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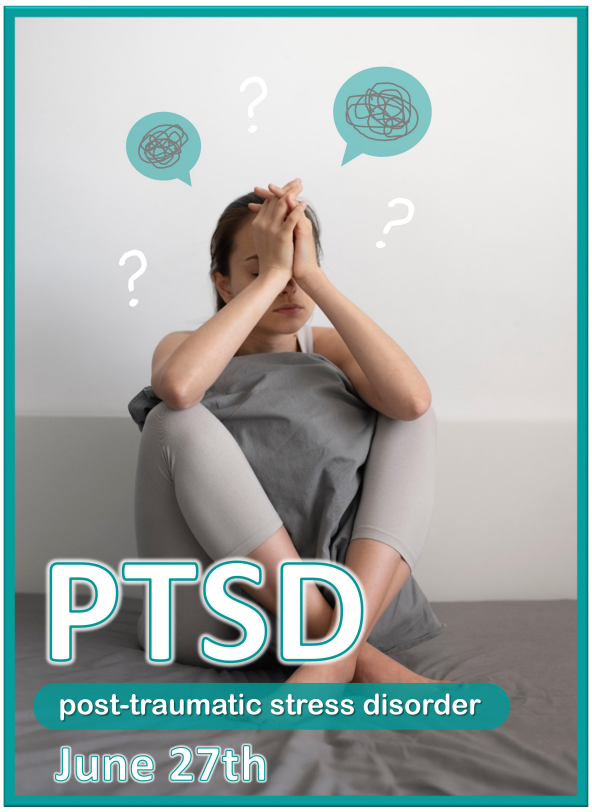

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CONTENTS / SADRŽAJ

HISTORY OF MEDICINE / ISTORIJA MEDICINE

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On the occasion of June 27, the day dedicated to post-traumatic stress disorder (PTSD), attention is drawn to this increasingly present, complex, disabling mental disorder that arises from experienced or witnessed trauma. Traumas that can cause PTSD can be various accidents (war conflicts, natural disasters, traffic accidents, sexual violence, abuse). PTSD causes both individual psychological consequences and consequences that represent a social "burden" (impairment of interpersonal relationships, reduction of work productivity). The more we know about PTSD and how to treat it, the more people affected by the disorder will know that help is available. Art therapy and drawings can serve as alternative means of expression and relief from trauma among veterans diagnosed with PTSD (you can read more on this topic in the paper by Mandić-Gajić and Špirić – DOI: 10.2298/VSP150512083M).

Povodom 27. juna, dana posvećenog posttraumatskom stresnom poremećaju (PTSP), podsećamo na ovaj, sve više prisutan, kompleksan, onesposobljavajući mentalni poremećaj koji nastaje usled doživljene traume ili traume čiji smo bili svedoci. Traume koje mogu izazvati PTSP mogu biti različite nesreće (ratni sukobi, prirodne katastrofe, saobraćajne nesreće, seksualno nasilje, zlostavljanje). PTSP uzrokuje i individualne psihološke posledice i posledice koje predstavljaju društveni "teret" (narušavanje međuljudskih odnosa, smanjenje radne produktivnosti). Što više znamo o PTSP i načinima njegovog lečenja, to će više osoba koje su pogođene ovim poremećajem znati da pomoć postoji. Art terapija i crteži mogu poslužiti kao alternativna sredstva izražavanja i oslobađanja od trauma među veteranima kod kojih je dijagnostikovan PTSP (više o ovoj temi možete pročitati u radu Mandić-Gajić i Špirić – DOI: 10.2298/VSP150512083M).

ORIGINAL ARTICLES

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Protective effect of dexmedetomidine combined with remifentanil on perioperative brain tissue in patients with severe traumatic brain injury and its influence on serum inflammatory markers

Zaštitno dejstvo deksmedetomidina u kombinaciji sa remifentanilom na perioperativno moždano tkivo kod pacijenata sa teškom traumatskom povredom mozga i uticaj na markere inflamacije u serumu

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Abstract

Background/Aim. Patients with a severe traumatic brain injury (TBI) demand intensive monitoring and treatment due to significant brain trauma or other accompanying causes, such as comorbidities or polytrauma. Patients with such injuries are under intense stress, leading to increased sympathetic excitability, and often experience agitation and pain. Appropriate sedation and analgesia are crucial for these patients, as they can reduce complications, mortality, and sequelae and improve quality of life. The aim of this study was to examine the impact of dexmedetomidine combined with remifentanil on postoperative sedation, analgesia, and cerebral oxygen metabolism in patients with TBI. **Methods.** A prospective, single-blind, randomized, controlled clinical study included 80 patients divided into two groups: a control group (CG) that received dexmedetomidine (n = 40) and an observation group (OG) that received dexmedetomidine combined with remifentanil (n = 40). **Results.** Compared to CG, OG demonstrated superior sedation and analgesia, reduced sedation and mechanical ventilation durations, and lower heart rate, mean arterial pressure, and respiratory rate. Additionally, OG showed statistically greater reductions in inflammatory markers and serum cortisol levels and higher β-endorphin levels. Cerebral oxygen metabolism indices also improved more in the OG postoperatively, although the differences were not statistically significant. **Conclusion.** Sedation and pain management strategy using of dexmedetomidine combined with remifentanil improved patient outcomes by speeding recovery and reducing physiological stress. Additional research is needed to determine the long-term effects of this combination on brain oxygen metabolism.

Key words:
analgesia; brain; craniocerebral trauma; deep sedation; dexmedetomidine; quality of life; remifentanil; treatment outcome.

Apstrakt

Uvod/Cilj. Pacijente sa teškom traumatskom povredom mozga (TBI) treba intenzivno pratiti i lečiti zbog značajne traume mozga odnosno drugih pratećih uzroka, kao što su komorbiditeti ili politrauma. Pacijenti sa takvim povredama su pod intenzivnim stresom, što dovodi do povećane ekscitabilnosti simpatikusa i čestog doživljaja uznemirenosti i bola. Odgovarajuća sedacija i analgezija ključne su za te pacijente jer mogu smanjiti komplikacije, mortalitet, posledice i poboljšati kvalitet života. Cilj rada bio je da se ispita uticaj deksmedetomidina u kombinaciji sa remifentanilom na postoperativnu sedaciju, analgeziju i cerebralni metabolizam kiseonika kod pacijenata sa TBI. **Metode.** Prospektivnom, jednostruko slepom, randomizovanom kontrolisanom kliničkom studijom obuhvaćeno je 80 bolesnika, podeljenih u dve grupe: kontrolnu grupu (KG) koja je primala deksmedetomidin (n = 40) i ispitivanu grupu (IG) koja je primala deksmedetomidin u kombinaciji sa remifentanilom (n = 40). **Rezultati.** U poređenju sa KG, IG je pokazala bolju sedaciju i analgeziju, skraćeno trajanje sedacije i mehaničke ventilacije, kao i sporiji srčani ritam, niži srednji arterijski pritisak i frekvenciju disanja. Takođe, u IG je utvrđeno statistički značajnije sniženje nivoa inflamacijskih markera i nivoa kortizola u serumu, kao i viši nivo β-endorfina. Takođe, indeksi cerebralnog metabolizma kiseonika su pokazali veće postoperativno

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poboljšanje u IG, mada razlika nije bila statistički značajna. **Zaključak.** Strategija sedacije i upravljanja bolom korišćenjem deksmedetomidina u kombinaciji sa remifentanilom poboljšava ishode lečenja pacijenata tako što ubrzava oporavak i smanjuje fiziološki stres. Potrebna su dodatna istraživanja da bi se utvrdili dugoročni efekti ove kombinacije na metabolizam kiseonika u mozgu.

Ključne reči:
analgezija; mozak; kraniocerebralne povrede; sedacija, duboka; deksmedetomidin; kvalitet života; remifentanil; lečenje, ishod.

Introduction

Patients with severe traumatic brain injury (TBI) need intensive monitoring and treatment due to severe brain trauma or other causes of severe comorbidity illness [1]. Patients with severe TBI experience heightened sympathetic excitability due to intense stress, often resulting in varying levels of agitation and pain, as demonstrated by numerous clinical studies [2, 3]. Undeniably, patients with severe craniocerebral injuries (CI) should receive appropriate sedation and analgesia, and this treatment is safe and effective [4, 5]. For patients with severe CI, appropriate sedation and analgesic treatment can prevent complications, significantly reduce mortality and sequelae, and enhance patients' quality of life [6, 7]. However, there are still many concerns and debates about the need for sedation and analgesia in clinical practice, and the implementation of sedation and analgesia in different hospitals is also very different. At present, the *status quo* of sedation and analgesia treatment for patients with CI is still in a difficult situation. Therefore, it is crucial to explore a better treatment strategy that can provide more solutions for the clinical management of agitated CI patients.

Frequent agitation and acute pain are more common in the postoperative period of patients with severe craniocerebral trauma (CCT), which can indirectly or directly cause TBI [8–10]. Effective sedation and analgesia management in severe CCT enhances patient comfort, reduces stress responses, and protects brain function [11]. Implementing analgesic treatment in patients with severe TBI remains a significant clinical challenge. The primary focus in administering sedation and analgesia to patients with TBI is ensuring safety, followed by emphasizing individualized treatment approaches [12, 13]. Some studies have shown that dextromethorphan injection can lead to dose-related sedation, analgesia, and anxiolysis, with minimal impact on hemodynamics and a mild effect on respiration. Additionally, it can cause a unique state of sedation that allows for arousal, which is very suitable for neurosurgical patients experiencing pain and agitation. However, there is a phenomenon of analgesic insufficiency [14–16]. Pure μ-receptor agonist remifentanil was characterized by a fast onset of action, high potency, and short half-life, which is expected to compensate for the analgesic deficiency of dexmedetomidine alone [17]. In short, the application of analgesic and sedative drugs is indispensable for patients with severe CI and those who have undergone surgery. Therefore, it is necessary to explore how to retain the favorable factors and avoid the harmful factors in order to better treat patients with severe CI and prolong their lives.

Numerous clinical studies indicate that hypertension and significant blood pressure fluctuations are critical risk factors for rebleeding in TBI patients. Hemodynamic fluctuations associated with hypertension can damage blood vessel walls, leading to arterial hardening and reduced elasticity, increasing the risk of rupture during severe blood pressure changes. Persistent high blood pressure following hemorrhage complicates the cessation of bleeding [18, 19]. In addition, the permeability of blood vessel walls increases, which leads to fluid leakage into the brain tissue. However, because the skull is a rigid, closed structure with limited capacity, this added volume raises intracranial pressure, potentially resulting in cerebral herniation [20].

The aim of this study was to examine the impact of dexmedetomidine combined with remifentanil on postoperative sedation, analgesia, and cerebral oxygen metabolism ($CMRO_2$) indices in patients with TBI.

Methods

Study design

This prospective, single-blind, randomized controlled clinical study included 80 patients with severe TBI admitted to the hospital's Intensive Care Medicine Department, Gansu University of Chinese Medicine, China. The patients were randomly divided into two groups using computer-generated values. This selection was based on pre-test results and relevant literature, with sample size estimated using PASS 11.0.7 software. The study was approved by the Ethics Committee of Gansu University of Traditional Chinese Medicine (protocol: GSUSYT; dated May 21, 2022). All experiments were performed in accordance with the Declaration of Helsinki.

Successive steps of treatment with indications for mechanical ventilation

Upon admission to the Intensive Care Unit (ICU), all patients received a standardized treatment protocol based on international guidelines for severe CI management. Mechanical ventilation was initiated in patients who met any of the following criteria: Glasgow Coma Scale (GCS) score ≤ 8 with signs of airway compromise; respiratory failure, indicated by a partial pressure of oxygen (PaO_2) < 60 mm of mercury (mmHg) or a $PaCO_2$ > 50 mmHg despite oxygen therapy; hemodynamic instability requiring vasopressor support; severe agitation compromising patient safety and treatment adherence; need for neuromuscular blockade due to increased intracranial pressure or refractory seizures.

Criteria for initiating analgesia and sedation

Analgesia and sedation were initiated based on predefined criteria, which included the following: Riker Sedation-Agitation Scale (SAS) score ≥ 5, indicating agitation requiring intervention; Non-Verbal Pain Scale (NVPS) score ≥ 3, suggesting significant pain; clinical signs of discomfort, such as tachycardia, hypertension, or excessive movement; prevention of secondary brain injury due to excessive stress responses (e.g., intracranial hypertension, metabolic derangements); facilitation of mechanical ventilation and patient-ventilator synchrony.

Inclusion criteria were as follows: patients who have had their diagnosis confirmed by imaging; patients with a GCS score of 1–8 to ensure feasible sedation and analgesia scores; patients requiring sedation and analgesia; those who have not herniated their brains and no history of allergy to sedative or analgesic medications.

Exclusion criteria were as follows: those who are critically ill and expected to die within 24 hrs; patients with difficult hemodynamic control; patients with significant hepatic, pulmonary, and renal insufficiency; patients with a history of cardiac disease, cerebral dysfunction, or diabetes mellitus; patients who are pregnant or breastfeeding; patients with brain stem injury and patients with penetrating brain injury or spinal cord injury.

Subgroups and interventions

The patients' basic treatment after admission was routinely handled in accordance with the guidelines for the treatment of craniocerebral diseases, and they were given treatment plans such as dehydration and diuresis, prevention and treatment of epilepsy, nutritional neurology, and maintenance of the stability of the internal environment. No significant differences were observed between the two groups regarding other treatments, including pharmacologic interventions and support for cardiopulmonary and other organ functions, except for variations in medications used for intervening factors. During the study period, a total of 115 patients with severe CI requiring sedation and analgesia were enrolled in our department. Of these, 12 died within 24 hrs, circulation could not be stabilized despite various treatments in 10 patients, and the families of 13 patients chose to discontinue treatment midway. A total of 80 participants were enrolled in the study, with patients needing sedation and analgesia included after assessment. These patients were randomly assigned using computer-generated numbers into two groups: the control group (CG), receiving dexmedetomidine alone (n = 40), and the observation group (OG), receiving dexmedetomidine combined with remifentanil (n = 40).

Discontinuation indications

Discontinuation of sedation and analgesia was considered under the following conditions: blood pressure stabilized at 65–100 mmHg (1 mmHg = 0.133 kPa); normal blood gas parameters including pH 7.35–7.45, partial pressure of carbon dioxide (PCO_2) 4.65–5.98 kPa, total carbon dioxide (TCO_2) 24–32 mmHg, partial pressure of oxygen (PO_2) 10.64–13.3 kPa, oxygen saturation ($SatO_2$) 3.5 kPa, actual bicarbonate 21.4–27.3 mmol/L, standard bicarbonate 21.3–24.8 mmol/L, residual base –3 to 3 mmol/L, and an anion gap of 8–16 mmol/L; stable respiratory function without any clinical indications for continued sedation or analgesia based on assessment scores; ventilatory exercises were initiated or preparation for weaning was underway; if the patient had died due to treatment failure, rendering further sedation or analgesia unnecessary.

Analgesic sedation evaluation

The degree of analgesic sedation of patients was assessed using NVPS and SAS. The analgesia and sedation objectives were established, with pain assessment conducted using the NVPS. This scale evaluates facial expression, activity, defensive action, and physiological indicators like blood pressure, heart rate, and respiratory rate on a 0–10-point scale. The analgesia target was set at an NVPS score of less than 3. Agitation was evaluated using the SAS rating scale, defining SAS ≥ 5 points as agitation. The goal of sedation was SAS < 5 points (SAS scoring scale: 1 point – unable to arouse; 2 points – very sedated; 3 points – sedated; 4 points – quiet and cooperative; 5 points – agitated; 6 points – very agitated; 7 points – dangerously agitated).

