YU ISSN 0042-8450

ВОЈНОСАНИТЕТСКИ ПРЕГЛЕД

Часопис лекара и фармацеута Војске Србије

Military Medical and Pharmaceutical Journal of Serbia

Vojnosanitetski pregled

Vojnosanit Pregl 2024; April Vol. 81 (No. 4): pp. 193–254.

Vojnosanitetski Pregled 2024 April Vol. 81 (No. 4): pp. 193–254.

Vol. 81, No. 4, 2024

VOJNOSANITETSKI PREGLED

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

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

ISSN 0042-8450
eISSN 2406-0720

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

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

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

Printed by: Vojna štamparija, Beograd, Resavska 40b

Vol. 81, br. 4, 2024

VOJNOSANITETSKI PREGLED

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

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

ISSN 0042-8450
eISSN 2406-0720
Open Access
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Adresa redakcije: Univerzitet odbrane, Medicinski fakultet Vojnomedicinske akademije, Centar za medicinske naučne informacije, Crnotravska 17, 11 040 Beograd, Srbija. Informacije o pretplati (tel.): +381 11 3608 997. E-mail (redakcija): **vsp@vma.mod.gov.rs**

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

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

Štampa: Vojna štamparija, Beograd, Resavska 40b

CONTENTS / SADRŽAJ

This April marks the 170th death anniversary of Karl Adolph von Basedow (28 March 1799 – 11 April 1854), a German physician who, in 1840, described the clinical trias – exophthalmos, goiter, and tachycardia – associated with hyperthyroidism (overproduction of thyroid hormones). A few years earlier, in 1835, some of these symptoms were described by an Irish physician, Robert James Graves. Today, it is known that Graves-Basedow disease is an organ-specific autoimmune disease in the pathogenesis of which numerous genetic and epigenetic factors, as well as environmental factors, are involved (viruses, smoking, stress, radiation, drugs, etc.). This disease occurs 5 to 10 times more often in women and is most often manifested between 30 and 60 years of age.

U aprilu ove godine obeležava se 170 godina od smrti Karla Adolfa fon Bazedova (28. mart 1799 – 11. april 1854), nemačkog lekara, koji je 1840. godine opisao klinički trijas – egzoftalmus, gušavost i tahikardiju – udružene sa hipertiroidizmom (povećanom produkcijom tiroidnih hormona). Nekoliko godina ranije, 1835. godine, pojedine od ovih simptoma opisao je irski lekar Robert Džejms Grejvs. Danas se zna da je Grejvs-Bazedovljeva bolest organ-specifična autoimunska bolest u čiju patogenezu su uključeni brojni genetski i epigenetski faktori kao i faktori okruženja (virusi, pušenje, stres, zračenje, lekovi, itd). Ova bolest se 5 do 10 puta češće javlja kod žena i najčešće manifestuje između 30. i 60. godine života.

ORIGINAL ARTICLES

UDC: 616-006.81-08::616-036.21
DOI: https://doi.org/10.2298/VSP231022010N

Insight into the management of patients with melanoma in times of the COVID-19 pandemic – a single-center experience

Uvid u lečenje obolelih od melanoma za vreme pandemije COVID-19 – iskustvo jednog centra

Jelena Nikolić*†, Marija Marinković*†, Mladen Jovanović*†, Ivana Mijatov*‡, Dragana Leković-Stojanov†, Stefan Kecman†

***University of Novi Sad, Faculty of Medicine, Novi Sad, Serbia; University Clinical Center of Vojvodina, †Clinic for Plastic and Reconstructive Surgery, ‡Clinic for Maxillofacial Surgery, Novi Sad, Serbia**

Abstract

Background/Aim. Despite all innovations in medicine, melanoma still has a rising incidence and high mortality and thus represents a significant challenge for the healthcare system. The pandemic of the coronavirus disease 2019 (COVID-19), spanning three years, redirected healthcare resources, suspended preventive programs, and strained the healthcare system, significantly impacting melanoma management. The aim of this study was to assess the influence of the COVID-19 pandemic on the treatment of melanoma patients. **Methods.** This retrospective study analyzed melanoma patients treated at a single tertiary care center over two distinct three-year periods: pre-COVID period (2017–2020) and the COVID-19 pandemic period (2020–2022). The following data were collected and compared: patient demographics, melanoma characteristics, time intervals from biopsy to surgery, and the share of thin and thick melanoma. **Results.** During the COVID-19 pandemic period, there was a 30% reduction in melanoma patients compared to the pre-COVID-19 period. The decline was most pronounced in 2020 when the state of emergency was introduced in the country. Thin melanoma percentage as a measurement of successful screening programs decreased significantly during the COVID-19 pandemic (11.57% vs. 24.01%), while median Breslow thickness remained stable. The distribution of different histological types was consistent across both periods. The average time from biopsy to surgery remained similar between the two periods, around 40 days. **Conclusion.** Despite challenges posed by the COVID-19 pandemic, melanoma remains a critical healthcare issue. This study emphasized the significance of prioritizing melanoma care in emergencies, ensuring patient identification and timely treatment in order to optimize survival and minimize treatment costs.

Key words:
biopsy; dermatologic surgical procedures; diagnosis; melanoma; covid-19; plastic surgery procedures.

Apstrakt

Uvod/Cilj. Uprkos svim inovacijama u medicini, za melanom se i dalje beleže porast incidence i visoka smrtnost, zbog čega predstavlja značajan izazov za zdravstveni sistem. Pandemija koronavirusne bolesti 2019 (COVID-19), koja je trajala tri godine, preusmerila je resurse zdravstvene zaštite, obustavila preventivne programe i opteretila zdravstveni sistem, što je uticalo na lečenje obolelih od melanoma. Cilj rada bio je da se proceni uticaj pandemije COVID-19 na lečenje obolelih od melanoma. **Metode.** Retrospektivnom studijom su obuhvaćeni oboleli od melanoma koji su lečeni u jednom tercijarnom zdravstvenom centru tokom dva trogodišnja perioda: pre-COVID-19 perioda (2017–2019) i perioda pandemije COVID-19 (2020–2022). Prikupljeni su i uporedeni sledeći podaci: demografske karakteristike bolesnika, karakteristike melanoma, vremenski interval od biopsije do radikalne hirurške intervencije i udeo tankih i debelih melanoma. **Rezultati.** Tokom perioda pandemije COVID-19, u odnosu na period pre pandemije, zastupljenost obolelih od melanoma je smanjena 30%. Najveći pad registrovan je tokom 2020. godine, u vreme uvedenog vanrednog stanja u zemlji. Procenat tankog melanoma, kao mera uspešnih programa skrininga, značajno se smanjio tokom pandemije COVID-19 (11,57% naspram 24,01%), dok se srednja debljina melanoma, prema skali Breslow, nije značajno promenila. Distribucija različitih histoloških tipova melanoma bila je nepromenjena u oba perioda. Prosečno vreme od biopsije do hirurškog zahvata ostalo je slično između dva perioda, oko 40 dana. **Zaključak.** Uprkos

Correspondence to: Jelena Nikolić, University Clinical Center of Vojvodina, Clinic for Plastic and Reconstructive Surgery, Hajduk Veljka 1, 21 137 Novi Sad, Serbia. E-mail: JELENA.NIKOLIC@mf.uns.ac.rs

izazovima koje je donela pandemija COVID-19, melanom ostaje kritično pitanje zdravstvene zaštite. Ovo istraživanje je istaklo značaj davanja prioriteta lečenju obolelih od melanoma u urgentnim okolnostima, kako bi se obezbedila rana identifikacija bolesnika i njihovo pravovremeno lečenje, u cilju njihovog boljeg preživljavanja i manjih troškova lečenja.

Ključne reči:
biopsija; hirurgija, dermatološka, procedure; dijagnoza; melanom; covid-19; hirurgija, rekonstruktivna, procedure.

Introduction

Melanoma is still one of the most important health care issues, with constantly rising incidence and highest mortality of all skin cancers. It represents not only important health problems but also critical economic concerns that must be considered while creating health strategies and estimating the direct cost of treatment.

In the last three years, the pandemic of coronavirus disease 2019 (COVID-19) has redirected our focus on a new threatening disease that has almost paralyzed healthcare systems worldwide. Our country was not spared of all the problems that other wealthier countries also faced on a daily basis. All human and technical resources were allocated for treating COVID-19 patients, as our priority was keeping the COVID-19 pandemic under control. In the meantime, all preventive and educational programs for the people at risk of getting melanoma, including the screening programs, were suspended. Keeping up with the strict protocols for melanoma treatment [obtaining a biopsy of suspected skin tumors, sentinel lymph node biopsy, and surgery scheduling] was sometimes impossible, clearly exceeding healthcare system availability.

All studies confirmed a strong impact of time to definitive treatment of melanoma on overall survival. Studies confirmed that delay of surgery beyond 29 days for stage I melanoma negatively affects the overall survival of the patient [1]. The current recommendation for definitive treatment of primary melanoma is 3 to 4 weeks after diagnosis in the United States and 4 to 6 weeks in Europe [2, 3]. Postponing adequate treatment will definitely have a negative impact on the course of the disease, the disease-free period, the cost of treatments, and most importantly, the survival of the patient.

Methods

This research was designed as a retrospective study that included patients who underwent melanoma surgery in a single tertiary care center (Clinic for Plastic and Reconstructive Surgery, University Clinical Center of Vojvodina, Serbia) in two distinct periods: three-year non-COVID-19 period 2017–2019 and three-year COVID-19 pandemic time 2020–2022. The study was approved by the Ethics Committee of the University Clinical Center of Vojvodina (No. 00-217).

We compared these two periods to search for the influence of the COVID-19 pandemic on the healthcare system and melanoma patients. Patient general information (age, gender), clinical and pathohistological characteristics of melanoma (localization, type of melanoma, maximal depth of tumor in mm, time lap between biopsy of melanoma and definitive radical surgery, share of thin melanoma with maximum depth lower than 1 mm, share of thick melanoma with maximum depth greater than 4 mm) were taken from medical documentation and compared between the two groups.

Statistical analysis was performed with the SPSS 20 software. Descriptive statistics were shown using mean and standard deviation. Wilcoxon test was used to determine the existence of statistically significant differences between the two dependent samples for variables that do not follow a normal distribution. Mann-Whitney independent samples *t*-test was used to determine the existence of statistically significant differences between the two independent groups for variables that do not follow a normal distribution. All tests were performed on a 0.05 significance level unless pointed differently.

Results

Results presented in Figure 1, in the three-year pre-COVID-19 period (2017–2019), show that 356 patients underwent melanoma surgery, 30% more than in the three-year COVID-19 time (2020–2022) when 273 patients underwent the same surgery. The results also show that there were no significant differences in gender distribution between the two cohorts, pre-COVID-19 and COVID-19 time (Chi-square test was 0.0249, $p = 0.87$).

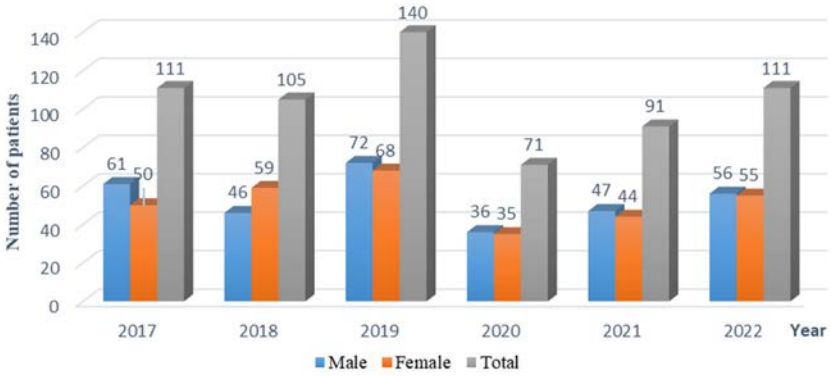

Fig. 1 – Gender distribution of patients in the pre-COVID-19 period (2017–2019) and the COVID-19 period (2020–2022).

Gender distribution in different age groups in the general sample (2017–2022) is presented in Figure 2. It shows that there is a significant difference in gender distribution with the dominance of women in younger age groups (< 50) compared with male dominance in older age groups (Chi-square test $p < 0.01$).

The number of patients who underwent melanoma surgery in 2020, according to the mount, is presented in Figure 3. There were no patients who underwent melanoma surgery in April in our hospital.

The median depth of melanoma was 2.5 mm, and, according to the Mood's median test, there was no statistically significant difference in the median values for melanoma depth *per* year, $p = 0.34$ (Table 1).

According to the Chi-square test, there was a significant difference in the presence of thin melanoma (maximal depth < 1 mm) in the pre-COVID-19 and COVID-19 time, but for a level of significance of $p < 0.1$ ($p = 0.065$) as presented in Tables 2 and 3.

The difference in the distribution of thick melanoma (> 4 mm) in pre-COVID-19 and COVID-19 periods is statistically significant ($p < 0.05$) (Table 3).

The distribution of different histological types of melanomas is presented in Table 4. According to the Chi-

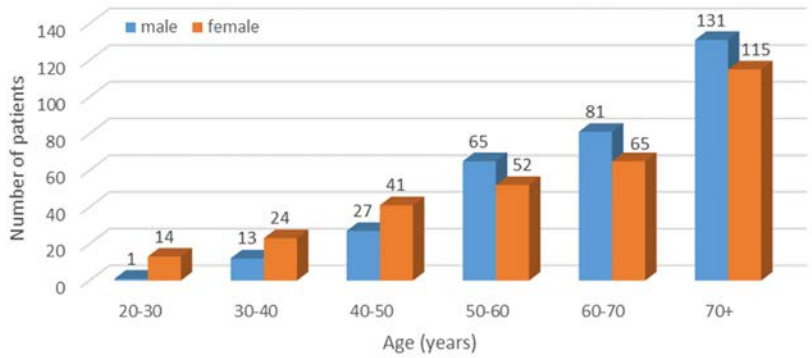

Fig. 2 – Gender distribution of patients in different age groups: dominance of women in younger age groups (< 50) vs. male dominance in older age groups ($p < 0.01$).

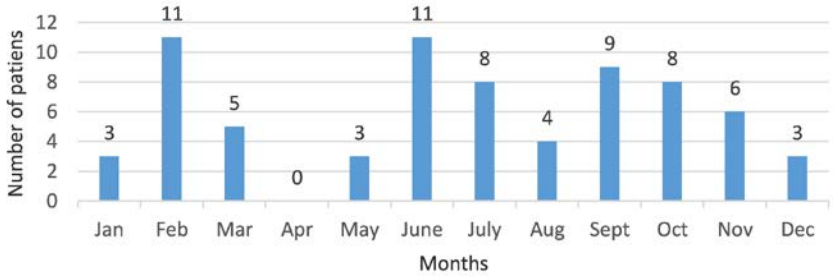

Fig. 3 – Number of patients who underwent melanoma surgery in 2020.

Table 1

Average depth of melanoma *per* year from 2017 to 2022

Parameter	Year					
	2017	2018	2019	2020	2021	2022
Melanoma depth (mm)	2.20	2.00	3.00	1.70	3.00	2.40

Values are presented as median values.

Table 2

Distribution of thin melanoma *per* year

Melanoma depth (mm)	Year					
	2017	2018	2019	2020	2021	2022
≥ 1	82	79	108	61	81	95
< 1	29	26	32	10	10	16
Total	111	105	140	71	91	111

Values are presented as numbers.

Table 3

Distribution of thin and thick melanoma in the pre-COVID-19 and COVID-19 period

Melanoma depth (mm)	Period (years)	
	2017–2019	2020–2022
Thin		
≥ 1	269 (75.56)	237 (86.81)
< 1	87 (24.44)	36 (13.19)
Thick		
> 4	134 (37.64)	77 (28.21)
≤ 4	222 (62.36)	196 (71.79)
Total, n	356	273

n – number. Values are presented as numbers (percentages).

Table 4

Distribution of different histological types of melanoma *per* year

Type of melanoma	Year						Total
	2017	2018	2019	2020	2021	2022	
Acral	2	4	5	6	2	7	26
In situ	5	2	3	1	–	2	13
LM	4	1	3	–	4	–	12
NM	37	32	44	23	41	41	218
SSM	63	66	85	41	44	61	360
Total	111	105	140	71	91	111	629

LM – *lentigo maligna*; NM – nodular melanoma; SSM – superficial spreading melanoma. Values are presented as numbers.

Table 5

Time interval between biopsy and radical surgery

Parameter	Year					
	2017	2018	2019	2020	2021	2022
Time interval (days)	40 ± 17	40 ± 19	45 ± 17	40 ± 34	37 ± 23	48 ± 35

Values are given as mean ± standard deviation.

square test, there is no statistically significant difference in the distribution of different types of melanoma during the pre-COVID-19 and COVID-19 periods ($p > 0.05$).

The time interval (in days) between biopsy and radical surgery in all examined cases is present in Table 5. Statistical analysis revealed that the average time from biopsy until radical surgery in the pre-COVID-19 period (2017–2019) was 42 days, and during the COVID-19 period (2020–2022), it was 41 days. There is no statistically significant difference in these data ($p = 0.87$). A statistically significant difference was seen only between the years 2021 and 2022 ($p = 0.04$).

Discussion

There are various perspectives when dealing with melanoma issues. Early diagnosis will obviously lead to better survival without the need to implement adjuvant therapies necessary for advanced stages. The economic burden of melanoma is also increasing, not only because of increased incidence but also because of the increased cost of treatments, primarily in advanced stages of the disease where expensive immunotherapy and targeted therapy are included. The figure that illustrates this change the best is the participation of adjuvant therapy in the overall cost of treatment of patients with melanoma. Chevalier et al. [4] published in their study from 2008, before revolutionary targeted therapy was introduced, that the chemotherapy available at that time accounted for only 17% of the total direct cost of melanoma treatment, whereas in a recent study from 2018, Buja et al. [5] stated that medical therapy accounts for 39.2% of all costs. In a situation where the healthcare system is financially overloaded with unexpected costs of treating a new threatening disease – COVID-19, it is the right moment to emphasize the importance of maintaining screening campaigns focused on detecting melanoma in the early stage in all circumstances.

Considering melanoma treatment, several new problems emerged during the COVID-19 pandemic. As the whole healthcare system was on alert, focused on COVID-19, all other non-COVID-19 medical problems were on hold, trying to avoid a crash of the system. All other segments of the healthcare system faced the necessary postponement of diagnostic and therapeutic procedures. The question for the medical community was how this temporary closure of the primary healthcare system for melanoma patients and difficult access to care would affect further clinical picture and outcome of these patients. In a great population-based modelling study conducted in England, Maringe et al. [6] tried to estimate the impact of the COVID-19 pandemic on cancer

mortality. They analyzed changes in the provision of cancer care during the pandemic, modifications of diagnostic pathways, and treatment schedules, focusing on the impact that delayed diagnosis would have on mortality and concluded that there is an increase in deaths due to cancer ranging from 4.8% for lung cancer to 16.6% for colorectal cancer. We can assume that a similar influence could be expected in melanoma patients as this highly malignant disease also requires early diagnosis in order to expect a good outcome. As shown in our study, there were 30% fewer melanoma patients in our hospital in the COVID-19 period compared to pre-COVID-19 time. Lallas et al. [7] also presented a similar decline of 36.4% during COVID-19 time in the number of melanoma patients in Greece. The drop in the number of patients was especially conspicuous in 2020, marking a 50% decline, as in that period, a state of emergency with a complete lockdown was declared. The state of emergency and lockdown was proclaimed in Serbia on March 15, and there were no patients who underwent melanoma surgery in April in our hospital. Similar trends could be seen in some other studies presenting the situation in their countries [7–9]. That certainly does not mean that melanoma as a disease "disappeared" during the COVID-19 pandemic. We suppose that people were focused on COVID-19, terrified of the deadly disease, and as melanoma does not hurt and does not look urgent, they postponed all preventive check-ups for later. Diagnosing thin melanoma requires preventive check-ups and skin biopsies, as those melanomas are usually unnoticeable. On the one hand, people postponed all "unnecessary" doctor visits, and on the other, primary medical care was mostly inaccessible for non-COVID-19 problems. Our results confirm this assumption, showing a significant decrease in the number of patients with thin melanoma compared to the pre-COVID-19 time (11.57% vs. 24.01%), even though average Breslow thickness in both cohorts did not significantly change as we expected. The majority of studies [7, 10, 11], unlike ours but like the one presented by Ungureanu et al. [12], representing the impact of the pandemic in Romania on melanoma management, marked a significant increase in the thickness of melanoma and an increased proportion of thick melanoma during the COVID-19 time. Unlike them, results presented by Ricci et al. [10], considering Italy, show a lower median thickness of melanoma during lockdown compared to the pre-COVID-19 period (0.66 vs. 0.88 mm). This could open discussion on the level of health education in different countries as it might tell us that people who are educated about health risks did not underestimate other health problems during the COVID-19 pandemic. Other studies also confirm delays in melanoma diagnosis, whose consequences are yet to be determined [12, 13]. A retrospective cohort study from five European cancer centers shows an overall increase in Breslow thickness and a significant delay in diagnosis of cutaneous melanoma [14]. Our results show that the distribution of different histological types of melanoma did not change over the years with the expected dominance of superficial spreading melanoma as described in the literature.

The question arises whether late diagnosis of melanoma, canceled screening programs, postponed radical surgical interventions after biopsy, and deprived adjuvant therapy due to hospital closures and quarantine measurements could lead to worse outcomes for patients and higher costs of treatment as a silent consequence of the COVID-19 pandemic. We shall see that in the coming years, but further studies will certainly address this problem; hence, we can try to change clinical practice during possible future pandemics. All epidemiological studies in the last decade showed a constant rise in new melanoma cases even without COVID-19, and no one could have predicted this disruption to the healthcare system and the damage that it would cause to other diseases, leading probably to the escalation of numbers in all cancer statistics [15, 16].

Our results show that the average time to definitive surgical treatment in this one-center study, before and during the COVID-19 pandemic, was similar (42 vs. 41 days). We initially expected to have a delay in radical surgery of melanoma during COVID-19 time. However, as all other elective surgeries in our hospital were canceled, oncological patients were the only patients who had at least some access to surgery. Luckily, this allowed us to keep the usual protocol for melanoma treatment.

Another unfortunate circumstance that connects these two diseases (melanoma and COVID-19) is the data suggesting that severe acute respiratory syndrome coronavirus 2 is more likely to affect older adult males with chronic comorbidities already at greater risk of melanoma [1, 17].

Employment of all medical staff in COVID-19 departments, regardless of specialty, along with quarantine measures and limited access to primary healthcare, led to some innovative solutions, like the introduction of telemedicine or some sort of teledermatology in diagnosing cutaneous malignancies. Although crisis drives innovation, we were very conservative about this idea and tried to maintain, if possible, all medical sources available for patients "face to face". Dealing with this problem, Gomolin et al. [18] clearly underline in their study the limit of teledermatology and that the consequences of falsely reassuring a person that they do not have melanoma can be fatal.

Conclusion

Following strict guidelines for melanoma treatment in times of pandemics can be challenging. As we can see with the COVID-19 pandemic, closing the health system for all other medical problems in an attempt to solve one problem creates confusion where people are uncertain about where to go and how to address their health issues. Time-sensitive nature of melanoma treatment requires that we keep our focus on this disease in all emergencies to enable expeditious patient identification and early treatment in order to provide the best survival and decrease avoidable melanoma deaths.

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Received on October 22, 2023
Accepted on January 9, 2024
Online First February 2024

ORIGINAL ARTICLE

UDC: 159.9:616-056.257-08-036
DOI: https://doi.org/10.2298/VSP231204012T

Higher degree of dysfunctional attitudes and beliefs and higher scores of frustration intolerance in women with unsuccessful weight loss

Veća izraženost disfunkcionalnih stavova i uverenja i veća netolerancija frustracije kod žena sa neuspešnim gubitkom telesne mase

Vesna Tepšić Ostojić*†, Zvezdana Stojanović*†, Tatjana Mraović‡, Danijela Ristić Medić§

Military Medical Academy, *Psychiatric Clinic, ‡Institute for Preventive Medicine, Belgrade, Serbia; †University of Defence, Faculty of Medicine of the Military Medical Academy, Belgrade, Serbia; §University of Belgrade, Institute for Medical Research, Center of Research Excellence in Nutrition and Metabolism, Group for Nutritional Biochemistry and Dietology, Belgrade, Serbia

Abstract

Background/Aim. Obesity is a chronic and relapsing condition, and since recently, it has been considered a global epidemic. Current guidelines for tripartite treatment of obesity emphasize a program of lifestyle modifications such as medical nutrition therapy, aerobic exercise, and behavioral intervention. The aim of the study was to evaluate whether specific psychological factors (general attitudes and beliefs and frustration tolerance) could be predictors of successful weight loss, i.e., factors that can be further addressed as part of the integrated therapy approach. **Methods.** A total of 84 consecutive overweight and obese female participants who were apparently healthy and homogeneous in age, level of education, and marital or employment status were prescribed the same medical nutrition therapy protocol as a single six-month therapeutic intervention. Prior to starting the therapy, the General Attitude and Belief Scale (GABS) and the Frustration Discomfort Scale (FDS) were administered. The participants were divided into two groups: successful (group A) and unsuccessful (group B). Group A, or the successful group [with the mean body mass index (BMI) ± standard deviation (SD) of 24.1 ± 5.81 kg/m^2], consisted of 40 participants who have reached the corresponding loss, i.e., they lost ≥ 10% of the starting body weight (BW). Group B, or the unsuccessful group (with the mean BMI ± SD of 28.51 ± 2.74 kg/m^2), consisted of 44 participants who have not reached the corresponding loss, i.e., they lost < 10% of the starting BW. **Results.** Participants in group B had significantly higher scores ($p < 0.05$) on the GABS subscales for the Need for Approval, Need for Comfort, and Other Downing. A statistically significant difference between groups ($p < 0.05$) was shown for FDS subscales of Emotional Intolerance, Entitlement, and Achievement Frustration. **Conclusion.** Results of our study showed that participants who were unsuccessful in medical nutrition therapy had a higher degree of dysfunctional attitudes and beliefs and higher scores of frustration intolerance when compared to successful participants. The recommendation, based on our results, would be to include psychotherapeutic techniques in the integrative obesity treatment, aimed at cognitive changes and increasing frustration tolerance.

Key words:
obesity; prognosis; psychology; surveys and questionnaires.

