial palmar arch at the level of the fourth common digital palmar artery and Kaplan's line.

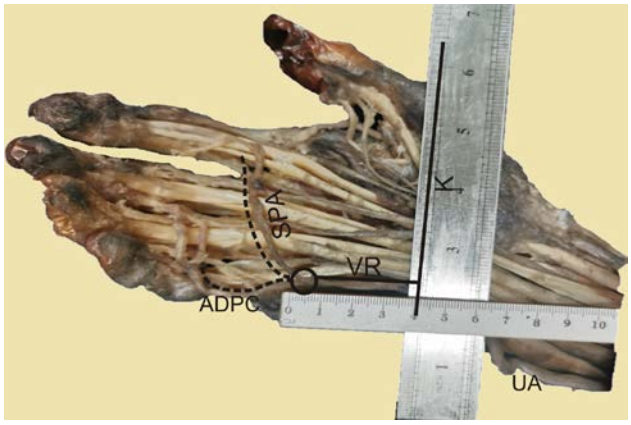

Fig. 2 – Methodology of measurement of the parameters examined in hand with incomplete type of the superficial palmar arch.
K – Kaplan's cardinal line; SPA – superficial palmar arch; UA – ulnar artery; ADPC – fourth common digital palmar artery; VR – vertical distance between the superficial palmar arch at the level of the fourth common digital palmar artery and Kaplan's line.

Statistical analysis of the data was performed in SPSS 11.0 using the Mann-Whitney *U* test, with the accepted level of statistical significance of $p < 0.05$.

Results

The results of our study are demonstrated in Tables 1 and 2. The complete type of the SPA was observed in 6 of 14 hands (42.86%), while the incomplete type was detected in 8 of 14 hands (57.14%). The average distance between the SPA (at the level of the fourth common digital palmar artery) and Kaplan's cardinal line measured 2.13 ± 0.32 cm in the group with the complete type. The same parameter took the value of 3.33 ± 0.87 cm in the group with the incomplete type of the SPA. A statistically significant difference was found by comparing the results between the two groups, with $p = 0.02$. Further investigation of data collected did not reveal a statistically significant difference between male and female cadavers, with $p = 0.65$.

Table 1

Results obtained by measuring the distance between the superficial palmar arch (SPA) and Kaplan's cardinal line

Cadaver number	SPA type	Distance between SPA and Kaplan's line (cm)
1	incomplete	3.8
2	complete	2.5
3	incomplete	3.7
4	incomplete	3.0
5	complete	2.1
6	complete	1.6
7	complete	2.0
8	incomplete	3.1
9	incomplete	3.5
10	complete	2.2
11	complete	2.4
12	incomplete	1.4
13	incomplete	4.0
14	incomplete	4.1

Table 2

Comparison of the distance between Kaplan's cardinal line and the superficial palmar arch (SPA) in groups with complete and incomplete type

SPA type	Distance between SPA and Kaplan's line (cm)	
	mean	SD
Incomplete (57.14%)	3.33	0.87
Complete (42.86%)	2.13*	0.32

SD – standard deviation.
***statistically significant decrease of the parameter examined in a group with complete type of the superficial palmar arch.**

A comparison of our results with those of other studies is shown in Table 3.

Table 3

Comparative analysis of incidence of complete and incomplete type of the superficial palmar arch (SPA) in the present and previous studies

SPA type	Coleman and Anson [10]	Janevski et al. [18]	Ikeda et al. [11]	Tagyl et al. [3]	Joshi et al. [19]	Present study
Complete	78.5%	42.4%	96.4%	75%	82%	42.86%
Incomplete	21.5%	57.6%	3.6%	25%	18%	57.14%

Discussion

The first aim of the present study was to analyze the variations in the formation of the SPA. This interesting feature of the SPA has intrigued a great number of scientists, whose first task was to describe the course, side branches, and relation of this blood vessel with adjacent elements. The development of modern medicine in the 20th and 21st century necessitated the systematization of previously collected knowledge with the purpose of its practical use in the fields of plastic, reconstructive, and microsurgery. This was how various classifications of the SPA were introduced. In the present study, the division between the complete and incomplete types was used. This classification appeared for the first time in the works of Jaschtschinski [9] at the end of the 19th century [17] and was elaborated further by Coleman and Anson [10] in the 20th century. Investigating the sample that comprised 650 hands, these two scientists observed 5 subgroups (A, B, C, D, and E) within the complete type and 4 subgroups (A, B, C, and D) within the incomplete type of the SPA [10].

The present study showed a moderately higher incidence of the incomplete type (57.14%) compared to the complete type (42.86%) of the SPA. Previous studies, however, found the opposite, i.e., the dominant occurrence of the complete type. Coleman and Anson [10] observed the complete type in 78.5% of cases and the incomplete type in 21.5% of cases, while Ikeda et al. [11] found the complete type in even 96.4% of specimens and the incomplete type in just 3.6% of specimens. On the other hand, data collected in the present study are in complete concordance with the results of the research on 500 hand angiograms conducted by Janevski et al. [18], with the recorded percentages of 42.4% for the complete and 57.6% for the incomplete type of the SPA.

The origin of the differences between the results of the present study and the results of some other studies is certainly multifactorial. At first, this discordance can be explained by the small size of our sample compared to other studies that were conducted on several hundreds of hands. Furthermore, ethnical differences in examined populations contribute for sure to the disparity of results [19]. Ultimately, because a subjective inspection of the sample was the method used in data collecting, differences in the interpretation of the complete and incomplete type of the SPA may be one of the causes of the differences in the results.

Unusual cases of the complete absence of the SPA [20] or double SPA [4] have been described in the literature. Other studies suggest that the median artery can contribute to the formation of both complete and incomplete types of SPA [21]. This blood vessel normally regresses during fetal development but sometimes persists and can be found in adults. Due to its close relationship with the median nerve, the persistent median artery can provoke the symptoms of carpal tunnel syndrome [22]. The aforementioned findings are extremely rare and were not observed in the present study.

Considering the variability of the SPA, there is a classification proposed by Gokhroo et al. [2] that is worth mentioning because it includes several criteria in the SPA typing, such as dominance, completeness, diameters of the ulnar and radial arteries, blood flow rate, and the existence of atherosclerotic lesions.

The second aim of the present study was to analyze the localization of the SPA in relation to the topographic landmarks of the hand. It is an incredibly important aspect in the examination of the SPA because topographic landmarks, such as palmar creases and Kaplan's cardinal line, are widely used to avoid injuries to this blood vessel during surgical procedures. Nevertheless, the distance between the SPA and Kaplan's cardinal line was inspected in a limited number of research papers. To the best of the author's knowledge, there is no study in which the given parameter was compared between the complete and incomplete types of the SPA.

The results of the present study revealed a greater distance between the incomplete type of the SPA and Kaplan's line (3.33 ± 0.87 cm) compared to the complete type (2.13 ± 0.32 cm). These data differ considerably from the results of the research led by Panchal and Trzeciak [23], where the average of the examined distance was measured at 1.18 ± 0.43 cm. The differences are slightly smaller when the results of the present study are compared to those of the research conducted by McLean et al. [16], in which the same parameter took the value of 1.53 ± 0.86 cm. This discordance can be explained by the differences in the interpretation of Kaplan's cardinal line, whose definition changed over time [24].

The cause of the observed differences in the distance between groups with the complete and incomplete types of the SPA is possibly found in the embryologic development of the hand vascular network, as is the case for the variations in the formation of this blood vessel [25]. It is evident that the incomplete type of the SPA, which was considered anomalous in the past, very often goes together with some other anatomical varieties, such as the absence of the palmaris longus muscle or persistent median artery [26]. Jaschtschinski [9] regarded the incomplete type of the SPA as an "atavistic feature", and it was proved that very rare variations of the SPA observed in humans represent the phylogenetic retention of the primitive vascular patterns found in other primates – chimpanzee, gorilla, orangutan, gibbon, and macaque.