Statistical analysis

Data were compiled using a specialized database and analyzed with SPSS 19.0 software. Prior to analysis, a normality test was conducted. Data following a normal distribution were presented as mean ± standard deviation, whereas non-normally distributed data were represented as median (95% confidence interval). Qualitative data were expressed as relative frequencies. Data following a normal distribution were analyzed using Analysis of Variance (ANOVA) or *t*-tests for quantitative variables and Chi-square tests for qualitative variables. Non-parametric tests were applied to both quantitative and qualitative data that did not follow a normal distribution. The test level was $\alpha = 0.05$, and the difference was considered statistically significant at $p < 0.05$ and also at $p < 0.01$.

Results

Clinical data of study patients

The baseline characteristics of the clinical patients in OG and CG, including gender, age, body mass index (BMI), time from injury to surgery, duration of surgery, diagnosis type, and the American Society of Anesthesiologists (ASA) classification, showed no significant differences. There were no statistically significant differences between the two groups in terms of gender ratio ($p = 0.676$), age ($p = 0.735$), BMI ($p = 0.198$), injury-to-operation time ($p = 0.603$), dura-

tion of surgery (p = 0.949), diagnostic type distribution (p = 0.922), and ASA grading (p = 0.885) (Table 1). This suggests that the two groups were comparable in terms of baseline characteristics and provides a good control basis for subsequent clinical studies or comparisons of treatment outcomes.

Assessment of the sedative impact of remifentanil on patients with craniocerebral injuries

The sedative impact of remifentanil on patients with CI was evaluated to assess the drug's impact on sedation and compare sedation score changes between OG and CG pre- and post-administration. The study found no significant difference in sedation effect scores between OG and CG prior to drug administration (t = 0.685, p = 0.495). OG exhibited significantly lower sedation effect scores compared to CG at 2 hrs (t = 3.273, p = 0.002), 4 hrs (t = 4.343, p < 0.001), and 12 hrs (t = 4.506, p < 0.001) post-administration. OG exhibited a significantly superior sedative effect compared to CG following drug administration (Table 2).

Effect of remifentanil on the time required for sedation and the duration of mechanical ventilation in patients with traumatic brain injury

The study results show that OG had a notably shorter sedation time than CG. Specifically, the mean sedation time for OG was 27.65 ± 5.36 min, while CG had a mean sedation time of 31.46 ± 5.81 min. The comparison yielded a t-value of 3.959, demonstrating a statistically significant difference between the groups (p = 0.0001). OG experienced a reduced mean mechanical ventilation time of 10.74 ± 4.13 hrs, in contrast to the CG mean of 13.15 ± 6.97 hrs. The t-value for mechanical ventilation time was 2.304, which was also statistically significant (p = 0.023) (Table 3). These findings suggest that the sedation protocol used in OG resulted in more rapid recovery and reduced the need for mechanical ventilation.

Evaluation of remifentanil's analgesic effectiveness in patients with craniocerebral injuries

Analgesic effect scores were compared between the OG and CG at various time points. Prior to dosing, pain scores

Table 1

Comparative analysis of clinical data between the observation and control groups

Parameters	Observation group (n = 40)	Control group (n = 40)	t/χ^2	p
Gender				
male	21	18	0.175	0.676
female	19	22		
Age, years	45–68 (56.45 ± 5.74)	43–69 (56.02 ± 6.37)	0.340	0.735
BMI, kg/m^2	22–28 (25.12 ± 1.58)	23–28 (25.51 ± 1.29)	1.297	0.198
Time from injury to surgery, hrs	3–18 (10.52 ± 3.76)	3–17 (10.12 ± 3.58)	0.523	0.603
Duration of surgery, min	89–184 (136.25 ± 23.74)	92–180 (135.94 ± 22.17)	0.065	0.949
Diagnosis				
epidural hematoma	16 (34.78)	14 (30.43)		
subdural hematoma	13 (28.26)	16 (34.78)		
brain contusion	12 (26.09)	11 (23.91)	0.487	0.922
simple comminuted depression fracture of the skull	5 (10.87)	5 (10.87)		
American Society of Anesthesiologists				
Phase I	21 (45.65)	20 (43.48)		
Phase II	16 (34.78)	17 (36.96)	0.145	0.885
Phase III	9 (19.57)	9 (19.57)		

BMI – body mass index; min – minutes.
All values are given as numbers (percentages) and range (mean ± standard deviation).

Table 2

Assessment sedative impact of remifentanil pre- and post-administration of the drug between the two groups of patients with traumatic brain injury

Group	Before dosing	After drug administration		
		2 hrs	4 hrs	12 hrs
Observation	5.51 ± 1.02	3.84 ± 0.82$^{\#}$	4.02 ± 0.75	4.05 ± 0.81
Control	5.34 ± 1.34	4.41 ± 0.85	4.79 ± 0.94	4.83 ± 0.85
t	0.685	3.273	4.343	4.506
p	0.495	0.002**	< 0.001***	< 0.001***

All values are given as mean ± standard deviation.
Note: $^{\#}p$ < 0.05 indicates the difference between the time before dosing and 2 hrs after drug administration in the observation group. ** p < 0.01 indicates the difference between the observation and control groups 2 hrs after drug administration.
***p < 0.001 indicates the difference between the observation and control groups, 4 or 12 hrs after drug administration.**

were comparable between OG (8.45 ± 1.76) and CG (8.58 ± 1.84), showing no significant difference. Notable differences were detected at 2, 4, and 12 hrs post-drug administration. After 2 hrs, the OG mean score was 3.44 ± 0.52, significantly lower than the CG, 4.51 ± 0.62 (t = 8.968, p < 0.001), indicating reduced pain in OG. After 4 hrs, OG had a mean score of 4.15 ± 0.66, compared to CG, 4.94 ± 0.91, with a t-value of 4.766 and a p-value of < 0.001, indicating a significant difference in pain reduction. At 12 hrs, OG had a mean score of 3.45 ± 0.71, while CG scored 4.53 ± 0.65. The t-value was 7.610, with p < 0.001, indicating a significant analgesic effect in OG (Table 4). OG consistently demonstrated a stronger analgesic effect than CG at all post-administration time points.

Analysis of vital signs in observation group and control group

Our data revealed notable variations in heart rate, mean arterial pressure, and respiratory rate between OG and CG, both before and after drug administration. Before drug administration, the two patient groups showed no significant differences in heart rate, mean arterial pressure, or respiratory rate. Heart rate, mean arterial pressure, and respiratory rate were significantly lower in OG compared to CG at both 6 and 12 hrs post-dosing. Six hrs post-drug administration, OG exhibited reductions in heart rate (117.41 to 89.24 beats/min), mean arterial pressure (98.74 to 85.41 mmHg), and respiratory rate (25.02 to 20.19 breaths/minute). At 12 hrs post-dose, OG heart rate was further reduced to 88.21 beats/minute, mean arterial pressure to 80.54 mmHg, and respiratory rate to 17.14 breaths/minute. In contrast, in CG, heart rate decreased from 116.94 to 95.54 beats/minute, mean arterial pressure decreased from 99.14 to 89.94 mmHg, and respiratory rate decreased from 25.43 to 22.20 breaths/minute at 6 hrs post-dose. In CG, heart rate decreased to 89.14 beats/minute, mean arterial pressure decreased to 86.17 mmHg, and respiratory rate decreased to 20.52 breaths/minute at 12 hrs post-dose (Table 5). OG expe-

Table 3

Effect of remifentanil on the time required for sedation and the duration of mechanical ventilation between the two groups of patients with traumatic brain injury

Group	Sedation time (min)	Mechanical ventilation time (hrs)	t/χ^2	p
Observation	27.65 ± 5.36	10.74 ± 4.13	3.959	< 0.001***
Control	31.46 ± 5.81	13.15 ± 6.97	2.304	0.023*

All values are given as mean ± standard deviation.
***p* < 0.05 indicates the difference between sedation and mechanical ventilation time in the control group. ***p* < 0.001 indicates the difference between sedation and mechanical ventilation time in the observation group.**

Table 4

Evaluation of remifentanil analgesic effectiveness in patients with TBI between the two groups

Group	Before dosing	After drug administration		
		2 hrs	4 hrs	12 hrs
Observation	8.45 ± 1.76	3.44 ± 0.52###	4.15 ± 0.66	3.45 ± 0.71
Control	8.58 ± 1.84	4.51 ± 0.62##	4.94 ± 0.91	4.53 ± 0.65
t	0.346	8.968	4.766	7.610
p	0.730	< 0.001***	< 0.001***	< 0.001***

TBI – traumatic brain injury.
All values are given as mean ± standard deviation.
Note: ##*p* < 0.01 indicates the difference between the time before dosing and 2 hrs after drug administration in the observation group. ###*p* < 0.001 indicates the difference between before dosing and 2 hrs after drug administration in the control group. ***p* < 0.001 indicates the difference between the observation and control groups 2, 4, or 12 hrs after drug administration.

Table 5

Analysis of vital signs before and after administration of remifentanil between the two groups of patients with TBI

Group/time after administration	Heart rate (beats/min)	Mean arterial pressure (mmHg)	Respiratory rate (breats/min)
Observation			
before dosing	117.41 ± 13.62	98.74 ± 9.85	25.02 ± 1.34
6 hrs	89.24 ± 7.25*	85.41 ± 8.27*	20.19 ± 1.50*
12 hrs	88.21 ± 10.25	80.54 ± 9.41b	17.14 ± 1.42b
Control			
before dosing	116.94 ± 15.98	99.14 ± 9.92	25.43 ± 1.41
6 hrs	95.54 ± 6.26	89.94 ± 9.12	22.20 ± 1.37
12 hrs	89.14 ± 11.19	86.17 ± 10.25	20.52 ± 1.59

TBI – traumatic brain injury.
All values are given as mean ± standard deviation.
Note: *p* < 0.05 indicates the difference between before dosing and 6 hrs after drug administration in the observation group.

rienced a more pronounced reduction in heart rate, mean arterial pressure, and respiratory rate compared to CG following drug administration, indicating a potentially faster recovery and a more significant impact on physiological indices.

Comparison of inflammatory stimulation indices between observation group and control group

Inflammatory stimulation indices were compared between OG and CG at various time points. Before dosing, both groups exhibited similar baseline levels of C-reactive protein (CRP), tumor necrosis factor (TNF)-α, interleukin (IL)-6, and IL-10, indicating comparable inflammation. OG showed significant decreases in all inflammatory markers 6 hrs after drug administration compared to baseline (CRP, TNF-α, IL-6, IL-10; $p < 0.01$ for each). Specifically, OG showed a 46.15% decrease in CRP, a 20.80% decrease in TNF-α, a 25.99% decrease in IL-6, and a 22.06% decrease in IL-10. In contrast, CG demonstrated a less pronounced reduction in these markers, with CRP decreasing by 33.22%, TNF-α by 15.97%, IL-6 by 22.60%, and IL-10 by 12.58%. Twelve hrs after drug administration, OG exhibited a significantly greater reduction in inflammatory markers than CG, with CRP levels decreasing by 78.39% ($p < 0.01$), TNF-α by 27.09% ($p < 0.01$), IL-6 by 33.32% ($p < 0.01$), and IL-10 by 30.17% ($p < 0.01$) (Table 6). The intervention in OG more effectively suppressed the inflammatory response at both 6- and 12-hr intervals compared to CG.

Analysis of serum cortisol and β-endorphin levels in observation group and control group

Evaluations were conducted to assess the variations in serum cortisol and β-endorphin levels between OG and CG at various time points surrounding drug administration. The findings indicated no significant pre-dosing difference in cortisol levels between the groups ($p = 0.352$). Cortisol levels in OG were significantly lower than those in CG at both 6 and 12 hrs post-drug administration ($p < 0.001$). There was no significant difference in β-endorphin levels between the groups prior to dosing ($p = 0.379$). At both 6 and 12 hrs post-administration, β-endorphin levels in OG were significantly elevated compared to CG ($p < 0.001$) (Table 7). The drug administration significantly decreased cortisol levels and increased β-endorphin levels in OG compared to CG.

The study found significant differences in cortisol levels between the two groups at 6 hrs ($t = 7.475$, $p < 0.001$) and 12 hrs ($t = 6.060$, $p < 0.001$) post-administration, with OG exhibiting lower levels than CG. Significant differences in β-endorphin levels were noted at 6 hrs ($t = 7.669$, $p < 0.001$) and 12 hrs ($t = 7.214$, $p < 0.001$) post-drug, with OG showing higher levels than CG. However, no significant differences were found in cortisol and β-endorphin levels between the groups prior to dosing (cortisol: $t = 0.936$, $p = 0.352$; β-endorphin: $t = 0.884$, $p = 0.379$) (Table 7). OG exhibited significantly reduced cortisol levels and increased β-endorphin levels compared to CG at both 6 and 12 hrs post-administration.

Table 6

Comparison of inflammatory stimulation indices before and after administration of remifentanil between the two groups of patients with TBI

Group/time after administration	CRP (mg/L)	TNF-α (pg/mL)	IL-6 (pg/mL)	IL-10 (pg/mL)
Observation				
before dosing	19.04 ± 6.52	122.63 ± 32.58	385.94 ± 65.21	96.37 ± 10.23
6 hrs	10.25 ± 2.13*	97.14 ± 13.41*	285.61 ± 35.69*	75.12 ± 12.84*
12 hrs	4.12 ± 1.74*	89.41 ± 12.04*	257.41 ± 31.26*	67.31 ± 13.15*
Control				
before dosing	20.17 ± 5.98	128.94 ± 35.12	390.94 ± 68.26	97.15 ± 10.98
6 hrs	13.47 ± 3.05	108.34 ± 15.26	302.59 ± 37.28	84.92 ± 14.87
12 hrs	6.08 ± 1.90	98.94 ± 15.69	280.47 ± 35.29	76.27 ± 17.83

TBI – traumatic brain injury; CRP – C-reactive protein; TNF – tumor necrosis factor; IL – interleukin.
All values are given as mean ± standard deviation.
Note: *$p < 0.05$ indicates the different levels of CRP or TNF-α between before dosing and 6 and 12 hrs after drug administration in the observation group.

Table 7

Analysis of serum cortisol and β-endorphin levels before and after administration of remifentanil between the two groups of patients with traumatic brain injury

Group	Cortisol (μg/L)			β-endorphin (ng/L)		
	before dosing	after administration		before dosing	after administration	
		6 hrs	12 hrs		6 hrs	12 hrs
Observation	276.94 ± 24.83	156.83 ± 20.14	142.63 ± 18.12	286.32 ± 32.94	348.12 ± 12.69	341.26 ± 10.85
Control	282.16 ± 28.52	192.36 ± 25.17	176.58 ± 23.94	292.14 ± 30.12	331.07 ± 14.25	324.17 ± 11.85
t	0.936	7.475	6.060	0.884	7.669	7.214
p	0.352	< 0.001***	< 0.001***	0.379	< 0.001***	< 0.001***

TBI – traumatic brain injury.
All values are given as mean ± standard deviation.
Note: ***$p < 0.001$ indicates the different levels of cortisol or β-endorphin between the observation and control groups 6 hrs or 12 hrs after drug administration.