Apstrakt

Uvod/Cilj. Gojaznost je hronično i relapsirajuće oboljenje, a od skoro se smatra i globalnom epidemijom. U trenutnim smernicama za trojno lečenje gojaznosti ističe se program modifikacije životnog stila koji obuhvata medicinsku nutritivnu terapiju, aerobne vežbe i bihejvioralnu intervenciju. Cilj rada bio je da se proceni da li specifični psihološki faktori (opšti stavovi i uverenja i tolerancija na frustraciju) mogu biti prediktori uspešnog gubitka telesne mase, odnosno faktori na koje je moguće uticati u integrativnom terapijskom pristupu. **Metode.** Ukupno 84 konsekutivne predgojazne i gojazne ispitanice, bez pridruženih bolesti ili stanja, homogene po godinama, obrazovanju, bračnom i profesionalnom statusu, dobile su jednak program medicinske nutritivne terapije, kao jedinu terapijsku intervenciju tokom šest meseci. Pre početka lečenja primenjene su Skala opštih stavova i uverenja (*General Attitude and Belief Scale* – GABS) i Upitnik frustracione netolerancije (*Frustration Discomfort Scale* – FDS). Ispitanice su podeljene u dve grupe: uspešnu (grupa A) i neuspešnu (grupa B). Grupa A, odnosno uspešna grupa [ispitanice sa srednjom vrednošću indeksa telesne mase (ITM) ± standardna devijacija (SD) 24,1 ± 5,81 kg/m^2] sastojala se od 40 ispitanica koje su dostigle odgovarajući gubitak, tj.

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

izgubile ≥ 10% početne telesne mase (TM). Grupa B, odnosno neuspešna grupa (ispitanice sa srednjom vrednošću ITM ± SD 28,51 ± 2,74 kg/m²) sastojala od 44 ispitanice koje nisu dostigle odgovarajući gubitak, tj. izgubile su < 10% početne TM. **Rezultati.** Ispitanice u grupi B imale su značajno više skorove ($p < 0,05$) na subskalama GABS Potreba za priznanjem, Potreba za komforom i Obezvređivanje drugih osoba. Statistički značajna razlika između grupa ($p < 0,05$) pokazana je za FDS subskale Emocionalna netolerancija, Pravednost i Frustracije vezane za postignuća. **Zaključak.** Rezultati našeg istraživanja su pokazali da su ispitanice koje su bile neuspešne u sprovođenju medicinske nutritivne terapije imale viši nivo disfunkcionalnih stavova i uverenja i više skorove frustracione netolerancije u poređenju sa onima koje su bile uspešne u tome. Preporuka, bazirana na našim rezultatima, mogla bi biti da se u integrativni model lečenja gojaznosti uključe i psihoterapijske tehnike, usmerene na kognitivne promene i povećanje tolerancije na frustraciju.

Ključne reči:
gojaznost; prognoza; psihologija; ankete i upitnici.

Introduction

Obesity is a chronic and relapsing condition defined as excess body fat in the body with body mass index (BMI) ≥ 30 kg/m^2 and is considered a global epidemic. It is associated with multiple comorbidities and requires long-term medical management [1–4]. Intrinsic factors that lead to obesity are now meeting with an increasingly obesogenic environment [4]. With a current trend of increasing morbidity and mortality due to obesity, life expectancy could decrease for the first time in modern history [1, 5] Other than the well-known physical disorders, there are many mental disorders, such as mood disorders, anxiety, and major depression commonly associated with obesity [3, 4]. There is substantial evidence of a bidirectional relationship between depressive disorders and obesity [3]. Obesity has been shown to reduce self-esteem, negatively impact quality of life, increase social anxiety, and promote avoidance behaviors [6]. The stigma surrounding obesity also has a considerable effect on educational, professional, social, and healthcare aspects of life [7].

The current guidelines [8, 9] for the treatment of overweight (BMI 25.0–29.9 kg/m^2) and obesity (BMI ≥ 30.0 kg/m^2) emphasize a program of lifestyle modifications for all individuals with a BMI index of 30 kg/m^2 and above or 25 kg/m^2 plus two weight attributed co-morbidities. Lifestyle modification is tripartite treatment with weight loss and consequent weight management being the core of every treatment program [9]. Calorie-restricted diet therapy [2, 8, 9] is the foundation of weight loss treatment as energy deficit is needed for weight loss. The second part of the treatment is aerobic exercise [10] along with the promotion of active leisure time and reduction of sedentary lifestyle. Last but not least, the aim of behavioral intervention is to facilitate the achievement of therapeutic goals of lower energy intake and higher energy expenditure. It is suggested that the education of obese individuals leads to recognition and modification of environmental stimuli and consequent food intake, therefore resulting in a change in dietary habits and level of physical activity. The intervention is accompanied by reinforcement of specific tasks like goal-setting and problem-solving strategies [11].

A neutral energy balance is required to restore weight gain and is a key factor in the long-term success of lifestyle modification treatment [12]. The set point theory suggests that some biological factors drive a person to overeat in order to maintain/regain their weight [13]. It is not easy to resist the urge to (over)eat in today's extremely obesogenic environment with easy-to-get palatable calorie-rich food that also promotes a sedentary lifestyle.

However, the plethora of studies [12, 14] suggests that many obese individuals are able to achieve and maintain weight loss through lifestyle modifications. It can be hypothesized that one of the possible answers for the individual differences in the results of lifestyle modifications in obesity treatment could be the specific cognitive mechanisms involved [15]. Therefore, the new treatments addressing cognitive mechanisms are evolving [16–19] and enhancing the tripartite lifestyle modification programs. Another possible answer could be that the more individualized approach takes into consideration psychiatric conditions, personality traits, or other psychological factors such as frustration tolerance that obese individuals do or do not possess [15]. This paper is a continuation of our previous research [20] in determining psychiatric and specific psychological factors that contribute to failure or success in weight loss treatment. Thus, the aim of this study was to further evaluate whether specific psychological factors (general attitudes and beliefs and frustration tolerance) could be the predictors of successful weight loss, i.e., factors that can be addressed in an integrated therapy approach.

Methods

In our previous paper [20], we explained in detail the selection of participants, anthropometric measurements, and medical nutrition therapy protocol of this prospective cohort study with two measurements. The sample size was based on the need to detect the connection between changes in the BMI in the six-month intervention time and the scores on the instruments used. The required minimum for a statistical significance level of 0.05 and statistical power of 0.8 was 84 participants. In summary, 84 consecutive overweight/obese female participants who were apparently healthy and homogenous in age, level of education, and marital or employment status were administered the same medical nutrition therapy protocol. After six months of calorie-restricted conventional diet therapy as the only therapeutic intervention, the participants were divided into two groups regarding the outcome: the successful group, further referred to as group A, and the unsuccessful group, or group B. Group A consisted of 40 (48%) participants who lost ≥ 10% of starting body weight (BW) (BMI 24.1 ± 5.81 kg/m^2), and group B included 44 (52%) participants who lost < 10% of starting BW (BMI 28.51 ± 2.74 kg/m^2). The research protocol was approved by the Ethics Committee, University Clinical Center of Serbia, Belgrade, Serbia (reference No. 10/2, from November 19, 2015) prior to data collection.

Psychopathology measures

To rule out possible psychiatric co-morbidities, all participants were interviewed by the same investigator with a standard psychiatric interview before participating in the study.

Two self-administered questionnaires were used for the purpose of the research. Participants had 60 min in a quiet place to fill out the following questionnaires: the 55-item General Attitude and Belief Scale (GABS 55) [21] and the Frustration Discomfort Scale (FDS) [22]. Both self-assessment questionnaires were validated in the language of the studied population and are listed in the Repository of Psychological Instruments in Serbian [23] (appendices 1 and 2).

GABS was used to assess basic rational and irrational beliefs that may influence the course and outcome of obesity. GABS 55 has parallel sets of rational and irrational statements. The statements were formulated to include irrational cognitive processes (e.g., demandingness, devaluation, general self-esteem, low frustration tolerance) and three additional domains: achievement, recognition, and comfort. Fifty-five statements are divided into seven subscales. The subscales include general attitudes and beliefs and statements referring to attitudes and beliefs at the time of assessment. The seven subscales are the following: Rationality, which consists of nine statements referring to rational/cognitive processes such as achievement, approval, fairness, and comfort; Self-downing, which consists of nine questions with the commonality being negative self-reporting and negative reporting of self in light of negative circumstances; Need for Achievement, which consists of nine questions referring to demandingness, intimidation, and low tolerance of frustrations, bearing in mind that all the items are focused on achievement; Demand for Fairness, which consists of nine questions that assess the fairness or consideration of a person's treatment by other people; Other Downing, which consists of three questions that measure negative assessment of other people; Need for Comfort, which consists of nine questions contrasting the importance of comfort apropos discomfort; Need for Approval, which consists of seven questions referring to the acceptance and likewise disapproval of the approval of others. Items are rated on a five-point scale of distress (ranging from "does not apply" to "completely applies to me").

FDS is a multidimensional instrument based on a theory [24, 25] that posits frustration intolerance as one of the two main causes of psychological disorders. This scale was used to assess possible intolerance or low tolerance of frustration, which could be the reason for the failure of the obesity therapy. It consists of 28 questions, i.e., four subscales of seven questions. Subscale 1 – Emotional Intolerance, including intolerance of emotional distress. Items include beliefs regarding the uncertainty, controllability, and aversiveness of emotion. Subscale 2 – Entitlement, including fairness and gratification. It is represented as the belief that one's desires must be met and that other people should indulge and not frustrate those desires. Subscale 3 – Discomfort Intolerance, including intolerance of difficulties, refers to the attitude that life should be easy, comfortable, and free of hassle. Subscale 4 – Achievement Frustration, including intolerance of thwarted goals with items aimed at assessing the intolerance of frustration, as opposed to loss of self-worth, following achievement goal failure. The questions refer to the time immediately before the assessment and are scored on a five-point distress scale (ranging from "not at all" to "fully existent").

Statistical analysis

Statistical data analysis was performed using IBM SPSS Statistics 22 (IBM Corporation, Armonk, NY, USA). Results were presented as mean ± standard deviation (SD), and the Mann-Whitney *U* test was used. All *p*-values less than 0.05 were considered significant.

Results

Average scores of particular domains obtained from the GABS 55 questionnaire are presented in Table 1. Results indicate that both groups had similar scores in the Rationality subscale only. Participants in group B had higher scores in all other subscales, particularly in the subscales Need for Approval, Demand for Fairness, and Other downing; the difference was statistically significant ($p < 0.05$).

Table 1

Rational and irrational beliefs

GABS 55 subscales	All participants (n = 84)	Group A (n = 40)	Group B (n = 44)	*p*
Rationality	30.01 ± 7.57 (9, 45)	30.00 ± 8.17 (9, 45)	30.02 ± 7.08 (9, 37)	0.989
Self-Downing	17.08 ± 4.73 (9, 30)	16.47 ± 4.09 (9, 27)	17.64 ± 4.55 (11, 30)	0.264
Need for Achievement	22.30 ± 7.78 (9, 45)	20.90 ± 8.81 (9, 45)	23.57 ± 6.56 (9, 37)	0.117
Need for Approval	15.87 ± 5.51 (7, 34)	14.55 ± 5.08 (7, 24)	17.07 ± 5.74 (7, 34)	< 0.05
Need for Comfort	21.77 ± 6.65 (9, 39)	20.33 ± 6.99 (9, 34)	23.09 ± 6.11 (10, 39)	0.056
Demand for Fairness	22.25 ± 7.3 (9, 42)	20.22 ± 7.12 (9, 33)	24.09 ± 7.05 (12, 42)	< 0.05
Other-Downing	6.62 ± 2.74 (3, 14)	5.98 ± 2.82 (3, 14)	7.20 ± 2.56 (3, 13)	< 0.05

GABS 55 – General Attitude and Belief Scale 55-item.
All values are given as mean ± standard deviation (minimum, maximum).

Table 2

Tolerance to frustration

FDS subscales	All participants (n = 84)	Group A (n = 40)	Group B (n = 44)	*p*
Emotional Intolerance	16.48 ± 5.38 (7, 34)	14.40 ± 4.88 (7, 24)	18.36 ± 5.15 (10, 34)	< 0.05
Entitlement	17.58 ± 5.49 (7, 31)	15.53 ± 4.67 (7, 23)	19.45 ± 5.56 (9, 31)	< 0.05
Discomfort Intolerance	17.46 ± 5.87 (7, 30)	16.22 ± 5.61 (7, 28)	18.59 ± 5.93 (7, 30)	0.065
Achievement Frustration	18.04 ± 5.69 (7, 33)	16.60 ± 5.85 (7, 31)	19.34 ± 5.27 (9, 33)	< 0.05

FDS – Frustration Discomfort Scale.
All values are given as mean ± standard deviation (minimum, maximum).

The average scores of particular domains obtained from the FDS questionnaire are presented in Table 2. A statistically significant difference between groups ($p < 0.05$) was shown for FDS subscales of Emotional Intolerance, Entitlement, and Achievement Frustration. Participants from group B also had higher scores on the Discomfort Intolerance subscale.

Discussion

Lifestyle modification is considered to be a very demanding form of pre-obesity and obesity therapy. There is evidence that coping difficulties include food deprivation, suffering from hunger, feelings of dissatisfaction, nervousness, tension, anxiety, fear of failure, and many other factors that need to be overcome for obesity therapy to be successful [3, 24, 25]. Our study showed that participants which have not reached the corresponding BW loss had a quantitatively higher degree of expression of dysfunctional attitudes and beliefs and a lower degree of expression of rational ones as measured by GABS 55. Almost all these results were present on most subscales of the applied instrument.

Analysis of subscale 4, related to the need for approval, i.e., receiving or not receiving approval or recognition from others [21, 26], shows that participants from group B had more pronounced irrational beliefs. This subscale includes statements such as "It is terrible when someone treats me without enough respect" or "It is terrible when someone important to me does not like me". The expression of such beliefs indicates a strong need for support and recognition in situations that can be difficult and demanding. Considering that the six-month program of medical nutrition therapy is difficult and demanding, the absence of an expected level of approval could be an important factor contributing to the failure to meet therapy goals.

Subscale 5 (like the FDS questionnaire) measures the need for comfort relating to the ease of living [21, 26]. Participants with strong demands for comfort will have difficulties in maintaining normal functioning when faced with life circumstances that imply discomfort. The same can be expected in situations involving adhering to demanding therapy procedures [27, 28]. Therefore, it is not unexpected that the scores for unsuccessful participants are close to or statistically significantly higher than the scores of the successful ones. An example of these phenomena would be statement number 17: "Daily problems, difficulties, or limitations annoy me terribly". In the demanding situation of a six-month therapy process, participants with such irrational beliefs had serious difficulties and distress that hindered them further in achieving the goals.

Subscale 6 refers to the demand for fairness [21, 26], i.e., the imperative that other people treat the individual with consideration and fairness. The characteristic rational thinking within this group is the following statement: "It is important to me that people are fair to me, but I see that they do not have to be". Such rational attitudes and beliefs are less present in subjects with unsuccessful medical nutrition therapy. This means that in situations where there is no positive reinforcement from others, it will be easy to give up on long-term proclaimed goals, such as adhering to medical nutrition therapy.

The last subscale of the applied questionnaire refers to other downing [21, 26], with the scores significantly higher in group B. This increase in scores indicates that in frustrating circumstances, these kinds of irrational attitudes and beliefs will lead to dysfunctional behavior, i.e., non-compliance with therapy advice.

In recent years, a more individual and refined approach to obesity therapy has been in development, focusing on concomitant cognitive change. Behavioral approach and therapy as a part of lifestyle modification are being "upgraded", shifting the focus to cognitive change and more individualized treatment [16, 19].

Results of basic research show the importance of cognitive processes in regulating (un)healthy eating habits [15]. A significant association is found in the results of clinical research as well [29]. Specific cognitive factors relating to lifestyle modification treatment are identified in different studies [18, 19]. Higher expected one-year BW reduction at the start of treatment [30, 31], starting motivation for treatment based on appearance [31], personal reference of BW seen as acceptable or disappointing [32], and dissatisfaction with treatment weight [15] are all considered to be some of the cognitive factors linked to treatment discontinuation. Participants from group B had a quantitatively higher degree of expression of dysfunctional attitudes and beliefs, and our results of general cognitive factors are in concordance with the specific ones. We were unable to compare them to other general cognitive factors measured by GABS 55 since we were unable to find any available and relevant published results.

The ability to tolerate frustration was measured by the FDS questionnaire [22, 24], based on the theory that frustration intolerance is a multidimensional concept. FDS subscale scores point out the specifics of reduced frustration tolerance in the group of unsuccessful participants. The Entitlement subscale refers to the need and indispensable requirement that desires be fulfilled and that other people help them without thwarting them [22, 24, 27]. Facets of this subscale (correctness and urgency of gratification) and their higher score in the women from group B indicate that they have a stronger perception of injustice and the impossibility of achieving gratification compared to the women from group A. This cognitive structure negatively affects the implementation of the required treatment and does not lead to quick gratification [27, 28]. In addition, the need for immediate gratification (as part of frustration intolerance) without receiving a quick and "fair" reward (which is not possible in the case of this type of treatment) is a significant factor that can contribute to the failure of treatment.

The Emotional Intolerance subscale and its elevated scores in women with unsuccessful outcomes indicate that they had a hard time bearing with negative affectivity, especially anxiety that occurred during the treatment. The above result speaks in favor of the fact that they could not adequately tolerate emotional distress as a consequence of implementing a restrictive diet. Emotional intolerance reflects the belief that emotional distress cannot be tolerated and must be avoided and controlled, along with the reduction of uncertainty [22, 24]. This is achieved by shifting priorities from distant goals to immediate affective regulation [33]. Therefore, it can be assumed that to avoid negative effects and their consequences, participants did not adhere adequately to the treatment protocol. All of the above indicates that during the treatment, it would be necessary to include psychotherapeutic techniques that would contribute to accepting negative affect and increasing emotional control. The Discomfort Intolerance subscale describes the demand for an easy and comfortable life [22, 24]. Nevertheless, in our study, the statistical analysis indicates that the comparison of subjects with successfully and unsuccessfully implemented medical nutrition therapy does not reach a statistically significant level. However, due to the existence of a difference in arithmetic means, it can be said that this factor is more pronounced in group B. An increase in scores on the Discomfort Intolerance subscale indicates that the respondents are more inclined to achieve short-term satisfaction at the expense of long-term goals [22, 24, 33]. In the case of our research, this would mean that long-term goal, i.e., weight loss, is subordinated to short-term goals that include ease and comfort. Subordinating long-term goals to short-term ones leads to inadequate implementation and/or discontinuation of therapy when faced with difficulties. The last subscale relates to Achievement Frustration. It implies the need and demand for a perfect result that does not suffer deviations and oscillations [22, 24, 34]. As the scores on this subscale were also significantly higher in the unsuccessful group of participants, this indicates that they probably had a problem during the course of the treatment, which necessarily implies oscillations in motivation and the achievement of short-term goals – "that every day of every month of the duration of the treatment, I must lose weight and that I must not give up even occasionally". Knowing the demands of medical nutrition therapy [3, 22-24] and occasional oscillations in reaching goals, it is clear that this factor can negatively affect the overall outcome of the treatment, i.e., lead to premature termination of therapy. Given that this is, to our knowledge, the first study that examined frustration intolerance using the FDS questionnaire in subjects who were undergoing medical nutrition therapy, we gave our opinions relating to the results we obtained. These opinions are based on the basic concepts of the Rational Emotive Behavior Therapy theory [22, 24, 35, 36] and the settings of the instrument used to measure frustration intolerance [24, 25].

This study has some limitations. First, we have not divided overweight and obese women into separate groups. However, some research data confirm the same weight loss in overweight and obese women during the same treatment period [10]. Secondly, this discussion is more theoretical because, to our knowledge, there have been no exact studies with published results using the specific questionnaires we applied that we could refer to for confirmation and guidance in this unexplored area of research.

Conclusion

Results of our studies show that participants who were unsuccessful in medical nutrition therapy had quantitatively higher degrees of irrational attitudes and beliefs and higher scores of frustration intolerance when compared to participants who were successful in medical nutrition therapy. They had significantly higher scores on the subscales of Emotional Intolerance, Entitlement, and Achievement Frustration and a higher score on the Discomfort Intolerance subscale. The participants from unsuccessful group had significantly higher scores on the subscales Need for Approval, Demand for Fairness, Other-Downing, Self-downing, Need for Achievement, and Need for Comfort when compared to successful group.

Therefore, based on our results, the inclusion of psychotherapy techniques in the integrative treatment of obesity can be recommended in order to promote cognitive change and increase frustration tolerance. The second refers to increasing the ability to delay gratification, the ability to accept and endure negative affectivity, especially anxiety and the overall discomfort that accompanies it, the ability to tolerate difficulties and efforts, and, finally, the ability to overcome perfectionism related to achieving daily goals during the treatment.

Acknowledgement

This study was supported by the Ministry of Science, Technological Development, and Innovation of the Republic of Serbia (No. 451-03-47/2023-01/200015).

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Received on December 4, 2023
Revised on January 10, 2024
Revised on January 18, 2024
Accepted on January 23, 2024
Online First March, 2024

Appendix 1

Upitnik opštih stavova i uverenja – skala GABS 55

Pažljivo pročitajte svaku tvrdnju i zaokružite broj pored tvrdnje koji najbolje pokazuje stepen u kojem se slažete sa navedenom tvrdnjom

		nimalo	malo	umereno	prilično	izrazito, snažno
1	Kada se u teškim životnim situacijama osećam loše ne doživljavam to kao užas, već nastavljam dalje sa životom.	1	2	3	4	5
2	Ubeđena sam da bih bila bezvredna osoba ako bih loše uradila stvari koje su mi značajne u životu.	1	2	3	4	5
3	Ne mogu da podnesem kada ne uspem u nekim važnim stvarima i neizdrživ mi je osećaj promašenosti.	1	2	3	4	5
4	Ne mogu podneti kada su drugi ljudi prema meni neuviđavni i kada me tretiraju nepravedno.	1	2	3	4	5
5	Stanja nelagodnosti, napetosti ili nervoze su mi neizdrživa i ne mogu da podnesem kada se tako osećam.	1	2	3	4	5
6	Ne mogu podneti kada se ne dopadam ljudima koji su meni značajni.	1	2	3	4	5
7	Ja vredim kao osoba čak i ako ne uradim dobro stvari koje su mi važne.	1	2	3	4	5
8	Kada se osećam neugodno, napeto ili nervozno mislim da je to dokaz moje bezvrednosti.	1	2	3	4	5
9	Ako mi se desi da ne uradim dobro stvari koje su mi značajne, doživljavam to kao pravu katastrofu.	1	2	3	4	5
10	Užasno je i grozno kada me ljudi iz mog okruženja tretiraju nepravedno.	1	2	3	4	5
11	Ne mogu da podnesem i neizdrživa su mi neka loša osećanja i unutrašnja stanja.	1	2	3	4	5
12	Strašno je kada se nekome ko mi je značajan ne dopadam.	1	2	3	4	5
13	Iako želim da uspem u onome što mi je važno, uviđam da ne moram nužno uspeti u tim stvarima.	1	2	3	4	5
14	Ako se ne dopadnem nekome ko mi je važan, to znači da sam ja nedopadljiva osoba.	1	2	3	4	5
15	Ja moram dobro uraditi ono što mi je važno i neću prihvatiti ako to ne uradim dobro.	1	2	3	4	5
16	Ljudi moraju biti pravedni prema meni i ne prihvatam njihovu nepravednost.	1	2	3	4	5
17	Užasno me nerviraju svakodnevni problemi, teškoće ili ograničenja.	1	2	3	4	5
18	Kada me neko tretira neuviđavno pomislim kako to pokazuje koliko loših i nepopravljivih ljudi ima na ovom svetu.	1	2	3	4	5
19	Prihvatam sebe i osećam se kao vredno ljudsko biće i onda kada me odbaci neko ko mi je značajan.	1	2	3	4	5
20	Ako ne uradim dobro stvari koje su mi jako važne, mislim da je glavni razlog moja lična neadekvatnost.	1	2	3	4	5
21	Užasno je kada čovek ne uradi dobro neke važne stvari u životu.	1	2	3	4	5
22	Grozno je kada me neko tretira bez dovoljno uvažavanja.	1	2	3	4	5
23	Neophodno mi je da imam lep život i ne mogu da prihvatim kada mi život nije dovoljno lep i prijatan.	1	2	3	4	5
24	Mislim da moram uvek da se dopadnem ljudima i jako teško bih prihvatila da se nekome ne dopadnem.	1	2	3	4	5
25	Ne volim kada me neko tretira bez uvažavanja, ali mogu to podneti.	1	2	3	4	5
26	Ako me odbaci neko ko mi je značajan, to znači da sam ja bezvredna osoba.	1	2	3	4	5
27	Ne mogu da podnesem kada ne uradim dobro stvari koje su mi važne.	1	2	3	4	5
28	Ne mogu da podnesem kada me ljudi ne tretiraju onako kako zaslužujem.	1	2	3	4	5
29	Neophodno mi je da imam smiren život i ne mogu da prihvatim životne teškoće.	1	2	3	4	5
30	Verujem da su ljudi koji me tretiraju nepravedno bezvredni i loši ljudi.	1	2	3	4	5
31	Šteta je ako se ne dopadam nekome ko se meni dopada, ali to nije strašno.	1	2	3	4	5
32	Ako me ne vole ljudi koji su mi značajni to pokazuje koliko sam bezvredna.	1	2	3	4	5
33	Od suštinske je važnosti da neke stvari u životu uradim dobro i stoga ih moram raditi dobro.	1	2	3	4	5
34	Moram biti poštovana i uvažavana i ne mogu da prihvatim nepoštovanje.	1	2	3	4	5
35	Užasno je kad čovek ima neprilike u životu i kada je zbog toga opterećen.	1	2	3	4	5
36	Ne bih podnela da me ne voli ili ne prihvata neko ko mi je značajan.	1	2	3	4	5
37	Mogu dobro da podnesem kada sam opterećena životnim problemima.	1	2	3	4	5
38	Ne bih vredela kao osoba ako bih imala više neuspeha u životu.	1	2	3	4	5
39	Nepodnošljiva mi je pomisao na neuspeh u nekim značajnim stvarima.	1	2	3	4	5
40	Grozno je kada je neko prema meni neuviđavan.	1	2	3	4	5
41	Mislim da je užasno kada se osećam loše, utučeno, napeto ili nervozno.	1	2	3	4	5
42	Od suštinske mi je važnosti da me vole i prihvataju ljudi koji su mi značajni.	1	2	3	4	5

		nimalo	malo	umereno	prilično	izrazito, snažno
43	Važno mi je da su ljudi prema meni pravedni, ali uviđam da ne moraju biti.	1	2	3	4	5
44	Kada u životu naiđem na teškoće ili imam neke probleme mislim da sam ja kriva i da to dokazuje koliko sam loša.	1	2	3	4	5
45	Za mene je prava katastrofa ako doživim neuspeh u važnim stvarima.	1	2	3	4	5
46	Ne mogu da podnesem kada me ljudi ne poštuju.	1	2	3	4	5
47	Ne mogu da podnesem kada naiđem na životne teškoće ili neprilike.	1	2	3	4	5
48	Neophodno mi je da budem prihvaćena i voljena od ljudi do kojih mi je stalo.	1	2	3	4	5
49	Želim da budem prihvaćena od ljudi do kojih mi je stalo, ali uviđam da ljudi ne moraju da me prihvataju samo zato što ja to želim.	1	2	3	4	5
50	Kada imam probleme i neugodnosti u životu mislim da manje vredim kao osoba.	1	2	3	4	5
51	Moram biti uspešna u stvarima koje su mi važne i ne prihvatam neuspehe.	1	2	3	4	5
52	Najvažnije mi je da me ljudi oko mene uvažavaju i poštuju.	1	2	3	4	5
53	Ne smem dozvoliti da se osećam utučeno ili nervozno i mislim da ne mogu prihvatiti loša osećanja.	1	2	3	4	5
54	Neizdrživo mi je ako me neko ko mi je značajan ne voli ili odbacuje.	1	2	3	4	5
55	Ako se ljudi prema meni ponašaju bez poštovanja to pokazuje koliko su loši.	1	2	3	4	5