The knowledge of the anatomy of the SPA has many clinical implications. On the one hand, some procedures re-

quire transradial access (TRA), e.g., cardiac catheterization and arterial cannulation. Cardiac catheterization is frequently used in the diagnosis or treatment of certain cardiovascular conditions, while radial arterial cannulation precedes hemodialysis or arterial blood gas analysis. On the other hand, the ultimate choice in some interventions may be complete removal of the radial artery, which serves as a coronary artery bypass graft (CABG), or taking part in vascularized skin flaps that are nowadays widely used in microsurgery [27]. In both cases, it is essential to assess the existence of collateral circulation in the hand before performing the procedures. The methods of evaluation include Allen's test, Doppler ultrasonography, pulse oximetry, or angiography [28]. It has been shown that people with the incomplete type of SPA are more prone to hand ischemia as one of the complications of the aforementioned procedures [29]. The same problem may occur even in persons with the complete type of SPA [30], which is explained either by the incapacity of the anastomotic vessels to ensure the needs of tissue in the newly created situation of compromised blood flow or by the existence of atherosclerotic lesions which narrow the lumen of the UA and disable adequate blood supply. Other researchers suggest that multiple anastomoses within the vascular bed of the hand increase the risk of profuse bleeding in case of traumatic injury of the blood vessel, but at the same time help faster recovery [31]. In addition to assessing the type of SPA, it is of tremendous importance to know its localization to avoid the iatrogenic injury of this blood vessel during some surgical operations on the hand, like carpal tunnel release (CTR). In this context, it is crucial to know the exact distance between the SPA and Kaplan's cardinal line, which represents the distal border of "the safe zone" in operations in the wrist area [23].

In the end, we must mention that the present study contains some limitations. Those are the small sample size which does not allow the generalization of the obtained results to the entire population, and the examination of the hands of just one side, so it was not possible to draw a conclusion about the existence of bilateral symmetry of the SPA.

Conclusion

The vascular network of the hand is organized in a very complex manner. Its dominant blood vessel is the SPA, typically formed by anastomosing of the UA and RA. However, this blood vessel is characterized by remarkable variability in its formation and localization to the topographic landmarks of the hand, such as Kaplan's cardinal line. Every surgeon should be aware of this variability while performing the interventions on hand, as it affects the course of the operation and the postoperative recovery. Our study reported a higher incidence of the incomplete type in comparison to the complete type of the SPA. It was also observed that there are differences in the distance between these two types of SPA and Kaplan's cardinal line. Because of that, the authors suggest that a thorough assessment of hand circulation should be performed prior to any invasive procedure to avoid hand ischemia as one of the possible complications. Finally, there is a need for further investigation in this field with the goal of the advancement of pre-existing knowledge. One of the directions in which future research could be taken is the examination of factors that influence the outstanding variability of the SPA.

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Giant exophytic Marjolin's ulcer of the lower leg after a gunshot wound

Gigantski egzofitični Marjolinov ulkus na potkolenici posle sklopetarne povrede

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Abstract

Introduction. Marjolin's ulcer is a skin malignancy that occurs on a scar or chronic wound. It most commonly occurs on a burn scar. Squamous cell carcinoma is the most common type of this tumor in more than 90% of cases. The rate of this rare malignant transformation is 1–2%. Marjolin's ulcer is more aggressive than other skin cancers. Wide excision is the treatment of choice. Recurrences are common. We present a large exophytic carcinoma of the lower leg as a rare form of this tumor according to the size and type together with reconstruction results. **Case report.** A 52-year-old man was presented with a large exophytic tumor on the left lower leg. The tumor was located at the site of the previous gunshot injury. The latent period was 22 years. Tumor size was 14 × 12 cm. Wide excision was performed (2-cm surgical margin), including the deep fascia, and the defect was closed by a split-thickness skin graft from the opposite thigh. Histology showed well-differentiated squamous cell carcinoma. There were no regional or distant metastases. One year after surgery, there was no recurrence of the tumor. **Conclusion.** Early diagnosis of Marjolin's ulcer and wide excision are mandatory. Surgical margins for excision should be 2 cm, and excision should include deep fascia. Multiple and repeated biopsies of chronic wounds are advised. There is no consensus on the staging of Marjolin's ulcer and lymph node dissection.

Key words:
burns; carcinoma, squamous cell; cicatrix; leg; reconstructive surgical procedures; wounds, gunshot.

Apstrakt

Uvod. Marjolinov ulkus je maligni tumor kože koji se javlja na ožiljku ili na hroničnoj rani. Najčešće se javlja na ožiljku od opekotine. Najčešći tip tumora je skvamocelularni karcinom koji se javlja kod više od 90% slučajeva. Učestalost te maligne transformacije je 1–2%. Marjolinov ulkus je agresivniji od drugih karcinoma kože. Metoda izbora u lečenju je široka ekscizija. Recidivi su česti. Prikazujemo veliki egzofitični karcinom potkolenice, retku formu tumora u odnosu na veličinu i tip tumora, sa rezultatom nakon radikalne operacije i rekonstrukcije. **Prikaz bolesnika.** Prikazan je muškarac, star 52 godine, sa velikim egzofitičnim tumorom na levoj potkolenici. Tumor je bio lokalizovan na mestu prethodne sklopetarne povrede. Latentni period je iznosio 22 godine. Dimenzije tumora bile su 14 × 12 cm. Urađena je široka ekscizija (2 cm od ivica tumora) zajedno sa fascijom, a defekt je pokriven transplantatom delimične debljine kože, uzetim sa suprotne natkolenice. Histološki nalaz je pokazao da se radilo o dobro diferentovanom skvamocelularnom karcinomu. Nisu bile prisutne regionalne ili sistemske metastaze. Godinu dana posle operacije nije bilo recidiva tumora. **Zaključak.** Osnove lečenja Marjolinovog ulkusa su rana dijagnoza i široka ekscizija. Ekscizija treba da bude 2 cm od ivica tumora sa uklanjanjem duboke fascije. Preporuka je da se kod hroničnih rana periodično sprovode multiple biopsije. Stavovi oko stadijuma Marjolinovog ulkusa i disekcije limfnih žlezda nisu usaglašeni.

Ključne reči:
opekotine; karcinom, planocelularni; ožiljak; potkolenica; hirurgija, rekonstruktivna, procedure; rana vatrenim oružjem.

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Introduction

Marjolin's ulcer (MU) is a term used for skin malignancy arising from a scar. This malignant transformation, which is rarely seen, is named after French surgeon Jean-Nicolas Marjolin. Initially, the term referred to squamous cell carcinoma (SCC) that occurs on a burn scar many years after the injury. Later, it was noticed that other types of skin malignancy can occur and that the tumor can occur not only on the scars from the injury but also on the chronic wounds and the sites of chronic inflammation [1]. MU is more aggressive than other skin cancers, with a metastasis rate of 27.5% [2]. The latent period or transition time is about 26 years [3]. Burns are the most common cause of malignant degeneration (68%). SCC occurs in 94% of cases [4]. MU is more common in less developed countries. The most common localization is the lower extremity in half of the patients. Men are more commonly affected than women (2 : 1). MU is most commonly seen at the age of 55 years [5]. The diagnosis of MU is based on anamnesis, clinical presentation, and histological findings after biopsy. Venous ulcers, pressure sores, and chronic hidradenitis are most often mentioned in the differential diagnosis. Different sizes of MU have been reported. Treatment is primarily surgical, with wide excision and skin grafting. Radiotherapy and chemotherapy are usually adjuvant or palliative treatments for advanced cases of MU. Recurrence occurs in about 16.7% of patients [6].

We present a large MU of the lower leg at the site of a previous gunshot injury, with giant proportions for the exophytic form of MU in this region.

Case report

A 52-year-old male patient presented with a large fungoid soft tissue mass on the posteromedial part of the left lower leg. The tumor was located at the middle and distal 1/3 of the lower leg, measuring 14 × 12 cm (Figure 1). The patient had a gunshot injury at the same site 22 years earlier, and the wound healed by secondary intention. The growth of the tumor was slow, accompanied by bleeding and infection. The pain was of medium intensity. There was no distal neurovascular deficit. Inguinal nodes were not enlarged. Radiography showed a great number of metal foreign bodies with no bone damage (Figure 2). *Pseudomonas aeruginosa* was isolated, and the patient was treated with amikacin. In general anesthesia, a wide tumor excision was performed, using margins of 3 cm. Underlying deep fascia was included. The defect was closed by a split-thickness skin graft from the opposite thigh (Figure 3). Histological analysis showed well-differentiated invasive SCC, with histological grade I and nuclear grade I (Figure 4). There were no tumor elements at the edges of the resection, and no tumor cells were found in excised muscle samples. No distant metastases were found either on additional examinations. The skin graft was well

Fig. 1 – Exophytic tumor on the left lower leg, measuring 14 × 12 cm.