Table 8

Analysis of pre- and postoperative cerebral oxygen metabolism indices between observation and control groups

Group	$SjvO_2$ (%)		Da-jvO_2 (ml/L)		$CERO_2$ (%)	
	pre-op	72hrs post-op	pre-op	72hrs post-op	pre-op	72hrs post-op
Observation	50.96 ± 3.42	61.37 ± 4.15#	5.39 ± 1.88	4.14 ± 1.09#	40.27 ± 7.54	29.17 ± 5.79#
Control	51.07 ± 3.29	58.21 ± 3.54#	5.41 ± 1.87	4.97 ± 1.15#	40.34 ± 7.26	32.40 ± 6.38#
t	0.180		0.058		0.052	
p	0.858		0.954		0.959	

$SjvO_2$ – jugular venous oxygen saturation; Da-jvO_2 – difference in arteriovenous oxygen; $CERO_2$ – cerebral oxygen extraction ratio; pre-op – preoperative; post-op – postoperative.
All values are given as mean ± standard deviation.
Note: #$p < 0.05$ indicates the different levels of SjvO2, Da-jvO2, or CERO2 between pre-op and 72 hrs *post*-op measurements in the observation and control groups.

Pre- and postoperative cerebral oxygen metabolism indices compared between the observation group and control group

Preoperative $CMRO_2$ indices showed no significant difference between OG and CG. Seventy-two hrs post-operation, OG showed significant improvements in both jugular venous oxygen saturation ($SjvO_2$) and cerebral oxygen extraction ratio ($CERO_2$) indices compared to preoperative levels, whereas CG exhibited significant improvement only in the $SjvO_2$ index. Additionally, the difference in arteriovenous oxygen (Da-jvO_2) indices decreased in both groups, with a more pronounced reduction in OG. Statistical analysis indicated no significant postoperative differences in $SjvO_2$, Da-jvO_2, and $CERO_2$ indices between the two groups (Table 8). The results of our study revealed that surgery had a certain effect on $CMRO_2$ indices in both groups, but the improvement of $CMRO_2$ in OG was more pronounced during the postoperative period. The difference lacked statistical significance, necessitating further research for verification.

Discussion

Patients with severe TBI require intensive monitoring and treatment due to trauma, hemorrhage, ischemic stroke, intracranial infection, brain tumors, or other conditions [21]. Severe trauma can cause irreversible central nervous system damage and activate sympathetic nerves, initiating a cascade of inflammatory responses. This leads to the release of numerous inflammatory mediators, which increase the permeability of the blood-cerebrospinal fluid barrier, resulting in cerebral edema and neuronal damage or apoptosis. Additionally, this disrupts the balance between pro-inflammatory and anti-inflammatory systems, potentially inducing systemic inflammatory response syndrome, which significantly impacts patient prognosis [22–25]. Currently, there are more clinical anesthesia programs for craniocerebral surgery; however, a unified treatment plan is still lacking. Although traditional anesthetics can meet the requirements for analgesia and sedation, they have no clear effect on the inflammatory progression following TBI and can suppress the patient's nervous system to some extent. This suppression may affect the accuracy and objectivity of neurological examinations and condition assessments [26, 27]. Therefore, effective sedation and analgesia management for patients with severe CCT is particularly critical.

Dexmedetomidine plays an effective role in analgesia, sedation, and anxiolysis and can also inhibit sympathetic activity, thereby placing the patient in a sleep-like state of arousal [28, 29]. However, some studies have indicated that dexmedetomidine alone is insufficient for analgesia and that fentanyl and propofol should be administered intermittently to maintain the best analgesic and sedative effects [30]. Remifentanil is a potent narcotic analgesic, comparable in potency to fentanyl and 60–80 times stronger than morphine. It shares a similar mechanism of action with morphine and is characterized by a rapid onset and no accumulation in the body during continuous infusion. This makes it suitable for prolonged intraoperative analgesia through continuous administration [31]. This study evaluated the use of remifentanil for postoperative sedation and analgesia in patients with severe CCT. Results indicated that OG had lower sedation and analgesia scores at 2, 4, and 12 hrs post-administration compared to CG. Additionally, OG exhibited lower heart rate, mean arterial pressure, and respiratory rate at 6 and 12 hrs post-administration. Remifentanil enhances sedation and analgesia in severe CCT patients with minimal impact on respiratory function. This may be because remifentanil, as a pure mu-opioid receptor agonist, has a strong analgesic effect, effectively compensating for the limited analgesic efficacy of dexmedetomidine and jointly maintaining a stable sedative-analgesic state.

In patients with severe CCT, the adrenergic sympathetic nervous system is significantly activated, exacerbating pain and oxidative stress injury due to various pathological factors [32]. Serum cortisol evaluates the degree of adrenergic activation and contributes to neuronal damage through the production of oxygen-free radicals, while β-endorphin expression inhibits the circulatory and respiratory centers, resulting in impaired regulation of the cardiovascular center [33]. The study indicates that 6 and 12 hrs post-drug administration, OG exhibited lower cortisol levels and higher β-endorphin levels compared to CG. This suggests that remifentanil effectively reduces cortisol and elevates β-endorphin levels during postoperative dexmedetomidine sedation and analgesia in patients with severe CCT. Remifentanil enhances central analgesia by inhibiting the release of endogenous opioid peptides and suppressing sympathetic-adrenomedullary activity,

thereby modulating cortisol and β-endorphin levels. Elevated levels of CRP, TNF-α, IL-6, and IL-10 are key indicators of inflammation, which can exacerbate brain tissue damage and cerebral vascular edema [34]. The study found that at 6 and 12 hrs post-drug administration, OG exhibited lower levels of CRP, TNF-α, IL-6, and IL-10 compared to CG, indicating that remifentanil effectively reduces inflammatory stress in patients with severe CCT. This may be attributed to the use of remifentanil in dexmedetomidine-based sedation and analgesia management, which enhances the overall sedative and analgesic effect. Effective sedation and analgesia can inhibit the release of inflammatory factors, suppress intracellular cyclic-phosphate adenosine, and limit lymphocyte proliferation, which in turn reduces inflammatory stimulation within the body. Remifentanil enhances sedation and analgesia, mitigates inflammatory responses, regulates cortisol and β-endorphin levels, and does not increase the incidence of postoperative cognitive dysfunction in postoperative care following severe CCT, making it a suitable option for clinical application [35].

In our study, we evaluated neurological recovery, GCS improvement, and ICU length of stay. Regarding the effects of dexmedetomidine, we analyzed its impact on sedation level, hemodynamic stability, intracranial pressure, cerebral perfusion pressure, or adverse events (e.g., hypotension and bradycardia). Our findings indicate that dexmedetomidine provided adequate sedation with minimal impact on cerebral perfusion pressure in mild/moderate TBI but required careful hemodynamic monitoring in severe cases.

Despite comprising just 2% of total body mass, the human brain has a high metabolic rate, with cerebral blood flow constituting 13.9% of cardiac output. Neurons are highly sensitive to energy disruptions, making the central nervous system particularly vulnerable to damage and $CMRO_2$ abnormalities following TBI. Currently, common clinical indicators for monitoring $CMRO_2$ include $SjvO_2$ [36], Da-jvO_2, and $CERO_2$ [37]. $SjvO_2$ serves as the "gold standard" for assessing $CMRO_2$, indicating overall cerebral blood flow and oxygen utilization, and significantly declines after TBI. Both Da-jvO_2 and $CERO_2$ effectively measure cerebral oxygen uptake [38] and indicate the extent of oxygen uptake or consumption by brain tissues [37]. In patients with TBI, the cerebral oxygen demand often exceeds supply, necessitating enhanced oxygen extraction from the blood, resulting in elevated Da-jvO_2 and $CERO_2$ levels. In addition, TBI causes central nervous system cells to produce inflammatory mediators, inducing a series of cascade reactions in the body. Certain inflammatory factors modulate leukocyte activation and aggregation *via* the blood-cerebrospinal fluid barrier, sustaining intracranial inflammation and contributing to the barrier's disruption and cerebral edema development [39]. CRP plays a crucial role in removing foreign bodies and necrotic tissues and serves as a key marker of inflammation and disease activity [40]. TNF-α, produced by activated macrophages, is a central nervous system mediator in immune and inflammatory responses. IL-6, derived from multiple cell types, can disrupt the blood-cerebrospinal fluid barrier, causing edema and worsening brain damage [41]. IL-8 acts as a chemokine facilitating neutrophil migration to inflammatory sites, contributing to secondary brain injury. The acute phase response of these inflammatory factors is directly proportional to the severity of brain tissue damage and accurately reflects the extent of secondary brain injury [42]. The study found that at 72 hrs post-operation, OG exhibited significantly improved $SjvO_2$, Da-jvO_2, and $CERO_2$ levels, along with reduced CRP, TNF-α, IL-6, and IL-10 levels compared to CG. Additionally, OG had a significantly higher rate of good prognosis, with all differences being statistically significant ($p < 0.05$). Dexmedetomidine combined with remifentanil is effective in the perioperative management of patients with severe TBI by reducing cerebral oxygen consumption, improving cerebral metabolic disorders, lowering levels of inflammatory factors, inhibiting inflammatory responses, and enhancing patient prognosis.

Conclusion

In summary, remifentanil enhances the postoperative sedative and analgesic effects of dexmedetomidine in intensive care unit patients with severe craniocerebral trauma, leading to reduced stress levels, diminished inflammatory response, stabilized vital signs, and minimal adverse effects, making it suitable for clinical application.

Conflicts of interest

The authors declare no conflict of interest.

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Synergistic effects of serum albumin and HDL cholesterol concentrations on serum oxidized LDL cholesterol concentration in obese individuals with normal glycoregulation and patients with type 2 diabetes mellitus

Sinergistički efekti koncentracije serumskog albumina i HDL holesterola na koncentraciju oksidovanog LDL holesterola u serumu gojaznih osoba sa normalnom glikoregulacijom i obolelih od dijabetesa melitusa tipa 2

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Abstract

Background/Aim. Serum albumin and high-density lipoprotein (HDL) cholesterol molecules have multiple physiological functions, including an antioxidant role in neutralizing the harmful effects of oxidized low-density lipoprotein (oxLDL). In obese individuals, albumin and HDL cholesterol molecules are unable to counteract the unfavorable effects of oxLDL cholesterol adequately. The aim of the study was to examine the functional relationships between oxLDL cholesterol, HDL cholesterol, and serum albumin. **Methods.** The study included 30 obese individuals with newly diagnosed type 2 diabetes mellitus (before and after a three-month treatment with metformin), 30 obese individuals with normal glucose tolerance, and 30 normal-weight subjects (control group). The groups were age- and sex-matched. **Results.** Both qualitative and quantitative changes in the levels of HDL cholesterol and albumin were detected among the groups. Statistically significant changes were found in the linear correlations between albumin and ox-LDL cholesterol among the study groups. Furthermore, by forming a synergistic influence of independent variables (HDL cholesterol and albumin), expressed through a complex polynomial of the dependent variable (oxLDL) of the quadratic type, statistically significant qualitative and quantitative changes in maximal oxLDL values were observed in all examined groups. **Conclusion**. The results of our study indicate a potential synergistic effect of albumin and HDL cholesterol in the prevention of oxidative damage, as well as a possible alteration in the quality of the ratio of these parameters in relation to oxLDL cholesterol molecules under conditions characterized by increased oxidative stress.

Key words:
albumins; diabetes mellitus, type 2; lipoproteins, hdl; lipoproteins, ldl; obesity; oxidative stress.

Apstrakt

Uvod/Cilj. Serumski albumin i molekuli *high-density lipoprotein* (HDL) holesterola imaju višestruke fiziološke funkcije, uključujući antioksidativnu ulogu u neutralisanju štetnih efekata oksidovanog lipoproteina niske gustine (*oxidized low-density lipoprotein* – oxLDL). Kod gojaznih osoba, molekuli albumina i HDL holesterola nisu u stanju da se adekvatno suprotstave nepovoljnim efektima oxLDL holesterola. Cilj rada bio je da se ispitaju funkcionalni odnosi između oxLDL holesterola, HDL holesterola i albumina u serumu. **Metode.** Studijom je obuhvaćeno 30 gojaznih osoba sa novodijagnostikovanim dijabetesom melitusom tipa 2 (pre i posle tromesečnog lečenja metforminom), 30 gojaznih osoba sa normalnom tolerancijom glukoze i 30 ispitanika normalne težine (kontrolna grupa). Grupe su bile podudarne po starosti i polu. **Rezultati**. Utvrđene su kvalitativne i kvantitativne razlike u nivoima HDL holesterola i albumina među grupama. Pokazane su statistički značajne promene u linearnim korelacijama između albumina i oxLDL holesterola među ispitivanim grupama. Takođe, formiranjem sinergističkog uticaja nezavisnih varijabli (HDL holesterola i albumina), izraženih kroz složeni polinom zavisne varijable (oxLDL) kvadratnog tipa, uočene su statistički značajne kvalitativne i kvantitativne

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promene u maksimalnim vrednostima oxLDL u svim ispitivanim grupama **Zaključak.** Rezultati našeg istraživanja ukazuju na mogući sinergistički efekat albumina i HDL holesterola u prevenciji oksidativnog oštećenja, kao i na mogućnost promene kvaliteta odnosa tih parametara u odnosu na molekule oxLDL holesterola u uslovima koje karakteriše povećan oksidativni stres.

Ključne reči:
albumini; dijabetes mellitus, insulin nezavisni; lipoproteini, hdl holesterol; lipoproteini, ldl holesterol; gojaznost; stres, oksidativni.

Introduction

Serum albumin has multiple physiological functions, including an antioxidant role in plasma and extracellular fluid. Conditions characterized by increased oxidative stress (OS) can reduce the capacity of albumin, resulting in modification of its structure. Increased levels of glycated albumin in diabetes mellitus (DM) may contribute to the development of chronic complications [1].

A decrease in high-density lipoprotein (HDL) cholesterol levels can be observed in different inflammatory disorders. The cause of this phenomenon is most likely the oxidative modification of HDL cholesterol during OS, with a consequent structural change of its molecule [2]. Although there is evidence of the existence of "dysfunctional HDL cholesterol", biomarkers for its monitoring in routine clinical practice have not yet been fully established [3, 4]. Both molecules participate in the reduction of oxidative and inflammatory damage by oxidized low-density lipoprotein (oxLDL) particles [5], which is well documented, but to our knowledge, their joint action has not been described in the previous literature.

This study aims to examine the functional relationships between oxLDL, HDL cholesterol, and serum albumin in obese individuals with newly diagnosed type 2 DM (T2DM) (before and after a three-month treatment with metformin), obese individuals with normal glucose tolerance (NGT), and normal-weight (NW) subjects.

Methods

Participants

The study was conducted at the Clinic for Endocrinology, Diabetes, and Metabolic Disorders of the University Clinical Center of Vojvodina, Serbia, and included 30 obese individuals with newly diagnosed T2DM before treatment (T2DMBT) and after a three-month treatment with metformin (T2DMAT) [mean values (MV) and standard deviation (SD) of body mass index (BMI) at the baseline was 34.41 ± 4.68 kg/m^2], 30 obese individuals with NGT (group ONGT) (37.37 ± 6.11 kg/m^2), and 30 NW subjects (control group) (23.34 ± 3.12 kg/m^2). The groups were age- and sex-matched. A detailed medical history was taken from all patients, and a physical examination and other detailed laboratory analyses were performed.