GABS 55 – ***General Attitude and Belief Scale*** **55-*item***

Appendix 2

Upitnik frustracione netolerancije

Pred vama se nalaze neke uobičajene misli i uverenja koje ljudi mogu da imaju kada su uznemireni ili frustrirani. Pročitajte svaku tvrdnju i ocenite koliko dobro ona opisuje vaša uverenja u ovim situacijama. Koristite skalu u sledećem značenju: odsutno-1; blago-2; umereno-3; jako-4; veoma jako-5.

		odsutno	**blago**	**umereno**	**jako**	**veoma jako**
1	Moram naći najlakši način da rešim problem, ne mogu da izdržim da se mučim.	1	2	3	4	5
2	Ne mogu da podnesem kada moram da čekam na stvari koje želim odmah.	1	2	3	4	5
3	Apsolutno moram da se oslobodim uznemiravajućih osećanja što brže, ne mogu da izdržim kada ona traju.	1	2	3	4	5
4	Ne mogu da podnesem da budem sprečena da ostvarim svoj puni potencijal.	1	2	3	4	5
5	Ne mogu da podnesem da radim zadatke koji mi se čine suviše teškim.	1	2	3	4	5
6	Ne mogu da podnesem kada se ljudi ponašaju protivno mojim željama.	1	2	3	4	5
7	Ne bih mogla da podnesem osećaj da gubim razum.	1	2	3	4	5
8	Ne mogu da podnesem frustraciju kada ne ostvarim svoje ciljeve.	1	2	3	4	5
9	Ne mogu da podnesem da radim nešto kada nisam za to raspoložena.	1	2	3	4	5
10	Ne mogu da podnesem ukoliko drugi ljudi stanu na put onome što želim.	1	2	3	4	5
11	Ne mogu da podnesem određene misli.	1	2	3	4	5
12	Ne mogu da tolerišem snižavanje sopstvenih standarda čak i kada bi to bilo korisno.	1	2	3	4	5
13	Ne mogu da podnesem kada moram da se teram da radim.	1	2	3	4	5
14	Ne mogu da podnesem kada me uzimaju „zdravo za gotovo“.	1	2	3	4	5
15	Ne mogu da izdržim situacije u kojima bih mogla da se osetim uznemireno.	1	2	3	4	5
16	Ne mogu da podnesem da završim sa poslom ukoliko nisam u potpunosti zadovoljna time kako sam ga obavila.	1	2	3	4	5
17	Ne mogu da izdržim opterećenje kada moram da uradim nešto odmah.	1	2	3	4	5
18	Ne mogu da podnesem kada moram da se povinujem zahtevima drugih.	1	2	3	4	5
19	Ne mogu da izdržim uznemirujuća osećanja.	1	2	3	4	5
20	Ne mogu da podnesem da radim nešto ukoliko nisam u stanju da to uradim dobro.	1	2	3	4	5
21	Ne mogu da izdržim da radim stvari koje zahtevaju dosta napora i muke.	1	2	3	4	5
22	Ne mogu da podnesem kada ja moram da se menjam, a drugi su u krivu.	1	2	3	4	5
23	Ne mogu da nastavim sa svojim životom, ili da budem srećna, ako se stvari ne promene.	1	2	3	4	5
24	Ne mogu da podnesem osećaj da nisam na visini nekog zadatka.	1	2	3	4	5
25	Ne mogu da podnesem kada moram da istrajavam u zadacima koji mi ne prijaju.	1	2	3	4	5
26	Ne mogu da podnesem kritiku, naročito onda kada znam da sam u pravu.	1	2	3	4	5
27	Ne mogu da podnesem da izgubim kontrolu nad svojim osećanjima.	1	2	3	4	5
28	Ne mogu da podnesem nikakav pad sopstvene samokontrole.	1	2	3	4	5

ORIGINAL ARTICLE

UDC: 159.9::616.31
DOI: https://doi.org/10.2298/VSP231209013M

The influence of psychological factors on the frequency and perception of post-endodontic pain

Uticaj psiholoških faktora na učestalost i percepciju postendodontskog bola

Marija Mijailović, Veljko Kolak

University Business Academy in Novi Sad, Faculty of Dentistry, Pančevo, Serbia

Abstract

Background/Aim. Post-endodontic pain (PEP) is associated with the presence of any uncomfortable feeling or sensitivity that occurs within a few hours or a few days after the endodontic treatment. The aim of this study was to evaluate the possible association between psychological factors and the frequency and perception of PEP. **Methods.** The study sample consisted of 140 patients with incisors, canines, or premolars indicated for root canal treatment (RCT) without periapical pathology. A single experienced endodontist was involved in the procedure, and the same clinical protocol was used for all the patients. Participants psychometric evaluation was done using the Depression Anxiety Stress Scale 21 (DASS-21). PEP levels were assessed using a Visual Analog Scale at different intervals (24, 48, and 72-hour intervals and after a week). **Results.** The presence of postoperative pain was reported in 63.6% of the respondents. The vast majority rated the pain intensity as mild, and the pain significantly decreased over evaluated time intervals. Participants who exhibited higher scores for depression, anxiety, and stress reported significantly higher pain intensity. The multivariate logistic regression analysis showed that tooth type ($p = 0.001$) and high anxiety score ($p = 0.035$) were directly associated with the occurrence of pain after therapy. **Conclusion.** Psychological factors, such as depression, stress, and anxiety, influenced pain perception after RCT treatment, and a high anxiety score was directly associated with the frequency of post-obturation pain.

Key words:
anxiety; depression; pain, postoperative; psychology; root canal therapy; stress, psychological; surveys and questionnaires.

Apstrakt

Uvod/Cilj. Postendodontski bol (PB) se opisuje kao neugodan osećaj ili bolna osetljivost koja se javlja u roku od nekoliko sati ili nekoliko dana posle endodontske terapije zuba. Cilj rada bio je da se proceni moguća povezanost između psiholoških faktora i učestalosti i opažanja PB. **Metode.** Uzorak studije činilo je 140 pacijenata čiji su sekutići, očnjaci ili premolari bili indikovani za lečenje kanala korena zuba (KKZ), bez prisustva periapikalne lezije. Terapijsku proceduru izvodio je isti terapeut, specijalista endodoncije, a identičan klinički protokol korišćen je za sve pacijente. Za psihometrijsku procenu korišćena je Skala stresa, anksioznosti i depresije (*Depression Anxiety Stress Scale* 21 – DASS-21). Intenzitet PB procenjivan je upotrebom Vizuelno analogne skale u različitm intervalima (24, 48 i 72 sata i nedelju dana kasnije). **Rezultati.** Prisustvo postoperativnog bola prijavilo je 63,6% ispitanika. Velika većina ispitanika ocenila je intenzitet bola kao blag, a bol se značajno smanjivao tokom ispitivanih vremenskih intervala. Učesnici studije sa ispoljenim višim stepenom depresije, anksioznosti i stresa prijavili su značajno veći intenzitet bola. Multivarijantnom logističkom regresionom analizom utvrđeno je da su tip zuba koji je lečen ($p = 0,001$) i visoki stepen anksioznosti ($p = 0,035$) bili direktno povezani sa pojavom bola posle terapije. **Zaključak.** Psihološki faktori, kao što su depresija, stres i anksioznost, uticali su na opažanje bola posle lečenja KKZ, a visok stepen anksioznosti bio je direktno povezan sa učestalošću pojave bola nakon terapije.

Ključne reči:
anksioznost; depresija; bol, postoperativni; psihologija; zub, lečenje korenskog kanala; stres; ankete i upitnici.

Introduction

Endodontic treatment (ET) is considered a kind of microsurgical procedure that requires great precision in a narrow space of root canals with limited direct-view access. The term "post-endodontic pain" (PEP) is a condition associated with the presence of any uncomfortable feeling or sensitivity that occurs within a few hours (hrs) or a few days after the ET [1]. The prevalence of postoperative pain (PP) reported in different studies varies from 13.1% to 64.7%. This wide variance was attributed to the study type (prospective or retrospective), sampling method, different kinds of assessing and defining

Correspondence to: Marija Mijailović, Faculty of Dentistry in Pančevo, Žarka Zrenjanina 179, 26 000 Pančevo, Serbia.
E-mail: marija.mijailovic.mm@gmail.com

PP concerning different criteria, preoperative diagnosis, or the time point when the pain was recorded [2]. The pain intensity can range from mild to intensive or severe, and it can last one day or sometimes several weeks. Patient dissatisfaction can sometimes be dominantly caused because of the occurrence of PP. Besides that, the occurrence of pain could be an indicator of some kind of pathology, and even more, may raise doubts about the long-term treatment success [3]. Previous studies evaluated common factors that could influence the occurrence of discomfort and pain after the ET. The most frequently investigated factors were inadequate instrumentation, apical extrusion of infected debris, irrigation solutions or intracanal dressing, missed canals, presence of traumatic occlusion, preoperative pain or periapical pathology, number of visits, etc. [4].

The International Association for the Study of Pain defined pain as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage" [5]. Later on, a more complete definition occurred, which included psychological, noxious transmission, and a very important modulatory component. By this definition, pain is "an unpleasant sensation associated with actual or potential tissue damage and mediated by specific nerve fibers to the brain, where its conscious appreciation may be modified by various factors" [6]. In general, pain presents an experience that is subjective and influenced by various factors, such as personality, behavior, and physical and psychological factors, and is, therefore, difficult to quantify and standardize [7]. Previous studies have demonstrated that emotional states, such as fear, anxiety, depression, or stress, can modulate human pain reactivity. Anxiety refers to agitation, impatience, irritability, or difficulty relaxing. Stress is characterized by tension, which is persistent, by a tendency to overreact to stressful events, and by a low threshold of becoming frustrated or upset. Depression is characterized by a low level of positive affect (lack of energy, dysphoria, anhedonia, and hopelessness). Dental fear and dental anxiety represent strong negative feelings related to different dental treatments and may result in the worsening of dental health by interfering with patients' compliance. Previous painful experiences and anxious episodes regarding dental procedures could also be related to the reported pain [6, 8]. However, there is still a lack of studies that investigate the role of anxiety and depression on pain perception concerning ET [9].

The aim of this study was to evaluate the possible association between psychological factors and the frequency and perception of PEP.

Methods

Study population

The participants were selected from the Department of Dentistry, Community Health Center in Ljig, Serbia, between February and June 2023. The study was performed following the ethical principles for medical research involving human subjects stipulated by the Declaration of Helsinki and approved by the institutional Ethics Committee (protocol No. 191/2023, from February 21, 2023). Oral and written informed consent was obtained from all participants.

G*Power 3.1 software (Heinrich Heine University, Dusseldorf, Germany) was used to determine the sample size, with the power set at 80% and the alpha level at 0.05. The mean and standard deviation (SD) values were taken from a previous study conducted by Çiçek et al. [10]. The sample volume was determined to be at least 109 participants.

The inclusion criteria for the study were the following: systemically healthy patients over 18 years of age who could understand the use of the pain scale, with incisors, canines, or premolars indicated for root canal (RC) treatment (RCT), diagnosed with symptomatic or asymptomatic irreversible pulpitis or pulp necrosis (with normal periapical tissues) and those which required RCT due to prosthetic reasons. The exclusion criteria were: patients under 18 years of age, pregnancies, contraindications for RCT due to systemic disease, immunosuppressed patients, reported intolerance to nonsteroidal anti-inflammatory drugs, patients with pacemakers, conditions which require antibiotic prophylaxis, reported medication with an anti-inflammatory or analgesic agent before treatment, patients undergoing orthodontic treatment, teeth with periodontal disease or periapical pathology, internal and external resorption, teeth with open apices, RC retreatment, calcified or extremely narrow canals, teeth with severe RC curvature or anatomic abnormalities, cases of inadequate obturation after treatment (inhomogeneous filling, short fillings, apical extrusion of obturation material). The final study sample was 140 after the exclusion of patients who did not meet the criteria.

Psychological status evaluation

The evaluation of the participant's psychological status was done using the Depression, Anxiety, and Stress Scale (DASS)-21, translated into the Serbian language. This scale represents the short version of the original 42-item DASS, created by Lovibond and Lovibond [11], and has been widely used for screening the symptoms of depression, anxiety, and stress (DAS) at different levels. The questionnaire contains 21 statements, divided into three subscales containing seven statements and measuring the three dimensions of negative emotional states – depression (DASS-D), anxiety (DASS-A), and stress (DASS-S). A 4-point Likert scale ranging from 0 ("does not apply to me at all") to 3 ("applies to me most of the time or always") was used to categorize the answers for each statement. The final score was calculated after multiplying the sum of 7 scores of each subscale by 2. The score for the three subscales was classified as normal, mild, moderate, severe, or extremely severe, as recommended by the original authors of the DASS-21. This scale was previously validated on the general adult population and student population in Serbia [12, 13].

Root canal treatment procedure

A single operator, an experienced endodontics specialist, performed all the diagnostic and treatment procedures to minimize or eliminate treatment individual variability be-

tween different operators. The same standard evidence-based protocol (shaping, cleaning, and filling the RC system) was used for all cases included in the study in order to standardize the ET. Before the treatment, local anesthesia with 2.5 mL of 2% lidocaine containing 40 mg/2 mL + 0.025 mg/2 mL adrenaline (Galenika a.d., Belgrade, Serbia) was administered. The working field was isolated using the rubber dam, and the round diamond burs were used to perform the access cavity. A modified step-back technique with Nickel-Titanium (NiTi) K-Files (FKG Dentaire, La Chaux-de-Fonds, Switzerland) was used for instrumentation. The coronal third of RC was shaped using Gates-Glidden drills (#1, 2, and 3) (Dentsply Maillefer, Ballaigues, Switzerland), and the working length was determined using an electronic apex locator (DPEX III, Guilin, Guangxi, China). The RCs were prepared to a master apical size of 40/0.02, the step-back technique was done with K-Files 45–55/0.02, and the depth of insertion was reduced by 1 mm. The irrigation was done using 2.5% sodium hypochlorite (NaOCl) (i-dental, Siauliai, Lithuania) with a 27-G needle, inserted 2 mm short of the working length between each file. The same volume of irrigant was used during each treatment. To remove the smear layer after RC preparation, approximately 3 mL of 17% ethylenediaminetetraacetic acid (i-dental, Siauliai, Lithuania) was used. The polymeric calcium hydroxide RC sealer (Sealapex™, Sybron-Kerr, Romulus, MI, USA) and the lateral compaction method were used for obturation at the same visit. In the case of multi-visit treatment, temporary RC filling was done using calcium hydroxide paste (Calxyl®, OCO Präparate GmbH, Dirmstein, Germany). A post-treatment radiograph was taken immediately after treatment to evaluate the quality of RC obturation.

Postoperative pain assessment

The occurrence and pain intensity after ET were evaluated using a Visual Analog Scale (VAS) at 24, 48, and 72-hour intervals and after a week. According to this scale, the pain level was numerically recorded in the range of 0–10. The participants were asked to indicate, on the scale, the point on the line that represents the intensity of pain at a given moment. The following VAS classification was used: 0 – no pain, 1–3 – mild pain, 4–6 – moderate pain, and 7–10 – severe pain. The operator explained to the patients in detail how to use the visual scale, and during the intervals, the patients were called by phone and asked to fill in the PP questionnaire.

Statistical analysis

For the analyses, the statistical software SPSS v20.0 (IBM Inc, USA) was used. Descriptive statistics for baseline demographic and clinical features were expressed as numbers and percentages for all categories. DASS-21 and VAS scale scores were expressed as percentages, with mean, SD, and minimum and maximum values. The analysis of the intensity of PP for the evaluated time intervals was performed using the Friedman test ($p \leq 0.05$). An Independent *t*-test was used to evaluate the difference in PEP intensity (mean ± SD) among DASS-21 categories at predetermined time points, at the significance level of $p \leq 0.05$. Evaluation of the association between the incidence of PEP and different variables at the subject level was done using logistic regression. First, univariate unconditional logistic regression analysis was performed, with each variable as an independent and the presence of PEP as a dependent variable. After that, the multivariate logistic analysis was performed, including only the variables with significant correlation as independent variables. The strength of association was presented by odds ratio with a 95% confidence interval at the significance level of $p \leq 0.05$.

Results

The present study included 140 participants of both genders over 18 years of age (mean age 39.23 ± 14.38, range 18–68). ET was conducted on 92 single-rooted and 48 double-rooted teeth. The demographic characteristics of the study population are presented in Table 1.

Table 1

Baseline demographic and clinical features

Variable	Values
Age, years	
18–40	80 (57.1)
41+	60 (42.9)
Gender	
female	60 (42.9)
male	80 (57.1)
Tooth	
single-rooted	92 (65.7)
double-rooted	48 (34.3)
Dental arch	
upper	102 (72.9)
lower	38 (27.1)
Preoperative pain	
asymptomatic	32 (22.9)
symptomatic	108 (77.1)
Pulpal vitality	
vital	116 (82.9)
necrotic	24 (17.1)

All values are expressed as numbers (percentages).

The results of the evaluation of participants' psychological indicators using the DASS-21 for each category (mean values, SDs, and score range) are presented in Table 2.

Table 2

DASS-21 scores of the study sample

Parameter	Mean ± SD	Range
Depression	5.74 ± 7.12	0–32
Anxiety	6.56 ± 7.56	0–30
Stress	11.03 ± 10.03	0–42
Total	23.27 ± 23.43	0–98

DASS-21 – Depression Anxiety Stress Scale-21; SD – standard deviation.

Normal scores for the DASS-21 subscales were obtained among the largest number of respondents (77.1%, 65.7%, and 68.6%, respectively), while 5.0%, 10.7%, and 8.6%, respectively, exhibited severe or extremely severe scores (Figure 1).

The presence of PP 24 hrs after obturation was reported in 63.6% of the respondents. The vast majority rated the pain intensity as mild, and only two respondents rated it as moderate 24 hrs after obturation. The intensity of PP significantly decreased over evaluated time intervals ($p < 0.001$). The frequency and intensity of PEP at predetermined time intervals are presented in Table 3.

The results of the comparison of post-obturation pain (POP) intensity among DASS-21 categories revealed that participants with moderate to severe scores in three categories reported significantly higher pain intensity at predetermined time points, except in the depression category after 72 hrs (Table 4).

The occurrence of POP was associated with tooth type ($p = 0.002$), high depression ($p = 0.042$), anxiety ($p = 0.005$),

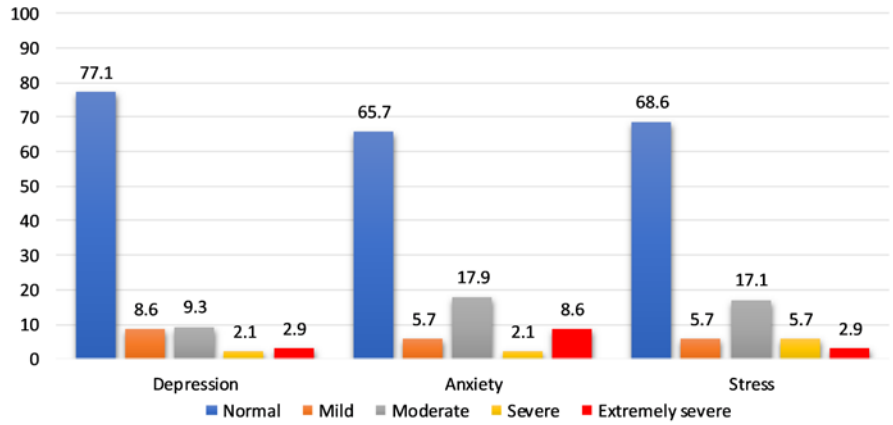

Fig. 1 – Distribution of the study participants according to the DASS-21 categories. DASS-21 – Depression Anxiety Stress Scale-21

Table 3

Frequency and intensity of post-endodontic treatment pain at predetermined time intervals

Time (intervals)	F	I	χ^2	*p*
After				
24 hrs	89 (63.6)	1.31 ± 1.19 (0–4)	121.76	< 0.001
48 hrs	68 (48.6)	0.59 ± 0.67 (0–2)		
72 hrs	20 (14.3)	0.14 ± 0.35 (0–1)		
7 days	4 (2.9)	0.03 ± 0.17 (0–1)		

F – number (percent) of participants who reported post-endodontic treatment pain; I – intensity of post-endodontic pain given as mean ± standard deviation (minimum-maximum) according to the Friedman test.

Table 4

Difference of post-endodontic treatment pain intensity among DASS–21 scale categories at predetermined time points

Time-point	Depression		*t*	*p*
	normal/mild	moderate/severe		
After				
24 hrs	1.13 ± 1.09	2.25 ± 1.12	4.2204	< 0.001*
48 hrs	0.50 ± 0.62	1.00 ± 0.65	3.3094	<0.005*
72 hrs	0.13 ± 0.34	0.20 ± 0.41	0.7849	0.4339
7 days	0.00 ± 0.00	0.20 ± 0.41	5.4380	< 0.001*
	Anxiety			
	normal/mild	moderate/severe		
24 hrs	1.00 ± 1.02	2.03 ± 1.17	5.1371	< 0.001*
48 hrs	0.40 ± 0.57	1.00 ± 0.64	5.4380	< 0.001*
72 hrs	0.08 ± 0.27	0.30 ± 0.46	3.4798	< 0.001*
7 days	0.00 ± 0.00	0.10 ± 0.30	3.3094	< 0.005*
	Stress			
	normal/mild	moderate/severe		
24 hrs	1.08 ± 1.08	1.92 ± 1.18	3.9342	< 0.001*
48 hrs	0.42 ± 0.57	1.00 ± 0.68	4.9873	< 0.001*
72 hrs	0.08 ± 0.27	0.33 ± 0.48	3.9713	< 0.001*
7 days	0.00 ± 0.00	0.11 ± 0.32	3.5797	< 0.001*

For abbreviation, see Table 2. Values are expressed as mean ± standard deviation.*significant at $p < 0.05$ (Student's *t*-test).

Table 5

Association between the incidence of post–endodontic treatment pain and different variables

Variable	Presence of PEP n (%)	Logistic regression analysis					
		univariate			multivariate		
		OR	95% CI	*p*-value	OR	95% CI	*p*-value
Gender							
female	35 (58.3)	0.674	0.34–1.35				
male	54 (67.5)	1		0.266			
Age, years							
18–40	54 (67.5)	1.484	0.74–2.97				
41+	35 (58.3)	1		0.266			
Preoperative pain							
symptomatic	60 (62.0)	0.743	0.32–1.72				
asymptomatic	22 (68.7)	1		0.489			
Pulpal vitality							
vital pulp	71 (61.2)	0.526	0.19–1.42				
necrotic	18 (75.0)	1		0.206			
Tooth							
single root	50 (54.3)	0.275	0.12–0.63		0.232	0.09–0.55	0.001*
two roots	39 (81.2)	1		0.002*	1		
Dental arch							
upper	69 (67.6)	1.882	0.88–4.02				
lower	20 (52.6)	1		0.103			
Visits							
single	26 (54.2)	0.544	0.26–1.12				
multiple	63 (68.5)	1		0.097			
DASS-21 Depression							
normal/mild	72 (60.0)	0.265	0.07–0.95		0.345	0.05–2.29	0.271
moderate/severe	17 (85.0)	1		0.042*	1		
DASS-21 Anxiety							
normal/mild	56 (56.0)	0.270	0.11–0.67		0.205	0.05–0.89	0.035*
moderate/severe	33 (82.5)	1	0.19–0.74	0.005*	1		
DASS-21 Stress							
normal/mild	60 (57.7)	0.329	0.13–0.82		0.616	0.09–3.91	0.607
moderate/severe	29 (80.5)	1		0.017*	1		

***significant at $p < 0.05$; OR – odds ratio; CI – confidence interval; PEP – post-endodontic pain. For other abbreviations, see Table 2.**

and stress ($p = 0.017$) scores after conducting univariate unconditional logistic regression analysis. The multivariate logistic regression analysis revealed that only tooth type ($p = 0.001$) and high anxiety score ($p = 0.035$) had a direct link with the occurrence of POP (Table 5).

Discussion

The final goal of RCT is to maintain the tooth, but this treatment among patients is frequently associated with some extent of pain before, during, and after the treatment. The multifactorial nature of pain perception is well known, and the expression of pain is related to the person's biology and psychology. Therefore, a better understanding of patients' psychological aspects, their anticipation, and their ability to cope with the dental procedure associated with pain could increase patients' confidence and contribute to the improvement of their level of care. In this regard, one should not only consider the individual perception of the treatment but also take into account the impact of the negative social connotations associated with the treatment [14].

The evaluation of participants' psychological status in this study was done using the DASS-21, which is widely used, and it turns out that it presents a model that can successfully recognize and separate symptoms related to DAS. It has been translated and adapted in a large number of countries on all continents and applied in different linguistic and cultural communities [15]. DAS mean values in the present study, as well as the percentages of participants with at least moderate levels for three categories (14.3%, 28.6%, and 25.7%, respectively), were very similar to the results from a recent study in Serbia, among dental students [13]. It should be mentioned that higher levels of DAS were recorded in the other study among the general adult population of Serbia. However, those results could be considered as expected, given the timing of the study, which was almost immediately after declaring the state of emergency due to the COVID-19 pandemic and after the residents of Serbia spent a month in lockdown [12].

The gold standard for measuring pain intensity is patient self-assessment. VAS was used in the present study to measure the level of pain after ET. In 1969, Aitken was among the first to discuss the measurement of feelings using VAS, and today, the VAS scale is widely used for measuring psychosocial subjective phenomena [16]. It is highly responsive, it does not burden the patients too much, it is

fairly simple, easy to manage the administration, and solved quickly. The presence of PP was reported by 63.6% of respondents in the present study. This incidence can be considered high, even though it should be mentioned that there is an extremely wide range of results in the literature regarding the frequency of PEP, probably due to the differences in pain assessment methods and definitions of pain, different inclusion criteria, different techniques used for ET, different quality assessment, the difference in host-related factors, as well as whether potentially present microbiological or iatrogenic factors were taken into account [17]. The reasons for this high percentage of reported PP could be due to the participants' individual perceptions or the fact that the manual RC instrumentation technique was used in the present study, which is commonly considered a significant factor. This outcome can be possibly associated with the results of previous studies that reported reduced periapical extrusion of infected debris in the case of rotary NiTi instrumentation [18]. It should be mentioned that there are studies that reported less frequency of pain when the modified step-back technique was used compared with the reciprocal and rotational techniques [10]. Pain intensity in the present study was low, namely around 1 or 2 points on the VAS scale. The pain reached its peak overall up to 24 hrs after the treatment, and the intensity significantly decreased over evaluated time intervals. Previously conducted studies reported the same results [10, 19, 20].