Fig. 2 – X-ray of the left lower leg, with a great number of foreign bodies after a gunshot injury.

Fig. 3 – Split-thickness skin graft two weeks after surgery.

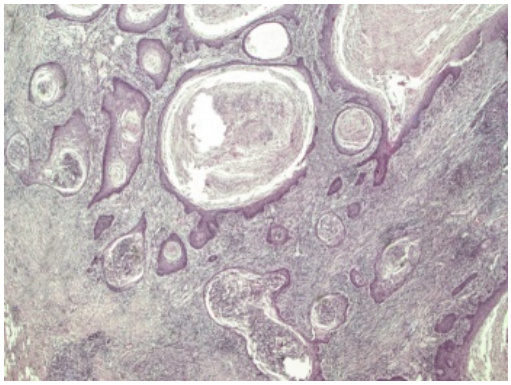

Fig. 4 –Well-differentiated squamous cell carcinoma with incomplete and complete keratinization in the form of horn pearls [hematoxylin and eosin staining, ×200].

consolidated, and the donor site healed well without complication. One year after the surgery, there was no recurrence of the tumor, and there was no regional and systemic metastasis.

Discussion

MU was named after the French doctor Jean-Nicolas Marjolin, who described an ulcer on a burn scar in 1828. In addition to the burn scar, which is the most common cause of MU (in 68% of cases), it can also occur at scars from other injuries, surgeries, chronic ulcers (vascular, pressure sore), chronic osteomyelitis, site of the previous frostbite, scars from skin grafts, donor sites of skin grafts, vaccine scars, fistulas (including AV fistulas), sinuses, chronic suppurative hidradenitis, radiodermatitis, discoid lupus erythematosus, pemphigus, herpes zoster, leprosy ulcer, etc. [4]. In general, MU occurs on scars from wounds that have healed by secondary intention, on chronic wounds, and on the skin with chronic inflammation with frequent disruption of the skin. There is a case of MU that was developed on the surface of the pleura several years after the treatment of empyema with the Eloesser flap [7].

The incidence of MUs is estimated to be 2% in post-burn scars and 1.7% in chronic wounds. It occurs more commonly in older age and is two times more common in men. In a study by Xiang et al. [8], among 140 patients with MU, the initial injury or disease was at the age of 1–75 years, and MU developed at the age of 15–85 years, averaging 53.3 years. The latency period is 11–41 years, with an average of 28.8 years. The age of the patient at the time of injury is in negative correlation with the length of the latency period. MU most often occurs on the lower leg (62%). Other sites are the head (16%), upper extremity (12%), and trunk (10%) [9]. SCC is found in more than 90% of patients with MU. Other malignant tumors are less common: basal cell carcinoma, melanoma, verrucous carcinoma, sebaceous cell carcinoma, fibrosarcoma, angiosarcoma, liposarcoma, leiomyosarcoma, osteosarcoma, dermatofibrosarcoma protuberance, malignant solitary fibrous tumor, etc. [10]. Rare localization and types of MU have been described, such as sebaceous cell carcinoma of the eyelid, 16 years after a chemical burn in a 68-year-old woman [11].

The histological finding of a well-differentiated type is characterized by islands of squamous cells with minimal cellular and nuclear atypia and rare pathological mitoses. Intercellular bridges are present, with emphasized keratinization. In the center of the tumor islands, lamellar concentric keratin masses-horn pearls are presented. Peritumoral and intratumoral stromal lymphocytic infiltrate is pronounced. Granulomatous formations around the foreign body are common in response to the presence of keratin. There are some differences between classic SCC of the skin (non-MU SCC) and SCC in MU (MU SCC). MU SCC occurs three times more often in men and at a slightly younger age (52 years), most often on the lower leg. Non-MU SCC is 1.1–1.7 times more common in men, usually occurs at the age of about 66 years, and most often on the head and neck, which is explained by exposure to UV radiation. Metastases in non-MU SCC occur less frequently (3–23%), and five-year survival is higher (61.5–94.6%). In one-third of patients, the tumor affects the muscle or bone (36%). The rate of lymphadenopathy is 23.6%, but metastases in lymph nodes are present in only 7% [9], which can be explained by a significant degree of inflammation and infection in the tumor region and obliteration of lymphatic channels by scar tissue. Metastases of MU can be lymphatic or systemic, most often in the lungs. The highest frequency of metastases in MU is noted at pressure sore (61%) [12]. Overall 3-year survival is 65–75% and 35–50% in metastatic MU [2].

Many authors have emphasized the process of malignant transformation in MU: reduction of circulation, obliteration of lymphatic channels, weakened epithelium cell junctions, epithelial implantation, releasing of local toxins, genetic interactions, and constant irritation by exogenous factors [13]. No theory has been confirmed, and the pathogenesis of MU is likely multifactorial. Many factors reduce immunological control, with increased potential for carcinogenesis. Reduction of circulation, obliteration of lymph vessels with scar tissue, and reduced activity of Langerhans' cells are significant in the development of MU. Toxic substances released by scar tissue may have mutagenic effects. A mutation in the p53 and fas genes was detected in MU, which is important in the regulation of cell apoptosis and homeostasis. Recent research has indicated the role of biomarkers as indicators for invasivity of MU [14]. It has been noted that there is an imbalance of molecules that regulate cell adhesion (claudin-1, E-cadherin, and desmoglein) due to which cell separation and biochemical transformation of cells are promoted. With the existing epithelial-mesenchymal transition (EMT) and increased production of extracellular components of the matrix (ECM) (increased expression of matrix metalloproteinases − MMPs), the so-called favorable tumor microenvironment (TME) can be developed. The invasion of tumor cells is also accelerated by certain molecules of cell signals (epidermal growth factor receptor, focal adhesion kinase, etc.) [14]. Li et al. [15] showed that survivin, a member of the apoptosis inhibitor protein family, plays a significant role in the formation of MU.

There are two clinical forms of MU, infiltrative and exophytic. The infiltrative type is formed easily, the degree of metastasis is high, and the prognosis is poor. Exophytic form grows slower, and the frequency of metastases is lower [16]. In a study performed on 56 cases of MU, Chalya et al. [17] described the presence of pain in 93% of patients and tumor infection in about 90% of patients. Tumor size ranged from 2 to 15 cm.

The largest infiltrative MU on the lower leg was described by Sakellariou et al. [18], which was 19 × 11 cm in diameter. The largest MU, in general, was described by Saraiya [19] as an ulcerative infiltrative form, measuring 43 × 23 cm. The MU we described in the paper was 14 × 12 cm in size, which ranks it as one of the largest exophytic MU compared to those described in the available literature.

Treatment of MU is primarily surgical: wide excision or amputation in advanced cases of MU in extremities. Because of the tumor size and the margins of excision, direct closure

is seldom possible. Skin graft covering is preferred. Local skin flaps are not advised, except in defects with exposed underlying tendon or bone. Split-thickness skin graft offers a good assessment of tumor recurrence. Lymph node dissection is controversial but can be considered with positive lymph nodes on physical or ultrasound examination. According to Metwally et al. [20], recurrence was noted as early as 3 months and as late as 25 years and can be associated with distant metastasis in some patients. Predictors of recurrence were age, nodal status, and type of defect reconstruction [20]. Adjuvant radiation and chemotherapy are indicated if surgical resection is not possible [4].

Conclusion

Early diagnosis, radical excision with adequate reconstruction, lymphadenectomy in case of enlarged regional lymph nodes, and adequate oncological protocol are the bases of the treatment of MU. Skin grafting of large and deep burns is mandatory. Periodic multiple biopsies are required in patients with chronic wounds, such as lower leg ulcers. We advise wide excision of MU, up to 2–3 cm from the tumor edges, together with the fascia and appropriate reconstruction and therapeutic dissection of lymph nodes in case of malignant lymphadenopathy. Radiotherapy and chemotherapy may be additional therapeutic procedures in metastatic MU.