Inclusion criteria for all study groups except the NW group were as follows: a BMI above 30 kg/m^2; patients who were not suffering from diseases that could influence the oxidative status (liver and kidney diseases, arterial hypertension, hyperlipidemia, etc.); non-smokers; patients who were not taking vitamin supplements or drugs with established effects on OS (statins, fibrates, angiotensin-converting enzyme inhibitors, calcium channel blockers, etc.). Exclusion criteria for all groups were the following: patients suffering from diseases that could influence the oxidative status (liver and kidney diseases, arterial hypertension, hyperlipidemia, etc.); smokers; patients taking vitamin supplements or drugs with established effects on OS (statins, fibrates, angiotensin-converting enzyme inhibitors, calcium channel blockers, etc.). T2DM patients who took therapy irregularly, occasionally smoked, or took drugs that affect the oxidative status of the organism were excluded from the study.

In all groups without T2DM, glucose levels were measured both fasting and 2 hrs after starting the oral glucose tolerance test, along with hemoglobin A1c (HbA1c) levels. Obese patients were included in the study based on their BMI values, while T2DM patients were included according to the American Diabetes Association criteria for the diagnosis of DM. Ethical approval for this study was granted by the Ethics Committee of the University Clinical Center of Vojvodina (approval No. 00-08-10).

Biochemical analysis

The HDL cholesterol levels were measured using a direct enzymatic colorimetric test with the Ultra HDL reagent kit (Abbot, USA) on the automated Architect ci4100 analyzer (Abbot, USA). Cholesterol concentration in oxLDL particles was determined manually using the oxLDL/MDA Adduct enzyme-linked immunosorbent assay (ELISA) kit (Immundiagnostik AG, Germany), following the ELISA method with an RT-2100C microplate reader and RT-2600C microplate washer (Rayto, China). HbA1c levels were determined using the latex agglutination-inhibition method with the HbA1c reagent kit (Abbot, USA), also on the automated Architect ci4100 analyzer (Abbot, USA). Serum albumin levels were determined during serum protein electrophoresis using the spectrophotometric bromine cresol green method on the Siemens Advia 1800 chemistry analyzer.

Statistical analysis

The basic parameters of MV and SD were calculated, and a distribution was established for each statistical group, verified using the Chi-squared test (Table 1). For the parameter HbA1c, the same procedure was applied: MV and SD were calculated, and the distri-bution was verified using the Chi-squared test (Table 2). Statistical analysis included parameter sets and corresponding tests of dispersive analysis using analysis of variance (ANOVA). A linear correlation

test was performed using Pearson's product-moment correlation coefficient (Tables 3 and 4). For the analytical expression of the relationship between parameters in obese individuals with T2DM before and after metformin treatment, regression analysis was applied. Changes in the values of correlation coefficients indicate the effects of metformin therapy. The significance of these changes was verified by Pearson's correlation coefficient test (Table 4).

Table 1

The basic parametric and non-parametric characteristics with ANOVA of HDL cholesterol, albumin, and oxLDL

Parameter/group	Mean value (ANOVA test)	Standard deviation	Distribution	*p*-values (Chi-square test)
HDL cholesterol (mmol/L)				
NW	1.5109[A]	0.3077	normal	0.4258
ONGT	1.1400[B]	0.2186	log-normal	0.1205
T2DMBT	**1.0475[C]**	0.2252	log-normal	0.3117
T2DMAT	**1.0669[C]**	0.2180	log-normal	0.1225
Albumin (g/L)				
NW	**47.6967[D]**	2.3946	uniform	0.6238
ONGT	**45.8540[D]**	3.3474	log-normal	0.0920
T2DMBT	45.4593[E]	2.8407	log-normal	0.2133
T2DMAT	**46.0807[D]**	3.0443	uniform	0.2933
oxLDL (ng/mL)				
NW	57.4194[F]	59.9692	exponential	0.1101
ONGT	111.7000[G]	75.9165	exponential	0.0689
T2DMBT	183.4594[H]	227.5207	exponential	0.1034
T2DMAT	129.6192[I]	117.1498	exponential	0.0708

ANOVA – analysis of variance; HDL – high-density lipoprotein; oxLDL – oxidized low-density lipoprotein; NW – normal-weight individuals; ONGT – obese individuals with normal glucose tolerance; T2DMBT – obese individuals with newly diagnosed type 2 diabetes mellitus (T2DM) before metformin treatment initiation; T2DMAT – obese individuals with newly diagnosed T2DM after a three-month metformin treatment.

***Note*: Between the values marked with identical capital letters, there is no significant difference according to the ANOVA test for the significance threshold of $p < 0.05$. There are no significant differences between the T2DMBT and T2DMAT groups in HDL values (marked with the capital letter C), while other differences are significant. There are no significant differences in the NW, ONGT, and T2DMAT groups in serum albumin values (marked with the capital letter D), while other differences are significant. All oxLDL values are significantly different between study groups.**

Table 2

The basic parametric and non-parametric characteristics of hemoglobin A1c (HbA1c)

Groups	Mean value of HbA1c (%) (ANOVA test)	Standard deviation	Distribution	*p*-values (Chi-square test)
NW	**5.1967[A]**	0.296439	normal	0.4493
ONGT	**5.5200[A]**	0.334781	normal	0.2102
T2DMBT	8.2696[B]	2.186131	normal	0.0526
T2DMAT	7.4176[C]	1.509281	normal	0.0382

For abbreviations, see Table 1.

***Note*: Between the values marked with identical capital letters, there is no significant difference according to the ANOVA test for the significance threshold of $p < 0.05$. There are no significant differences between the NW and ONGT groups in HbA1c values (marked with the capital letter A), while other differences are significant.**

Table 3

Correlations between HDL cholesterol and oxLDL with Pearson tests

		HDL cholesterol			
		NW	ONGT	T2DMBT	T2DMAT
oxLDL	NW	***r* = +0.2900**[Fig 1A]	$p = 0.1394$	$p = 0.0905$	$p = 0.1235$
	ONGT		***r* = +0.0037**[Fig 1B]	$p = 0.4115$	$p = 0.4518$
	T2DMBT			***r* = -0.0628**[Fig 1C]	$p = 0.4573$
	T2DMAT				***r* = -0.0308**[Fig 1D]

Fig – figure. For other abbreviations, see Table 1.

***Note*: On the diagonal of Table 3, the values of linear correlations (marked with the letter *r*) of HDL and oxLDL (bold and underlined values) are given. The correlation graphs are shown in Figure 1 (A for the NW group, B for the ONGT group, C for the T2DMBT group, D for the T2DMATx group). Outside the diagonal are the values of the results of the Pearson correlation test (*p*-value) differences are significant for $p < 0.05$. There are no significant differences between correlations.**

Table 4

Correlations between serum albumin and oxLDL with Pearson tests

		Serum albumin			
		NW	ONGT	T2DMBT	T2DMAT
oxLDL	NW	***r* = -0.1840**$_{\text{Fig 2B}}$	p = 0.0642	***p = 0.0186***	p = 0.3122
	ONGT		***r* = +0.2310**$_{\text{Fig 2B}}$	p = 0.2978	***p = 0.0281***
	T2DMBT			***r* = +0.3656**$_{\text{Fig 2C}}$	***p = 0.0073***
	T2DMAT				***r* = -0.3096**$_{\text{Fig 2D}}$

Fig – figure. For other abbreviations, see Table 1.
***Note*: On the diagonal of Table 4, the values of linear correlations (marked with the letter *r*) of albumin and oxLDL (bold and underlined values) are given. Correlation graphs are shown in Figure 2 (A for NW, B for ONGT, C for T2DMBT, D for T2DMAT). Outside the diagonal are the values of the results of the Pearson correlation test (*p*-value); differences are significant for $p < 0.05$, italic bold values.**

Based on empirical data, theoretical two-dimensional approximation functions were established, with HDL cholesterol and albumin as independent variables and oxLDL as the dependent variable. The reliability of the approximation was established by correlation. Based on the application of double integrals, the volume of the dependent variable defined by approximate functions was calculated over the domain D, representing the real intervals of the empirical independent variables, as well as over the maximum domain D_{max}. This double integral model was applied to investigate the synergistic effect of HDL cholesterol and albumin on oxLDL. The significance was set at 0.05 for all parametric and non-parametric verifications, and at 0.10 for the Pearson test. The results were calculated using classical mathematical analysis methods, specifically double integrals, without reliance on commercial software.

Results

The basic parametric and non-parametric characteristics, along with ANOVA test results for HDL cholesterol, albumin, and oxLDL, are presented in Table 1, and for HbA1c in Table 2. Table 1 illustrates the distribution dynamics and differences in MVs of the examined parameters. The HDL cholesterol group primarily followed a log-normal distribution, with the lowest levels observed in newly diagnosed T2DM patients and those undergoing treatment. Serum albumin levels tended toward a uniform distribution, with the lowest values found in newly diagnosed T2DM patients. Quantitative statistical significance was given between MVs, precisely indicated by different superscript capital letters. Values marked with the same capital letter showed no statistically significant difference (ANOVA test and significance threshold set at $p = 0.05$ were emphasized). Qualitative differences between study groups were expressed in the types of distributions. The significance threshold was set at $p = 0.05$, and all distributions were verified using the Chi-squared test, with confirmed verification in all cases ($p > 0.05$). If different verified distributions were identified among groups, a significant qualitative difference was established. Qualitative changes in distribution types were observed for HDL cholesterol (Normal and Log-normal) and serum albumin [Even (Uniform) and Log-normal] between groups, whereas no qualitative changes were found in oxLDL distributions (exponential distribution of all groups) (Table 1). For the parameter HbA1c, quantitative differences were observed between groups, but there were no qualitative changes as all cluster distributions were verified as normal ($p > 0.05$) (Table 2).

The linear correlation coefficients between HDL cholesterol and oxLDL across the different studied groups are presented in Table 3 and Figure 1. It can be seen that there was a change in the direction of the linear regression, especially in individuals with newly diagnosed T2DM before metformin treatment initiation. At a significance threshold of $p = 0.10$, the Pearson test revealed a statistically significant change in correlation between the T2DMBT group and the NW group. Table 3 displays the correlation coefficients along the main diagonal, representing relationships within the same group, while the values symmetrical to the diagonal represent the Pearson test results indicating changes in correlations between groups. These findings are illustrated graphically, with group indices as follows: 01 – NW; 02 – obese normal glucose tolerance; 03 – T2DM before therapy; 04 – T2DM during metformin therapy.

The linear correlation coefficients between serum albumin and oxLDL in the different studied groups are given in Table 4 and Figure 2. Figure 2 shows the exceptional correlation dynamics in serum albumin. From the NW group to ONGT and the T2DMBT, there was a significant positive change in the angle of linear regression, which returned to the initial value after therapy with metformin. Changes in correlation coefficients were significant. In Table 4, the main diagonal displays correlation coefficients within each group, while the other values represent Pearson test results that indicate the dynamics of changes between groups. The indices on the graphs are highlighted (01 – NW, 02 – obese normal glucose tolerance, 03 – T2DM before therapy, 04 – T2DM during metformin therapy).

Differences in parametric and non-parametric characteristics (Table 1), as well as variations in correlations (Tables 3 and 4), provided the foundation for further investigation.

In this research, a two-dimensional function was applied, with oxLDL as the dependent variable and HDL cholesterol and serum albumin as independent variables. Figures 3–6 illustrate both empirical data and approximated theoretical distributions of the two-dimensional functions, with oxLDL as the dependent variable and HDL cholesterol and alb-

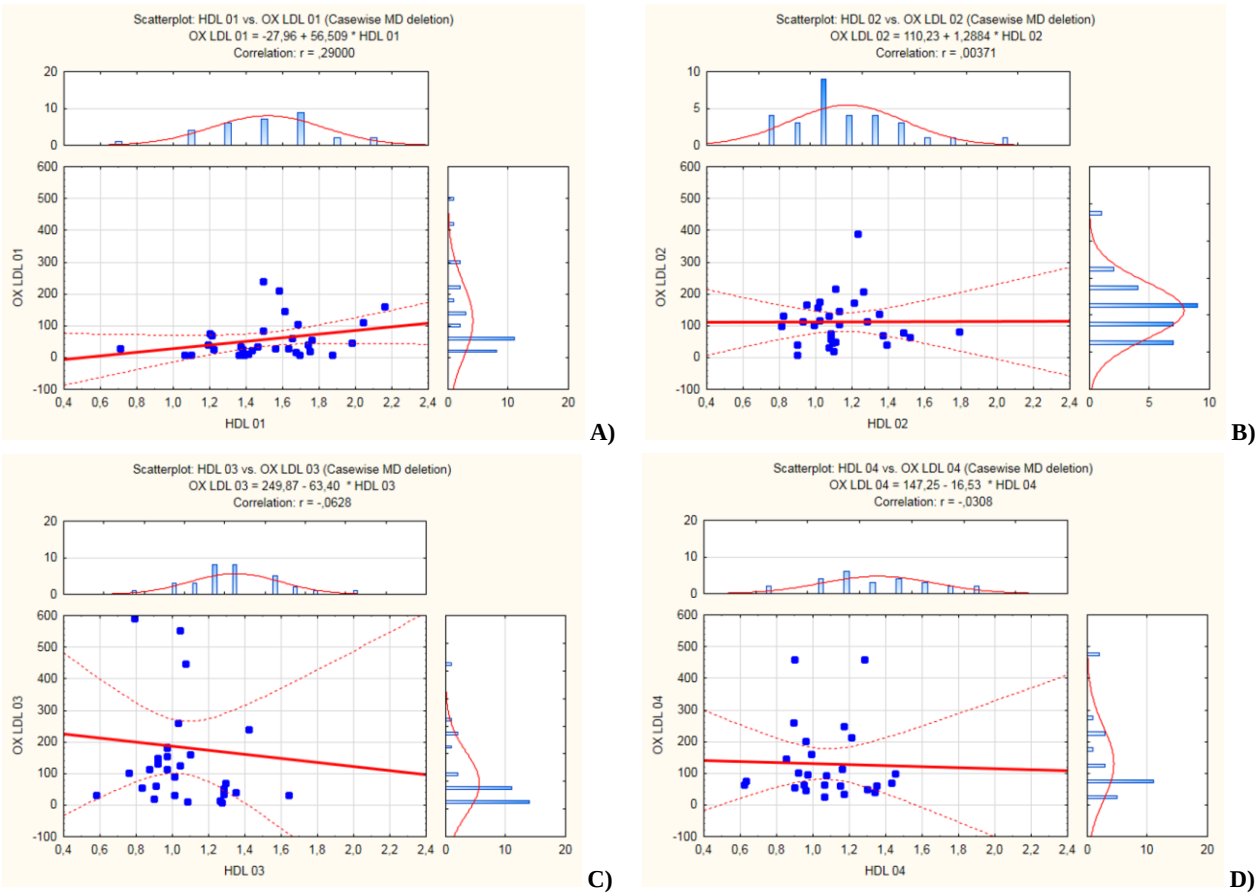


Fig. 1 – The linear correlations of HDL cholesterol and oxLDL in the examined groups [A – 01 – normal weight, B – 02 – obese normal glucose tolerance, C – 03 – type 2 diabetes mellitus (T2DM) before therapy, C – 04 – T2DM during metformin therapy].
MD – missed data. For other abbreviations, see Table 1.
***Note*: Changes in the slope (coefficient) of the linear regression express the dynamics of the HDL and oxLDL relationship between the groups. The changes in the correlations of these groups were not statistically significant, with a note that they approach a statistically significant difference, especially in the T2DM group before therapy and the control group ($p = 0.0905$).**

umin as independent variables. Approximate functions, where x stands for HDL cholesterol and y for serum albumin, are shown in all the Figures. In the theoretical function, the independent empirical variables (HDL cholesterol as variable x and serum albumin as variable y) are analytically expressed. The theoretical oxLDL data were obtained when the empirical values of HDL cholesterol (as variable x) and serum albumin (as variable y) were included in the theoretical function. The theoretical distribution function of oxLDL for each group is given in Figures 3–6. Based on empirical data, it was not possible to perform an analysis of the migration of oxLDL maximum between groups. That is why it was necessary to approximate empirical data with theoretical data, i.e., with a function that can be applied as in an integral calculation.