POP intensity at predetermined time points was significantly higher among participants of the present study with moderate to severe scores for DAS in comparison to ones with normal or mild scores. Maggirias and Locker [21], in their research, confirmed the important role of psychological factors in pain perception. It has already been proven that the cognitive component must be considered when evaluating the presence of pain and assessing its intensity, taking into account recall of previous experiences and learning. Anxiety and depression can also affect the perception of pain [22]. In the medical literature, depression has been extensively studied as a predictor of PP, as well as a correlation between depression and the patient's assessment of VAS and pain [23]. However, there are conflicting conclusions. In dentistry, depression has not been widely explored as a predictor for PP. Yang et al. [24], in their study, found that participants with diagnosed depression reported greater levels of pain related to ET. Khademi et al. [9] evaluated the pain perception during RCT of patients diagnosed with symptomatic irreversible pulpitis and the potential influence of psychological factors. The results indicated that neuroticism traits and depression were associated with higher pain levels. Nevertheless, the authors concluded that in spite of the introduction of certain models, the direct association remained uncertain. The association between high levels of stress and conditions related to increased orofacial pain levels, such as bruxism, is well-established [13]. A recent study demonstrated that stress can modulate pain perception and the other way around, indicating the bidirectional association. However, the difference between acute and chronic stress should be emphasized because acute stress can even affect a reduced perception of pain, while, on the contrary, chronic stress can affect an increased perception over time. The authors of the study concluded that increased exposure to stress may represent an increased risk for more pronounced pain perception [25]. There is a lack of literature data regarding the potential influence of stress on the occurrence and perception of PEP. In the research by Sana et al. [26], a significant correlation was recorded between pain and stress, and it has been concluded that increased stress levels before ET have increased procedural pain perception during and after treatment.

Gender, tooth type, preoperative pain, single/multiple visits, irrigants, and their activation, different medications, instrumentation technique, obturation material and techniques, and the vitality of the pulp were some of the factors that were related to the incidence of PEP in different literature. The conclusion from the literature review was that this condition is multifactorial. Time presents an important factor in the evaluation of PEP, and different factors are interdependent and interrelated [27]. Multiple logistic regression models in the present study revealed that only tooth type and high anxiety score were associated with the occurrence of POP. The significant association with the tooth type is probably expected and might be due to the canal anatomy complexity and increased number of canals in double-rooted teeth. Moreover, the chairside time could be influenced by the difference in teeth anatomy, which could also affect PP [28]. Except for the fact that a larger number of RCs and canal anatomy complexity increases the risk that some of the potentially significant factors exert their influence on the occurrence of intraoperative and PEP, the differences between short and long treatments could be confounded by tooth type. The possible explanation could be the progressive decrease of the anesthetic effect, together with the increase of the patient's anxiety as the intervention extended [29]. Glennon et al. [30] reported a higher level of PP after the ET of molars in comparison with anterior and premolar teeth, with the explanation that debridement is more difficult due to the complexity of the RC system or the simple fact that a larger number of RCs increases the possibility of postoperative complications. The relationship between pain and anxiety before, during, and after dental treatment has already been reported in the literature. The nature of the relationship is dynamic, resulting from the correlation between expected and perceived pain [31]. Studies have also reported that higher levels of pain after dental treatment were recorded among participants with higher scores on dental anxiety and pain scales [6]. In their study, Wu et al. [31] found that the important factor for anxiety and post-treatment pain reduction after emergent ET of teeth with symptomatic irreversible pulpitis was the level of pain relief expectation before the treatment. A positive correlation between the experienced pain and dental anxiety among patients with irreversible pulpitis was also reported by Dou et al. [32], while Murillo-Benítez et al. [33] found that during RCT, moderate or intense levels of intraoperative pain were more than twice as likely felt by anxious patients. Likewise, it should be noted that there were studies that indicated that anxiety was not a significant factor for pain perception at any phase of ET [9].

The present study has some limitations. The sample size matched the calculation performed before starting the research, but it is not large enough for generalizing the findings of the study. A single operator performed all the treatments using the same clinical protocol for all the patients, all in order to minimize or eliminate intraoperative factors important for the treatment outcome. However, previous studies presented some differences concerning the incidence and intensity of PEP dependent on different instrumentation techniques, irrigation agents and techniques, or obturation techniques and sealer types [1, 34, 35]. Bearing in mind that the results of the present study indicated a significant association of PEP with tooth type, further studies should be conducted, divided into different groups (with a single canal and with two or more canals) for comparison. The sample included both vital and nonvital teeth. Although the presence of different pulp pathology could affect the occurrence and intensity of PEP, the authors decided to form the sample without classification into individual diagnoses, relying on the results of previous studies, indicating that the effect of the pulp vitality remains inconclusive [27, 36]. Further on, socially desirable answers cannot be completely avoided in the case when the participants complete the questionnaires on their own [37]. Likewise, a single instrument (VAS scale) was used to measure the intensity of pain. Although it is widely used and results in high rates, some authors claim that PP intensity can be more precisely measured when more than one scale is used in order to compare the relationships between each intensity scale and obtain a more accurate measure [19].

Conclusion

The results of the present study indicated that psychological factors, such as depression, anxiety, and stress, had an influence on pain perception after the RCT. Among evaluated psychological factors, a high anxiety score was significantly associated with the occurrence of post-obturation pain. Additional studies need to be done in the future, bearing in mind the lack of evidence regarding the association between psychological factors and personality characteristics with the perception of pain in the field of endodontology.

Conflict of interests

The authors declare no conflict of interest.

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Received on December 9, 2023
Revised on January 19, 2024
Revised on January 28, 2024
Accepted on February 6, 2024
Online First March 2024

ORIGINAL ARTICLE

UDC: 614.2:616-083.98]::614.8.028.4
DOI: https://doi.org/10.2298/VSP231229015G

Hospital professional staff awareness of hospital plans for the provision of teams for medical support in case of disasters

Upućenost bolničkog profesionalnog osoblja u bolničke planove formiranja ekipa za medicinsko zbrinjavanje u slučaju katastrofa

Mariya Georgieva Georgieva*, Rostislav Stefanov Kostadinov*, Mariya Anastasova Semerdjieva-Filipova†, Elena Vladimirova Valkanova*

Medical University of Plovdiv, Faculty of Public Health, *Department of Epidemiology and Disaster Medicine, †Department of Health Care Management, Plovdiv, Bulgaria

Abstract

Background/Aim. Resuscitation and surgical medical aid are the most important medical procedures in disaster relief operations. The limited time frame available for life-saving activities in such circumstances demands that resuscitation and surgical medical teams be established and trained for timely reaction and disaster medical support prior to disaster occurrence. The aim of the study was to analyze the awareness among the hospital medical staff in the Plovdiv Region of the hospital response plan in case of disaster, which concerns surgical and resuscitation team composition and tasks for disaster medical support. **Methods.** An anonymous survey, consisting of 55 questions about hospital staff awareness of the medical activities described in the hospital disaster medical support plan, was conducted between July and September 2019 among 295 hospital medical professionals in the Plovdiv Region, Bulgaria. Descriptive statistics and Pearson's χ^2 test were used in the statistical analysis of results. **Results.** The survey showed that the majority of medical staff demonstrated complete ignorance regarding the presence, number, and tasks of the specialized medical teams planned for hospital disaster response. Employees of multidisciplinary hospitals for active treatment were more familiar with the aforementioned plans than their colleagues from the university multidisciplinary hospitals. **Conclusion.** Insufficient awareness about human resources foreseen for disaster medical support negatively impacts the hospital surge capacity, leading to inadequate medical assistance in such cases. Hospital tactical disaster preparedness must be ameliorated by conducting a more rigorous training program among medical staff related to the planned hospital activities in case of disastrous events, especially for those working in emergency departments, intensive care units, and surgical departments.

Key words:
bulgaria; disaster medicine; disaster planning; medical staff, hospital; surveys and questionnaires.

Apstrakt

Uvod/Cilj. Reanimacija i hirurška medicinska pomoć su najvažnije medicinske aktivnosti u procedurama pomoći u katastrofama. Ograničeno vreme za spasavanje života u takvim okolnostima nameće potrebu da se timovi za reanimaciju i hirušku medicinsku pomoć uspostave i obuče za pravovremeno reagovanje i medicinsku podršku u slučaju katastrofe pre nego što se katastrofa dogodi. Cilj rada bio je da se analizira upućenost bolničkog medicinskog osoblja, u regionu Plovdiva, u planove bolničkog reagovanja koji se tiču sastava hirurških i reanimacijskih timova i zadataka medicinske pomoći u slučaju katastrofe. **Metode.** Anonimna anketa, koja se sastojala od 55 pitanja i koja je trebalo da pruži informacije o tome koliko je bolničko osoblje upoznato sa medicinskim aktivnostima opisanim u bolničkim planovima medicinske podrške u slučaju katastrofe, sprovedena je od jula do septembra 2019. godine i obuhvatila je 295 medicinskih stručnjaka u bolnicama u regionu Plovdiva, u Bugarskoj. U statističkoj analizi rezultata korišćeni su deskriptivna statistika i Pearson-ov χ^2 test. **Rezultati.** Anketa je pokazala da većina medicinskog osoblja nije znala ništa u pogledu postojanja, broja i zadataka specijalizovanih medicinskih timova planiranih za bolničko reagovanje u slučaju katastrofe. Zaposleni u multidisciplinarnim bolnicama za aktivno lečenje bili su u većoj meri upoznati sa pomenutim planovima u odnosu na svoje kolege iz univerzitetskih multidisciplinarnih bolnica. **Zaključak.** Nedovoljno znanje o ljudskim resursima koji su predviđeni za pružanje medicinske pomoći u uslovima katastrofa negativno utiče na povećanje kapaciteta bolnica,

Correspondence to: Mariya Georgieva Georgieva, 15-A "Vasil Aprilov" Blvd., Plovdiv 4002, Bulgaria.
E-mail: mari.georgieva@mu-plovdiv.bg

što dovodi do neadekvatne medicinske pomoći u takvim situacijama. Taktička spremnost bolnica u slučaju katastrofa mora biti poboljšana sprovođenjem rigoroznijeg programa obuke medicinskog osoblja u vezi sa planiranim bolničkim aktivnostima u slučaju katastrofalnih događaja, posebno za one koji rade u odeljenjima hitne medicinske pomoći, jedinicama intenzivne nege i hirurškim odeljenjima.

Ključne reči:
bugarska; medicina katastrofa; katastrofe, planiranje; kadar bolnički; ankete i upitnici.

Introduction

Most disasters are characterized by severe consequences for the population due to diverse life-threatening injuries. Mechanical injuries include head and body traumas, such as closed head and brain injury, fractures of the limbs, fractures of the skull and the spine, chest fractures, and severe injuries of the chest, abdomen, and/or pelvis. Traumatized casualty management requires multidisciplinary resuscitation, intensive care, and surgical medical aid. Therefore, disaster medical response teams must be prepared to provide appropriate medical treatment for casualties, including a wide variety of resuscitative and surgical procedures [1–4].

In the occurrence of head injuries and severe injuries of the chest, abdomen, and/or pelvis, due to the urgency of required surgical procedures, disaster medical support has to provide urgent life-saving resuscitation along with surgical procedures under anesthesia. Therefore, surgeons and anesthesiologists are the main performers of medical support during the early medical response. The number of foreseen disaster surgical and resuscitation teams are key indicators for surge capacity [3, 5, 6].

In circumstances of limited time for adequate medical support in eventual disaster scenarios, effective, timely, and adequate medical assistance in such situations demands the formation of disaster support teams prior to a crisis or disaster occurrence. Proper team formation implies that the team members know each other and that they have undergone the same type and amount of theoretical and practical training [7].

The aim of the study was to analyze the awareness among hospital medical specialists in the Plovdiv Region, Bulgaria, of the concept of the hospital disaster response plan, surgical and resuscitation team composition, and tasks for disaster medical support.

Methods

An anonymous survey consisting of 55 questions was conducted in two multidisciplinary hospitals for active treatment and one university multidisciplinary hospital for active treatment between July and September 2019 among hospital medical professionals in the territory of the Plovdiv Region. For each medical specialist, the survey purpose was to self-determine the level of their awareness and medical readiness for responding in case of a disaster. The survey cards, along with brief introductions, were presented to the medical staff by an interviewer. Medical specialists who expressed willingness to participate in the study received the questionnaire and were kindly asked to fill it in a paper form.

Based on the different hospital types and their role during an eventual disaster medical support, multidisciplinary hospitals for active treatment were chosen to participate in the study. The reason for this choice was the fact that, in case of a disaster, the casualties are to be evacuated to the nearest hospital, which can provide life-saving therapeutic and surgical assistance to casualties with multiple traumas, and the chosen types of hospitals were in accordance with these prerequisites. The hospitals included in the study were: the University Multidisciplinary Hospital for Active Treatment "Plovdiv" – Plovdiv, Multidisciplinary Hospital for Active Treatment "Asenovgrad" – Asenovgrad, and Multidisciplinary Hospital for Active Treatment "Dr. Kiro Popov" – Karlovo. They were selected randomly from a list of existing hospitals in the Plovdiv Region.

Written permission to conduct the study has been sent from the hospital managers in response to application forms sent to them. Application forms with incoming numbers were sent to the directors of the following institutions: two application forms, No. B-1024, from August 6, 2019, and No. P-800, from July 6, 2019, were sent to the executive director of the University Multidisciplinary Hospital for Active Treatment "Plovdiv" – Plovdiv; request No. 1305, from July 10, 2019, was sent to the manager of the Multidisciplinary Hospital for Active Treatment "Asenovgrad" – Asenovgrad, and application form No. 748 from July 9, 2019, was sent to the manager of the Multidisciplinary Hospital for Active Treatment "Dr. Kiro Popov" – Karlovo.

A total of 310 medical specialists were surveyed, but due to incomplete and missing data on some of the survey cards, 15 participants dropped out. Therefore, the actual number of participants was ultimately 295. The number of medical professionals that participated in the study formed 8.6% of all medical personnel (general population) in the multidisciplinary hospitals for active treatment in the Plovdiv Region, and was representative in relation to the general population.

Data processing and quantitative analysis were conducted using the specialized software platform IBM® SPSS® 21.0 (IBM Corporation, Armonk, New York, USA). Descriptive statistics were used to calculate the relative percentages. Pearson's χ^2 test was applied in testing hypotheses for a statistically significant relationship between the studied factorial and performance traits. Graphic analysis was applied to illustrate processes and phenomena, certain regularities, or dependencies. Microsoft Office Excel 2013

was used for tabular and graphical analysis. The value of $p < 0.05$ was considered statistically significant for all analyses.

Results

Most of the surveyed hospital staff (n = 265, 89.8%) demonstrated complete ignorance regarding the presence of specialized medical teams in the hospital disaster response plans. Only a small part (n = 39, 15.3%) of the medical professionals knew about the planned resuscitation teams. Only 41 (13.9%) participants were informed of the number of surgical teams the hospital must provide in case of a disaster (Figure 1).

The most aware of disaster resuscitation teams were the medical personnel with medical practice between 1 and 10 years; 25 (21.7%) of them knew about their hospital plans for the required teams ($p = 0.041$, $\chi^2 = 8.24$) (Figure 2).

There was a significant statistical difference in awareness about resuscitation and surgical teams regarding the hospital type. Employees in multidisciplinary hospitals for active treatment were more knowledgeable; 29 (27.9%) of them were aware of their hospital resuscitation teams (Figure 3), while only 16 (8.4%) of their colleagues working in the university multidisciplinary hospitals for active treatment were informed about disaster plans ($p = 0.001$, $\chi^2 = 19.82$).

Similarly to their knowledge about the resuscitation teams, the medical staff was unfamiliar with the number of surgical teams planned in case of a disaster. Twenty-one (20.2%) specialists in multidisciplinary hospitals for active treatment were informed about the number of surgical teams, while 20 (10.5%) of their colleagues from the university multidisciplinary hospital for active treatment provided a positive answer (Figure 4). Although a small part of the medical staff was generally informed about the planned surgical capabilities, the difference between medical specialists working in multidisciplinary hospitals for active treatment and those working in the university multidisciplinary hospital was statistically significant ($p = 0.033$, $\chi^2 = 5.31$).

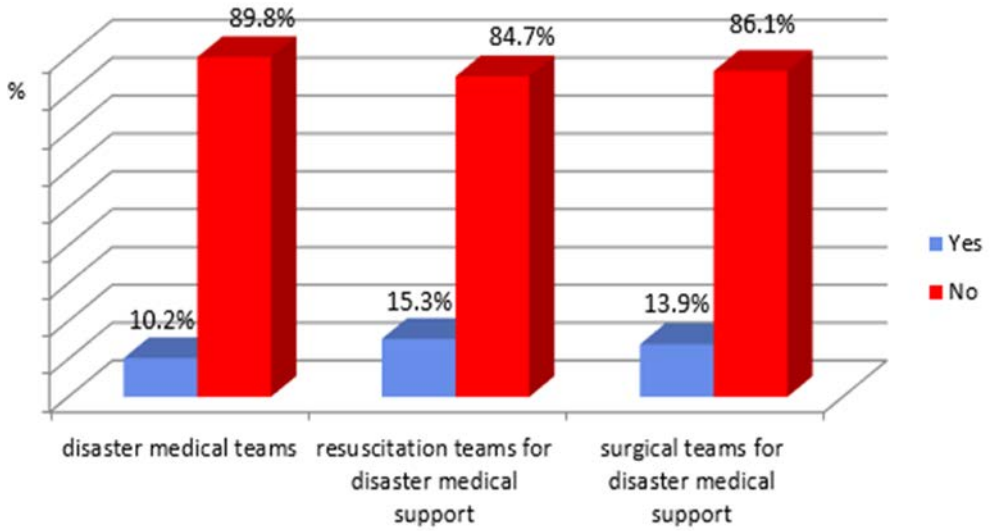

Fig. 1 – Hospital staff awareness of hospital disaster medical teams.

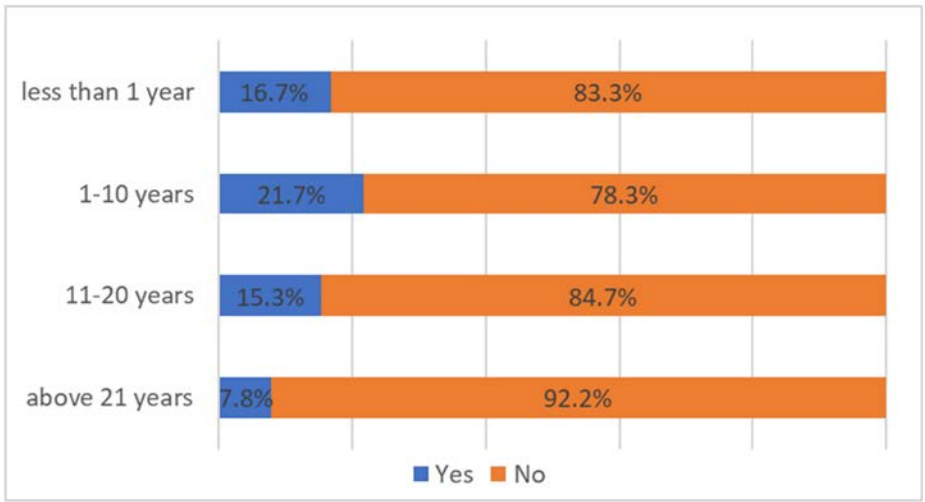

Fig. 2 – Hospital staff awareness of resuscitation teams for disaster medical support depending on duration of employment.

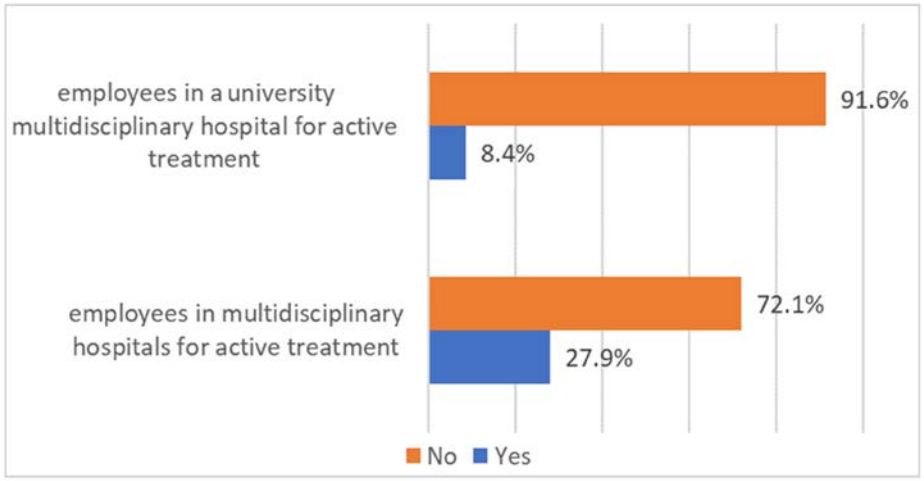

Fig. 3 – Hospital staff awareness of resuscitation teams for disaster medical support depending on the workplace.

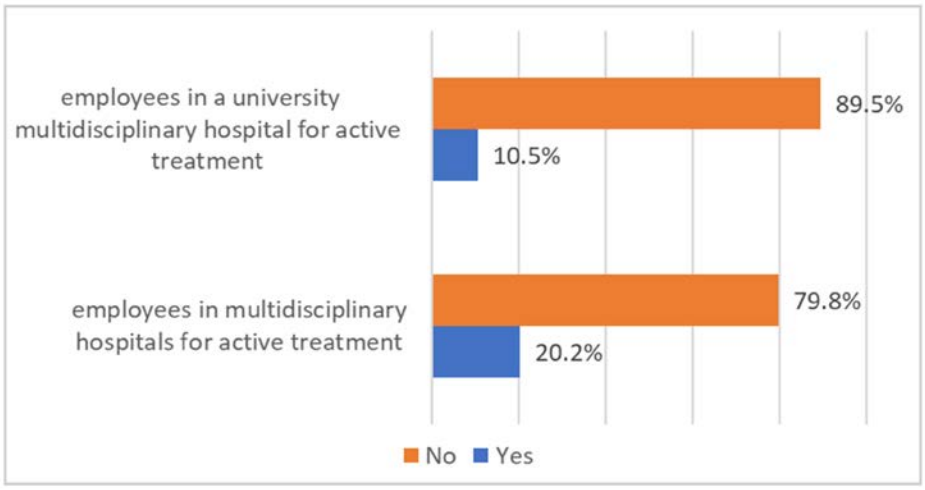

Fig. 4 – Hospital staff awareness of surgical teams for disaster medical support depending on the workplace.

Discussion

As the hospitals are the final stage of medical support in case of a disaster, it is necessary for them to be prepared to act adequately and promptly to save as many casualties as possible. Due to the nature of disasters and the limited time available to attend to the casualties, disaster planning of medical means and capabilities is required. Good preparedness presupposes preliminary organization of the number and the type of medical teams the hospital may provide in case of a disaster [8–11]. Most of the hospital personnel have shown complete ignorance regarding the presence of specialized medical teams, as well as the number of surgical and resuscitation teams planned in the hospital disaster medical plan, even though all of this was provided in the plan of the hospital they have worked in. Poor awareness of medical staff for disaster response negatively affects surge capacity. The main hospital medical teams required in case of a disaster caused by overpressure and/or a blast wave are surgical and resuscitation teams, as most casualties would require surgical intervention, necessitating the use of anesthesia [7, 12]. Most of the medical staff was not familiar with the number of surgical teams planned in case of a disaster, even though in two of the hospitals where the study was conducted, the disaster medical support plan indicated the formation of one main surgical team and one spare team. Although generally only a small part of the medical staff of the region was informed about the planned surgical capabilities, the medical specialists working in multidisciplinary hospitals for active treatment were more familiar than the ones in the university multidisciplinary hospitals. Similar results regarding awareness related to the preparedness of providing surgical teams in disaster circumstances were found in other studies [13]. Insufficient awareness suggests insufficient medical staff disaster preparedness.

Except for administering anesthesia, anesthesiologists would also perform other medical procedures necessary for disaster medical support, as they have more experience and skills than their colleagues from other medical specialties in the following fields: airway management, intravenous catheterization, resuscitation, trauma, and critically ill

patient management, analgesia, administration of antidotes/medications and performing transfusion protocols. In case of a disaster, anesthesiologists may be assigned to emergency departments where they could perform triage, assist their colleagues in airway management, obtain vascular access, perform cardiopulmonary resuscitation, and treat those affected by chemical or biological agents. If casualties with complications such as acute renal failure, shock, disseminated intravascular coagulopathy, or rhabdomyolysis were present, then anesthesiologists in coordination with anesthesiology nurses would treat these casualties in intensive care units. That is why it is necessary to plan both main and additional resuscitation teams in case of a disaster. An additional anesthesia team would provide additional help when/if needed, replace the main team, and provide breaks for anesthesiologists of the main team, working 24 to 48 hrs (two days approximately) [13–15].

The hospitals participating in the study have also planned the use of one main and one additional resuscitation team in case of a disaster scenario; the number of resuscitation teams was described in the plans of both hospitals, but the medical specialists did not know about them. The low awareness level about the planned disaster resuscitation teams is worrying. Inadequate knowledge about the human resources that would be recruited to treat casualties in an eventual disaster can lead to inadequate medical support.

The medical staff with working experience between 1 and 10 years was the most informed about resuscitation teams compared to the rest of the employees, but even their level of knowledge was disturbing. The low awareness of medical professionals with working experience of under one year can be explained by their recent appointment to the job. However, the other medical specialists have had long enough medical practice in the institutions they worked in to get familiar with the disaster medical support plans of the hospital and to have participated in the creation of the plans. Their lack of awareness is unacceptable because well-trained personnel are a prerequisite for efficient hospital disaster preparedness. The interviewed medical staff could not be classified as well-trained.

Conclusion

The low level of awareness about the planned medical teams for disaster medical support is disturbing. Insufficient awareness about human resources to be used in disaster medical support is a prerequisite for inadequate medical provision and negatively impacts surge capacity. Surge capacity can be improved by ensuring adequately trained medical specialists. Medical personnel should undergo a rigorous training program and tabletop exercise, especially the ones working in emergency departments, intensive care units, and surgical departments, at least twice a year to increase the hospital's tactical disaster preparedness.