Acknowledgement

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CASE REPORT

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Unusual manifestation of gastric mucormycosis in a patient with rheumatoid arthritis

Neobična manifestacija gastričnog tipa mukormikoze kod bolesnice sa reumatoidnim artritisom

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Abstract

Introduction. Mucormycosis is a life-threatening opportunistic infection whose incidence has significantly risen during the last two decades. The gastrointestinal form is very rare, with the stomach as the most common site of infection, followed by the colon and ileum. Risk factors include uncontrolled diabetes mellitus, corticosteroid use, and organ transplantation. We report a patient with a history of rheumatoid arthritis who has developed gastrointestinal mucormycosis. To the best of our knowledge, this is the first such case reported in the literature. **Case report.** A 53-year-old female patient with a prior medical history of rheumatoid arthritis was admitted to the hospital due to persisting diarrhea. Physical examination revealed diffuse abdominal tenderness to palpation without meteorism and peritoneal signs. Laboratory results demonstrated systemic inflammation, so antibiotic therapy was administered. Abdominal computed tomography findings revealed inflammation of the rectum and the left colon. Colonoscopy findings were indicative of Crohn's disease. Additionally, the patient had developed profuse rectal bleeding and consequently underwent emergency surgery. Subtotal colectomy with ileostomy and partial gastrectomy was performed. The patient's condition rapidly worsened after the operation, and she died due to multiorgan failure. Histologic findings of resection specimens discovered chronic active colitis and extensive gastric necrosis associated with dense mixed inflammatory infiltration and numerous non-septate and 90-degree branching *hyphae*. Diagnosis of invasive gastric mucormycosis was obtained, but unfortunately, several days after the patient's death. **Conclusion.** It is very important to obtain high awareness among clinicians of this deadly infection to achieve a prompt diagnosis and effective therapy.

Key words:
arthritis, rheumatoid; diagnosis; diagnosis, differential; digestive system; histological techniques; mucormycosis.

Apstrakt

Uvod. Mukormikoza je oportunistička infekcija koja ugrožava život, čija je incidenca u značajnom porastu u poslednje dve decenije. Gastrointestinalni tip ove infekcije se javlja veoma retko, i u njemu je želudac najčešće mesto infekcije, a slede ga kolon i ileum. Faktori rizika su nekontrolisani dijabetes melitus, upotreba kortikosteroida i transplantacija organa. Prikazujemo bolesnicu sa istorijom reumatoidnog artritisa koja je dobila gastrointestinalnu formu mukormikoze. Prema našim podacima, ovo je prvi takav slučaj opisan u literaturi. **Prikaz bolesnika.** Bolesnica sa reumatoidnim artritisom, stara 53 godine, primljena je u bolnicu zbog perzistentne dijareje. Fizičkim pregledom ustanovljena je difuzna osetljivost stomaka na palpaciju, bez znakova meteorizma i peritonitisa. Labaratorijski nalazi su ukazali na sistemsku inflamaciju, tako da je uključena antibiotska terapija. Snimanjem kompjuterizovanom tomografijom nađeni su znaci inflamacije rektuma i levog kolona. Kolonoskopija je ukazivala na Kronovu bolest. Pored toga, bolesnica je razvila profuzno krvarenje iz rektuma i ubrzo je podvrgnuta hitnoj hirurškoj intervenciji. Izvršena je subtotalna kolektomija sa ileostomom i parcijalna gastrektomija. Nakon operacije, stanje bolesnice se naglo pogoršalo i umrla je od multiorganske disfunkcije. Patohistološkom analizom hirurškog materijala otkriven je hronični aktivni kolitis i opsežna nekroza želuca sa infiltracijom gustim mešovitim inflamatornim infiltratom i brojnim hifama, neseptiranim i razgranatim pod pravim uglom. Ustanovljena je

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mukormikoza, ali, na žalost, tek nekoliko dana nakon smrti bolesnice. **Zaključak.** Veoma je značajno skrenuti pažnju na ovu smrtonosnu infekciju, kako bi se postigla brza dijagnoza i uspešna terapija.

Ključne reči:
artritis, reumatoidni; dijagnoza; dijagnoza, diferencijalna; digestivni sistem; histološke tehnike; mukormikoza.

Introduction

Mucormycosis is a life-threatening opportunistic infection whose incidence has significantly risen during the last two decades [1–3]. Usually, it is caused by *Rhizopus oryzae* from the order Mucorales, which belongs to the Mucormycetes class [4]. Mucorales fungi are thermotolerant molds that are ubiquitous and widely found in forms of infective spores in organic substrates, such as bread, fruits, fermented milk, vegetable matter, alcoholic drinks, soil, and animal excreta [5, 6]. The infection is acquired by inhalation, ingestion, or inoculation of spores onto disrupted skin and mucosa. Spores invade previously damaged epithelium and penetrate through endothelial cells, causing thrombosis and massive tissue necrosis [7]. Therefore, invasive mucormycosis is classified as rhinocerebral syndrome, pulmonary, cutaneous, gastrointestinal, disseminated, and uncommon presentations [5].

The gastrointestinal form is very rare, with the stomach as the most common site of infection, followed by the colon and ileum [8]. Risk factors include uncontrolled diabetes mellitus, defects in phagocyte function, corticosteroid use, organ or stem cell transplantation, administration of deferoxamine in patients receiving hemodialysis, and iron overload [4, 9]. Trauma, burns, and surgery can be predisposing factors in immunocompetent hosts, as well [10].

We report a patient with a history of rheumatoid arthritis who has developed gastrointestinal mucormycosis. To the best of our knowledge, this is the first such case reported in the literature.

Case report

A 53-year-old female patient with a prior medical history of rheumatoid arthritis was admitted to the hospital due to persisting diarrhea presenting as 5 to 6 watery stools per day, which had lasted for 7 days, accompanied by dysuria. Her previous medical therapy included 20 mg prednisone per day and 10 mg methotrexate per week. The patient appeared pale, dehydrated, and lethargic. She did not have a fever. Her blood pressure was 95/50 mmHg with a heart rate of 95 beats per minute. Physical examination revealed diffuse abdominal tenderness to palpation without meteorism and peritoneal signs. The following laboratory results demonstrated systemic inflammation, leukopenia, hypoglycemia, hypoalbuminemia, and kidney dysfunction: C-reactive protein 229.6 mg/L (normal range 0–5 mg/L); procalcitonin 43.09 ng/L (normal < 0.05 ng/mL); white blood cells count 1.03×10^9/L (normal range 3.40–9.70 × 10^9/L); glucose 3.1 mmol/L (normal range 3.9–6.1 mmol/L); albumin 21 g/L (normal range 35–52 g/L); urea 13.9 mmol/L (normal range 2.5–6.7 mmol/L); creatinine 260 μmol/L (normal range 58–96 μmol/L); total protein 42 g/L (normal range 64–83 g/L). Other laboratory test results were unremarkable. Soon afterward, there was an overall impairment of general physical condition. The patient became hemodynamically unstable and developed acute respiratory failure, so she was intubated and put on mechanical ventilation along with inotropic support. Antibiotic therapy, including ciprofloxacin, metronidazole, then meropenem, vancomycin, and colistin, was administered intravenously (iv). Due to leukopenia, thrombocytopenia, and anemia [white blood cell count 1.9×10^9/L; platelets 16×10^9/L (normal range 150–400 × 10^9/L); hemoglobin 74 g/L (normal range 120–160 g/L)], filgrastim, units of platelet concentrate and units of reticulocyte were given. Blood culture was positive for *Pseudomonas aeruginosa*. Chest radiography findings indicated progression to respiratory distress syndrome and, therefore, intravenous methylprednisolone was initiated. Significant improvement in the patient's overall condition resulted in the withdrawal of the mechanical ventilation on day 14. During further examination, abdominal computed tomography findings revealed presacral abscess, inflammation of the rectum and the left colon, and free intraperitoneal fluid. Hence, colonoscopy was indicated, and it discovered multiple small ulcers in the rectum, edematous, erythematous mucosa in the colon, with multiple, partially fused erosions, serpiginous ulcers, and individual pseudopolyps. Mucosa had a cobblestone appearance, and the ileocecal valve was distorted. Therefore, these endoscopic findings were indicative of Crohn's disease. Several biopsies were taken, and pathohistological features correlated with acute colitis and were not conclusive for Crohn's disease. Stool samples were negative for *Salmonella spp*, *Shigella spp*, *Campylobacter spp*, *Yersinia enterocolitica*, and *Clostridium difficile* toxin. Urine culture showed 100,000,000 colony forming units (CFU) *per* mL of urine and positivity for *Pseudomonas spp*. Antibiotic, corticosteroid, and supportive therapy was continued. Nevertheless, there was an exacerbation of the patient's condition with loss of consciousness, seizures, and respiratory failure, so mechanical ventilation was initiated again. Additionally, the patient had developed profuse rectal bleeding and consequently underwent emergency surgery. Intraoperative findings revealed necrosis of the transverse colon and posterior wall of the stomach with clear ascitic fluid. For that reason, subtotal colectomy with ileostomy and partial gastrectomy was performed. However, even with the aggressive surgical and medical approach, the patient's condition rapidly worsened after the operation, and she passed away due to multi-organ failure on the 57th day of hospitalization. Histologic findings