In the NW group, there was no pronounced maximum of oxLDL values, with a high agreement between empirical and theoretical data. In the remaining groups, there is an obvious migration of the maximum levels of oxLDL (highlighted in red on the abscissas of the independent variables HDL and serum albumin in Figures 4–6).

In the NW group, the correlation between empirical and theoretical data was $r = +0.4551$, while in the ONGT group, the correlation was $r = +0.4241$. The maximum oxLDL levels were observed at higher values of HDL cholesterol, $\in[1.2, 1.8]$, and higher values of serum albumin, $\in[46, 52]$. In this zone, the oxLDL MV was 129.32, and in the complementary zone, it was 97.51. A statistically significant difference between these values was established using the ANOVA test ($p = 0.0193$).

In the T2DMBT group, the correlation between empirical and theoretical data was $r = +0.5291$. The maximum oxLDL levels were observed at lower values of HDL$\in[0.8, 1.2]$, and higher values of serum albumin$\in[46, 52]$. In this zone, the oxLDL MV was 302.56 and 182.62 in the complementary zone. A statistically significant difference between the stated values was established by the ANOVA test ($p = 0.0439$).

In the T2DMAT group, the correlation between empirical and theoretical data was $r = +0.5193$. The maximum oxLDL levels were found at higher values of HDL$\in[1.2, 1.8]$, and lower values of serum albumin$\in[38,44]$. In this zone, the

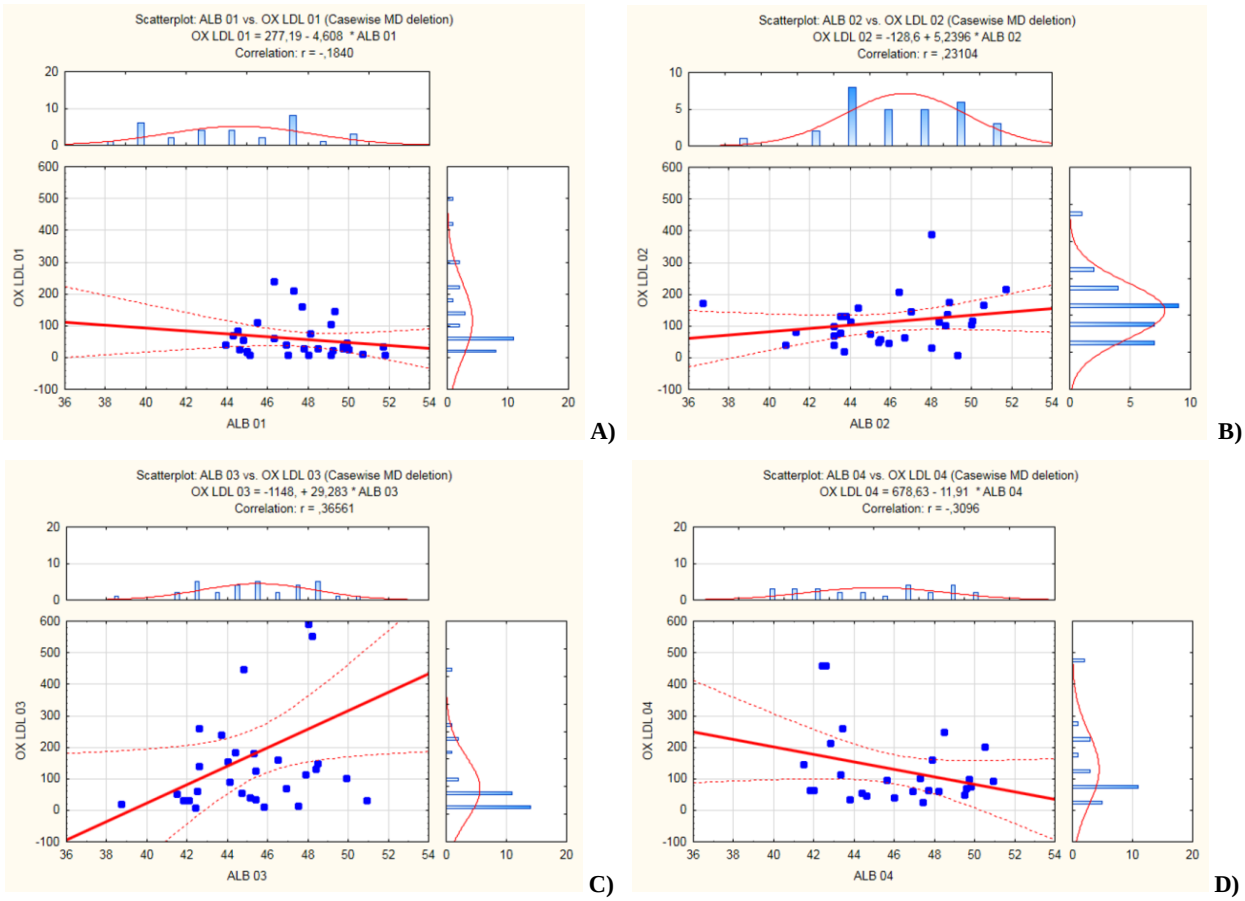


Fig. 2 – The linear correlations of albumin and oxLDL in the examined groups [A – 01 – normal weight, B – 02 – obese normal glucose tolerance, C – 03 – type 2 diabetes mellitus (T2DM) before therapy, C – 04 – T2DM during metformin therapy].
MD – missed data. For other abbreviations, see Table 1.

***Note*: Changes in the slope (coefficient) of the linear regression express the dynamics of the serum albumin and oxLDL relationship between the groups. Metformin therapy reduces the relationship between these two parameters to a relationship that is statistically in agreement with the control group. Changes in correlations were significant. Correlations of obese patients without diabetes and T2DM patients before therapy are significantly different compared to the control group and T2DM patients during metformin therapy (see Table 4).**

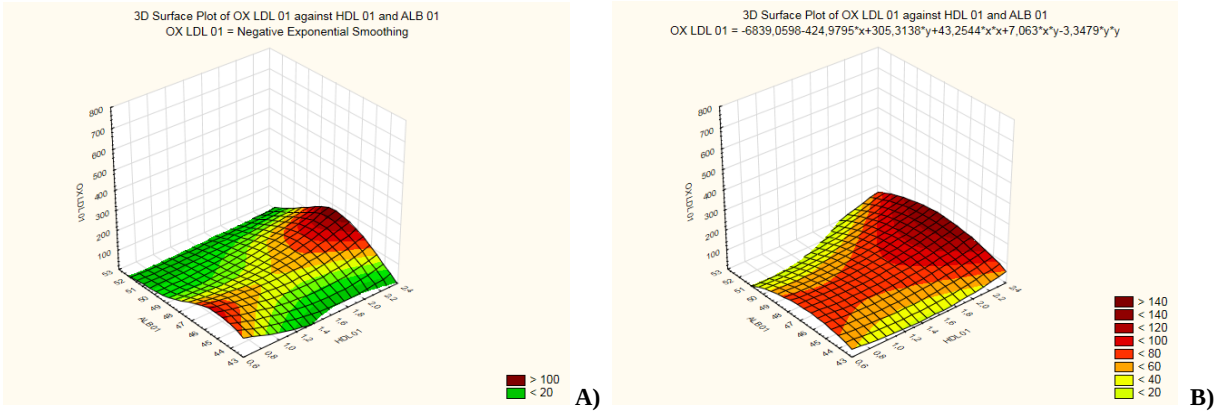
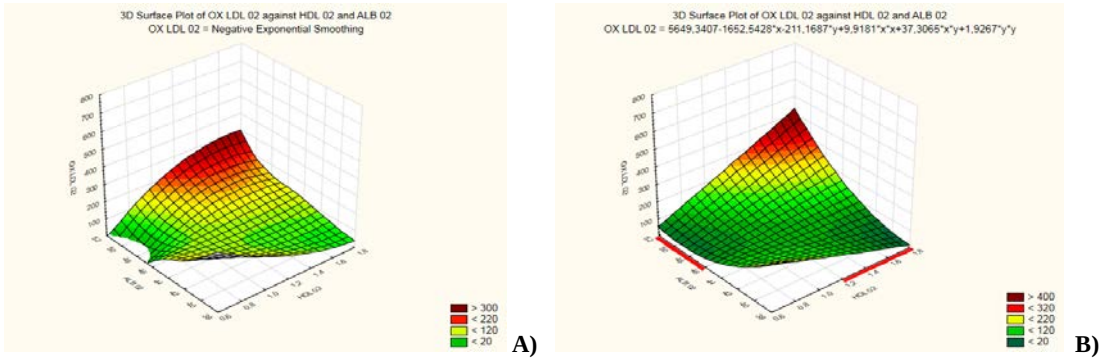


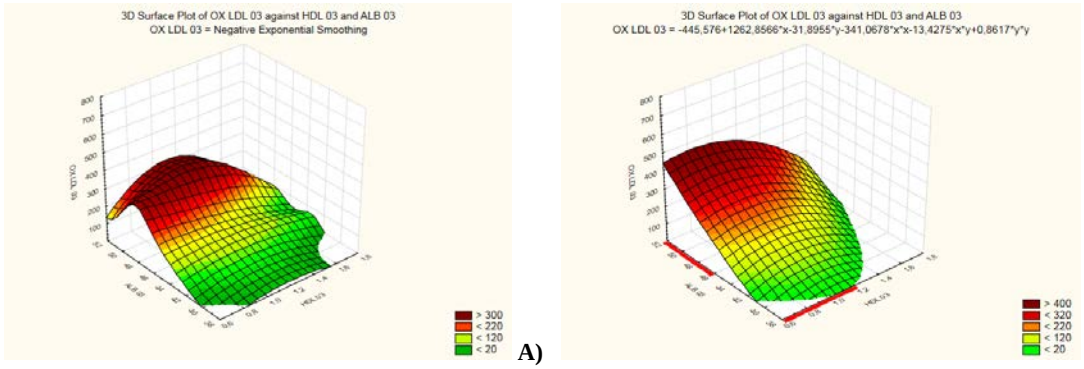
Fig. 3 – Distribution of A) empirical and B) theoretical data in the group of normal weight subjects, two-dimensional function of oxLDL as dependent variable and HDL cholesterol and albumin as independent variables.
ALB – albumin. For other abbreviations, see Table 1.



A) B)

Fig. 4 – Distribution of A) empirical and B) theoretical data in the group of obese individuals with NGT, two-dimensional function of oxLDL as dependent variable (HDL cholesterol and albumin as independent variables). ALB – albumin; NGT – normal glucose tolerance. For other abbreviations, see Table 1.

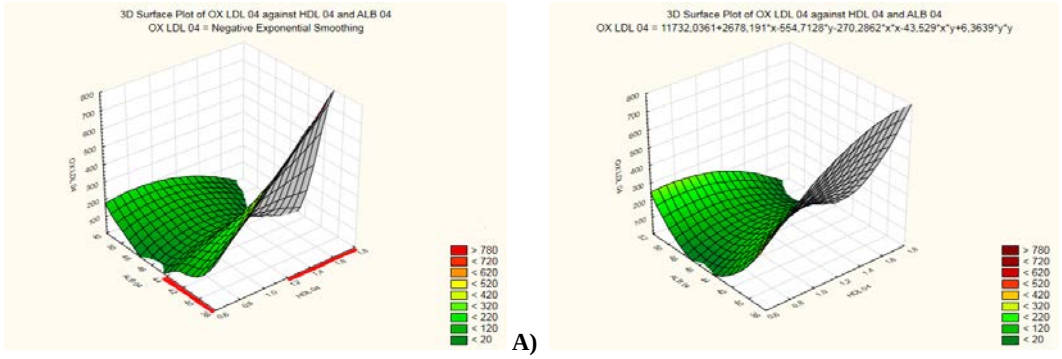
***Note*: The P_{max} area (maximum oxLDL values) in the ONGT group was found at high HDL cholesterol and high albumin values (the red line on the ordinates indicates the maximum oxLDL values). The volume under the graph of the function and above the surface P_{max} is 2.30 times greater than the volume over the complementary domain in which the oxLDL values are lower (see Table 5).**



A) B)

Fig. 5 – Distribution of A) empirical and B) theoretical data in the group of obese individuals with T2DM prior to metformin treatment initiation, two-dimensional function of oxLDL as dependent variable (HDL cholesterol and albumin as independent variables). ALB – albumin. For other abbreviations, see Table 1.

***Note*: The P_{max} area (maximum oxLDL values) in the T2DMBT group is found at low HDL cholesterol values and high albumin values (the red line on the ordinates indicates the maximum oxLDL values). The volume under the graph of the function and above the surface P_{max} is 3.49 times greater than the volume over the complementary domain in which the oxLDL values are lower (see Table 5).**



A) B)

Fig. 6 – Distribution of A) empirical and B) theoretical data in the group of obese individuals with T2DM after a three-month metformin treatment, two-dimensional function of oxLDL as dependent variable (HDL cholesterol and albumin as independent variables). ALB – albumin. For other abbreviations, see Table 1.

***Note*: The P_{max} area (maximum ox LDL values) in the T2DMAT group is found at high HDL cholesterol values and low albumin values (the red line on the ordinates indicates the maximum oxLDL values). The volume between the plot of the function and surface P_{max} is 3.19 times greater than the volume over the complementary domain where the oxLDL values are lower (see Table 5). Observe that the applied metformin therapy completely inverted the maximum values of oxLDL in relation to the values of HDL cholesterol (low values before the therapy and high values after the therapy), as well as serum albumin (high values before the therapy and low values after the therapy with metformin).**

oxLDL MV was 336.85, and in the complementary zone, it was 112.35. A statistically significant difference between the stated values was established using the ANOVA test ($p = 0.0065$).

This procedure proved the qualitative characteristic of oxLDL peak migration as a function of HDL cholesterol and serum albumin. This two-dimensional dependence can therefore be declared as synergistic.

Further on, we can calculate the quantity of this same relationship with double integrals, calculating the volume under the theoretical distribution of the two-dimensional function dependent variable oxLDL from independent variables HDL cholesterol and serum albumin. All volumes are calculated for the total domain D, which is standardized for all groups: HDL∈[0.6, 1.8] and low values of albumin∈[38,52]. The maximum domain is already specified for each group individually.