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Received on December 29, 2023
Accepted on February 6, 2024
Online First March 2024

ORIGINAL ARTICLE

UDC: 616.24-036.81-037
DOI: https://doi.org/10.2298/VSP230925016T

Comparison of DECAF score and DECAF+Lactate score in the prediction of mortality in patients with acute exacerbation of COPD

Poređenje skora DECAF i skora DECAF+Laktat u predikciji mortaliteta kod bolesnika sa akutnim pogoršanjem HOBP

Gulcan Tanrikulu*, Ramazan Guven†, Semih Korkut†, Utku Murat Kalafat‡, Dilan Duz§, Akkan Avci‖, Serkan Dogan‡

***Silivri State Hospital, Emergency Medicine Clinics, Istanbul, Turkey; Health Science University, †Istanbul Cam and Sakura City Hospital, Department of Emergency Medicine, ‡Istanbul Kanuni Sultan Suleyman Research and Training Hospital, Department of Emergency Medicine, Istanbul, Turkey; §Mimar Sinan Buyukcekmece State Hospital, Emergency Medicine Clinics, Istanbul, Turkey; ‖Health Science University, Adana City Research and Training Hospital, Department of Emergency Medicine, Adana, Turkey**

Abstract

Background/Aim. Chronic obstructive pulmonary disease (COPD) is a chronic process that progresses with exacerbations. Various studies are carried out to predict mortality. Among the routine tests used to monitor and treat disease in the emergency department, special efforts are made to find those that are meaningful and diagnostic. The aim of the study was to compare the DECAF score and DECAF+Lactate score and examine the significance of the DECAF+Lactate score in predicting mortality in critically ill patients presenting with COPD exacerbation. **Methods**. This prospective multicentric study included 435 patients recruited from two centers. Patients who presented to the emergency department with acute COPD exacerbation and fit the definition of critically ill according to the quick Sequential Organ Failure Assessment (qSOFA) were included in the study. The prognostic values of the scores were compared using the receiver operating characteristic (ROC) curve analysis. The efficiency of scoring 28-day mortality was compared with logistic regression analysis. **Results**. For 435 patients, sensitivity, specificity, and area under the curve (AUC) were calculated for lactate, DECAF score, and DECAF+Lactate score, which were statistically significant in the ROC curve analysis for predicting mortality: 50%, 90.2%, 0.711, odds ratio (OR): 0.622 [95% confidence interval (CI): 1.573–2.203]; 57.6%, 64.3%, 0.654, OR: 0.618 (95%CI: 1.501–2.291); 60.1%, 75.4%, 0.744, OR: 0.790 (95%CI: 1.826–2.659), respectively. Each unit increase in the DECAF+Lactate score increased the risk of mortality by 2.203. **Conclusion**. As a result of our study, we believe that the DECAF+Lactate score is a more effective scoring system than the DECAF score as a predictor of mortality in critically ill patients with COPD exacerbation.

Key words:
critical illness; lactates; mortality; prognosis; pulmonary disease, chronic obstructive; sensitivity and specificity; severity of illness index.

Apstrakt

Uvod/Cilj. Hronična opstruktivna bolest pluća (HOBP) je hroničan proces koji napreduje sa egzacerbacijama. Da bi se predvidela smrtnost obolelih, sprovode se razne studije. Među rutinskim testovima koji se koriste za praćenje i lečenje bolesti u hitnoj pomoći, posebno se nastoji da se pronađu testovi koji su smisleni i dijagnostički. Cilj rada bio je da se uporedi skor DECAF i skor DECAF+Laktat i ispita značaj skora DECAF+Laktat u predikciji smrtnosti kritično obolelih bolesnika sa pogoršanjem HOBP. **Metode.** Prospektivnom multicentričnom studijom obuhvaćeno je 435 bolesnika iz dva centra. U studiju su uključeni bolesnici koji su se javili u odeljenje hitne pomoći sa akutnim pogoršanjem HOBP i koji su odgovarali definiciji kritično bolesnih prema skoru *quick Sequential Organ Failure Assessment* (qSOFA). Prognostičke vrednosti skorova upoređene su korišćenjem analize *receiver operating characteristic* (ROC) krive. Efikasnost bodovanja 28-dnevnog mortaliteta upoređena je korišćenjem logističke regresione analize. **Rezultati.** Za 435 bolesnika izračunati su osetljivost, specifičnost i *area under the curve* (AUC) za laktate, DECAF i DECAF+Laktat skorove, koji su bili statistički značajni u ROC analizi za predikciju mortaliteta: 50%, 90,2%, 0,711, *odds ratio* (OR):

Correspondence to: Ramazan Guven, Health Science University, Istanbul Cam and Sakura City Hospital, Department of Emergency Medicine, Basaksehir/Istanbul/Turkey. E-mail: drramazanguven@gmail.com

0,622 [95% *confidence interval* – CI: 1,573–2,203]; 57,6%, 64,3%, 0,654, OR: 0,618 (95%CI: 1,501–2,291%); 60,1%, 75,4%, 0,744, OR: 0,790 (95%CI: 1,826–2,659), redom. Svako povećanje jedinice u skoru DECAF+Laktat povećavalo je rizik od smrtnosti za 2,203. **Zaključak.** Kao rezultat naše studije, verujemo da je skor DECAF+Laktat efikasniji sistem bodovanja od skora DECAF, kao prediktor mortaliteta kod kritično obolelih osoba sa pogoršanjem HOBP.

Ključne reči:
kritična stanja; laktati; mortalitet; prognoza; pluća, opstruktivne bolesti, hronične; osetljivost i specifičnost; bolest, indeks težine.

Introduction

Chronic obstructive pulmonary disease (COPD) is a treatable and preventable respiratory disease characterized by airflow limitation and respiratory symptoms due to permanent damage to the airway and alveoli, which may also be caused by exposure to harmful particles and/or gases and lung development abnormalities [1]. COPD is a chronic process that progresses with exacerbations. An exacerbation is an acute increase or worsening of the symptoms of the disease [2]. Although COPD exacerbation is a generalization by definition, COPD and COPD exacerbation is a condition that differs according to time and patient and has different treatment options.

Various studies are carried out to predict mortality and morbidity and to regulate treatment options for this disease, which has high mortality and morbidity and is increasing day by day. Studies are trying to find easier and cheaper methods. In these studies, researchers are especially trying to find meaningful and diagnostic tests, among the routine ones, for monitoring and treating the disease in the emergency department.

Lactate from laboratory tests is one of the most used parameters for mortality and morbidity [3]. It is also used in critical patients, sepsis, septic shock patients, hemorrhagic shock due to trauma or various reasons, evaluation of the patient, prediction of mortality, and evaluation of response to treatment [4]. In the patient follow-ups, lactate, which tends to increase, should be remarkable. For clinicians, lactate levels of 4 and above are associated with high-risk mortality [5].

In all studies, it was aimed to predict the clinical course and mortality with the blood values performed on the patients. Mortality is divided into short-term and long-term mortality according to the time of occurrence. DECAF (Dyspnea, Eosinopenia, Consolidation, Acidemia, atrial Fibrillation) score is a prognostic tool for routine estimation of mortality and morbidity in COPD [6]. DECAF score provides superiority in clinical decision and prognostic performance [7].

The aim of this study was to compare the DECAF score and DECAF+Lactate score and examine the effectiveness of the DECAF+Lactate score in predicting the mortality of critically ill patients with COPD exacerbation.

Methods

Study design and data collection

The Ethics Committee of the Istanbul Kanuni Sultan Suleyman Research and Training Hospital approved the study (Decision No. KAEK/2020.07.154). The study was performed according to the recommendations set by the Declaration of Helsinki on Medical Research involving Human Subjects and Good Clinical Practice guidelines. The study involved recruited patients from August 1, 2020, to December 31, 2021. Informed consent was obtained from all patients included in the study. Data and materials are reachable from hospital automation information systems.

As a criterion for inclusion in the study, the qSOFA score of the patients at the time of admission was accepted as 2 or above. Patients with worsening treatment for an existing infection, known malignant disease, renal failure, hematological or rheumatological disease, or additional diagnoses that could change the lactate value were excluded from the study.

A total of 502 patients with acute exacerbation of COPD who met the definition of critically ill (qSOFA $\geq$ 2), 228 from one and 274 from the other center, were included in the study. Eighteen of 502 patients were excluded from the study because they did not want treatment for various reasons, and their relatives left the emergency department to apply to another center. Twenty-eight of them could not be diagnosed with COPD from e-pulse (personal health record system in our country) or their past records, and 21 of them could not be found to have a mortality status. A total of 435 patients who met the inclusion criteria were included in the study (Figure 1).

Gender, background (chronic diseases), drug use, clinical follow-up, and 28-day mortality information of the patients were recorded.

On the first admission, fever, pulse, systolic and diastolic pressures, respiratory rates, Glasgow Coma Scale (GCS) values, and fingertip oxygen saturation (SpO_2) measurements of the patients taken before treatment were recorded. Furthermore, eosinophil values from hemogram parameters obtained from patients on the first admission and lactate and pH values obtained from arterial blood gas were recorded.

Score calculations

When calculating the DECAF score of the patients, the presence of dyspnea, eosinopenia, consolidation, acidosis, and atrial fibrillation (AF) was scored. Patients with a respiratory rate of 30 and above were included in the 5b group in the expanded modified Medical Research Council scale, and those below 30 were included in the 5a group. For eosinophil count, values < 0.05×10^9/L were accepted as eosinopenia.

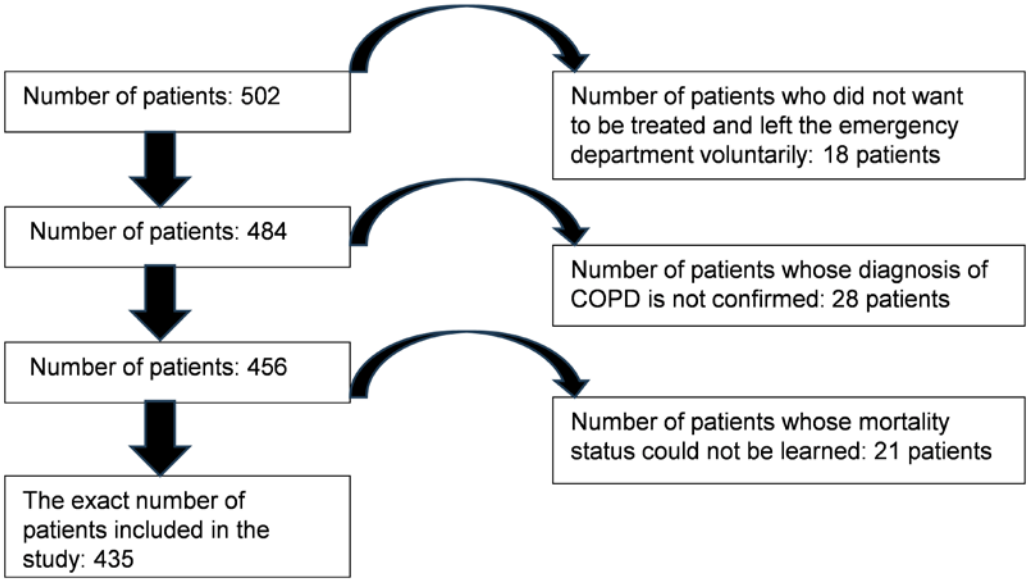

Fig. 1 – Flow chart of patients.

The performed posterior-anterior chest radiographs were evaluated by emergency medicine specialists for consolidation. A pH value below 7.3 was considered significant for acidosis. Electrocardiograms (ECGs) taken for the detection of AF were evaluated by emergency medicine specialists. DECAF score was calculated by adding one point each for 5a dyspnea, eosinopenia, consolidation, acidosis, and AF detection, and two points for the 5b dyspnea group. Patients with a DECAF score between 0–1 were included in the mild risk group, those with a score of 2 were included in the intermediate risk group, and patients with a score of 3 and above were included in the high-risk patient group for mortality. Lactate values for the DECAF+Lactate score, which we planned in order to have a more effective scoring than the DECAF score in our study, were calculated by adding 0 points for the score 0–2, 1 point for 2–4, and 2 points for > 4 to the DECAF scores.

Statistical analysis

The obtained data were analyzed in the SPSS Statistics 26.0 (IBM Inc., New York, USA) program. The Kolmogorov-Smirnov test was used to test the normality of the distribution of data. Categorical data are displayed as numbers and percentages. Continuous variables that comply with normal distribution are shown as mean ± standard deviation, and continuous variables that do not comply with normal distribution are shown as median (interquartile range). Pearson's Chi-square test was used to compare categorical data. Continuous variables were subjected to pairwise group analysis using the Mann-Whitney *U* test and independent sample *t*-test. Logistic regression analysis and receiver operating characteristic (ROC) curve analyses were performed to determine the effect of independent variables, found to be significant between groups, on mortality. The value of $p < 0.05$ was considered statistically significant.

Results

A total of 435 patients, 261 (60%) men and 174 (40%) women, whose vital signs met the criteria of critically ill patients [quick Sequential Organ Failure Assessment (qSOFA) ≥ 2] with COPD exacerbation and who applied to the emergency medicine clinic of the two tertiary care training and research hospitals, were included in our study. When the known chronic comorbidities of the patients were examined, we found that the most common diseases were hypertension in 220 (50.6%) patients, diabetes mellitus in 120 (27.6%) patients, and congestive heart failure in 82 (18.9%) patients.

When the regular drug use of the patients was questioned, we found that 388 (89.2%) patients used it for their chronic diseases, and 47 (10.8%) did not use it at all. During the follow-up, we found that 37 (8.5%) patients died in the emergency department, 268 (61.6%) were admitted to the intensive care unit, 28 (6.4%) were discharged, and 38 (8.7%) left the hospital for different reasons. When the 28-day mortality status of the patients was examined, we found that 158 (36.3%) patients died and 277 (63.7%) survived. In the analysis of patients' DECAF parameters, we found dyspnea in 116 patients, eosinopenia in 115, consolidation in 301, acidosis in 398, and AF in 120 patients (Table 1).

When we calculate the DECAF scores of the patients, 3 (0.7%) patients were in the mild-risk group, 78 (17.9%) were in the medium-risk group, and 354 (81.4%) were in the high-risk group.

When we grouped the patients according to mortality, we found a significant relationship between these groups and the DECAF risk group and its subgroups ($p < 0.001$). Again, we found a statistically significant relationship between mortality and dyspnea, eosinopenia, consolidation, and acidosis ($p < 0.001$, $p < 0.001$, $p < 0.001$, $p = 0.021$, respectively). No significant correlation was found between the presence of AF and mortality in the patients ($p = 0.325$) (Table 1).

When we examined the vital parameters and scores of the patients, we found that in the "survivors" group, heart rate, respiratory rate, lactate, DECAF, and DECAF+Lactate scores were statistically significantly higher than in the "non-survivors" group ($p < 0.001$). Again, we found statistically significantly lower systolic and diastolic blood pressures, SpO_2, GCS, and eosinophil values in the group with mortality compared to the group without mortality ($p < 0.001$). When we compared the high-risk group with 3 or more points according to the DECAF score and other patients, we found that body temperature, heart rate, respiratory rate, lactate, and DECAF+Lactate values in the high-risk group were statistically significantly higher compared to the group with lower DECAF score ($p = 0.01$, $p = 0.001$, $p < 0.001$, $p = 0.016$, $p < 0.001$, respectively). We found that the systolic and diastolic blood pressures, SpO_2, GCS, and eosinophil counts of the patients in the high-risk group were statistically significantly lower than in the other group ($p < 0.001$) (Table 2).

ROC curve analysis was performed for independent variables with statistical significance for the presence of mortality. Cut-off, sensitivity, and specificity values of variables with the statistically significant area under the curve (AUC) were calculated (Tables 3 and 4). It was observed that the AUC of the DECAF+Lactate score was higher than DECAF and lactate, and the odds ratio (OR) was higher. It was determined that each unit increase in the DECAF+Lactate score increased the mortality risk by 2.203 (OR: 2.203; 95% CI: 1.826–2.659).

Table 1

Characteristics of the study participants

Parameter	Survivors (n = 277)	Non-Survivors (n = 158)	*p*-value
Gender			
male	165 (37.9)	96 (22.1)	0.807
female	112 (25.7)	62 (14.3)	
Diabetes melllitus	69 (15.9)	51 (11.7)	0.098
Hypertensio arterialis	135 (31)	85 (19.5)	0.310
Coronary artery disease	67 (15.4)	50 (11.5)	0.092
Congestive heart failure	40 (9.2)	42 (9.7)	**0.002**
Acute renal failure	7 (1.6)	4 (0.9)	0.613*
Chronic renal failure	26 (6)	20 (4.6)	0.286
DECAF score			
0–2	67 (15.4)	14 (3.2)	**< 0.001**
≥ 3	210 (48.3)	144 (33.1)	
Dyspnea (respiratory rate), ≥ 30/min	56 (12.9)	60 (13.8)	**< 0.001**
Eosinopenia (eosinophils < 0.05×10^9/L)	55 (12.6)	60 (13.8)	**< 0.001**
Consolidation	178 (40.9)	123 (28.3)	**0.003**
Acidosis (pH < 7.3)	247 (56.8)	151 (34.7)	**0.021**
AF (including history of AF)	72 (16.6)	48 (11)	0.325

DECAF – Dyspnea, Eosinopenia, Consolidation, Acidemia, atrial Fibrillation; AF – atrial fibrillation. All values are given as numbers (percentages). Pearson Chi-square test; *Fisher's exact test.

Table 2

Analysis of vital signs, DECAF, and DECAF+Lactate scores according to the presence of mortality

Parameters	Survivors (n = 277)	Non-survivors (n = 158)	*p*-value	[1]DECAF score 0–2 (n = 81)	[2]DECAF score ≥ 3 (n = 354)	*p*-value
Body temperature (°C)	36.70 [0.60]	36.70 [0.80]	0.636	36.70 [0.45]	36.80 [0.80]	**0.010**
Heart rate (*per* minute)	87 [20]	96 [24.25]	**< 0.001**	84 [17]	90 [22]	**0.001**
Systolic blood pressure (mmHg)	125.36 ± 27.01	110.37 ± 28.04	**< 0.001**	130.20 ± 22.28	117.56 ± 29.02	**< 0.001**
Diastolic blood pressure (mmHg)	80.45 ± 14.75	73.68 ± 17.83	**< 0.001**	82.44 ± 13.27	76.98 ± 16.70	**0.002**
SpO_2 (%)	88 [9]	80 [15]	**< 0.001**	90 [8]	84 [12.25]	**< 0.001**
Respiration rate (*per* min)	27 [4]	28 [6]	**< 0.001**	26 [3]	28 [4]	**< 0.001**
Glasgow Coma Scale	13 [1]	13 [1]	**< 0.001**	14 [1]	13 [2]	**0.001**
Eosinophil count (10^3 µL)	0.71 ± 0.63	0.37 ± 0.47	**< 0.001**	0.81 ± 0.68	0.53 ± 0.57	**0.001**
Lactate (mmol/L)	1.84 [1.01]	2.98 [3.13]	**< 0.001**	1.84 [1.05]	2.14 [1.65]	**0.016**
DECAF Score	3.19 ± 0.93	3.80 ± 1.05	**< 0.001**	1.96 ± 0.19	3.75 ± 0.82	**< 0.001**
DECAF+Lactate score	3.66 ± 1.10	4.87 ± 1.39	**< 0.001**	2.46 ± 0.65	4.47 ± 1.17	**< 0.001**

Data that were not normally distributed were shown as median [interquartile range] and the Mann-Whitney *U* test was used. Normally distributed data are given as mean ± standard deviation and an Independent sample *t*-test was used.

SpO_2 – oxygen saturation. For other abbreviations, see Table 1.

[1] – mild/intermediate risk group of patients; [2] – high-risk patient group.

Table 3

ROC curve analysis results of scorings and lactate values

Test variables (cut-off)	AUC	Sensitivity %	Specificity %	NPD %	PPD %	*p*-value	Accuracy %
DECAF (≥ 3.5)	0.654	57.59	64.26	72.65	47.89	< **0.001**	61.84
Lactate (≥ 3.07 mmol/L)	0.711	50.00	90.25	75.99	74.53	< **0.001**	75.63
DECAF+Lactate (≥ 4.5)	0.744	60.13	75.45	76.84	58.28	< **0.001**	69.89

ROC – receiver operating characteristic; AUC – area under the ROC curve; NPD – negative predictive value; PPD – positive predictive value

Table 4

Logistic regression analysis results of scorings and lactate values

Test variables (cut-off)	β	*p*-value	OR	95% CI	
				lower bound	upper bound
DECAF	0.618	< **0.001**	1.855	1.501	2.291
Lactate	0.622	< **0.001**	1.862	1.573	2.203
DECAF+Lactate	0.790	< **0.001**	2.203	1.826	2.659

OR – odds ratio; CI – confidence interval.

Discussion

Lactate is one of the parameters used in the diagnosis and treatment effectiveness of critically ill patients. In our study, the effectiveness of lactate in demonstrating mortality in patients presenting with COPD exacerbation was discussed. DECAF+Lactate scoring is formed by adding lactate to the DECAF scoring, which is used to predict mortality in critically ill patients presenting with COPD exacerbation. The effectiveness of the DECAF+Lactate scoring in predicting mortality was investigated. DECAF+Lactate score was found to be more effective in predicting mortality.

In a study in which mortality scoring of 2,645 COPD patients was performed, it was found that 47% of the patients could not leave their homes without assistance, 29.8% had consolidation, and 17.9% had acidosis [6]. In a study examining patients diagnosed with COPD exacerbation, the prevalence of arrhythmia was reported as 97%. In the study where 24-hour Holter ECG monitoring results were recorded, the most common arrhythmias were ventricular premature beats, while permanent AF was detected in 30.3% of patients [8]. We think the presence of consolidation and acidosis in our study differs from the literature because we included only critical patients in the study rather than all COPD exacerbations.

It has been observed that the DECAF score can be scored on admission and can accurately predict the risk of death [6]. In a cohort study, the DECAF score was found to be a better predictor of mortality than the CURB-65 score [9]. In COPD exacerbations with pneumonia, DECAF was also reported as a stronger score for predicting hospital mortality [10]. In a study examining COPD exacerbation, DECAF and CURB-65 were found to have similar sensitivity, but DECAF was shown to be more specific for mortality than CURB-65 [11]. In our study, the DECAF score was significantly higher in the mortality group, and the fact that we used it instead of CURB-65 also receives support from the literature.

When we grouped the patients according to the presence of mortality, it was found that the heart rate, respiratory rate, lactate, DECAF, and DECAF+Lactate scores were statistically significantly higher in the group with mortality compared to the group without mortality. Again, systolic and diastolic blood pressures, SpO_2, GCS, and eosinophil values were found to be statistically significantly lower in the group with mortality compared to the group without mortality. In a systematic review, it was reported that the target systolic blood pressure was 70 mmHg and above in critically ill patients, and hypotension lasting longer than 24 hrs was found to be associated with increased mortality [12]. It is more clearly mentioned in the literature that hypotension causes an increase in myocardial damage, acute kidney injury, and death rates in critically ill and septic shock patients [13]. In a study conducted with 736 intensive care patients, intermittent systolic, diastolic, and mean blood pressures were measured. In critically ill hypotensive adult patients, the mean blood pressure of 65 mmHg and below has been shown to be excessive [14].

It has been reported that the risk of cardiovascular events is higher in patients diagnosed with COPD compared to the other population. That being said, approximately 30% of patients with COPD die for these reasons, and the risk of myocardial infarction in these patients increases in the days following acute exacerbation [15]. It has been observed that increased oxidative stress and inflammation during exacerbation may lead to an increased incidence of cardiovascular events. Additionally, DM, which is frequently seen with COPD, has been associated with high mortality in hospitalizations [16]. We think that the comorbidities present in COPD patients are the main reason for the high mortality of the disease.

When we compare the high-risk group with 3 or more points according to the DECAF score and the other patients, body temperature, heart rate, respiratory rate, and lactate values in the high-risk group were found to be statistically significantly higher when compared to the group with a low-

er DECAF score. The systolic and diastolic blood pressures, SpO_2, GCS, and eosinophil counts of the patients in the high-risk group were found to be statistically significantly lower than the other group. DECAF is a risk stratification tool designed to estimate the risk of death in COPD patients in acute exacerbation [10]. The fact that the DECAF score was designed for mortality estimation explains these statistical differences. In our study, a significant correlation was found between mortality and DECAF score.

In a meta-analysis, sensitivity, specificity, and AUC of the DECAF score for 30-day mortality were calculated and reported to be 71%, 75%, and 0.79, respectively [7]. In our study, the sensitivity and specificity values of the DECAF score for the estimation of mortality were 57.6% and 64.3%, respectively, and AUC was 0.65. In our study with critically ill patients, we think the difference in results depends on our patient group.

ORs were determined by performing ROC curve analysis for the independent variables and regression analysis for mortality for the independent variables that we found statistically significant for the presence of mortality. It was determined that the DECAF+Lactate score, which we designed as a predictor of mortality in COPD exacerbation patients, had higher AUC and ORs than the DECAF score and lactate. It was found that each unit increase in the DECAF+Lactate score increased the mortality risk by 2.203.

The limitations of our study include the evaluation of the direct radiographs of the patients by emergency medicine specialists, the lack of standardization, and the inability to perform Cox regression analysis due to the lack of daily mortality follow-up of the patients. The fact that the number of patient applications due to the COVID-19 pandemic continued to rise during the period when the study was conducted can also be considered a limitation. It should also be acknowledged that more comprehensive studies are needed to add a new scoring system to the literature.

Conclusion

Each unit increase in the DECAF+Lactate score increases the risk of mortality by 2.203. As a result of our study, we believe that the DECAF+Lactate score is a more effective scoring system than the DECAF score as a predictor of mortality in critically ill patients with COPD exacerbation.

Conflict of interest

The authors of this paper have no conflict of interest, including specific financial interests, relationships, and/or affiliations relevant to the subject matter or materials included.

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Received on September 25, 2023
Revised on January 10, 2024
Revised on January 28, 2024
Accepted on February 6, 2024
Online First March 2024

ORIGINAL ARTICLE

UDC: 616.133:616-004.6
DOI: https://doi.org/10.2298/VSP221028014K

The expression of renin-angiotensin system components in human carotid plaque

Ekspresija komponenti renin-angiotenzin sistema u humanom karotidnom plaku

Ana Kolaković*, Maja Bundalo*, Tamara Djurić*, Igor Končar†‡, Aleksandra Stanković*, Maja Živković*

University of Belgrade, *Institute of Nuclear Sciences "Vinča", Department of Health and Environmental Research, Laboratory for Radiobiology and Molecular Genetics, ‡Faculty of Medicine, Belgrade, Serbia; †University Clinical Center of Serbia, Clinic for Vascular and Endovascular Surgery, Belgrade, Serbia

Abstract

Background/Aim. The renin-angiotensin system (RAS) is linked to the development of atherosclerosis (As), including its initiation and progression. Besides the well-known angiotensin-converting enzyme (ACE), two newer RAS family members are related to vascular remodeling – ACE2 as a homolog of ACE and collectrin [transmembrane protein 27 (TMEM27)] as a homolog of ACE2. Up to now, a limited number of studies have examined the expression of these RAS components in advanced carotid plaque (CP) tissue based on the sex of the patients and plaque phenotypes (PPs). There are two ultrasonographically defined PPs – the hypoechogenic plaque (HoP) and the hyperechogenic plaque (HerP) phenotype. The aim of the study was to investigate whether there was a correlation between the expression of RAS components in the CP and the sex and PPs of patients. **Methods.** We examined 74 patients with advanced CP who underwent carotid endarterectomy. The intraplaque expression of RAS components was determined with the real-time polymerase chain reaction, using the TaqMan® gene expression assays and Western blot. A two-way ANOVA followed by a *post-hoc* Tukey test was performed for the statistical analysis of results. **Results.** No interaction was recorded between the sex of the patients and PPs in influencing the relative expression of ACE and TMEM27 messenger RNA (mRNA) ($p > 0.05$). In 56.06% of plaque samples, no expression of ACE2 mRNA was detected. Among the plaques where ACE2 mRNA expression was detected, its expression level was higher in females with the HoP phenotype compared to females with the HerP phenotype ($p < 0.001$). In patients with the HoP phenotype, females had higher expression of ACE2 mRNA than males ($p < 0.05$). In the male study group, ACE protein levels were significantly lower in the HoP phenotype compared to the HerP phenotype ($p < 0.001$). Females with the HoP phenotype had significantly higher ACE protein levels than males with the HoP phenotype ($p < 0.0001$). **Conclusion.** Our results revealed alterations in the expression levels of ACE and ACE2, at the mRNA and protein levels, in advanced carotid As. These alterations are impacted by sex and PP and may indicate a switch from the balanced RAS/ACE/ACE2 axis in the healthy blood vessel to the unbalanced axis in vascular remodeling due to As.