of resection specimens discovered chronic active colitis and extensive gastric necrosis associated with dense mixed inflammatory infiltration and numerous non-septate and 90-degree branching *hyphae* (Figures 1–4). Diagnosis of invasive gastric mucormycosis was obtained, but unfortunately, several days after the patient's death. An autopsy was not performed.

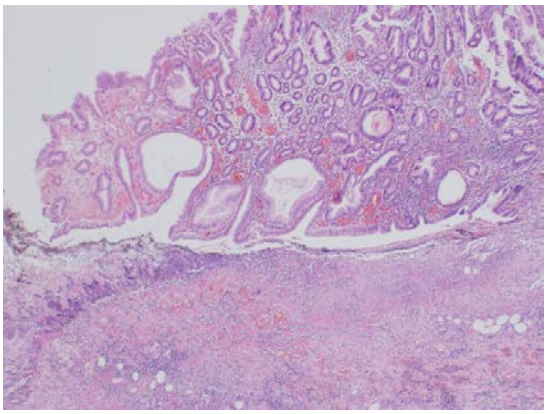

Fig. 1 – Ulceration of the gastric mucosa (hematoxylin and eosin, ×4).

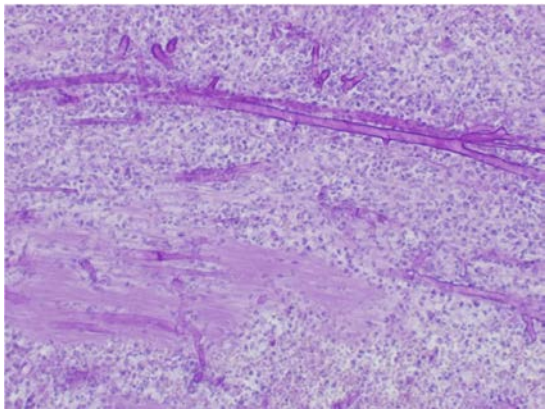

Fig. 2 – Broad non-septate *hyphae* with branching (periodic acid-Schiff, ×20).

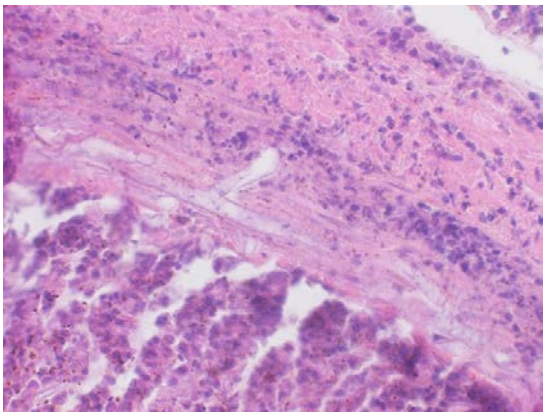

Fig. 3 – Angioinvasion by *hyphae* (hematoxylin and eosin, ×40).

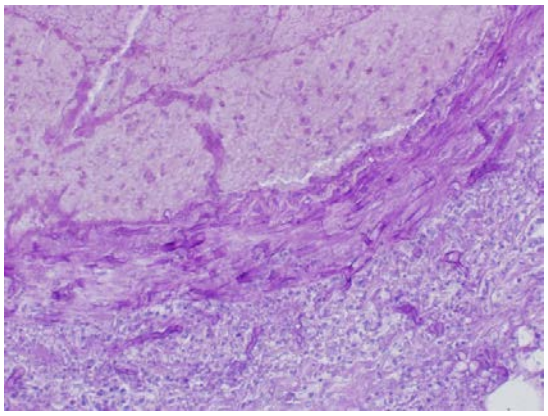

Fig. 4 – Angioinvasion by *hyphae* (periodic acid-Schiff, ×20).

Discussion

Mucormycosis is the second most frequent fungal infection in immunocompromised patients, but recently it has also been detected in immunocompetent hosts [5]. Our patient was on corticosteroid and immunomodulatory therapy (prednisone and methotrexate), which is not unusual for the development of fungal infection. However, to the best of our knowledge, she is the first patient who had developed gastrointestinal mucormycosis on the background of rheumatoid arthritis. Symptoms of gastrointestinal mucormycosis could be unspecific and vague, and they include nonspecific abdominal pain, intraabdominal abscess, distention associated with nausea and vomiting, fever, and hematochezia [11]. Our patient had severe diarrhea with watery and bloody stools, which led clinicians to suspect colitis and perform a colonoscopy. Nausea, vomiting, or other stomach-related symptoms were absent, so gastroscopy was not carried out. The endoscopic appearance of colonic mucosa had been consistent with Crohn's disease, although histopathological diagnosis did not confirm Crohn's colitis. There are few reported cases of this rare infection misdiagnosed as inflammatory bowel diseases (IBD) [12, 13]. Further differential diagnosis includes tumor, appendicitis, and typhlitis [14–17]. Gastric mucormycosis is often represented as ulceration with or without perforation and is much less common as a plaque-like lesion or exophytic ulcerated mass [18–20].

Therefore, with unspecific symptoms and unusual presentation, diagnosis is very difficult to achieve, and it requires a high rate of clinical suspicion and early multiple biopsies or surgical resection of the infected organ. Histopathological analysis of biopsied material is based on hematoxylin and eosin-stained tissue sections showing characteristic broad aseptate *hyphae*, which frequently have irregular and 90 degrees branching [5]. In our case, histpathological examination of the colonic biopsy specimens showed signs of acute inflammation. Mucormycosis was detected only in the surgical specimen of the resected stomach. An autopsy was not performed, so we did not establish disseminated mucormycosis.

Disseminated mucormycosis is documented in only 3% of cases [21]. Usually, hematogenous spreading occurs from the lungs and is less common from the alimentary tract, sinuses, and cutaneous lesions. Clinical presentation is unspecific and includes a wide range of symptoms (cerebral infection, kidney failure, hepatosplenomegaly, paralytic ileus).

The mortality rate is very high, over 90%, and diagnosis is often made on autopsy [22].

In the case of gastrointestinal mucormycosis, serial targeted biopsies from the rectum, colon, terminal ileum, and stomach are necessary. However, diagnosis is often delayed. There are no specific serologic markers, and microbiological tests are unreliable, with positive cultures in only 50% of cases [15]. Polymerase chain reaction (PCR) methods are still in the research phase, and their benefit is only in confirmation of histopathological diagnosis [5].

Despite aggressive medical treatment, the mortality of gastrointestinal mucormycosis is very high, approximately 85%, due to delayed diagnosis [21].

Only 25% of cases of gastrointestinal mucormycosis are diagnosed antemortem [21], so a high level of clinical suspicion and prompt diagnosis is crucial for reducing mortality.

The best therapeutic approach for mucormycosis (in general and especially for gastrointestinal mucormycosis) is a combination of antifungal therapy and extensive surgery. According to recommendations of the European Society of Clinical Microbiology and Infectious Diseases and the European Confederation of Medical Mycology (ESCMID), the treatment of choice is *iv* administration of liposomal amphotericin B at a dose of at least 5 mg/kg daily [23, 24]. Amphotericin is the medicine that has the best penetration through tissue and the least side effects. For patients who are not responding to amphotericin, posaconazole and other broad-spectrum azole are advised [5]. Extensive surgical removal of all necrotic tissue is also required. Frozen sections are often recommended for clear resection margins. And finally, it is necessary to withdraw or reduce immunosuppressive drugs and deferoxamine and to regulate hyperglycemia and acidosis in diabetic patients [8].