For the NW group (with no pronounced maximum of oxLDL), the total volume under the two-dimensional function was:

$$D_{NWH} = \int_{0,6}^{1.8}\int_{38}^{52}(-6{,}830.05 - 424.97x + 305.31y + 43.25x^2 + 7.06xy - 3.34y^2)dxdy = 528.58$$

For the ONGT group, the total volume under the two-dimensional function and declared maximum (red highlight on abscissas) was:

$$D_{ONGT} = \int_{0,6}^{1.8}\int_{38}^{52}(5{,}649.34 - 1{,}652.54x - 211.16y + 9.91x^2 + 37.30xy + 1.92y^2)dxdy = 1{,}900.27$$

$$D^{max}_{ONGT} = \int_{1,2}^{1.8}\int_{46}^{52}(5{,}649.34 - 1{,}652.54x - 211.16y + 9.91x^2 + 37.30xy + 1.92y^2)dxdy = 732.65$$

For the T2DMBT group, the total volume under the two-dimensional function and the maximum (red highlight on abscissas) was:

$$D_{T2DMBT} = \int_{0.6}^{1.8}\int_{38}^{52}(-445.57 + 1{,}262.85x - 3.89y - 341.06x^2 - 13.42xy + 0.8617y^2)dxdy = 14{,}878.77$$

$$D^{max}_{T2DMBT} = \int_{0.6}^{1.2}\int_{46}^{52}(-445.57 + 1{,}262.85x - 3.89y - 341.06x^2 - 13.42xy + 0.8617y^2)dxdy = 7{,}261.26$$

For the T2DMAT group, the total volume under the two-dimensional function and the maximum (red highlight on abscissas) was:

$$D_{T2DMAT} = \int_{0.6}^{1.8}\int_{38}^{52}(11{,}732.03 + 2{,}678.19x - 554.71y - 270.28x^2 - 43.52xy + 6.36y^2)dxdy = 3{,}277.19$$

$$D^{max}_{T2DMAT} = \int_{1,2}^{1.8}\int_{38}^{44}(11{,}732.03x - 554.71y - 270.28x^2 - 43.52xy + 6.36y^2)dxdy = 1{,}525.41$$

The total area of the domain D in all groups is equal to P = (1.8–0.6) × (52–38) = 16.8. The area of the maximum oxLDL in the groups ONGT, T2DMBT, and T2DMAT is standardized and equal to $P_{max} = 3.6$, as shown in Figures 4, 5, and 6, where the red-marked zones on the HDL cholesterol and serum albumin axes indicate the regions of maximum intensity. From the previous results, we can introduce the resultant of the intensity of the synergic effect as a quotient of the total volumes of oxLDL levels, which we denote by D on the reference surface P, i.e., $\rho = D/P$ (Table 5). The interpretation of this value is somewhat analogous to the MVs (129.32, 302.56, and 336.85, respectively, previous ANOVA tests). The basic problem of dimensional alignment is conditioned by the synergistic effect in complex metabolic relationships (HDL cholesterol expressed with mmol/L, serum albumin expressed with g/L, and oxLDL expressed with ng/mL). Nevertheless, the numerical expression of the synergistic effect is obvious – the quotient of these relations for each group and relation to the group with the highest intensity (T2DMBT group) is:

$\rho_{NW} = 528.58/16.8 = 31.46$ ($R_{NW} = 28.14$)
$\rho_{ONGT} = 1{,}900.27/16.8 = 113.11$ ($R_{ONGT} = 7.82$)
$\rho_{T2DMBT} = 14{,}878.77/16.8 = 885.64$ ($R_{T2DMBT} = 1.00$)
$\rho_{T2DMAT} = 3{,}277.19/16.8 = 195.07$ ($R_{T2DMAT} = 4.54$)

The T2DMBT group had an R_{NW} 28.14 times higher intensity of synergistic effect than the NW group, R_{ONGT} had 7.82 times higher intensity of synergistic effect than the ONGT group, and R_{T2DMAT} had 4.54 times higher intensity of synergistic effect than the T2DMAT group.

Table 5

Calculation of the ratios of the domain of the maximum and the complementary domain in groups

Groups	D_{max}	D	P_{max}	$D–D_{max}$	$P–P_{max}$	ρ_{max}	ρ_{com}	ρ_{max}/ρ_{com}
ONGT	732.65	1,900.27	3.6	1,167.62	13.2	203.5139	88.45606	2.30
T2DMBT	7,261.26	14,878.77	3.6	7,617.51	13.2	2017.017	577.0841	3.49
T2DMAT	1,525.41	3,277.19	3.6	1,751.78	13.2	423.725	132.7106	3.19

D – the volume of the double integral over the entire surface domain; D_{max} – the volume of the double integral on the domain of maximal oxLDL values; P_{max} area of the domain of maximum (red highlight area); $\rho_{max} = D_{max}/P_{max}$, synergistic resultant of serum albumin and HDL cholesterol over the maximal domain; $\rho_{com} = (D–D_{max})/(P–P_{max})$, synergistic resultant of serum albumin and HDL cholesterol over the complementary domain; ONGT – obese individuals with normal glucose tolerance; T2DMBT – obese individuals with newly diagnosed type 2 diabetes mellitus (T2DM) before metformin treatment initiation; T2DMAT – obese individuals with newly diagnosed T2DM after a three-month metformin treatment. For other abbreviations, see Table 1.

***Note*: The standardized area (domain) P_{max}, which represents the maximum values of oxLDL, changes position between the tested groups. In the ONGT group, the maximum values of oxLDL were found at high HDL cholesterol and high albumin values (Figure 4). In the T2DMBT group, the maximum values of oxLDL were found at low HDL cholesterol and high albumin values (Figure 5). In the T2DMAT group, the maximum values of oxLDL were found at high HDL cholesterol and low albumin values (Figure 6). Two-dimensional linear correlations (Figures 1 and 2) contain indications of P_{max} migrations. The three-dimensional representation in the Figures above clearly highlights the migrations of P_{max}. A more detailed description is given in the following graphics.**

Comparison of the intensity and MV of the synergistic effect of the independent variables HDL cholesterol and serum albumin on oxLDL in the maximum zones and complementary zones can be obtained when the volume under the function and the maximum zone are divided by the corresponding surfaces. The results are presented in Table 5, excluding the NW group, as it lacks a pronounced peak zone. In the zones of maximum, the intensity of oxLDL was formed by the dominant synergistic effect of HDL cholesterol and serum albumin.

In the ONGT group, the intensity in the maximum domain was 2.30 times higher than the complement zone (maximum domain participation 69.7%); in the T2DMBT group, it was 3.49 times higher than the complement zone (maximum domain participation 78.4%), and in the T2DMAT group, it was 3.19 times higher than the complement zone (maximum domain participation 78.7%) (Table 5).

It should be noted that metformin therapy reduced the intensity of the synergistic effect of independent variables by 3.54 times. However, in the maximum zone of oxLDL, there was a minimal reduction from 3.49 to 3.19, which in total participation represents only a few percent success, i.e., although this is indisputably proven, statistically significant (Table 1, ANOVA test) reduction of oxLDL with the use of therapy. Nevertheless, the application of therapy had a dominant effect on one group of patients, but in the maximum zones of oxLDL, this undeniable influence did not produce the expected results. In other words, there was a group of patients who reacted more inertly to therapy and the reduction of oxLDL levels.

Discussion

The results of our study revealed the existence of the following: a) both qualitative (different verified groups distributions) and quantitative (statistical differences in MVs) changes in HDL cholesterol in the examined groups and the incapability of metformin treatment to restore those changes to the pattern observed among NW individuals; b) both qualitative and quantitative changes in albumin in the examined groups and the capability of metformin treatment to restore only the quality (therapy restores the distribution of serum albumin to uniform one as in the control group) but not the quantity to the pattern observed among NW healthy individuals; c) only quantitative changes in oxLDL in the examined groups and the incapability of metformin treatment to restore those values to the levels observed among NW individuals

The most likely cause of the quantitative decrease and qualitative change of HDL cholesterol particles is their transformation from an antiatherogenic to a proatherogenic form under the influence of systemic OS and chronic inflammation [6]. The dysfunctionality of HDL cholesterol particles, already present in obesity, becomes exacerbated with the onset of hyperglycemia, which causes additional oxidative damage through glycation processes. It has been hypothesized that the reduced values of HDL cholesterol in conditions of elevated OS represent a purposeful response [7]. Moreover, previous studies have shown that HDL cholesterol particles undergo oxidative modification much faster than LDL cholesterol particles [8]. Interestingly, a recent epidemiological study demonstrated that high HDL cholesterol levels may also represent a risk factor for cardiovascular disease (CVD), despite the fact that HDL cholesterol is recognized as an antiatherogenic lipoprotein [9, 10].

Additionally, no statistically significant changes in the correlations between HDL cholesterol and oxLDL were observed across the examined groups. However, there was a tendency towards statistically significant change in correlations among obese individuals with T2DM prior to metformin treatment initiation and NW subjects. For the significance threshold of $p = 0.10$, a significant change in correlation compared to the NW group was observed in the T2DM group prior to therapy based on the Pearson test.

On the other hand, significant changes in the correlations between serum albumin and oxLDL were observed across the examined groups. A significant change in the ratio of linear correlations of these two parameters indicates the existence of biochemical dynamics and underscores the effect of metformin treatment on the improvement of the albumin oxidative status in the setting of T2DM.

Obese individuals with NGT and those with T2DM prior to metformin treatment initiation had an exactly proportional ratio of oxLDL and albumin, which did not differ significantly. However, metformin treatment reduced the linear correlation ratio of oxLDL and albumin to the linear correlation level observed among NW subjects. The observed shift in these two parameters indicates a change in the nature of the ratio of oxLDL and albumin in conditions characterized by increased OS (T2DM before metformin treatment) and after treatment initiation.

The most significant finding relates to the maximum values of oxLDL, which were established in obese individuals with NGT and continuously appeared among obese T2DM individuals, both prior to and after metformin treatment initiation. The basic quality was determined by the different locations of the oxLDL maximal values, which highlighted the unique dynamics of the synergistic influence of HDL cholesterol and albumin on oxLDL values. This synergistic influence is also significantly different from their individual influences established by linear correlations. The unique dynamic refers to the intense migration of maximum oxLDL values in the groups, where we observed different relationships and values of the investigated parameters of oxLDL cholesterol, HDL cholesterol, and albumin.

Namely, among NW subjects, the highest values of oxLDL were observed at high HDL cholesterol values and MVs of albumin, which shows greater engagement of HDL cholesterol compared to albumin in states characterized by lower OS levels, as well as the existence of functional preservation of both HDL cholesterol and albumin. On top of that, the absolute value of oxLDL was significantly lower among NW subjects compared to all other examined groups.

On the other hand, in obese individuals with NGT (Figure 4), the highest oxLDL values (the red line on the ordinates indicates the maximum oxLDL values in each group) were observed with high values of both HDL cho-

lesterol and albumin, which shows greater involvement of albumin in states of increased OS. The decrease in absolute HDL cholesterol values in this group may represent a useful adaptive mechanism due to the potential transformation of antiatherogenic into proatherogenic HDL cholesterol particles under the influence of systemic OS and chronic inflammation in obesity. Hypothetically, at this point, albumin molecules may already show dysfunction due to their oxidative modification, as significantly elevated levels of oxLDL are associated with high albumin values. Additionally, the absolute oxLDL value in obese individuals with NGT was four times higher compared to NW subjects.

Furthermore, among obese individuals with T2DM prior to the initiation of metformin treatment, the highest values of oxLDL were observed alongside lower values of HDL cholesterol and high values of serum albumin, demonstrating a greater involvement of albumin, but also the existence of additional oxidative and inflammatory stress caused by T2DM onset, which further deepens the compensatory decrease in the HDL cholesterol level. As observed in obese individuals in general, the absolute value of oxLDL was higher among obese individuals with T2DM prior to metformin treatment initiation than in NW subjects.

Finally, among obese individuals with T2DM after metformin treatment initiation, significantly higher oxLDL values were observed at high values of HDL cholesterol and low values of albumin. This finding indicates a complete inversion of the ratio of these parameters caused by the initiation of treatment. The unfavorably high value of HDL cholesterol points out the persistence of systemic inflammation but also indicates a partial recovery, returning its quantitative levels closer to the ones observed among obese individuals with NGT. The presence of low albumin values underscores a partial recovery and lower levels of OS (significantly higher levels of serum albumin in this group than in group T2DMBT) due to a quicker recovery than HDL molecules. Low albumin values may also occur due to consumption and degradation during antioxidant neutralization of oxLDL. Additionally, this suggests a functional association between albumin and the neutralization of oxLDL, as lower albumin values were associated with higher levels of oxLDL.

These findings demonstrated the existence of functional dependence on the examined parameters and a significant change in the value of linear correlations between the examined groups, thus suggesting different relationships between HDL cholesterol, albumin, and oxLDL depending on the level of oxidative damage. Additionally, albumin showed a more significant functional recovery compared to HDL cholesterol in relation to oxidative damage after metformin treatment initiation in the setting of T2DM. This model undeniably proved the special dynamics of oxLDL's maximum migration due to the synergistic influence of HDL cholesterol and serum albumin, as a special qualitative feature, which according to our knowledge, was not previously observed or investigated.

The potential therapeutic application of albumin in states of increased OS should be limited to those conditions in which high albumin levels would not favor further oxidative damage. Based on the findings of our study, this could follow the metformin treatment lasting for at least three months. Additionally, therapeutic strategies aimed at increasing HDL cholesterol levels should not be implemented before achieving adequate glycemic control, due to the potentially unfavorable effects of high levels of oxidatively modified HDL cholesterol, which may lead to an increase in oxLDL values.

Albumin is involved in redox reactions non-specifically, owing to the fact that its concentration in the extracellular compartment is very high, while renewal occurs in about twenty days. Due to its flexible structure, albumin is also easily modulated. Undoubtedly, all these properties of albumin should be considered in the development of treatments for illnesses and disorders associated with OS [1]. Glycated and oxidatively modified albumin significantly contributes to the pathogenesis of DM and other diseases. Recent data indicate that albumin is a major blood plasma protein that represents a molecular "core" and a link between various tissues and organs, essential for the health of the entire organism. The ratio of oxidized albumin to total albumin can be increased in liver disease, DM, and CVD [1, 11]. Among individuals with T2DM, deleterious vascular effects could originate from a complementary mechanism of action, including higher levels of OS biomarkers alongside the loss of antioxidant capacity of albumin. This underscores the importance of considering albumin quality in maintaining homeostasis between glycoxidation and the antioxidant system [12].

As far as compensatory reduction of dysfunctional HDL cholesterol is concerned, it has been demonstrated that acrolein modification of HDL cholesterol produces a dysfunctional particle that may promote atherogenesis by impairing its cholesterol transport function [13]. Recent studies measuring other indices of HDL cholesterol, such as the functionality, size, or number of its particles, revealed that HDL-lipid hydroperoxides (LOOH) (HDL-LOOH) and HDL-triglyceride (HDL-TG) represent clinically available markers for predicting approximate risks of CVDs [14].

Although both HDL cholesterol and albumin have a protective effect in preventing oxLDL modification and consequent progression of atherosclerosis and other complications, it turns into its opposite during HDL cholesterol and albumin oxidative damage in obesity and DM.

The strength of this study shows the synergistic effect of HDL cholesterol molecules and albumin in neutralizing the negative effects of oxLDL. In the presented study, these relationships change in the study groups according to the levels of OS, inflammation, and the longevity of the investigated parameters.

Limitations of the study

The limitations of this study are the small study group and the relatively short follow-up time of the subjects. Qualitative determination of glycosylated and oxidized fractions of HDL cholesterol and albumin molecules could contribute to further investigations in this field.