Key words:
carotid artery diseases; gene expression; plaque, atherosclerotic; proteins; renin angiotensin system; rna, messenger.

Apstrakt

Uvod/Cilj. Renin-angiotenzin sistem (RAS) povezan je sa razvojem ateroskleroze (As), uključujući njen nastanak i progresiju. Pored dobro poznatog angiotenzin-konvertujućeg enzima (*angiotensin-converting enzyme* – ACE), dva nova člana RAS familije povezana su sa remodelovanjem zidova krvnih sudova – ACE2 kao homolog ACE i kolektrin [*transmembrane protein* 27 (TMEM27)] kao homolog ACE2. Do sada je mali broj studija ispitivao ekspresiju komponenti RAS sistema u tkivu uznapredovalog karotidnog plaka (KP) u odnosu na pol bolesnika i fenotip plaka (FP). Postoje dva tipa KP definisana primenom ultrazvuka – fenotip hipoehogenog plaka (HoP) i fenotip hiperehogenog plaka (HerP). Cilj rada bio je da se ispita da li postoji korelacija između ekspresije komponenti RAS u KP i pola i FP bolesnika. **Metode.** Ispitano je 74 bolesnika sa uznapredovalim KP koji su bili podvrgnuti operativnoj proceduri karotidne endarterektomije. Ekspresija komponenti RAS sistema u tkivu plaka utvrđena je lančanom reakcijom polimeraze u realnom vremenu (*real-time polymerase chain reaction*) primenom eseja TaqMan® tehnologije i metode *Western blot*-a. Dvosmerna analiza varijanse i Tukey *post-hoc* test korišćeni

Correspondence to: Ana Kolaković, University of Belgrade, Vinča, Institute of Nuclear Sciences – National Institute of the Republic of Serbia, Department of Health and Environmental Research, Laboratory for Radiobiology and Molecular Genetics, Mike Petrovića Alasa 12–14, 11 351 Belgrade, Serbia. E-mail: anakolakovic@vin.bg.ac.rs

su za statističku obradu rezultata. **Rezultati.** Nije utvrđena interakcija FP i pola bolesnika u uticaju na relativnu ekspresiju informacione RNK (iRNK) za ACE i TMEM27 ($p > 0,05$). U 56,06% uzoraka plaka nije detektovana ekspresija iRNK za ACE2. U plakovima u kojima je detektovana ekspresija iRNK za ACE2, njen nivo bio je viši kod žena sa HoP u poređenju sa ženama sa HerP ($p < 0,001$). U grupi bolesnika sa fenotipom HoP, žene su imale viši nivo iRNK za ACE2 nego muškarci ($p < 0,05$). U grupi muškaraca, nivoi ekspresije ACE proteina bili su značajno niži u fenotipu HoP u poređenju sa HerP ($p < 0,001$). Žene sa fenotipom HoP imale su značajno viši nivo ACE proteina u poređenju sa muškarcima sa HoP ($p < 0,0001$). **Zaključak.** Naši rezultati pokazali su da postoje promene nivoa ekspresije ACE i ACE2, na nivou proteina i iRNK u uznapredovaloj karotidnoj As. Te promene zavise od pola i FP i mogu ukazivati na to da balans ose RAS/ACE/ACE2 koji postoji u zdravom krvnom sudu postaje poremećen tokom remodelovanja zida krvnog suda usled As.

Ključne reči:
aa.carotis, bolesti; geni, ekspresija; aterosklerotički plak; proteini; renin-angiotenzin sistem; rnk, informaciona.

Introduction

Atherosclerosis (As) is a chronic inflammatory disease of the arterial wall characterized by the development of atherosclerotic lesions (plaques) that can interrupt the blood flow in vessels but also be vulnerable to rupture [1]. The characterization of carotid plaque (CP) morphology by non-invasive ultrasound (US) may provide insight into the composition of the plaque and its structure [2]. Dominantly echolucent, hypoechogenic plaques (HoPs) of the carotid artery (CA), which are lipid-rich with increased macrophage density [3], may confer a higher risk for clinical complications (ischemic events) compared to dominantly echogenic plaques (hyperechogenic plaques – HerP) of the CA which are rich in fibrous tissue and sometimes calcification [4]. Recently, it has been shown that plaques stratified by their echogenicity, as determined by the US, have molecular signatures attributed to iron homeostasis, calcification, the balance of cell survival, and lipid transdifferentiation of the smooth muscle cell (SMC) [5].

The renin-angiotensin system (RAS) has a significant role in the regulation of numerous physiological functions, including blood pressure (BP), electrolyte balance, inflammation, oxidative stress, fibrosis, and cell proliferation. Therefore, RAS is integrally associated with the progression of As, remodeling of blood vessel walls, and plaque stability [6]. The RAS expression in various tissues highlights the importance of this system in tissues in which angiotensin (Ang) II, as the key active peptide, could be generated locally [7]. The discovery of Ang-converting enzyme (ACE) 2, a homolog of ACE [8, 9], demonstrated that the RAS functions through two distinct routes with opposing effects: the traditional ACE/Ang II/Ang III type1 receptor (AT1R) pathway and the novel ACE2/Ang(1-7)/mitochondrial assembly (Mas) receptor 1 (MasR1) axis pathway. ACE2 is known as an endogenous antagonistic regulator of the RAS, which inhibits detrimental Ang II signaling. It reduces BP by hydrolyzing Ang II into the vasodilator Ang(1-7) and has anti-inflammatory effects on the cardiovascular system [10].

Deletion of ACE2 in As-prone mice and apoE-deficient (ApoEKO) mice promotes upregulation of pro-inflammatory mediators of As: monocyte chemoattractant protein (MCP)-1, tumor necrosis factor (TNF)-α, interleukin (IL)-6, vascular cell adhesion molecule (VCAM)-1, matrix metalloproteinase (MMP)-9 and MMP-2 in aorta/artery tissues [11, 12]. On the other hand, overexpression of ACE2 in other animal models of As either decreases the level of Ang II, ACE, and AT1R protein in atherosclerotic lesions [13] or prevents early atherosclerotic lesion formation [14] and increases plaque stability [15].

ACE is expressed in multiple tissues [16], various cells in blood vessels, and human atheroma [17], whereas its homolog, ACE2, is expressed in endothelium, macrophage foam cells, and vascular SMC [18]. The development of As is delayed by ACE2 activation [18], and adverse vascular remodeling is prevented [19].

Collectrin [also known as transmembrane protein 27 (TMEM27)], a homolog of ACE2, is another new molecule that belongs to the RAS family that has a role in endothelial dysfunction, as shown in collectrin mice [20]. TMEM27 regulates arterial BP [20] and is expressed in the vascular endothelium and kidney [21].

Previously, the variation in the expression of some RAS family members, e.g., the MasR1, in human CP tissue was related to different PPs [22]. Moreover, treatment with the oral formulation of Ang(1-7) enhances a more stable phenotype in atherosclerotic CP [22], which is important as it is known that ACE2 is the main enzyme in Ang(1-7) synthesis. ACE2 messenger RNA (mRNA) is expressed in both early and advanced human carotid atherosclerotic lesions in another study, suggesting no significant differences according to the progression of As [18]. However, the ACE2 expression was not detected in some studies [17, 22], and thus, it requires further research. ACE expression was localized in atherosclerotic CA [23, 24], and other atheromas [17] but not related to CA phenotype [22]. The TMEM27 was detected previously in carotid tissue in our preliminary study [24].

Sex and gender play an important role in cardiovascular research when addressing disease prevalence, risk factors, diagnostic evaluation, and overall health outcomes [25]. Recently, sex differences in atherosclerotic PPs have been described (lipid-rich plaques in males, fibrous plaques in females) [26]. RAS activity is also modulated by androgens and estrogens and reveals sex-specific cardiovascular pathologies [27, 28]. Sex differences in ACE activation have been suggested [28, 29]. Likewise, sex differences in ACE and ACE2 modulation of Ang(1-7) levels in males and females have been suggested [30, 31]. This implies that men and women should be studied separately by sex in cardiovascular re-

search, especially in research on ACE and ACE2 expression in CP.

To our knowledge, there are no studies that investigated the expression of RAS components in advanced CP tissue depending on sex. Therefore, the goal of this study was to compare the expression of RAS family members (ACE, ACE2, and TMEM27) in advanced CP tissue regarding sex and different PPs (HerP/HoP).

Methods

Carotid atherosclerotic tissue specimens and ultrasound evaluation of the carotid artery

In the current investigation, we tested CP tissue specimens (n = 200) consecutively collected from patients who underwent carotid endarterectomy (CEA). The US evaluation of the CAs included the bifurcations as well as the common CA and internal CA. The North American Symptomatic Carotid Endarterectomy Trial method was used to measure the degree of stenosis, which was greater than 70%, and define the presence of plaque in the CAs [32]. According to Gray-Weale et al. [33], we have classified advanced CP tissue as HerP (dominantly echogenic) and HoP (dominantly echolucent) phenotypes. Patients' tissue samples were collected two weeks after the US examination. Tissue samples were immediately frozen in liquid nitrogen after being obtained from the patient and were kept at -80 °C. In this study, 74 tissues from patients not receiving ACE inhibitors or Ang receptor blockers antihypertensive treatment underwent further processing for RNA and protein extraction.

The Ethics Committee of the University Clinical Center of Serbia evaluated and approved the research protocol (No. 136/8, from July 21, 2016). Participants who took part in the study gave their informed consent.

Measurement of biochemical parameters

After being admitted for the planned CEA, biochemical analyses were performed *via* the hospital's routine laboratory protocol for all patients, as previously described [34].

Reverse transcription and quantitative real-time PCR

As previously reported, RNA was extracted, its amount and structural integrity were assessed, and reverse transcription was carried out [28]. After RNA quality assessment, only specimens with high RNA quality (n = 66) were converted to complementary DNA (cDNA).

For detection and quantification of ACE, ACE2, and TMEM27, TaqMan®Gene Expression assays were utilized, as follows: Hs00174179_m1, Hs00222343_m, and Hs00252907_m1, respectively (Applied Biosystems, Foster City, CA). The relative mRNA levels of ACE, ACE2, and TMEM27 were normalized to 18s rRNA (Hs99999901 s1), which was used as an endogenous control. All reactions were performed on a Real-time 7500 system according to recommended protocols (ABI, Foster City, CA).

Extraction of proteins and Western blot

We employed the techniques previously described in detail [28] to prepare tissue lysates from carotid tissue specimens (n = 20), determine the protein concentration, and prepare samples for Western blot. Primary antibodies (sc-20791 and sc-20998, Santa Cruz Biotechnology, USA, dilution 1:200) were incubated with polyvinylidene difluoride membranes to detect the presence of ACE and ACE2 proteins, respectively. Membranes were then washed and incubated with an appropriate secondary anti-rabbit antibody (dilution 1:10,000). The membranes were stained with Ponceau S (Sigma-Aldrich P3504) as a loading control for the Western blot. This reversible Ponceau S staining has been proven as the adequate methodology for assessing equal gel loading in Western blot [35]. Using ImageJ software (NIH, Bethesda, USA), signals produced by enhanced chemiluminescence reagents on films and signals obtained on membranes were scanned, and protein levels were quantified afterward.

Statistical analysis

Statistica Version 8.0 software (Stat Soft Inc., Tulsa, Oklahoma) and GraphPadPrism Version 6.0 software (GraphPad Software Inc., San Diego, CA), were used for statistical analysis. Unless otherwise noted, the results for continuous variables were reported as means with standard error of the mean. For all continuous variables, the Kolmogorov-Smirnov test was used to check for a normal distribution. Unpaired Student's *t*-test was used to compare mean values for continuous variables with normal distribution, while the Mann-Whitney *U* test was used for variables with skewed mean value distribution. The Chi-square test was applied for categorical variables. To ascertain whether the sex and PPs, as well as their interactions, alter the expression of RAS components, we performed a two-way ANOVA for multiple comparisons followed by the Tuckey *post-hoc* test. Differences with a two-tailed alpha-probability *p*-value of 0.05 or less were considered statistically significant. Using $2^{-\Delta CT}$ [36], the relative gene expression levels of the ACE, ACE2, and TMEM27 genes were calculated and normalized to 18s rRNA.

Results

Patients with carotid atherosclerosis

Table 1 shows the demographic, biochemical, and clinical parameters with regard to the classification of US-defined PPs (HerP vs. HoP). Biochemical parameters in the blood that were measured, including inflammatory and hemostasis mediators (IL-6, C-reactive protein, plasminogen, fibrinogen, plasminogen activator inhibitor-1, factor VII, antithrombin III, von Willebrand factor, and protein C), did not differ between the two different kinds of PPs. Age, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, triglycerides, total cholesterol levels, lipoprotein(a), white blood cell counts, red blood cell counts, and platelet counts were similar between the two groups with different PPs.

Table 1

Demographic, biochemical, and clinical parameters of patients regarding ultrasonographically defined plaque phenotypes

Parameters	Plaque phenotype		*p*-value
	HerP (n = 49)	HoP (n = 17)	
Age (years)	63.93 ± 8.84	67.82 ± 7.38	0.11▪
Sex			
male	32 (65.31)	14 (82.35)	0.22#
female	17 (34.69)	3 (17.65)	
Total cholesterol (mmol/L)	5.63 ± 1.09	5.79 ± 1.54	0.76▪
LDLC (mmol/L)	3.47 ± 0.86	3.62 ± 1.13	0.71▪
HDLC (mmol/L)	1.26 ± 0.27	1.17 ± 0.29	0.43▪
Triglycerides (mmol/L)	1.97 ± 0.99	2.18 ± 0.87	0.91*
Lipoprotein(a) (mg/dL)	35.16 ± 44.63	31.26 ± 47.55	0.88*
Hypertension	43 (87.76)	17 (100.00)	0.17#
Treatment with statins	10 (20.41)	4 (23.53)	0.88#
Antiplatelet therapy	49 (100)	17 (100)	0.99 #
Smoking	43 (87.76)	16 (94.12)	0.58#
Cerebrovascular insult	9 (18.37)	0 (0.00)	0.05#
Transient ischemic attack	7 (14.29)	4 (23.53)	0.40#
Coronary comorbidity	18 (36.73)	9 (52.94)	0.27#
Symptomatic[¤]	19 (38.78)	8 (47.06)	0.59#
Diabetes mellitus	10 (20.41)	5 (29.41)	0.47 #
PAOD	13 (26.53)	4 (23.53)	0.77 #
Glucose (mmol/L)	7.31 ± 3.20	6.43 ± 2.45	0.51*
ApoA-I (g/L)	1.81 ± 0.34	1.82 ± 0.38	0.99▪
ApoB (g/L)	1.14 ± 0.18	1.27 ± 0.34	0.82*
Factor VII (g/L)	115.42 ± 34.56	102.38 ± 24.62	0.37▪
Fibrinogen (g/L)	5.64 ± 1.83	5.84 ± 1.97	0.79▪
vWf (IU/dL)	171.50 ± 46.82	150.63 ± 35.73	0.30▪
Plazminogen (g/L)	138.88 ± 21.78	136.44 ± 19.72	0.78▪
D-dimer (mg/L)	0.19 ± 0.20	0.22 ± 0.17	0.73*
PAI-1 (U/mL)	4.26 ± 1.01	4.22 ± 1.38	0.94▪
Antithrombin III (g/L)	106.88 ± 13.16	103.89 ± 16.88	0.62▪
Protein C (g/L)	126.35 ± 34.58	118.78 ± 34.38	0.60▪
C-reactive protein (mg/L)	5.48 ± 4.55	4.53 ± 3.49	0.93*
Interleukin-6 (pg/mL)	6.07 ± 6.71	3.96 ± 3.43	0.53*
White blood cells ($\times 10^9$/L)	7.83 ± 1.50	8.97 ± 2.69	0.61*
Red blood cells ($\times 10^{12}$/L)	4.49 ± 0.35	4.78 ± 0.27	0.07*
Platelets ($\times 10^9$/L)	285.41 ± 78.37	250.11 ± 62.18	0.25▪
TAS (mmol/L)	1.60 ± 0.13	1.62 ± 0.13	0.72▪
Superoxide dismutase (IU/L)	1,397.78 ± 161.79	1,430.11 ± 150.30	0.64▪
Glutathione peroxidase (IU/L)	46.58 ± 16.27	64.93 ± 27.17	0.05▪
Glutathione reductase (IU/L)	57.61 ± 10.93	58.06 ± 10.21	0.92▪

HerP – hyperechogenic plaque; HoP – hypoechogenic plaque; LDLC – low-density lipoprotein cholesterol; HDLC – high-density lipoprotein cholesterol; PAOD – peripheral arterial occlusive disease; ApoA-I – apolipoprotein A-I; ApoB – apolipoprotein B; vWf – von Willebrand factor; PAI-1 – plasminogen activator inhibitor-1; TAS – total antioxidative status.

[¤] Patients who previously had symptoms of ipsilateral stroke or transient ischemic attack of the carotid artery territory.

▪ Student's *t*-test; # Pearson Chi-square test; *Mann-Whitney *U* test.

Results are presented as mean ± standard deviation or numbers (percentages).

We also analyzed mean values of the parameters of oxidative stress in plasma and did not find any difference in these parameters between HerP and HoP (Table 1).

ACE, ACE2, and TMEM27 gene expression in carotid plaque tissues

We examined the *ACE* gene expression in human CP tissue in relation to PPs [HerP phenotypes (n = 49) vs. HoP phenotypes (n = 17)] and sex [males (n = 46) vs. females (n = 20)]. The ACE mRNA level in CP tissue was not significantly different between different US-defined PPs (Figure 1). We also analyzed the gene expression of *ACE* in CP tissue with regard to sex and found no difference. Two-way ANOVA did not reveal a significant effect of sex and PP interaction on ACE mRNA expression ($p = 0.727$).

We detected the *ACE2* gene expression in 29 CP samples out of the 66 (43.94%) CP samples. Considering that in 56.06% of samples ACE2 mRNA was not detected, we checked if the distribution of sex and PPs was different

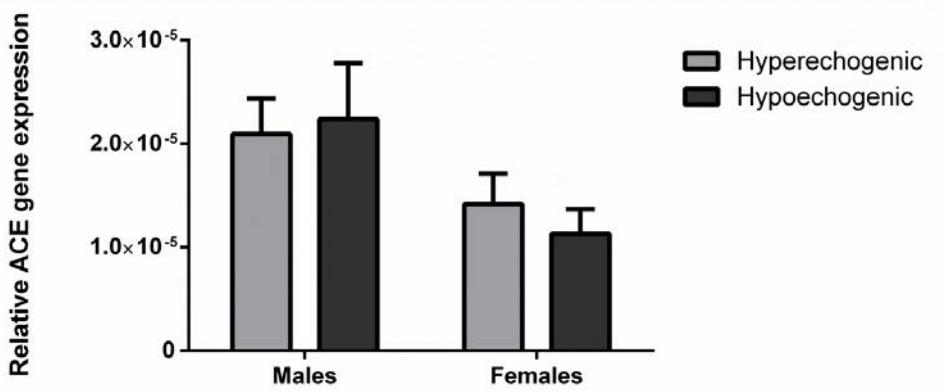

Fig. 1 – Relative angiotensin-converting enzyme *(ACE)* gene expression in carotid plaque (CP) tissue. **There was no significant difference in *ACE* gene expression between plaque phenotypes: males – HerP phenotypes (n = 32) vs. HoP phenotypes (n = 14), $p = 0.99$; females – HerP phenotypes (n = 17) vs. HoP phenotypes (n = 3), $p = 0.99$. The cDNA from CP tissue samples was used as a template in RT-qPCR for relative quantification of intraplaque mRNA expression of ACE. For each sample, the expression level of ACE mRNA was normalized to the housekeeping gene for 18S rRNA. The statistical significance was assessed by two-way ANOVA, followed by a *post-hoc* Tukey test. The results are expressed as $2^{-\Delta CT}$ values. Values are presented as mean ± standard error of the mean. For other abbreviations, see Table 1.**

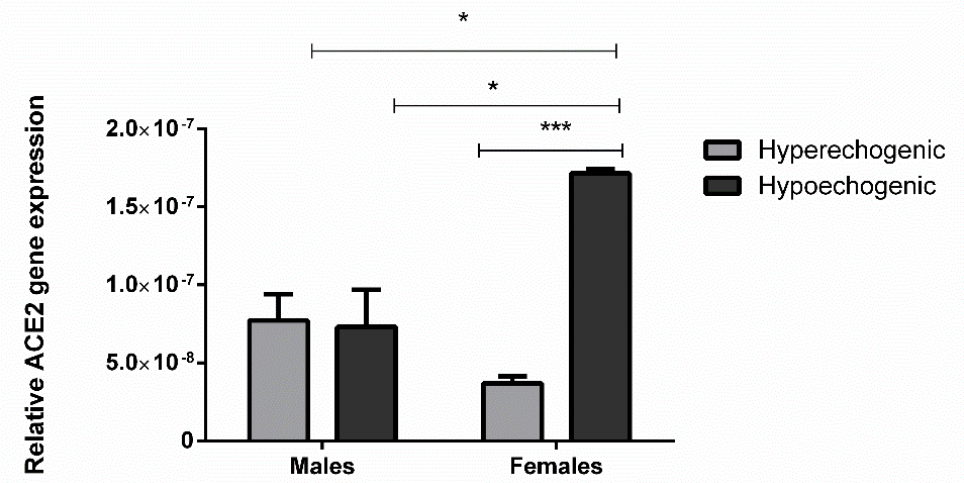

Fig. 2 – Relative angiotensin-converting enzyme *(ACE)2* gene expression in carotid plaque tissue. ***** significant difference between females with HoP phenotypes (n = 3) vs. females with HerP phenotypes (n = 9), $p < 0.001$; *significant difference between females with HoP phenotypes (n = 3) vs. males with HoP phenotypes (n = 11) and vs. males with HerP phenotypes (n = 6), $p < 0.05$. The cDNA from CP tissue samples was used as a template in RT-qPCR for relative quantification of intraplaque mRNA expression of ACE2. For each sample, the expression level of ACE2 mRNA was normalized to the housekeeping gene for 18S rRNA. The statistical significance was assessed by two-way ANOVA, followed by a *post-hoc* Tukey test. The results are expressed as $2^{-\Delta CT}$ values. Values are presented as mean ± standard error of the mean. For other abbreviations, see Table 1.**

between groups with and without detected expression. We did not find any significant differences in distributions (Chi-square test, $p = 0.63$). Hence, we compared the *ACE2* gene expression in HerP phenotypes (n = 20) vs. HoP phenotypes (n = 9) in males (n = 17) and females (n = 12). Significant correlations between sex and PPs and gene expression were found using two-way ANOVA ($p = 0.001$). Females with HoP CP had significantly higher levels of *ACE2* gene expression than females with HerP, according to a *post-hoc* analysis (Tuckey *post-hoc* test) ($p = 0.0007$) (Figure 2). Furthermore, we detected significantly higher *ACE2* gene expression in the HoP phenotype of females in comparison to males with the HoP phenotype ($p = 0.02$). There was no statistically significant difference between HerP and HoP CP tissue in the male patient group ($p = 0.99$).

The TMEM27 mRNA expression was not significantly different either with regard to sex or the US-defined PPs (Figure 3). Sex and PPs did not significantly interact, according to two-way ANOVA ($p = 0.61$). We analyzed the *TMEM27* gene expression in a total of 65 human CP tissue samples with regard to the PPs – HerP phenotypes (n = 49) vs. HoP phenotypes (n = 16), and sex – males (n = 45) vs. females (n = 20).

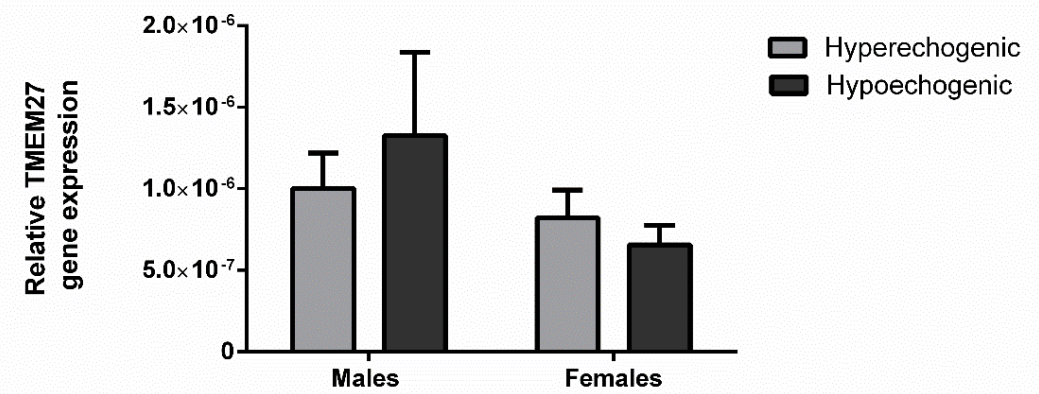

Fig. 3 – Relative transmembrane protein 27 (*TMEM27)* gene expression in carotid plaque tissue. There was no significant difference in *TMEM27* gene expression between plaque phenotypes: males – HerP phenotypes (n = 32) vs. HoP phenotypes (n = 14), $p = 0.86$; females – HerP phenotypes (n = 17) vs. HoP phenotypes (n = 3), $p = 0.99$. The cDNA from CP tissue samples was used as a template in RT-qPCR for relative quantification of intraplaque mRNA expression of TMEM27. For each sample, the expression level of TMEM27 mRNA was normalized to the housekeeping gene for 18S rRNA. The statistical significance was assessed by two-way ANOVA, followed by a *post-hoc* Tukey test. The results are expressed as $2^{-\Delta CT}$ values. Values are presented as mean ± standard error of the mean. For other abbreviations, see Table 1.

ACE and ACE2 protein expression detected by Western blot

To analyze whether ACE and ACE2 protein levels were altered regarding PPs (HerP vs. HoP) and sex (males vs. females), we performed a Western blot analysis. Two-way ANOVA revealed a significant correlation between sex and PPs and the ACE protein expression ($p < 0.0001$). Significantly higher ACE protein level was found in males with HerP phenotype of CP in comparison to males with HoP phenotypes ($p = 0.0007$, Tuckey *post-hoc* test). Furthermore, we detected higher ACE protein levels in females with HoP phenotypes than in males with the same PP ($p < 0.0001$) (Figure 4).