Additionally, the ESCMID recommends prophylactic administration of fluconazole in any patient with recent abdominal surgery and recurrent gastrointestinal perforations or anastomotic leakages, considering these conditions create a great risk for developing invasive candidiasis, which can be a life-threatening fungal infection as well as mucormycosis [25, 26].

Conclusion

Bearing in mind the high mortality of gastrointestinal mucormycosis, it is very important to obtain high awareness among clinicians of this deadly infection to achieve a prompt diagnosis and effective therapy. Furthermore, considering that diagnosis is dependent on biopsy, it is necessary to develop new noninvasive rapid tests to establish mucormycosis as urgently as possible and to avoid the worst outcome.

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CASE REPORT

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Facial nerve palsy as the first sign of late breast cancer metastasis to the temporal bone

Paraliza facijalnog nerva kao prvi znak kasne metastaze karcinoma dojke u temporalnu kost

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Abstract

Introduction. Late metastases of malignant tumors in the temporal bone are very rare lesions. They can be asymptomatic for a long time and usually manifest themselves in the form of hearing loss, dizziness, tinnitus, and paralysis of the facial nerve. Modern radiological diagnostics and explorative surgery with biopsy are essential for diagnosis. **Case report.** We present a rare and unusual case of a 66-year-old female patient with facial nerve paralysis that appeared as the first sign of metastatic breast cancer in the temporal bone 10 years after treatment. A sudden hearing loss and dizziness occurred six months later, and the value of CA 15-3 was elevated. Scintigraphy pointed to susceptible metastatic deposits of the axial skeleton without lesions in the temporal bone. Finally, repeated computed tomography revealed osteolytic changes in the temporal bone six months after that. Immunohistochemical analysis of mastoid tissue samples confirmed that it was a breast cancer metastasis. One year after palliative radiotherapy and oral hormone therapy, a patient had a good general condition with a better function of the facial nerve. **Conclusion.** A high degree of clinical suspicion sometimes requires repeated radiological diagnostics in order to detect osteolytic metastatic changes in the temporal bone but also in other bone structures within the hematogenous dissemination of the malignant disease.

Key words:
breast neoplasms; facial nerve diseases; immunohistochemistry; neoplasm metastasis; temporal bone; tomography, x-ray computed; treatment outcome.

Apstrakt

Uvod. Kasne metastaze malignih tumora u temporalnoj kosti su veoma retke lezije. One dugo mogu biti asimptomatske i obično se manifestuju gubitkom sluha, vrtoglavicom, zujanjem u ušima i paralizom facijalnog nerva. Savremena radiološka dijagnostika i eksplorativna hirurgija sa biopsijom neophodni su za dijagnozu. **Prikaz bolesnika.** Prikazujemo redak i neobičan slučaj 66-godišnje bolesnice sa paralizom facijalnog nerva koja se pojavila kao prvi znak metastatskog karcinoma dojke u temporalnoj kosti 10 godina nakon lečenja. Nagli gubitak sluha i vrtoglavica pojavili su se šest meseci kasnije, a vrednost CA 15-3 bila je povišena. Scintigrafija je ukazala na moguće metastatske depozite osovinskog skeleta, bez lezija u temporalnoj kosti. Konačno, ponovljena kompjuterizovana tomografija otkrila je osteolitičke promene temporalne kosti, šest meseci nakon toga. Imunohistohemijska analiza uzoraka tkiva iz mastoida potvrdila je da se radilo o metastazi karcinoma dojke. Godinu dana nakon palijativne radioterapije i oralne hormonske terapije, bolesnica je bila dobrog opšteg stanja, sa boljom funkcijom facijalnog nerva. **Zaključak.** Visok stepen kliničke sumnje ponekad zahteva ponavljanu radiološku dijagnostiku kako bi se otkrile osteolitičke metastatske promene u temporalnoj kosti, ali i na ostalim koštanim strukturama, u okviru hematogene diseminacije maligne bolesti.

Ključne reči:
dojka, neoplazme; facijalni nerv, bolesti; imunohistohemija; neoplazme, metastaze; temporalna kost; tomografija, kompjuterizovana, rendgenska; lečenje, ishod.

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Introduction

Temporal bone metastases are rare lesions, and they usually originate from malignant breast, lung, kidney, stomach, and prostate tumors [1, 2]. The metastases found 10 years after the initial treatment of the malignant disease are especially rare, and then they are defined as late metastases [3]. The most common way of metastases formation is the hematogenous dissemination of malignant cells to the temporal bone marrow, while disease spreading mechanisms through cerebrospinal fluid, lymphatic and perineural pathways, or directly from the surrounding areas have been described as well [2, 4–6]. The petrous apex, internal auditory meatus, and mastoid cavity are the areas of the temporal bone most commonly affected by metastatic changes [2, 4].

In addition to potentially being asymptomatic for a long time, temporal bone metastases usually manifest themselves in symptoms and signs corresponding to different diseases of the ear and the adjacent areas [2, 4, 5, 7, 8]. Contemporary imaging methods like high-resolution computed tomography (HRCT) and magnetic resonance imaging (MRI) uncover pathological changes in the temporal bone and the adjoining areas. However, differentiation of tumorous and inflammatory changes based on radiological findings sometimes proves to be very difficult, while differential diagnostic dilemma can be eliminated only through biopsy, histopathological and immunohistochemical analyses of the tissue samples [5, 8–10].

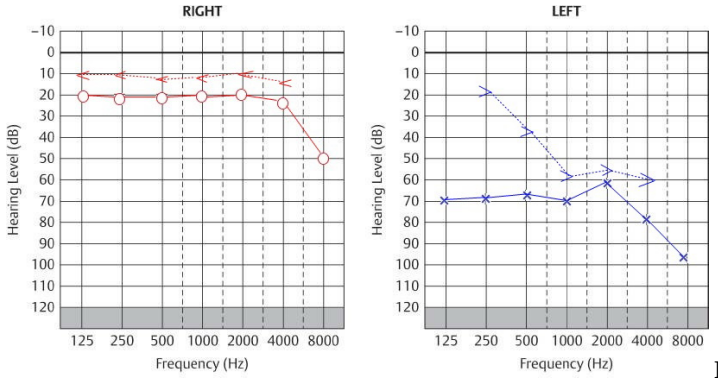
We present a rare and unusual case of a 66-year-old female patient with facial nerve palsy as the first sign of breast cancer metastasis to the temporal bone 10 years after initial treatment.

Case report

A 66-year-old female patient was admitted to the Clinic of Otorhinolaryngology and Maxillofacial Surgery of the Univeristy Clinical Center of Serbia in Belgrade due to facial nerve paralysis on the left that had appeared 12 months earlier. From the patient's medical history, we found out that the left ear was previously operated on twice (Tympanoplasty type 1) due to chronic tubotympanic otitis media. We also found out that the patient had undergone radical surgery using the Madden technique due to right breast cancer 11 years earlier (*Carcinoma lobulare*, high grade, G III NG III). It was then discovered intraoperatively that the primary tumor had broadly infiltrated the fat tissue of the lateral portion of the breast with a pronounced multifocality. No signs of regional metastases were detected in the axillary lymph nodes (0/27). Six cycles of chemotherapy with cyclophosphamide, 5-fluorouracil sodium, and epirubicin were given postoperatively as well as radiotherapy (RT). The patient was then given oral hormone therapy with tamoxifen for five years. She had regular control checkups for 10 years without signs of relapse of the malignant disease.

The current problems started 12 months earlier with the appearance of pain in the retroauricular area and sudden complete paralysis of the facial nerve on the left (Grade VI on the House-Brackmann scale). There were no signs of inflammation or any data on the possible trauma or herpes infection. HRCT of the temporal bones and endocranium was conducted, and the findings were normal. The patient was treated with corticosteroids and physical therapy, however, without improvement.