Conclusion

The results of our study indicate a potential synergistic effect of albumin and high-density lipoprotein cholesterol in the prevention of oxidative damage, as well as a possible alteration in the quality of the ratio of these parameters in relation to oxidized low-density lipoprotein cholesterol molecules under conditions characterized by an increased oxidative stress. In this context, the focus should be moved from albumin and high-density lipoprotein cholesterol quantity to their quality, particularly in terms of their oxidative modifications, provided that further studies are required to elucidate their relationship with cardiometabolic disorders. Future prospective studies enrolling a large number of participants are needed to confirm these assumptions.

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Hyperinflammation readily resolved by dexamethasone in pediatric patients with hematological malignancies

Hiperinflamacija uspešno otklonjena deksametazonom kod pedijatrijskih bolesnika obolelih od hematoloških maligniteta

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Abstract

Background/Aim. Hyperinflammatory syndromes are a relatively rare phenomenon, but can be life-threatening for children suffering from malignancy. Hemophagocytic lymphohistiocytosis (HLH) is perhaps the best recognized of several such hyperinflammatory syndromes that share a key aspect of pathogenesis – the "cytokine storm". However, conditions that resemble HLH but do not fully meet its diagnostic criteria are not uncommon and often present a diagnostic challenge. The aim of this study was to examine clinical features, disease course, and response to dexamethasone treatment in children with hematological malignancy and hyperinflammation. **Methods**. This retrospective observational study analyzed medical records of 11 children (four females and seven males; median age 10.8 years, range 3.1–16.3 years) investigated for potential hyperinflammation during treatment for a hematological malignancy at the University Children's Hospital in Belgrade, Serbia, from January 2023 to July 2024. Relevant clinical and laboratory parameters were retrieved (serum triglyceride concentration was measured in ten children), as well as data on potential triggers, dexamethasone treatment, and treatment outcome. **Results.** All children were febrile. Bicytopenia/pancytopenia was noted in six (54.5%), and splenomegaly in two (18.2%) children. Bone marrow aspiration was performed in nine children, and no hemophagocytosis was observed. Serum triglyceride concentration was elevated in one (10.0%) child. Fibrinogen levels were above 1.5 g/L in all cases, and ferritin levels exceeded 500 μg/L in ten (90.1%) children. Two (18.2%) children had soluble interleukin-2 receptor (sIL-2R) above 2,400 IU/mL (the median sIL-2R level was 1,041 IU/mL, range 396–9,069 IU/mL, and the interquartile range was 1,012 IU/mL). Only one child met five of the eight HLH-2004 criteria. A potential viral, bacterial, or fungal trigger was identified in eight children. Eight children were treated with dexamethasone, resulting in the rapid resolution of the hyperinflammatory episode. **Conclusion**. In the diagnostic work-up of a febrile child with a hematological malignancy, one should always consider an inflammatory condition that may respond favorably to glucocorticoid treatment.

Key words:
adolescent; child; diagnosis, differential; drug therapy; febrile neutropenia; inflammation; lymphohistiocytosis, hemophagocytic; treatment outcome.

Apstrakt

Uvod/Cilj. Hiperinflamatorni sindromi su relativno retka pojava, ali mogu ugroziti život dece obolele od maligniteta. Hemofagocitna limfohistiocitoza (HLH) je najpoznatija od nekoliko takvih hiperinflamatornih sindroma koji dele ključni aspekt patogeneze – „citokinsku oluju". Međutim, stanja koja podsećaju na HLH, ali ne ispunjavaju u potpunosti njene dijagnostičke kriterijume, nisu retka i često predstavljaju dijagnostički izazov. Cilj rada bio je da se ispitaju kliničke karakteristike, tok bolesti i odgovor na lečenje deksametazonom kod dece koja imaju hematološke malignitete i hiperinflamaciju. **Metode.** Retrospektivnom opservacionom studijom analizirane su istorije bolesti jedanaestoro dece (četiri devojčice i sedam dečaka; medijana uzrasta 10,8 godina, raspon 3,1–16,3 godine) ispitivane zbog potencijalne hiperinflamacije tokom lečenja od hematološke maligne bolesti na Univerzitetskoj dečjoj klinici u Beogradu, Srbija, od januara 2023. do jula 2024. godine. Prikupljeni su relevantni klinički i laboratorijski parametri (koncentracija triglicerida u serumu izmerena je kod desetoro dece), kao i podaci o potencijalnim okidačima, lečenju deksametazonom i ishodu lečenja. **Rezultati.** Sva deca bila su febrilna. Bicitopenija/pancitopenija je zabeležena kod šestoro (54,5%), a splenomegalija kod dvoje (18,2%) dece. Aspiracija koštane srži obavljena je kod devetoro dece, a hemofagocitoza nije uočena. Koncentracija triglicerida u serumu bila je povišena kod jednog (10,0%) deteta. Nivoi

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fibrinogena bili su iznad 1,5 g/L kod sve dece, a nivoi feritina nadmašili su 500 μg/L kod desetoro (90,1%) dece. Kod dvoje (18,2%) dece nivo solubilnog receptora za interleukin-2 (sIL-2R) bio je iznad 2 400 IU/mL (njegova medijana iznosila je 1 041 IU/mL, raspon 396–9 069 IU/mL, a interkvartilni opseg 1 012 IU/mL). Samo jedno dete ispunilo je pet od osam HLH-2004 kriterijuma. Potencijalni virusni, bakterijski ili gljivični okidač identifikovan je kod osmoro dece. Osmoro dece lečeno je deksametazonom, uz brzo okončanje naleta hiperinflamacije. **Zaključak.** U dijagnostičkoj obradi febrilnog deteta koje ima hematološki malignitet uvek bi trebalo razmotriti zapaljensko stanje koje povoljno reaguje na lečenje glukokortikoidima.

Ključne reči:
adolescenti; deca; dijagnoza, diferencijalna; lečenje lekovima; neutropenija, febrilna; zapaljenje; limfohistiocitoza, hemofagocitna; lečenje, ishod.

Introduction

Hemophagocytic lymphohistiocytosis (HLH) is a severe hyperinflammatory condition that may occur as a primary disorder or secondary to a range of diseases of diverse etiology (infectious, autoimmune, malignant, or metabolic) [1]. A common pathogenetic feature of all forms of HLH is a disturbance (either transient or permanent) in the function of cytotoxic lymphocytes, believed to compromise the timely termination of immune responses to a wide variety of noxae and stimuli, thereby fostering an uncontrollable amplification of inflammatory cascades at the level of mononuclear phagocytes [2]. While primary HLH most often presents in early childhood and features a clearly defined genetic etiology, secondary HLH may be diagnosed at any age, and its relationship to a potential genetic predisposition is much more complex [3].

Secondary HLH is relatively infrequent in children with malignancy, as compared to adults [4]. However, HLH is just one of several nosological entities comprising the wider group of hyperinflammatory syndromes that share important aspects of pathogenesis, such as an excessive and uncontrollable release of proinflammatory cytokines (i.e., cytokine storm) [5]. While HLH is a well-defined condition, mostly diagnosed using the HLH-2004 criteria issued by the Histiocyte Society [6], demarcation from other hyperinflammatory states may often be difficult in clinical practice, particularly since hallmarks of HLH (or its diagnostic criteria) may not all be present initially or concurrently. Thus, clinicians are often confronted with patients suffering from poorly defined hyperinflammation with a wide spectrum of clinical features that may only remotely resemble HLH, but still dictate a thorough investigation [7]. This spectrum of clinical presentation is reflected in a spectrum of response to anti-inflammatory treatment – many children with hyperinflammation may respond to glucocorticoids alone.

The aim of this study was to examine clinical features, disease course, and response to dexamethasone treatment in children with hematological malignancy and hyperinflammation.

Methods

This retrospective observational study reviewed the medical records of 11 children (four females and seven males, median age 10.8 years, range 3.1–16.3 years), who were investigated upon the request of the treating physician while being treated at the University Children's Hospital in Belgrade, Serbia, between January 2023 and July 2024. The patients were treated for a hematological malignant disorder, including: acute lymphoblastic leukemia (3 children), acute myeloid leukemia (excluding acute promyelocytic leukemia) (3 children), acute promyelocytic leukemia (1 child), mixed-phenotype acute leukemia (1 child), myelodysplastic syndrome (2 children), and anaplastic large cell lymphoma (1 child). The study was approved by the Ethics Committee of the University Children's Hospital in Belgrade, Serbia (No. 16/264, dated November 21, 2024).

All children were in full remission at the time of the inflammatory episodes under investigation. We retrieved relevant clinical data (presence of fever and splenomegaly) and laboratory findings [complete blood count with leukocyte differential, C-reactive protein (CRP), fibrinogen, triglycerides, ferritin, and soluble interleukin-2 receptor (sIL-2R)], as well as information on potential triggers of the hyperinflammatory reaction, dexamethasone treatment, and the obtained response/outcome. The patients were evaluated according to the HLH-2004 diagnostic criteria [6]. The diagnosis of HLH was established by the presence of either a molecular diagnosis consistent with HLH or five out of eight of the following criteria: fever > 38.5 °C; splenomegaly; cytopenia (bicytopenia or pancytopenia) (at least two of the following three: hemoglobin < 9 g/dL, platelets < 100×10^9/L, or neutrophils < 1.0×10^9/L); hypertriglyceridemia and/or hypofibrinogenemia (fasting triglycerides > 3.0 mmol/L or fibrinogen ≤ 1.5 g/L); hemophagocytosis in bone marrow, spleen, liver, lymph nodes, or other tissues; low or absent natural killer cell activity; serum ferritin concentration ≥ 500 μg/L and sIL-2R ≥ 2,400 U/mL.

Due to the small sample size, no statistical inferences were possible, and only descriptive statistics were used, except in determining the correlation between the number of HLH-2004 criteria fulfilled and sIL-2R serum concentration, where Spearman's rank correlation coefficient (r_s) was applied.

The response to glucocorticoid treatment was assessed based on clinical improvement (cessation of fever) and laboratory parameters.

Results

Individual patient data are displayed in Table 1. All children were febrile at the time of investigation, while six (54.5%) had bicytopenia or pancytopenia as *per* HLH-2004 criteria. Splenomegaly was present in two (18.2%) children. Bone marrow aspiration was performed in nine patients, and no signs of hemophagocytosis were found. CRP was elevat-

ed in all children [range 18.4–307.0 mg/L; interquartile range (IQR): 150.8 mg/L; reference range (RR): 0–5 mg/L]. Serum triglyceride concentration was measured in ten children and was elevated in one (10.0%; upper limit of RR: 3.5 mmol/L). None of the children had a fibrinogen level below the HLH-2004 cut-off of 1.5 g/L; in fact, in nine (81.8%) children, it was elevated (upper bound of RR: 4.6 g/L). The median fibrinogen level was 9.5 g/L (IQR: 8.5 g/L). Serum ferritin concentration was above 500 μg/L in ten (90.1%) children, with a median ferritin level of 1,483.5 μg/L (range 182.5–6,260.5 μg/L; IQR: 3,545.0 μg/L). Only two (18.2%) children had sIL-2R levels above 2,400 IU/mL. The median sIL-2R level was 1,041.0 ± 2,617.9 IU/mL (range 396–9,069 IU/mL).

Only one child met five of the eight HLH-2004 criteria. Another two children met four criteria, while most children in our series (six) fulfilled three. One child met two criteria, and another one fulfilled only one (Table 2).

Table 1

Basic characteristics, hematological malignant disorders, and laboratory findings of patients

Patient No.	Age (years)	Gender	sIL-2R (IU/mL)	Dg.	Fev	Spl	Hem	Cyt	Fer (μg/L)	Trig (mmol/L)	Fib (g/L)	CRP (mg/L)
1	16.2	f	9,069	MDS	+	+	n.p.	no	1,390.9	1.05	9.0	140.4
2	16.3	m	4,198	AML	+	–	–	yes	5,320.0	2.06	10.9	197.9
3	10.6	m	1,288	MPAL	+	–	–	no	1,485.3	1.58	3.9	18.4
4	6.2	m	519	ALL	+	–	n.p.	yes	3,157.9	1.29	9.6	128.2
5	13.4	f	440	APL	+	–	–	yes	3,181.3	3.53	9.5	68.9
6	3.8	f	539	AML	+	–	–	yes	1,031.8	0.73	6.6	81.3
7	14.8	m	447	ALCL	+	–	–	yes	6,260.5	1.94	14.5	307.0
8	10.8	f	396	MDS	+	–	–	no	182.5	0.50	10.5	29.1
9	16.0	m	1,386	ALL	+	+	–	no	849.2	2.67	5.9	137.6
10	3.5	m	1,041	ALL	+	–	–	yes	1,253.8	2.58	2.2	28.0
11	3.1	m	1,431	AML	+	–	–	no	4,273.6	N.p.	11.9	230.0

No. – number; f – female; m – male; sIL-2R – soluble interleukin-2 receptor; Dg. – diagnosis; Fev – fever; Spl – splenomegaly; Hem – hemophagocytosis on bone marrow aspiration; Cyt – cytopenia; Fer – ferritin; Trig – triglycerides; Fib – fibrinogen; CRP – C-reactive protein; MDS – myelodysplastic syndrome; AML – acute myeloid leukemia; MPAL – mixed-phenotype acute leukemia; ALL – acute lymphoblastic leukemia; APL – acute promyelocytic leukemia; ALCL – anaplastic large-cell lymphoma; n.p. – not performed.
All values are expressed as numbers.

Table 2

Fulfilment of HLH-2004 criteria, potential triggers, treatment, and outcome of patients

Patient No.	Age (years)	Gender	HLH Cr	Potential Trigger	Treatment	Outcome
1	16.2	f	5	EBV infection/reactivation (PCR-confirmed)	Dexamethasone	Resolution
2	16.3	m	4	None apparent	Dexamethasone	Resolution
3	10.6	m	2	Bacterial infection of CVK	Dexamethasone and antibiotics	Resolution
4	6.2	m	3	Bacterial pneumonia with pleural effusion	Dexamethasone and antibiotics	Resolution
5	13.4	f	3	Pulmonary aspergillosis	Dexamethasone and antimycotics	Resolution
6	3.8	f	3	*Pneumocystis jiroveci* pneumonia	Antibiotics	Resolution
7	14.8	m	3	None apparent	Dexamethasone and antibiotics	Resolution
8	10.8	f	1	None apparent	[1] None	Resolution
9	16.0	m	4	Crural abscess and anal fissure	Antibiotics	Resolution
10	3.5	m	3	Septicemia (*Escherichia coli*)	Dexamethasone and antibiotics	Resolution
11	3.1	m	3	Septicemia (*Streptococcus sanguinis*)	Dexamethasone and antibiotics	Resolution

HLH – hemophagocytic lymphohistocytosis; No. – number; HLH Cr – number of HLH-2004 criteria met; f – female; m – male; EBV – Epstein-Barr virus; PCR – polymerase chain reaction; CVK – central venous catheter.
[1] Note: one child underwent spontaneous resolution.

There was a significant correlation between sIL-2R level and the number of criteria fulfilled ($r_s = 0.7$, $p = 0.016$).