Two-way ANOVA of ACE2 protein levels showed that the effects of sex and PPs were not significant (Figure 5) and also that their interactions were not significant ($p = 0.86$).

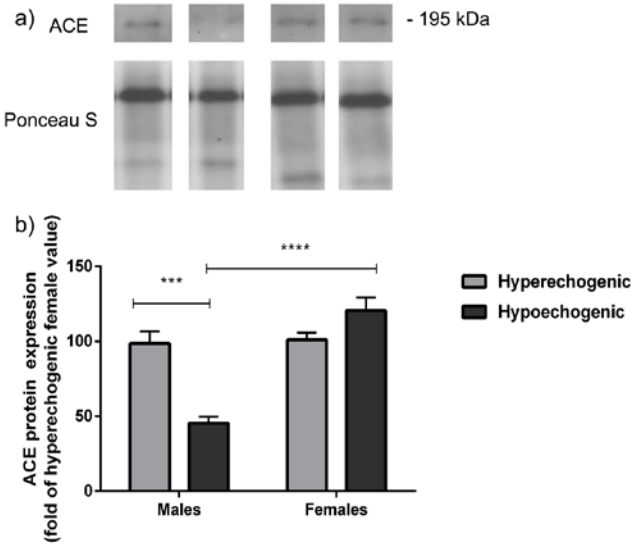

Fig. 4 – Western blot of angiotensin-converting enzyme *(*ACE*)* protein expression in carotid plaque tissue: a) representative image of Western blot: band No. 1 – HerP males; band No. 2 – HoP males; band No. 3 – Herp females; band No. 4 – HoP females; b) graphic display of Western blot results. Ponceau S staining was used as a loading control. * significant difference between males with HoP phenotypes (n = 4) vs. males with HerP phenotypes (n = 4), $p < 0.001$; **** significant difference between males with HoP phenotypes vs. females with HoP phenotypes (n = 6), $p < 0.0001$. The statistical significance was assessed by two-way ANOVA, followed by a *post-hoc* Tukey test. In all tests, the differences with two-tailed alpha-probability and $*p < 0.05$ were considered statistically significant. Results are presented as mean percent change ± standard error of the mean. For abbreviations, see Table 1.**

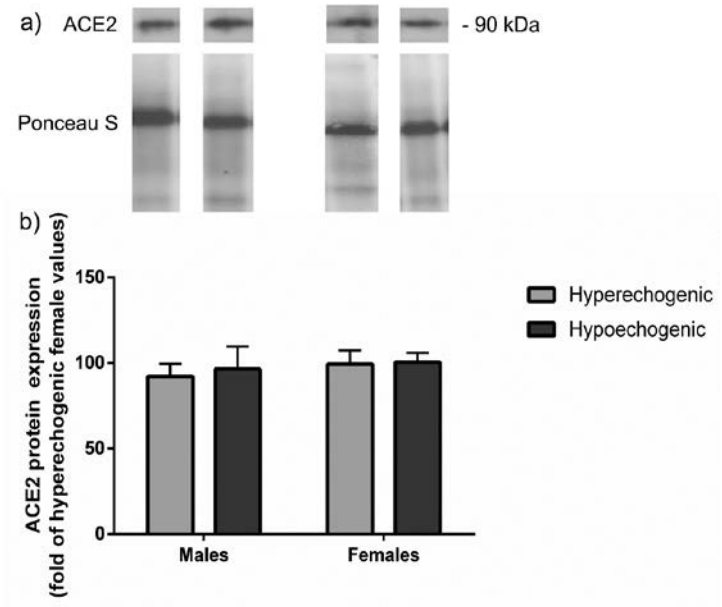

Fig. 5 – Western blot of angiotensin-converting enzyme *(*ACE*)*2 protein expression in carotid plaque tissue: a) representative image of Western blot: band No. 1 – HerP males; band No. 2 – HoP males; band No. 3 – Herp females; band No. 4 – HoP females; b) graphic display of Western blot results.
Ponceau S staining was used as a loading control. There is no significant difference in ACE2 protein expression between plaque phenotypes in males or females, $p > 0.05$. The statistical significance was assessed by two-way ANOVA, followed by a post-hoc Tukey test. Results are presented as mean percent change ± standard error of the mean.
For abbreviations, see Table 1.

Discussion

Ang II plays a role in As development, including the onset, progression, and destabilization of atherosclerotic lesions [37–39]. The discovery of the new ACE2/Ang(1-7)/MasR1 axis has challenged the role of the traditional ACE/Ang II/AT1R axis in some complications of chronic cardiovascular diseases [40, 41]. Moreover, RAS works *via* two opposing arms, resulting in either proatherogenic or atheroprotective effects. These effects may be influenced by the types of Ang peptides available and their levels, which are produced by two enzymes, ACE and ACE2, as well as the locally expressed receptors. A recent study established how sex affects circulating levels of the ACE2/Ang(1-7)/MasR1 axis in coronary As [42]. Since recent findings emphasize the significance of sex as an important variable in cardiovascular disease and genomics [43–45], our goal was to elucidate whether tissue intraplaque expressions of RAS family enzyme components, ACE, ACE2, and TMEM27, differ depending on PPs and sex. In our study group, we revealed a significant effect of sex and PPs on the expression levels of intraplaque ACE proteins. Lower protein levels of ACE were found in HoP compared to HerP in the male study group. Looking at the HoP phenotype only, the expression of the *ACE* gene was significantly lower in males than in females. ACE mRNA levels generally showed a trend of higher expression in males than in females, although not significant compared to females. Previous data [22] showed no difference in ACE mRNA intraplaque levels in asymptomatic vs. symptomatic patients. There could be several reasons for such discrepancies in results. In the current study, we do have a higher number of males than females, although the ratio of HerP vs. HoP is not different regarding sex. Yet, the number of females is limited, which is common in consecutive sample collections and represents one of the major study limitations. In addition, in males, As starts approximately ten years earlier than in females, and females in our study are of similar age as males, leading to a possibility of the presence of systemic prominent As. Likewise, there is a higher proportion of females who are symptomatic in comparison to males and with a greater percentage of coronary comorbidity in females (although not significantly) in our study group. It could contribute to a burden on the intraplaque expression of *ACE* in females with HoP in this study. However, it should be noted that plaques in males are more often associated with plaque hemorrhage and clinical events [46]. Sex is associated with the presence of atherosclerotic plaque hemorrhage, and it reshapes the relation between plaque hemorrhage and cardiovascular outcome [46], potentially biassing the study group in terms of surviving prior events.

It has been known for a while that PPs could be different among the sexes. It was shown that males develop unstable PPs, rich in inflammatory intraplaque content, more often than females [47], which is in agreement with the higher number of males in the HoP group. It is still an intriguing topic leading to novel research on the transcriptomic level of sex differences in atherosclerotic PPs (lipid-rich plaques in men,

fibrous plaques in women) [26]. In addition, the recent study shows that an increase in macrophage ACE in atherosclerotic mice reduces the As in comparison to wild-type mice [48], suggesting a new ACE effect, which they claim to be independent of Ang II. Since the mRNA and protein expression in our study are not in alignment, it is noteworthy that the correlation between expression levels of protein and mRNA in mammal tissue is not always high [49–51]. Many processes occur after mRNA forming: *post*-transcriptional, translational, and protein degradation regulation, so steady-state transcript abundances only partially predict protein abundances [52]. Indeed, a previous deep proteome and transcriptome analysis of 29 healthy human tissues revealed a strong difference between mRNA and protein quantities and that protein expression was often more stable across tissues than that of transcripts [53].

Bearing in mind the complexity of As as an inflammatory process, differences in expression might exist in intraplaque inflammatory cells infiltrate, state of activation, and interaction of different cell populations invading CP [54]. Histological analyses of plaques have revealed differences in cell species, inflammation, and neovascularization status between males and females [47]. Furthermore, using data on DNA methylation status in cells, it was discovered that female plaques contain more potential SMC-like cells, whereas male plaques contain more immune-like cells (macrophages, mast cells, T cells, B cells) [55].

Although ACE is expressed in a variety of cell types, it has been demonstrated that ACE produced by SMC directly causes As in both male and female mice, independently of BP and circulating ACE activity [56]. According to those recent findings, females in our study may have more SMC than expected in the same type of plaques compared to males, as well as possible different methylation status in cells that could reflect the observed sex differences in the ACE protein level.

There is still missing knowledge about the role and activation of the ACE2/Ang(1-7)/MasR1 axis in carotid atheroma instability. Numerous harmful phenotypes associated with cardiovascular disease may be inhibited by the activation of this RAS counter-regulatory axis [57]. It was proposed that the beneficial effect of ACE2 in thrombosis could be due to a concomitant increase in the production of Ang(1-7) and degradation of Ang II [40, 58]. Both ACE2 mRNA and protein expression were present in all layers of the vessel wall. In addition, ACE2 protein was present in human veins, non-diseased mammary arteries, and atherosclerotic arteries. In human carotid atherosclerotic lesions, ACE2 mRNA expression was present in an early and advanced lesion [18]. However, in a previous study, ACE2 mRNA expression was very weak in human CP [22]. ACE2 protein was expressed in macrophages, SMC, and in the endothelial cells of *vasa vasorum* from atherosclerotic CP [18]. Overexpression of ACE2 in the rabbit model of As was shown to reduce the size of atherosclerotic lesions, stabilize already existing lesions, and inhibit lesion growth at early stages of the disease but not at advanced stages [15]. Even the local pattern of shear stress forces in blood vessels affects the expression of ACE2 in atherosclerotic plaques [59]. It was shown that ACE2 activation improves endothelial function *in vivo* [60], attenuates the formation of thrombi, and decreases platelet adhesion to injured vessels [61]. The intraplaque levels of ACE2 mRNA in our study were significantly different among the groups regarding sex and PPs, as shown in our preliminary study [24] and herein. However, 44% of plaques in our study had detectable mRNA. Previously, in some studies, ACE2 expression was not detectable [17], while others detected a very weak level of ACE2 mRNA in human CP, only in a few specimens [22]. It is not clear why ACE2 is not detectable in a certain percentage of plaques. In our study group, we found significantly higher intraplaque ACE2 mRNA levels in females with HoP compared to females with HerP and in comparison to males. This result must be taken with caution since the group of females with HoP is rather small. Previously it was found that ACE2 protein levels did not differ with regard to PPs and were similar in all stages of As [18]. Furthermore, data so far point to the possibility that ACE2 functions as a tissue-specific negative feedback regulator of the activated RAS [18]. Due to the capacity of ACE2 to exert cardioprotective effects shown in animal models [62], its role requires further studies in humans.

Regarding levels of TMEM27 mRNA, we did not detect any significant differences concerning sex and plaque type. To our knowledge, we were the first to detect TMEM27 mRNA levels in intraplaque tissue of CAs. A previous study showed that TMEM27 was expressed in the primary endothelial cells of the lungs and regulated the balance of nitric oxide and superoxide [20]. This result suggested that activation of TMEM27 might be beneficial in the regulation of BP by affecting nitric oxide bioavailability and vascular damage [63]. Still, more functional research is needed to determine the involvement of TMEM27 in As.

Our study has several limitations. First of all, since this was a single-center study with a small sample size of patients, additional research, including a bigger sample size, is required. Second, the asymptomatic patient group in our study was more frequently diagnosed during a routine CA US. Some of the individuals in the study also had concomitant conditions, such as coronary artery disease, diabetes mellitus, and/or hypertension. At the moment, our findings related to the expression of components of the RAS gene family were detected in the overall intraplaque tissue, so the cellular source of these components is not known. We did not evaluate tissue and circulating levels of ACE and ACE2 simultaneously, thus failing to test the hypothesis that the increase in tissue ACE will be reflected in increased circulating activity. Likewise, we did not measure protein levels of TMEM27.

Conclusion

In summary, our study may provide additional information regarding the relationship between the intraplaque expression of components of the RAS family based on sex and PP in advanced carotid As. The main novelties of this study

are sex specific differences in the expression of RAS components in different CP phenotypes and the detection of TMEM27 mRNA levels in the CP. Further studies are needed to resolve and elucidate sex-specific differences and mechanisms involved in complex RAS components interplay in As.

Acknowledgement

The research was funded by the Serbian Ministry of Education, Science and Technological Development (grant No 451-03-66/2024-03/200017).

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Received on October 28, 2022
Revised on November 20, 2023
Revised on January 12, 2024
Accepted on January 21, 2024
Online First March 2024

C A S E R E P O R T

UDC: 616-007.1:616-006-053.2
DOI: https://doi.org/10.2298/VSP240103011T

Solitary basaloid follicular hamartoma: a report of two cases and a brief review of the literature

Solitarni bazaloidni folikularni hamartom: prikaz dva bolesnika i kratak pregled literature

Danica Tiodorović*†, Andrija Jović*, Sladjana Cekić*, Nataša Vidović‡, Tatjana Radević§, Željko Mijušković§‖

University Clinical Center of Niš, *Clinic of Dermatovenereology, ‡Center for Pathology and Pathological Anatomy, Niš, Serbia; †University of Niš, Faculty of Medicine, Niš, Serbia; §Military Medical Academy, Department of Dermatology and Venereology, Belgrade, Serbia; ‖University of Defence, Faculty of Medicine of the Military Medical Academy, Belgrade, Serbia

Abstract

Introduction. Basaloid follicular hamartoma (BFH) is a rare benign follicular malformation often clinically misdiagnosed. Patients with BFH demonstrate a variety of clinical manifestations and associated abnormalities. BFH may be a familial, congenital, or acquired condition with a localized or generalized distribution. Several clinical variants of generalized BFH are recognized, which may be associated with a diverse spectrum of abnormalities. **Case report.** We present two cases of solitary BFH in pediatric patients. The first patient was a 16-year-old boy with a history of autism spectrum disorder, admitted to our department for consultation due to a harmless dermal nevus on his face. The second patient was a six-year-old girl presented with a ten-month history of an asymptomatic, skin-colored papule located on the right nasolabial fold that gradually increased in size over time. Both cases were documented dermoscopically, and they were presented with a brief overview of the current literature. **Conclusion.** Considering the overlapping clinical, histological, and dermoscopic features of BFH with other benign and malignant lesions, its incidence in pediatric patients is probably higher than what is reported in the current literature.

Key words:
dermoscopy; diagnosis; immunohisochemistry; hamartoma; histological techniques.

Apstrakt

Uvod. Bazaloidni folikularni hamartom (BFH) je retka benigna folikularna malformacija koja se često pogrešno dijagnostikuje. Bolesnici sa BFH ispoljavaju različite kliničke manifestacije i udružene abnormalnosti. BFH može biti familijarna, kongenitalna ili stečena malformacija, sa lokalizovanom ili generalizovanom distribucijom. Opisano je nekoliko kliničkih varijanti generalizovanih BFH, koje mogu biti udružene sa širokim spektrom abnormalnosti. **Prikaz bolesnika.** Prikazujemo dva slučaja solitarnog BFH kod pedijatrijskih bolesnika. Prvi bolesnik je 16-godišnji dečak sa istorijom poremećaja iz spektra autizma, koji je primljen na odeljenje na konsultaciju zbog bezopasnog nevusa na licu. Drugi bolesnik je šestogodišnja devojčica sa desetomesečnom istorijom asimptomatske papule boje kože na desnoj nazolabijalnoj brazdi, koja se vremenom uvećavala. Oba slučaja su dermoskopski dokumentovana i prikazana uz kratak pregled aktuelne literature. **Zaključak.** Imajući u vidu kliničku, patohistološku i dermoskopsku sličnost BFH sa drugim benignim i malignim lezijama, njegova incidenca u pedijatrijskoj populaciji je verovatno viša nego što je u aktuelnoj literaturi prikazano.

Ključne reči:
dermoskopija; dijagnoza; imunohistohemija; hamartom; histološke tehnike

Introduction

Basaloid follicular hamartoma (BFH) is a benign follicular malformation often clinically misdiagnosed. Although observed as a relatively rare hamartoma, BFH is probably underreported due to a variable clinical presentation that may lead to diagnostic difficulties. BFH may be a congenital, familial, or acquired condition. Several clinical variants of BFH have been described, including the localized (solitary and multiple lesions) and generalized

Correspondence to: Danica Tiodorović, University Clinical Center of Niš, Clinic of Dermatovenereology, Bld. Dr. Zorana Djindjica 48, 18 000 Niš, Serbia. E-mail: danica.dr@gmail.com

forms, with the latter being mostly associated with systemic syndromes and various diseases such as myasthenia gravis, systemic lupus erythematosus (SLE), cystic fibrosis (CF), and alopecia [1–7]. Herein, we report two cases of solitary BFH in pediatric patients.

Case reports

Case 1

A 16-year-old boy with a history of autism spectrum disorder was admitted to our Department for consultation due to a harmless dermal nevus on his face. According to his mother, his past medical and family history was unremarkable. During the total body skin examination, a small, slightly pigmented papule was noticed on his lower back (Figure 1A). A dermoscopic examination of the lesion highlighted the presence of bluish-gray dots and globules over the brownish background with a linear irregular vessel (Figure 1B). The excisional biopsy was performed with a presumptive diagnosis of basal cell carcinoma (BCC). Standard histopathological (HP) methods [hematoxylin and eosin staining and immunohistochemical (ICH) analysis] were used for diagnostic purposes. Monoclonal mouse antibody against Bcl-2 (clone 124, DAKO, ready-to-use), CD34 (clone QBEnd 10, DAKO, ready-to-use), and CD10 (clone 56C6, DAKO, ready-to-use) were applied for ICH analysis after 4 μm thick, paraffin-embedded tissue selection was deparaffinized in xylene and rehydrated in graded ethanol series. In brief, tissue selections were treated with citrate buffer (pH 6.0) in a microwave oven for 20 min for antigen retrieval. After endogenous peroxidase activity block and rinsing with phosphate-buffered saline, each slide was incubated with primary antibody for one hour. Overall, an HP examination revealed numerous cords and strands of basaloid cells arranged in a radial and anastomosing pattern over the scant fibrous stroma (Figure 2A). Basaloid cells showed subtle peripheral palisading without nuclear pleomorphism, mitotic activity, and the presence of

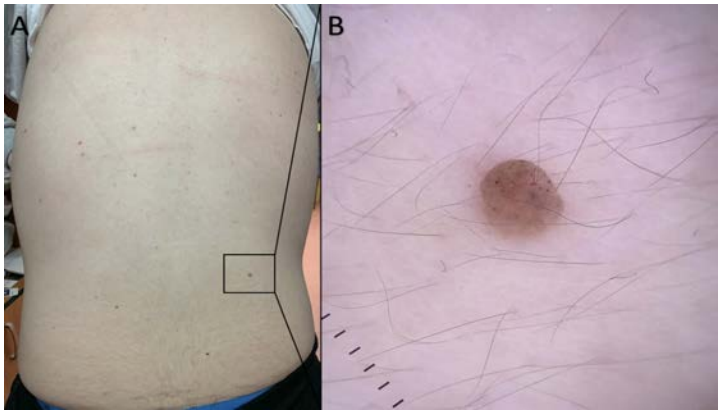

Fig. 1 – Case 1: clinical and dermoscopic features of basaloid follicular hamartoma in a boy: A) solitary papule on his right lumbar region; B) multiple blow globules and linear vessels observed on dermoscopy.

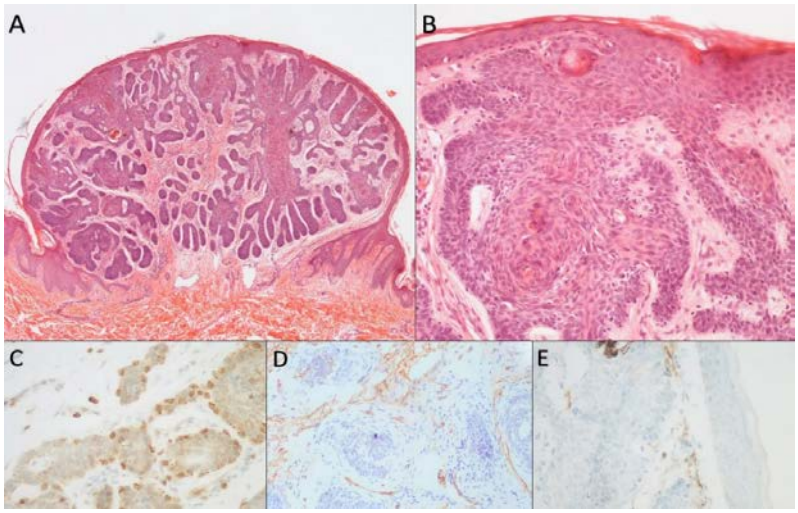

Fig. 2 – Case 2: histopathological and immunohistochemical (ICH) features of basaloid follicular hamartoma: A) cords and strands of basaloid cells arranged in a radial and anastomosing pattern [hematoxylin and eosin (HE) staining, ×4]; B) basaloid cells with subtle peripheral palisading without nuclear pleomorphism, mitotic activity, and the presence of cell necrosis (HE staining, ×10); C) Bcl-2 positivity in peripheral basaloid cells (ICH, ×20); D) CD34 positivity in stromal cells (ICH, ×20); E) CD10 positivity in stromal cells (ICH, ×20).

cell necrosis (Figure 2B). Additionally, ICH analysis revealed positivity of peripheral basaloid cells for Bcl-2 (Figure 2C), as well as CD34 and CD10 stromal cells positivity (Figure 2 D and E). Overall, those features were consistent with a diagnosis of BFH.

Case 2

A six-year-old girl presented with a ten-month history of an asymptomatic, skin-colored papule located on the right nasolabial fold (Figure 3A) that gradually increased in size over time. During a dermoscopy assessment, linear irregular vessels were noticed over the whitish-pinkish background (Figure 3B). Based on the clinical and dermoscopic features alone, suspicion of skin adnexal neoplasm was considered. An excisional biopsy was performed, and the HP examination revealed the presence of cords and strands of bland-looking basaloid cells with a branching and anastomosing pattern in the superficial dermis (Figure 4A). IHC staining for Bcl-2, CD34, and CD10 were obtained, showing Bcl-2 positivity in peripheral neoplastic cells (Figure 4B) and both positive staining for CD34 and CD10 in stromal cells (Figure 4 C and D) (method previously described). Considering HP and IHC features, a diagnosis of BFH was made.

Discussion

BFH, originally described in 1969 by Brown et al. [8] and later termed by Mehregan and Baker [9] in 1985, is a rarely encountered follicular malformation with diverse clinical presentations. According to genetic studies, the development of BFH involves a mutation in the patched homolog (PTCH) gene located on the chromosome band 9q23, a tumor suppressor gene also implicated in the pathogenesis of basal cell nevus syndrome (BCNS) [10, 11]. Namely, the PTCH gene product acts as a part of the receptor for the sonic hedgehog (SHH) protein, which has a fundamental role in numerous aspects during embryonic development. The PTCH protein forms a receptor

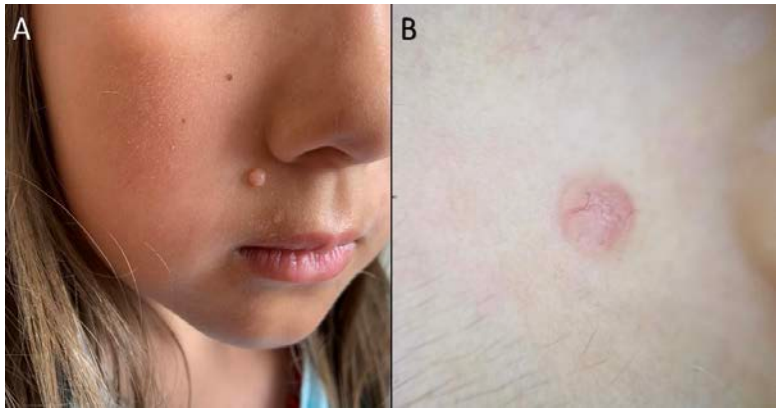

Fig. 3 – Clinical and dermoscopic features of basaloid follicular hamartoma in a girl: A) solitary non-pigmented papule on the right nasolabial fold; B) linear irregular vessels over the whitish-pinkish background observed on dermoscopy.

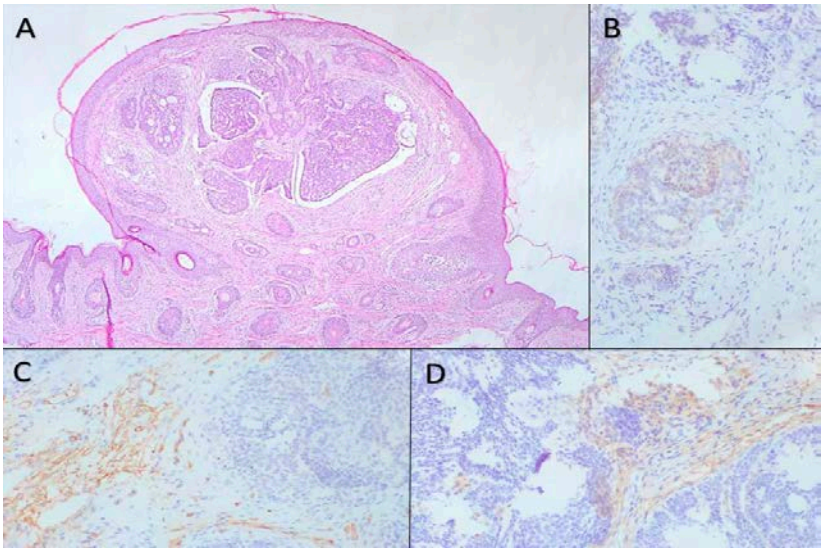

Fig. 4 – Histopathological and immunohistochemical (IHC) features of basaloid follicular hamartoma: A) cords and strands of basaloid cells arranged in a radial and anastomosing pattern with subtle peripheral clefting (hematoxylin and eosin staining, ×4); B) Bcl-2 positivity in peripheral basaloid cells (ICH, ×20); C) CD34 positivity in stromal cells (ICH, ×20), and D) CD10 positivity in stromal cells (ICH, ×20).

complex with a transmembrane signaling protein smoothened (SMO). In the absence of SHH protein, the PTCH receptor prevents the transduction of the downstream signal through the inactivation of SMO. In contrast, when SHH binds to the PTCH receptor, it releases the inhibition of SMO, which in turn leads to the upregulation of hedgehog target genes by transcription factors in the Gli family [10, 11]. Activation of this signaling pathway may lead to increased cell proliferation, resulting in abnormal growth and lesion formation [3, 10, 11].