A sudden hearing loss in the left ear and the appearance of vertigo occurred six months later. Audiological and vestibulological examination showed a severe mixed hearing impairment and severe damage to the sense of balance in the left ear while hearing in the right ear was normal (Figure 1). Scintigraphy of the skeleton with the technetium-99m-labelled 3,3-diphosphono-1,2-propanodicarboxylic acid (99mTc-DPD) indicated increased accumulation in the right shoulder joint area, left clavicle, left humerus, both femurs, left ischium, and sacral bone. The distribution of the radiopharmaceuticals in all segments of the vertebral column was noticeably non-homogeneous, with increased accumulation in the thoracic vertebrae bodies. Based on the scintigraphic features, the changes described may have corresponded to the secondary deposits (Figure 2). The value of tumor marker CA 15-3 was elevated and amounted to 54.5 U/mL (normal range: < 25 U/mL). The laboratory analyses recorded reduced values of the following parameters: erythrocytes 3.24 × 1,012/L (normal range: 3.80–5.00 ×



A) **B)**

Fig. 1 – A pure tone audiogram showed normal hearing in the right ear and severe mixed hearing loss in the left ear; frequency in hertz (Hz) is shown on the abscissa and hearing level in decibels (dB) is shown on the ordinate.

1,012/L), hemoglobin 96 g/L (normal range: 110–180 g/L), and hematocrit 0.31 L/L (normal range: 0.35–0.47 L/L). Moreover, elevated values of the following parameters were recorded: erythrocyte sedimentation rate 102 mm/h (normal range: < 30 mm/h), alkaline phosphatase (ALP) 876 U/L (normal range: 64–153 U/L), and lactate dehydrogenase (LDH) 313 U/L (normal range: < 241 U/L). MRI findings of temporal bones and endocranium were normal again. Lung radiography and echosonography examination of the left breast, both axillae, and abdomen did not reveal pathological changes. Physical therapy was continued with another cycle of corticosteroid therapy, followed by a temporary improvement in the facial nerve function for two months.

HRCT of the temporal bone, which was repeated 12 months after the initial paralysis of the facial nerve, uncovered osteolytic changes in the base of the skull, the petrous apex of both pyramids, *tegmen tympani* on the left with non-homogeneous shading in the mastoid cavity, perifacial and perilabirynthine cells (Figure 3).

The preoperative otomicroscopy finding indicated that it was an inactive tubotympanic otitis on the left, with a small perforation in the rear lower quadrant of the eardrum. An explorative mastoidectomy with epitympanotomy was performed. Intraoperatively, it was noted that the bone was osteolytic, sequestered, and dehiscent in the *tegmen* antri region with the presence of fibrous and adherent tumor tissue in the peridural region, around the labyrinth, and along the course of the facial nerve. Histopathological and immunohistochemical analyses of tissue samples have shown that it was metastatic breast cancer. Tumor cells were positive for cytokeratin 7 (CK7), GATA-3, estrogen receptor (ER) (Figure 4), progesterone receptor (PR) and negative for CK20, CDX-2, TTF-1, PAX-8, WT-1, mamoglobin, p40, CK5/6, TM, p63 (not shown). Rare single cells are positive for GCDFP15 (not shown).

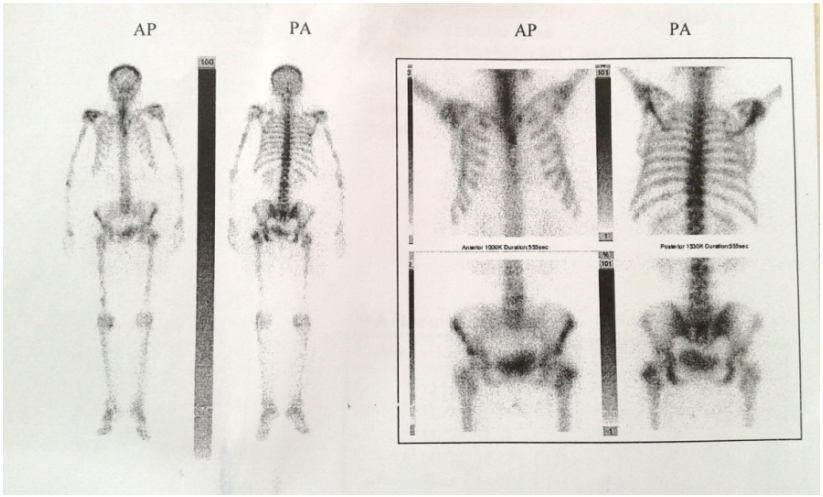


Fig. 2 – Scintigraphy of the skeleton with the suspected metastatic changes in the right shoulder joint area, left clavicle, left humerus, both femurs, left ischium, and sacral bone.

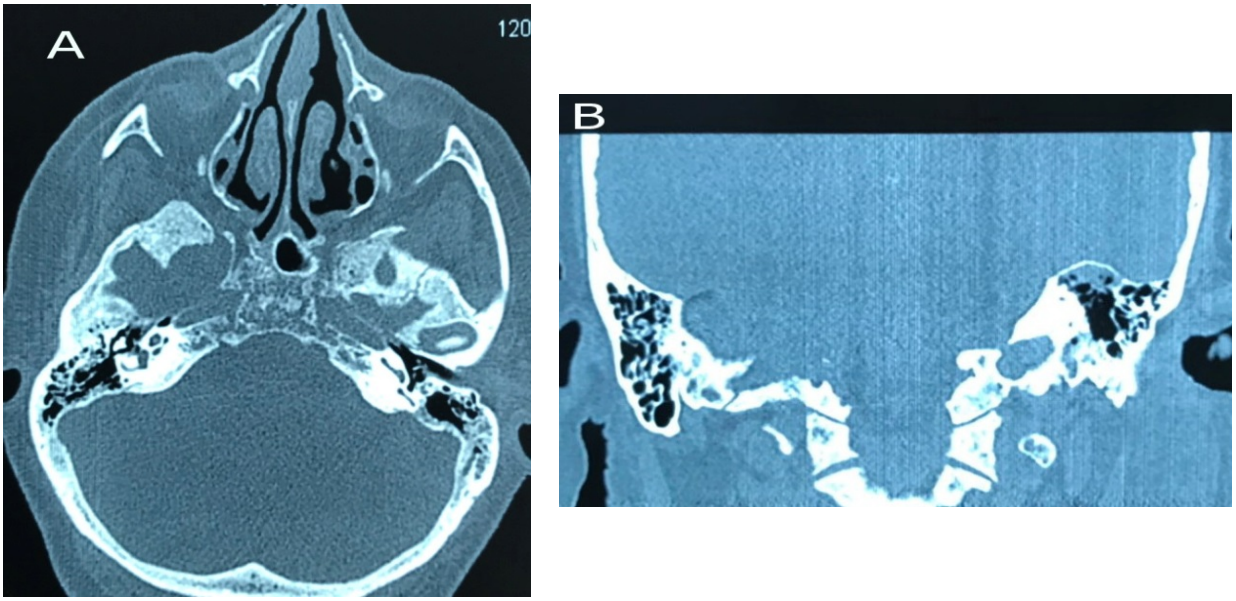


Fig. 3 – Computed tomography (CT) scan in axial (A) and coronal (B) plane showed osteolytic changes in the skull base, petrous apexes, and the left temporal bone.