Patients with BFH demonstrate a variety of clinical manifestations and associated abnormalities. BFH may be a familial, congenital, or acquired condition with localized or generalized distribution. In the context of localized disease, BFH may be present as a solitary lesion or display a linear and/or unilateral arrangement of multiple lesions [1–6]. Most described cases of localized BFH were situated on the scalp and face, although other locations, including the trunk and extremities, are also possible. Lesions usually clinically appear as a skin-colored to brown papule, plaque, or patches of alopecia in the case of scalp involvement [1–8]. Contrary to localized forms, generalized BFHs are commonly associated with a diverse spectrum of abnormalities. Several clinical variants of generalized BFH are recognized, including the following: a sporadic form of multiple BFHs without a systemic disease; an acquired form associated with alopecia and autoimmune diseases, including myasthenia gravis and SLE; a familial form with autosomal dominant inheritance with or without associated abnormalities, including multiple milia, comedo-like lesions, hypotrichosis, hypohidrosis, palmar and/or plantar pits (also known as generalized BFH syndrome); finally, a congenital form of multiple BFHs associated with alopecia and CF [3–7]. Additionally, BFH may occur in association with genodermatoses such as Bazex-Dupre-Christol syndrome, BCNS, and Happle-Tinschert syndrome (unilateral and segmental BFHs occurring along Blaschko's lines) [1–7].

Depending on the clinical presentation alone, a broad list of differential diagnoses should be considered. Dermal melanocytic nevi, BCC, trichoepithelioma, trichilemmoma, sebaceous hyperplasia, seborrheic keratosis, syringoma, acrochordons, and angiofibroma should be ruled out in case of solitary BFH. Linear BFH may be misdiagnosed as linear epidermal nevus, linear lichen striatus, and linear morphea, while generalized BFH may mimic BCNS, tuberous sclerosis, Cowden syndrome, Rombo syndrome, and multiple trichoepitheliomas [1, 11].

From HP aspects, BFH is composed of radial and anastomosing cords and strands of basaloid cells that display an epithelial attachment and/or arise from follicles. The basaloid cells are typified by bland morphology without pleomorphic nuclei, mitotic activity and cell necrosis. If the presence of peripheral palisading is observed, it should be focal and lacking in the degree typically seen within BCC. Beyond those observations, keratin cysts may be seen inside basaloid cords. Lesions of BFH are superficial and only appear where normal hair follicles are present. Therefore, interfollicular and deeper reticular dermis are not affected. The surrounding stroma is scant and consists of eosinophilic compact collagen and a small number of fibrocytes. While minimal retraction clefts between neoplastic tissue and stroma are occasionally reported in BFH, this feature is typical for BCC or trichoepithelioma. Regarding the IHC, BFH has a low proliferative rate, which can be demonstrated with Ki-67 expression in a small number of cells. Bcl-2 positivity may be observed in the outermost basaloid cells of BFH, while CD34 and CD10 are both expressed within stromal cells [11, 12].

Regarding HP, lesions that should be differentiated from BFH include BCC, trichoepithelioma, and folliculocentric basaloid proliferation. The differential diagnosis between BFH and BCC may be challenging, given the overlapping features of cords and stands of basaloid cells in both lesions. However, in BCC, basaloid cells reveal an increased mitotic activity, single-cell necrosis, as well as pronounced palisading and clefting. Furthermore, the neoplastic basaloid nests of BCC tend to involve the interfollicular dermis and destroy pre-existing hair follicles. BCC has been reported to display a higher Ki-67 mitotic index, prominent Bcl-2 staining, and a lack of expression of CD34 in stromal cells. Abundant stroma with numerous fibrocytes, prominent keratin cyst formation, and the presence of papillary mesenchymal bodies in trichoepithelioma allow a straightforward differentiation from BFH. A folliculocentric basaloid proliferation is a reactive proliferation of mantle epithelium that occurs in the skin adjacent to BCC. HP features that favor the diagnosis of folliculocentric basaloid proliferation over BFH include a vertically oriented basaloid proliferation with a surrounding prominent basement membrane and the absence of both keratin cysts and direct epidermal attachment [11, 12].

Although HP findings are consistent in all clinical variants of BFH, the dermoscopic appearance of this rare hamartoma is quite variable [13–18]. Namely, dermoscopic features of BFH are scarce and limited to a few case reports. Mauleón et al. [14] were the first ones to report a dermoscopy of BFH, which displayed an unspecific structureless blue pattern. Other reported dermoscopic features of BFH include a structureless brown pattern [15], a papillomatous and cobblestone pattern with brown-black globules, dots, and comedo-like openings, bluish-gray and brownish globules over the pinkish background with linear irregular vessels [13, 18]. Recently, Besagni et al. [18] published three pediatric patients of BCNS with multiple BCCs and BFHs, revealing dermoscopic features of bluish-gray globules and nests within both types of lesions and typical arborizing vessels in BCCs. They highlighted the vascular pattern as a possible dermoscopic clue when differentiating between those lesions [18]. However, in both of our cases, the vascular pattern was apparent and could now support that observation.

Conclusion

We reported two cases of solitary BFH in pediatric patients, whose incidence is probably higher than what is reflected in the current literature considering overlapping clinical, histological, and dermoscopic features of other benign and malignant lesions, particularly BCC. BFH should be considered in the differential diagnosis of solitary lesions in the pediatric population.

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Received on January 3, 2024
Revised on January 22, 2024
Accepted on January 23, 2024
Online First February 2024

HISTORY OF MEDICINE

UDC: 616.89-008.441.3(497.1)
DOI: https://doi.org/10.2298/VSP240109021M

The use of psychoactive substances in the Kingdom of Serbs, Croats, and Slovenes (1918–1929) and the Kingdom of Yugoslavia (1929–1941)

Korišćenje psihoaktivnih supstanci u Kraljevini Srba, Hrvata i Slovenaca (1918–1929) i Kraljevini Jugoslaviji (1929–1941)

Aleksandar Medarević*, Kristina Djordjić†

***Institute of Public Health of Serbia "Dr. Milan Jovanović Batut", Belgrade, Serbia;**
†University of Belgrade, School of Dental Medicine, Belgrade, Serbia

Key words:
drug addiction; history of medicine; history, 19th century; serbia.

Ključne reči:
narkomanija; istorija medicine; istorija, 19 vek; srbija.

Introduction

Until the beginning of the 70s of the last century, the use of psychoactive substances for non-medical purposes in Yugoslavia was not considered a severe problem [1]. Reports on the frequency of drug use were particularly sparse for the period before World War II [a]. Nowadays, illegal production and drug trafficking, as well as facilitating the use of drugs, are punishable by law, and their long-term use is considered to lead to addiction inevitably. Untreated addiction progresses into an illness and a serious social problem. However, it was not always like that. Both doctors and pharmacists were well aware of the health effects of morphine, semi-synthetic alkaloids, and cocaine in the years after World War I. Still, the benefits of using such substances were thought to outweigh their side effects [2]. Thus, opium was applied primarily as a medicine against pain and stomach cramps, most often in the form of an injection, but also as a powder, tincture, syrup, ointment, or patch. Morphine was the most potent analgesic and anesthetic, without which surgical operations were impossible. A semi-synthetic derivative of opium, diacetylmorphine (known by its Heroin® trademarked name), was promoted as a cough suppressant, especially in the case of tuberculosis, but also as a hypnotic. Cocaine was used in the treatment of morphine addiction to reduce pain as well as to increase physical and mental functions [3].

[a]The term used was applied on purpose to avoid the trap of analyzing facts from the past in light of the contemporary picture of drug (ab)use.

Legal production of psychoactive substances in the Kingdom of Yugoslavia

The poppy has grown in the Kingdom of Yugoslavia territory since the Neolithic [4]. Frisk states in his dictionary that the word "poppy" has a Slavic (perhaps Serbian) or Germanic origin [5]. Homer mentions the poppy when he sings of the death of the Trojan hero Gorgition, brother of Hector and son of Priam: "He bent drooping his head to one side, as a garden poppy bends beneath the weight of its yield and the rains of springtime; so his head bent slack to one side beneath the helm's weight [6]."

In southern Serbia, poppy cultivation ceased at one point and was restored in the 1880s. The reasons for its renewed popularity were the possibility of export, the growing needs of Europe, the increasingly strict Asian anti-opium policy, and the decline in the profitability of cotton cultivation [7]. In the Kingdom of Yugoslavia, poppies were grown in Southern Serbia (current North Macedonia), mainly in Tikveš, where it became the main agricultural product along with wine [8]. The plant was grown in private rural households, which contributed to Yugoslavia being the largest producer of opium in Europe after Turkey. Yugoslav opium was considered high-quality, given that the morphine content in it was 8–14%, which was higher than in the case of competing Turkish opium (8% of morphine) and the incomparably lowest-quality Asian opium (3–5%) [9].

Opium production was prone to oscillations, as it was heavily influenced by domestic and foreign buyers and resellers and depended on international politics [10]. In areas un

Correspondence to: Aleksandar Medarević, Institute of Public Health of Serbia "Dr. Milan Jovanović Batut", Dr. Subotića 5, 11 000 Belgrade, Serbia. E-mail: aco.batut@gmail.com

der poppy, yields and prices varied greatly. However, despite all the problems, the poppy was an essential product of Southern Serbia and a source of well-being for entire villages and towns [11]. Larger villages earned the same income as entire regions in other parts of the Kingdom. Due to its profitability, it was said that the poppy was "the gold of Southern Serbia" [12]. The most significant part of the production was intended for export: only export value to the United States was equal to the entire Vardarska Banovina budget [9]. Non-exported quantities of opium were processed in domestic factories, two of which were leading "Skopska alkaloid factory S. & F. Ognjanović" (which changed its name to "Alkaloid" after 1945) and "Chemical Products Factory in Hrastnik d. d." from Celje [13].

Where poppies were not grown, industrial hemp (*Cannabis sativa*) was cultivated. Hemp occupied about one-third of the area under industrial plants in Yugoslavia, making the Kingdom the world's third-largest crop producer after the Union of Soviet Socialist Republics and Italy. Hemp was mainly grown for the growers' needs. Fibers and woody mass were the most important hemp products [14]. Over time, domestic hemp earned a bad reputation due to its low content of tetrahydrocannabinol and the name it shares with Indian hemp (*Cannabis indica*). Therefore, its production in the post-war Federal People's Republic of Yugoslavia almost ceased entirely.

Despite the mass production of both poppy and hemp, there was almost no abuse among the producers. The producers knew that poppy was a "dangerous plant". However, chroniclers wrote that "not a single evil came from working with the poppy" [11]. Exceptionally, Stanojević [15] stated that farmers in Southern Serbia gave their children poppy seeds to make them sleep. In his brochure "Excessive enjoyment and delusions to the detriment of health" from 1929, Milorad Dragić [16] described the effects of alcohol, nicotine, and caffeine on humans. He wrote: "Fortunately, the use of cocaine is not known to our people. Neither is the use of opium. Both of these means are known to a tiny part of the inhabitants of the city".

Beginnings of non-medical use of psychoactive substances

During World War I, psychoactive drugs were used for medical and non-medical purposes, issued by state military and civilian authorities, but also obtained by soldiers and civilians [17, 18]. Sedatives such as alcohol, morphine, and opium help to overcome physical and emotional pain, remove fears, and soften the experience of war [3]. Opioid dosing practices at the time were inconsistent and unclear [17]. Furthermore, the wounded procured and used opium themselves, which often led to death due to overdose. During the war, monitoring the amount of given analgesics was impossible. This practice of long-term administration of opioids inevitably led to the emergence of addiction. Additionally, opium misuse was widespread among healthcare workers who had access to opioids and other drugs [3].

Stimulants, such as cocaine and alcohol, were often deliberately administered in small amounts to soldiers at the front, as these substances allowed them to focus, calm down, and show heroism in bloody battles. The habit of regular cocaine use was brought to Yugoslavia by refugees from the Russian Empire, where abuse of this substance had already spread among the civilian population during the war and revolution. In the first years after the end of the war, thousands of Russian emigrants immigrated to Yugoslavia. Their life in a new country, despite the support of the state and King Alexander himself, was challenging [19]. It seems that the difficult living conditions and the loss of hope of returning to the homeland contributed to the further spread of cocaine abuse. Over time, some Russians became involved in international criminal gangs of drug producers and smugglers, whose centers were in Paris, Vienna, Marseille, Thessaloniki, and Constantinople [9]. The leaders of these gangs were mainly Jews, Greeks, and Armenians, who used their business connections for drug trafficking [20]. In consulting various sources, we did not come across any indication that such illegal drug dealings took place under the auspices of armed paramilitary organizations, as depicted in a contemporary series [21].

Use of psychoactive substances in Belgrade

Most archival data on the use of these substances was related to Belgrade, the capital of Yugoslavia. There were several salons in the capital where "men and women with strange passions" gathered and where cocaine was used and hashish was smoked [22]. Those salons were located on today's Kralja Aleksandra Boulevard, Knez Mihailova Street, Skopljanska Street (today Nušićeva Street), around Đeram Market, and in a luxurious bar on Terazije (probably the Claridge Club, i.e., Krsmanović house) [23, 24]. They gathered a motley company of "failed actors, journalists, ladies from higher circles, even ministers at their disposal" [24]. During the 1920s, the police often tolerated the appearance of these circles, probably because they were made up of an influential clientele. Almost all salon employees were Russians. A salon with a similar purpose also existed in Novi Sad [25]. As police raids became more frequent, salons moved to the outskirts, even to smaller towns. Thus, the police in Ivankovo near Vinkovci discovered a club for pleasure, which a certain Evgeni Nikolski owned. The club was intended for members of higher Belgrade circles, as well as wealthy Russian emigrants, who held cocaine weekend sessions there [24].

Journalists visited such clubs several times, bringing readers a detailed description of the sessions: "Midnight was approaching. The salons were full. The session has started. Ladies took out gold and silver powder cases and took white powder. Men did the same. Those, on the other hand, who could not tolerate cocaine, took morphine. Hashish was smoked." Not all guests were able to afford cocaine. The poor, especially ladies of Russian origin, went around the guests begging for drugs. At the end of the session, the hosts "brought glasses of some strong drink, brought from India" to soften the effects of the narcotic and slowly lead the users

into abstinence. The state after the drug stopped working was very unpleasant for the user. One of those present at the session described to the journalist such a state after the cocaine stopped working: "It seems that someone is watching me through a hole in the attic. A terrible feeling comes over me, and I have to inject myself with at least one ampoule of morphine [22]."

It is known that the mixture of alcohol and cocaine alters the individual effects of these substances in terms of enhancing the desired outcomes [26]. Cocaine users in interwar Belgrade also noticed this. Therefore, they tried their best not to let their habits be reflected in their profession. As an example, we present a part of an article from the *Pravda* newspaper (1904–1941) about Belgrade cocaine users, more specifically about a famous Belgrade ballerina, whom cocaine helped maintain the appearance of a double life: on the one hand, an alcoholic and regular drug user, and on the other, a respected member of society: "That evening, in precisely one hour, she had to appear in one of the more difficult parties. Moreover, she is drunk now. - I will be sober on stage. - After that, the ballerina took a dark jar from her purse. White tiny crystals flashed. Cocaine! She took a few deep inhalations of the white powder. The companions looked on in wonder. However, after a few minutes, the young artist was sober. Only the tired look revealed that she had not slept for two nights. She played very well, and no one could notice anything unusual about her. Just after the performance had ended, the fatigue broke her down. They took her home [27]."

At the beginning of the 1930s, Belgrade had about 300,000 inhabitants, so all the long-term drug users mostly knew each other, and all the devastating consequences of using these substances could be seen through the examples of others [28]. However, the embrace of the drug was too strong for many to end its use. One experienced cocaine user, a young man in his early twenties, the son of respectable parents, described the grip of cocaine in the following words: "You are mistaken if you think that I do not know the terrible consequences of my actions. I am aware of that. […] I know I am broken. Nevertheless, what can I do! I cannot give it up. It is stronger, much stronger than my will. I tried it out of curiosity, and that turned out badly for me [27]." When describing the causes of addiction, Dr. Sztraka pointed out that "curiosity" is the most common cause among health workers [29]. There was a well-known case of a paramedic at the state hospital in Belgrade, a long-term addict who died suddenly and whose body was found after three days of intensive searching, and no less than in the hospital library [30].

Professor of forensic medicine Milovan Milovanović, in his paper from 1932, stated that deaths due to acute cocaine poisoning were not rare in Yugoslavia. In contrast, registered deaths among long-term cocaine users were sporadic. Possible reasons are the efforts of families and friends to hide such cases from the public, inadequate police-medical investigation, the coroner's ignorance of the signs of use, and the unavailability and insufficient sensitivity of toxicological analyses. At the same time, the transition from injection therapy to inhalation, according to Milovanović, resulted in a smaller amount of the active substance being introduced into the body. Therefore, physiological functions were less impaired, with a smaller mortality risk. In his paper, Milovanović describes three cases of chronic cocaine poisoning with anamnestic data and forensic medical findings – the first two cases date from September 1929, while the third was recorded in March 1931. The author describes the corpses of the deceased in detail, with a description of the changes that years of cocaine, morphine, tobacco, and alcohol use left on the organs. Each report is accompanied by an anamnesis of the development of addiction, in which one can see how this vice gradually destroyed the addict's body and soul, permanently destroying their psychophysical and social well-being. The first two deceased were Russian emigrants, both officers in their thirties, who ended their lives in Belgrade as vagrants. The first was involved in the cocaine resale in the city as a member of a criminal quartet. Both of them brought the habit of using cocaine from the front. The third deceased was a Yugoslav, a student in his early twenties, the son of a university professor, who died in the family home. He used cocaine for a short time, less than two years. They got cocaine from drugstores and pharmacies, from hotel doorkeepers and resellers. The money was obtained from stealing, begging, and borrowing from relatives and friends. They started unsuccessful treatment several times. Before their death, they spent time in abandoned buildings, shantytowns, brothels, or on the street. The elderly Russian died of pneumonia, and the Yugoslav died of tuberculosis [28].

Two activities closely related to drug use were the slave trade and prostitution [31]. The connection between these three forms of crime in the Balkans has survived to the modern age [23]. Promising them safe accommodation and salary, criminals recruited young girls in Belgrade [17]. Other girls entered the world of prostitution and drugs through friends or lovers [17, 23]. Some worked as escorts for wealthy gentlemen, while others served as public servants in salons for honored guests. One such salon was the "Kod Malog Orlića", a tavern on Južni Boulevard, which today represents the broader center of the city, but then it was the capital's outskirts [23]. The tavern served as a cover for a brothel and a salon where cocaine users, mostly Russian men and women, consumed the white powder. In a raid carried out in 1928, the police rescued ten girls from the hands of a criminal gang. The owner of the bar was also the gang leader who pimped out girls for his guests for money. In addition to female prostitution, male prostitution also flourished in pre-war Belgrade. Men were described as "favorite agents of modern cocainism". Along with prostitution, various sexual tendencies were also common in these circles [23].

Some physicians, pharmacists, and their assistants were involved in illegal drug trafficking and prostitution. A medical doctor from Knez Mihailova Street was known for regularly visiting "a madam" in Palmotićeva Street, in whose apartment "*her protégés*" received morphine injections. Before the war, the home of another doctor in Sarajevska Street was turned into a brothel and club, where selected members enjoyed cocaine, opium, and morphine [32].

Use of psychoactive substances in Skopje

The second city for which we found the most information is Skopje, the seat of the Vardar Banovina and one of the largest cities in Yugoslavia. Released from the centuries-long Ottoman occupation in 1912, Skopje preserved its traditional oriental appearance in the interwar period. This was especially obvious in the Muslim part of the town, which consisted of narrow streets surrounded by high walls and wooden screens. Just behind some of those high walls, hidden from unwanted views, in the gardens of the once-rich aghas and beys, Indian hemp was grown [24]. Hashish was procured from Thessaloniki, and perhaps the most popular derivative of Indian hemp was the so-called "asrar" (i.e., charas), a petrified resin obtained from freshly harvested flower buds of living Indian hemp [33]. Unlike asrar, hashish is obtained from previously harvested or dried flowers. Asrar was most often smoked in a pipe, between layers of dry (on the bottom) and moist (on the top) tobacco. It was usually consumed socially, with the pipe passed from hand to hand with characteristic deep inhalations. Others smoked asrar in hookahs, and after just a few puffs, the opiate caused hallucinations in the user. Hashish and asrar were sold to gentlemen and ladies from the posh circles of Skopje in hidden places such as coffee grinders in Porečka Street, shantytowns around Mustafa Pasha Mosque, around Kuršumli Khan, and in Kujundžija Bazaar [b] [33, 34]. Muslims in Skopje often smoked hashish and asrar during bazaar breaks, from noon to three in the afternoon, with the inevitable strong, black coffee, which mixed with the sweet taste of narcotics [35]. With more frequent police raids, private houses in Skopje's Čair district became a trading hub [33].

The journalist from "Vreme" newspaper reported on the trial of the "dangerous drug addict" from Skopje, Jaʿfar Ismailović, who had decades of experience in selling hashish and nefes (kief, cannabis powder that is added to a pipe filled with tobacco). Ismailović spent part of his youth in Asia, where he got in touch with opium, hashish, nefes, and other narcotics. In 1934, the police raided Jaʿfar-agha's apartment in Relje Krilatice Street, where they found several motionless young men smoking nefes with two women and Ismailović himself in a trance. "He has been green in the face with very sunken eyes and a droopy lower lip." The old Ismailović gave the journalist the impression of a person with mental health problems, which was also recorded in the reports from the trial. In his defense, the accused said he was not guilty "because he got used to it from his youth and cannot live without nefes". In his testimony, one of the present young men described the session in which he participated after the agha promised them that "they will see many beautiful women in their dreams and that they will be pleased": "We were reclining on the asura. We lay down; Jaʿfar-agha filled his pipe and took the first drag. After that, he handed the pipe to the first person beside him so it went in order. Then we smoked another pipe, but I already could not see anything clearly around me. When I stretched, I saw a jug of water and knocked it over with my foot. I still remember that the pie dish seemed like a huge jar, and the water pouring out was like a big stream. After that, everything flowed like honey. I don't know anything else, namely, I remember, Mr. Judge, but I am ashamed to tell what I saw". Enjoyment was not punishable under the laws, so Jaʿfar-agha was sentenced to a symbolic penalty of suspended sentence and reimbursement of court costs. Having already served this sentence in custody, Ismailović left the courtroom as a free man, probably for a new dose of asrar [34].

Another case in Skopje, which turned into an affair in 1934, was the temporary absence from the stage of the prima donna of the Skopje theatre, Mary Podhraski [36]. In a series of articles, the actress herself publicly admitted that she had used cocaine, obtained from the local pharmacist no more than six months, and was, therefore, being treated in the Belgrade sanatorium of Dr. Stojimirović [c] under the diagnosis of depression: *dépression nerveuse* (*cocaionom et morphion*) [37–39]. The manager of the Skopje theatre, Vojinović, had to get involved in the affair and spoke of Meri Podhraska as an "extraordinary actress" esteemed by all her colleagues. Indeed, she was the only professional actress in the theatre, so she was forced to play all the leading female roles in the repertoire. In the continuation of the announcement, the manager describes the mental state of his diva, which arose as a result of a turbulent life outside the theatre: "Miss Podhraski herself wanted to leave Skopje and look for a cure for her headache, which she had been complaining about for the last few days. She could not sleep at night. She constantly talked about suicide and cried for the slightest thing [39]." However, even after returning from Belgrade, Podhraski was often absent from the scene for health reasons. Merchants and powerful patrons gifted her with narcotics, which, according to gossip, she continued to use [36]. This excellent actress, a star of the Zagreb, Novi Sad, and Sarajevo theatres, to whom both God and nature "gave everything: beauty, voice, expression, sensitivity" but with a shallow education, died on February 9, 1942, in Banja Luka as the prima donna of the Croatian State Theatre [36]. "Alcohol and opium destroyed her young body", Stanoje Dušanović wrote in his recollections. "Alcohol and drugs ruined her", said Pavle Bogatinčević. Svetolik Nikačević, her colleague from the stage, wrote in his memoirs "She fancied life excessively, and above all, she fancied the love" [36]. She died at the age of 33.

Treatment of psychoactive substance misuse

Data on addiction treatment is even scarcer than data on drug use. It is also understandable if we consider the frequency of the problem and the stigma among users and doctors to speak publicly about this problem. It seems that the wealthier ones, among whom there were the most beneficiar-

[b]Kujundžija is the old name for a goldsmith's craftsman who makes, repairs, or modifies jewelry and small decorative items from gold, silver, and other precious metals.

[c]Dr. Dušan Stojimirović (1870–1955) was one of the most respected psychiatrists in the country and the personal physician of Crown Prince Djordje.

ies, used the services of private doctors and sanatoriums, like Mrs. Podhraski; the rest chose state hospitals. Data from state hospitals includes the shortcoming that patients treated for drug abuse were recorded together with patients treated for alcohol abuse [40, 41].

In the Kovin Special Hospital, in the first decade of its operation (from 1924 to 1934), out of 1,800 people admitted for hospital treatment, only one person was treated under the diagnosis of drug addiction, while 37 patients had problems with alcohol [40]. In the Vrapče State Hospital in Zagreb in 1930, out of 2,096 patients, 32 had issues with alcohol and seven with drugs, of which all had a morphine addiction [41]. According to the estimate of the hospital director, Dr. Alexey Kulzhenko, one to two men and one woman a year began treatment for morphine misuse during the period between 1929 and 1932. In the early 1930s, about 35% of all psychiatric patients in Yugoslavia were treated at Vrapče Hospital. Based on this, we can estimate the frequency of this health problem in the entire country using data from one hospital [42].

Physicians from Zagreb, Dr. Koloman Sztraka and Dr. Radoslav Lopašić, according to their testimonies, successfully treated opium addiction with the mandatory cessation of drug consumption at the beginning of treatment. Dr. Sztraka used Dicodide® (generic name – dihydrocodeine) as a substitution, while cardiac symptoms were treated using Cardiazol® (generic name – pentetrazol). He applied substitution alternately with saline physiological solution as a placebo and psychotherapeutic support. Dr. Sztraka wrote about the importance of understanding each patient and the psychological phenomena behind addiction: "We doctors must get to know the patient psychologically from the ground up. Energetic and strong words, in addition to other treatments, are of the greatest importance. The doctor must penetrate to the bottom of the patient's soul; he must lift his tired spirit because, unfortunately, the character of these patients is not strong, and there is a danger that they will again reach for that poison and become its victim again. (...) We have to explain that the patient faces unforeseen risks and consequences when they fall back to their previous state; that is, if they retake morphine, they will finally perish!" When the desire appeared, the patient had to see a doctor who would prescribe one of the three therapies: replacement medicine, placebo, or psychological support. In the beginning, the daily number of visits was in double digits, but over time, it decreased and after about a month of abstinence, the patient would be considered cured [29, 43].

Conclusion

The Kingdom of Serbs, Croats, and Slovenes was a major producer of high-quality opium, as well as a crossroads of legal and illegal trade in psychoactive substances. However, abuse was rare and exclusively in the largest cities, where it was presented as a status symbol in the high society. Opium, opium derivatives, and cocaine were most commonly consumed. People with addiction problems were treated in private sanatoriums and state hospitals. The results of the treatment depended primarily on their ability to maintain abstinence in the circle in which they acquired this life-threatening habit.

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Received on January 9, 2024
Accepted on February 20, 2024
Online First March 2024

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

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