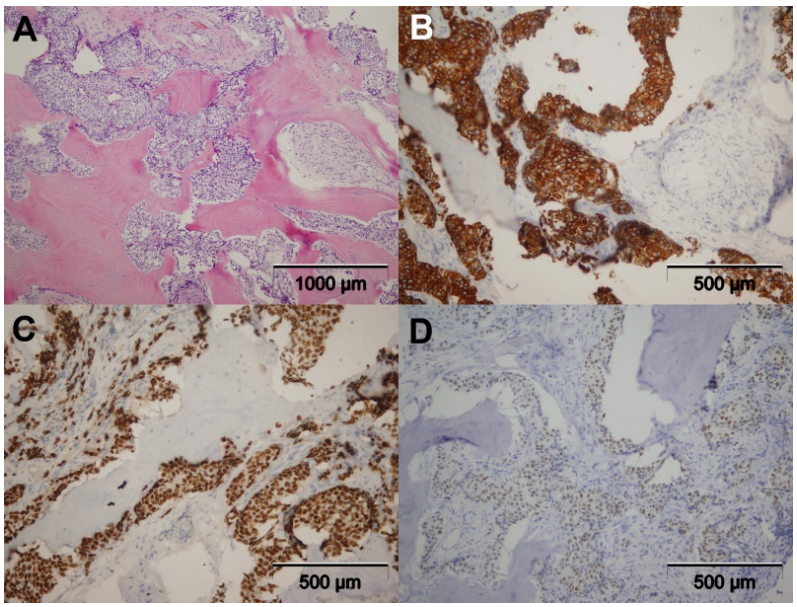


Fig. 4 – Histopathological and immunohistochemical findings of mastoid tissue samples. Bone marrow is infiltrated with medium sized cells with indistinct borders, abundant, eosinophilic, or clear cytoplasm. Nuclear polymorphism is mild. Chromatin is evenly dispersed, with small nucleoli in some tumor cells. Tumor growth is diffused with formation of rare tubules. Hematoxylin-eosin (A); Tumor cells are positive for cytokeratin 7 (CK7) (B), GATA-3 (C) and estrogen receptor (ER) (D).

By the decision of the Oncology Consilium for malignant breast disease, palliative RT with therapeutic dose (TD) 40 Gy was applied to the area of the left temporal bone with further application of oral hormone therapy (anastrozole). Following the RT, there was a gradual improvement in the function of the mimic facial muscles, and 12 months later, during the last control examination, we established a facial nerve paresis Grade III on the House-Brackmann scale.

Discussion

Reviewing the world literature from 1902 to 1994, Streitmann and Sismanis [2] recorded 139 cases of metastatic tumors in the temporal bone, and about 25% of metastases originated from breast cancer. Metastatic disease in the temporal bone may be asymptomatic for a long time and undetected for several reasons. Routine examination of the ear, audiological screening, and radiological diagnostics of the temporal bone are not commonly carried out during the evaluation and monitoring of patients with malignant diseases. In addition, it is well known that the otic capsule is the strongest bone in the human body, and the facial nerve is protected by the Fallopian canal. Based on autopsy results, Gloria-Cruz et al. [4] have detected metastases in the temporal bone in 22% of patients who primarily had no disseminated malignant disease. Although metastatic changes were found in both temporal bones in 62% of the cases, as many as 36% of the patients had no otological symptoms. Streitmann and Sismanis [2] also state that approximately 30% of the patients with unilateral metastases in the internal auditory meatus had no symptoms, and the disease was detected accidentally either by radiological methods or by autopsy. Since histopathological examination of the temporal bone is not routinely performed during the autopsy in patients treated for malignant diseases, some authors believe that the prevalence of metastatic tumors in the temporal bone is underestimated [2, 4].

Symptoms and signs of metastatic disease in the temporal bone are non-specific and may correspond to various inflammatory, systemic, or specific illnesses. Therefore, it is very important to take a detailed anamnesis and perform a complete physical examination. According to some studies, hearing loss is one of the most common symptoms of metastatic disease in the temporal bone, which is present in 40% of patients [4]. Metastases in the temporal bone can also be manifested by vertiginous disorders, as well as very unusual symptoms and signs that resemble temporal arteritis [7, 8]. Extremely rare, metastases of malignant tumors manifest themselves as a soft-tissue mass in the external ear canal [9].

There are numerous causes of facial nerve palsy, most notably Bell's palsy, herpes zoster oticus, chronic suppurative otitis media, and cholesteatoma. According to literature data, the incidence of facial nerve paralysis in the temporal bone metastases ranges from 15% to 50% [4, 11, 12]. Studies have shown that tumor invasion of the Fallopian canal does not necessarily lead to facial nerve paralysis, and the clinical presentation correlates well with the degree of tumor infiltration into the nerve fibers [13, 14]. Some histological studies have shown that the complete paralysis of the facial nerve was present only in the tumor invasion through the epineural layer, which was reported in about 50% of cases [13, 14]. It is also

important to pay attention to the clinical presentation of the facial nerve paralysis that does not necessarily have a sudden onset and progressive character. Breadon et al. [15] describe two cases of breast cancer metastases in the temporal bone that had been clinically manifested in the form of recurrent paralysis of the facial nerve.

According to some authors, facial nerve paralysis with otalgia and periauricular edema represents a highly suspicious triad of symptoms that can indicate the presence of metastases in the temporal bone [11]. Thus, the occurrence of the individual or associated symptoms and their clinical course depend on the localization and extension of the metastatic lesion in the temporal bone, as well as whether it is a unilateral or bilateral presentation of the disease [2].

The finding of temporal bone destruction on the CT scan is always highly suspicious but not pathognomonic for malignant tumors and may correspond to other pathological conditions, such as cholesteatoma, necrotizing otitis externa, or paraganglioma. In suspicion of metastatic disease, attention should be paid to the typical localization of osteolytic lesions in correlation with a medical history, laboratory, clinical, and audiological findings. MRI is a superior method for differentiating the soft-tissue changes in the temporal bone and detection of endocranial propagation of the pathological process. In addition, it is possible to display the entire intratemporal course of the facial nerve in detail. Therefore, MRI is recommended in the unusual clinical course of facial nerve palsy. Scintigraphy of the skeleton and positron emission tomography are also very important in detecting metastases and disseminated malignant diseases [7].

The case of our patient is interesting for many reasons. Namely, she had a history of chronic suppurative otitis media on her left side, and she had previously undergone two operations in other institutions. The facial nerve paralysis had appeared on the same side. However, as it was an inactive process, the possibility of otogenic facial nerve paralysis was excluded. Furthermore, there were no other known etiological factors, and CT findings of the temporal bone were normal. Despite the emergence of new symptoms in the form of dizziness and hearing impairment, elevated values of CA 15-3, ALP, and LDH, repeated imaging diagnostics did not reveal pathological changes in the temporal bone even six months later. The finding of scintigraphy was highly suspected of metastatic disease of the axial skeleton but did not indicate any temporal bone lesion. In addition, our patient did not have any of the skeletal-related events such as bone pain, pathological fracture, spinal cord compression, or tumor-induced hypercalcemia [16]. Interestingly, there was a temporary improvement in facial nerve function afterward, as described by some authors in the literature [10, 15]. Although everything indicated a likely late hematogenous dissemination of the malignant disease, it was necessary to conduct HRCT of the temporal bone repeatedly in order to detect osteolytic metastatic changes in typical localization.

Treatment of patients with breast cancer relapse in the form of metastases in the temporal bone depends on the locoregional status, systemic expansion of the malignant disease, the general condition of the patient, histopathological findings, and previous therapy. The temporal bone region is mostly treated with palliative RT in combination with chemotherapy and hormone therapy [5, 17].

Literature data point to the absence of temporal bone metastases if the primary malignant tumor is discovered early and treated adequately [4]. Our patient had a local advanced malignant tumor of the breast, and the histopathological type of invasive lobular carcinoma is, according to some studies, an independent risk factor for the appearance of metastases in the bones [18]. Detection of metastatic disease in the temporal bone is often an indicator of the presence of distant metastases in other locations within hematogenous dissemination [4, 5, 7], which was confirmed by scintigraphy of the skeleton in our case. Our patient had been in complete remission for ten years. In the literature, there are cases of recorded metastatic breast cancer in the temporal bone as much as 33 years after the initial treatment [8]. Therefore, some authors emphasize that the follow-up period for patients treated for breast cancer must continue even after the routine period of ten years, with regular control of tumor markers [3].

Conclusion

Although late metastatic tumors in the temporal bone are very rare, they should be considered as a differential diagnosis in cases of otological symptomatology, which is refractory to therapy, and facial nerve paralysis that has a prolonged or unusual clinical presentation. This particularly applies to the elderly population and patients who have a positive history of malignancy, regardless of how long the treatment period has elapsed. A high degree of clinical suspicion sometimes requires repeated imaging diagnostics in order to detect osteolytic metastatic changes in the temporal bone, but also in the other bone structures within the hematogenous dissemination of the malignant disease. The occurrence of otologic symptoms that are usually a late sign of the systemic metastatic disease, as well as the time that is sometimes needed to establish a definitive diagnosis are only some of the factors that negatively affect the overall prognosis of these patients.

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