In eight children, a potential trigger was identified, while the remaining three exhibited no conceivable trigger for a hyperinflammatory episode (Table 2). Five of the presumed triggers were bacterial, two were fungal (including *Pneumocystis jirovecii*), and one was viral in nature [Epstein-Barr virus (EBV)]. Eight children were treated with dexamethasone (10 mg/m^2 for 7 to 10 days, initiated within three days from the onset of the febrile episode), with rapid resolution of their hyperinflammation. Of the remaining three, two were successfully treated by antibiotics (the child with *Pneumocystis jirovecii* and another with crural abscess and anal fissure), while one case resolved spontaneously (Table 2). In four of the eight children treated with dexamethasone (50.0%), a recurrent hyperinflammatory episode occurred one to eight months later, which again rapidly resolved upon another course of dexamethasone. All children were alive at the time of manuscript preparation (median follow-up 10 months, range 4–22 months).

Discussion

In a previously published series of 45 pediatric patients from our institution, investigated for hyperinflammation of all etiologies over a ten-year period, 11 (24.4%) patients had a hematological malignancy, of whom only one (9.1%) met the HLH-2004 criteria for HLH [8]. Given that only one patient in the present series (also consisting of eleven patients) met the same criteria (again, 9.1%), the proportion of children with true HLH among those exhibiting signs of potential hyperinflammation that may prompt the clinician to order appropriate investigation appears to be fairly constant. However, such instances have obviously become more frequent, as judged by the respective length of the time periods covered by the two series comprising an equal number of patients (ten years vs. two years). This may well be due to increased awareness of hyperinflammatory complications during the treatment of hematological malignancies, as well as enhanced diagnostic capacities. The rarity of true HLH in children with malignancy is in line with published reports on its incidence, such as the work of Löfstedt et al. [9] in Sweden, who retrospectively found only nine confirmed pediatric cases between 1997 and 2018. The annual incidence in malignancy-affected adults, on the other hand, was found to equal or exceed 0.62 *per* 100,000. It is, however, reasonable to assume that these figures, since they pertain to HLH alone, represent merely the tip of the iceberg of hyperinflammation.

At the time, all children in our series were undergoing aggressive chemotherapy; all were febrile, and most (10/11, 90.1%) had a ferritinemia above the HLH-2004 threshold. Therefore, it can be said that all children had febrile neutropenia, highlighting the principal diagnostic quandary in hyperinflammation arising during treatment of a hematological malignancy: how to differentiate an infectious complication from pure uncontrolled inflammatory response, given that clinical findings may be identical [10]. This dilemma is compounded by the fact that probable or confirmed presence of an infectious agent, in itself, does not preclude the possible diagnosis of a hyperinflammatory state [11]. Furthermore, the diagnostic value of splenomagaly and cytopenias is quite problematic in this setting, as these findings may result from the underlying condition and/or its treatment [12]. It is notable that the only child in our series who met the HLH-2004 criteria (patient number 1) had a polymerase chain reaction-confirmed reactivation of EBV infection and was therefore confronted with a potent HLH-triggering agent, apparently no less relevant in children undergoing treatment for malignancy than in the general population [13].

It is notable that patient number 1, a child with apparent EBV-associated HLH, was successfully treated with dexamethasone alone, as were seven of the ten children who met fewer than five HLH-2004 criteria. Among these seven children, one had pulmonary aspergillosis and was also treated with antimycotics (patient number 5), four had bacterial infections and received antibiotics (patients 3, 4, 10, and 11), while two had no apparent trigger (patients 2 and 7); however, patient number 7 also received antibiotics as part of the pre-emptive approach for febrile neutropenia. Of the three patients not treated with dexamethasone (patients 6, 8, and 9), two were eventually found to suffer from infections curable by antimicrobial treatment alone (*Pneumocystis jirovecii* pneumonia and crural abscess/anal fissure, respectively), while in the remaining child, initial inflammatory symptoms and signs resolved spontaneously. This was also the only patient in the series not exhibiting a ferritin level above 500 μg/L. Nevertheless, the inclusion of this patient in the diagnostic work-up for hyperinflammation (as with the other two children with "pure" infection) could still be justified by reasonable excess of caution, since in pediatric hematology and oncology, hyperinflammatory complications can progress rapidly and may be fatal if treatment is delayed [14]. Although it is impossible, in the absence of large controlled studies, to say how much glucocorticoid treatment actually contributed to the observed favorable outcome, it is conceivable that these time-tested antiinflammatory agents with a complex and multifaceted set of mechanisms of action at the very least accelerated the resolution of the (hyper)inflammation [15]. It is also noteworthy that some children meeting the HLH-2004 criteria have previously been reported to respond well to glucocorticoid therapy alone, or in combination with intravenously administered immunoglobulins [16]. In addition, the fact that four of our patients had a repeated hyperinflammatory episode that cleared upon another course of dexamethasone indirectly attests to a probable existence of a relative genetic predisposition [17]. Our experience supports the notion that in the setting of an inflammatory condition arising in pediatric malignancy, HLH-2004 criteria *per se* would not be a sound basis for treatment decisions. Moreover, differential diagnosis between potential sepsis and some form of systemic inflammatory response syndrome remains a constant issue of concern [18]. This could perhaps be resolved by an in-depth investigation of the inflammatory response at the cytokine level, such as the application of proteomics to look into interferon-γ signatures [19], once such diagnostic modalities become more widely available.

Another notable point is that sIL-2R was elevated above the HLH-2004 threshold of 2,400 IU/mL in only two patients (the first and second patient) – the child with apparent EBV-associated HLH and another one without an identified trigger, but with four criteria met. This is in line with the reported sensitivity and specificity of sIL-2R level measurement [20, 21]. However, most children in our series displayed moderately elevated sIL-2R values, which correlated significantly with the number of criteria fulfilled. This is somewhat expected, since HLH-2004 criteria have generally been devised to detect hyperinflammation. Nevertheless, moderately elevated plasma levels clustering around the midpoint between physiological and HLH-associated range may not be without significance in patient work-up. This raises the question whether a lower threshold for sIL-2R level might be diagnostically useful in detecting more broadly defined hyperinflammation in complex clinical settings. Ideally, the answer should be determined through appropriately designed studies in large patient populations, endowed with sufficient statistical power.

Finally, although the HLH-2004 protocol was not applicable to our patient series as such, it still offered us a useful orientation tool for diagnostic investigation. The possibility of an overlap between HLH and other (hyper)inflammatory states, along with the inherently diachronic nature of its diagnostic criteria, is explicitly acknowledged in the Protocol [22], where it is stated that not all patients meet the criteria, and many may indeed do so only later in the course of the disease. This additionally strengthens the case regarding hyperinflammation that we encountered as a type of pathophysiological process related to, yet distinct from, HLH, which, fortunately, proved to be fully treatable by dexamethasone.

Conclusion

An inflammatory state responding well to glucocorticoid treatment should always be considered a possibility in the work-up of a febrile condition arising during the treatment of pediatric hematological malignancies, whether or not an apparent infectious trigger is present.

Conflict of interest

The authors declare no conflict of interest.

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Pattern of maxillofacial trauma in children and adolescents: a three-year retrospective study

Obrazac maksilofacijalne traume kod dece i adolescenata: trogodišnja retrospektivna studija

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Abstract

Background/Aim. Understanding the epidemiological aspects associated with maxillofacial traumas (MFTs) is necessary to develop effective preventive and protective strategies. The aim of this study was to assess the age-related characteristics and pattern of MFTs in patients under 18 years of age. **Methods.** A retrospective study analyzed clinical data from individuals aged 0–18 years with MFTs presenting to the emergency department over a three-year period. Data were related to epidemiological aspects such as age, gender, types of trauma, and type of bone fracture. A descriptive statistics of a data set and a χ^2 test for categorical variables were used. **Results.** A total of 418 patients with MFT were included in this retrospective study. The mean age of the patients was 8.5 ± 5.5 years, and the mean Glasgow Coma Scale score was 14.7 ± 1.4. The most common type of bone fracture was cranial fracture (87.31%). The most common reasons for admission were assault (24.8%) in male patients and fall (79.2%) in female patients (p = 0.009 in both cases). Admissions peaked in August (11%), on weekdays (73.6%), and between 4:00 pm and midnight (49.4%). Consultations were requested for 63.4% of the cases, and the most frequent requests were at the Plastic Reconstructive and Aesthetic Surgery Department (51.67%), especially for females (54.7%), and due to falls (67.4%). More consultation requests were sent for the patients from the 0–6 age group (66.5%). Conservative treatments were applied to 61% of all cases. The patients from the 0–6 age group (58.9%) were treated more frequently in surgical and intensive care units (p = 0.001), while the patients from the 7–12 (40%) and 13–18 (35.7%) age groups received conservative treatment more frequently (p = 0.001). The majority of patients aged 0–3 years (49.7%) were treated in surgical and intensive care units (p < 0.05). **Conclusion.** In pediatric patients in Türkiye, MFTs were most prevalent in the 0–3 age group, and MFTs were most often caused by falls. The fractures most frequently involved cranial bones. The findings of the study provide significant insights for defining preventive and health-promoting policies.

Key words:
adolescent; age factors; child; maxillary fractures; sex factors; türkiye; wounds and ınjuries.

Apstrakt

Uvod/Cilj. Za razvoj efikasnih preventivnih i zaštitnih strategija neophodno je razumevanje epidemioloških aspekata povezanih sa maksilofacijalnim traumama (MFT). Cilj rada bio je da se procene karakteristike vezane za uzrast i obrazac MFT kod pacijenata mlađih od 18 godina. **Metode**. Retrospektivnom studijom analizirani su klinički podaci osoba uzrasta 0–18 godina koje su zadobile MFT i javile se u hitnu pomoć tokom tri godine. Podaci su se odnosili na epidemiološka obeležja kao što su starost, pol, vrste traume i tip preloma kostiju. Korišćeni su deskriptivna statistika skupa podataka i χ^2 test za kategoričke varijable. **Rezultati**. Retrospektivnom studijom obuhvaćeno je ukupno 418 pacijenata sa MFT. Prosečna starost pacijenata iznosila je 8,5 ± 5,5 godina, a srednji skor Glazgovske skale kome 14,7 ± 1,4. Najčešća vrsta preloma bila je fraktura lobanje (87,31%). Najčešći razlozi za prijem bili su napad (24,8%) kod pacijenata muškog pola, a kod pacijenata ženskog pola pad (79,2%) (p = 0,009 u oba slučaja). Najveći broj prijema bio je u avgustu (11%), radnim danima (73,6%) i između 16 časova i ponoći (49,4%). Konsultacije su zatražene za 63,4% slučaja, a najčešći zahtevi bili su na Odeljenju plastične, rekonstruktivne i estetske hirurgije (51,67%), posebno za pacijente ženskog pola (54,7%) i zbog padova (67,4%). Za pacijente uzrasta 0–6 godina (66,5%) poslato je više zahteva za konsultacije. Konzervativni tretman primenjen je kod 61% svih pacijenata. Pacijenti uzrasta 0–6 godina (58,9%) češće su lečeni u hirurškim jedinicama i jedinicama intenzivne nege (p = 0,001), dok su pacijenti uzrasta 7–12 godina (40%) i 13–18 godina (35,7%) češće lečeni konzervativno (p = 0,001). Većina pacijenata

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uzrasta 0–3 godine (49,7%) lečena je u hirurškim jedinicama i jedinicama intenzivne nege ($p < 0,05$). **Zaključak**. Kod pedijatrijskih pacijenata u Turskoj, najčešće MFT bile su kod pacijenata uzrasta 0–3 godine, a MFT su najčešće bile izazvane padom. Prelomima su najčešće bile zahvaćene kosti lobanje. Nalazi studije pružaju značajne uvide u definisanje preventivnih politika, kao i politika koje unapređuju zdravlje.

Ključne reči:
adolescenti; životno doba, faktor; deca; maksila, prelomi; pol, faktor; turska; povrede.

Introduction

Maxillofacial trauma (MFT) is relatively rare in children [1]. However, traumatic brain injury is currently the leading cause of disability and mortality among children worldwide [2]. The etiology and characteristics of pediatric MFT vary by region due to differences in social, environmental, and economic factors [3].

Physiologically, children are particularly vulnerable to facial fractures due to the flexibility of their developing skeletons, the presence of unerupted teeth, and the lack of paranasal sinus pneumatization compared to adults [4]. MFTs are significant because children's faces are difficult to protect during falls, and such injuries can adversely affect maxillofacial development and dental health.

Inappropriate treatment of MFT in children may lead to complications such as temporomandibular joint ankylosis and developmental problems [5]. These concerns are compounded by the potential for severe long-term effects, which can result in functional impairments and negatively impact the quality of life for affected individuals [1]. Understanding the epidemiological aspects associated with MFT is essential for developing effective clinical treatment strategies.

This study aimed to analyze the type and incidence of MFT in individuals aged 0–18 years.

Methods

The retrospective study was conducted at a tertiary hospital. It was approved by the Ethics Committee of Gaziantep University, more precisely, the Non-Interventional Clinical Research Ethics Committee (No. 2024/61, from March 13, 2024). The research adhered to the Declaration of Helsinki and good clinical practice guidelines. Medical records were reviewed by matching treatment and diagnosis codes within the hospital information management system.

The study included all injured individuals aged < 18 years, regardless of gender, who presented with a history of trauma and had complete medical records of clinical and radiographic diagnoses during the specified study period. Individuals with incomplete hospital records were excluded from the analysis. Data from the Gaziantep Training and Research Hospital Emergency Department (ED) were examined for cases admitted between April 12, 2021, and April 28, 2024. Out of 2,355 cases, 1,617 had a history of MFT, with 417 cases meeting the inclusion criteria for the study. The patients were categorized into six age groups: 0–3 years old (infants), 4–6 years old (preschoolers), 7–9 years old (school-age children), 10–12 years old (preadolescents), 13–15 years old (early adolescents), and 16–18 years old (teenagers). The age groups were combined into three broader categories for a more balanced representation: preschool period (0–6 years old), school period (7–12 years old), and adolescents (13–18 years old).

The hospital records of all eligible patients were reviewed and classified into the following categories: age and gender of the patient, type of trauma, presence and type of bone fractures, mandibular fracture sites, consulting department, type of treatment, date and time of admission, and the Glasgow Coma Scale (GCS) scores. The type of fractured cranial bone was not recorded separately as frontal, parietal, temporal, occipital, sphenoid, and ethmoid bones. Due to the fast registration system implemented in the ED, all the data about cranial bones were recorded under the more general term "cranial bone fracture". In the current study, treatment procedures in which surgical interventions were not performed during the MFT treatment were considered conservative treatment. The GCS scoring system comprised three parameters: eye-opening, verbal response, and motor response. The GCS scores were calculated by summing the points from each parameter, ranging from 3 to 15 points. The GCS was categorized as follows: a score ≤ 8 indicated severe injury (coma), 9–12 suggested moderate damage (a pre-coma state), and 13–15 represented mild trauma (the patient is conscious).

Descriptive statistics for the data obtained from the study were presented, including the mean, standard deviation, median, minimum, and maximum values for numerical variables, along with frequency and percentage analyses for categorical variables. The Chi-square test was employed to compare categorical variables. Analyses were conducted using the statistical software SPSS v. 22.0 (Chicago, USA), with a significance level set at $p < 0.05$.

Results

The distribution of age groups, bone fractures, and trauma reasons is given in Table 1.

The mean age was 8.5 ± 5.5 years (median 8 years, minimum 1, maximum 18). In the current study, there were 312 males and 106 females, and the male-to-female ratio was 2.94 : 1. The mean GCS score was 14.7 ± 1.4 (median 15, minimum 3, maximum 15). The most commonly diagnosed type of bone fracture among those presenting to the ED was cranial fracture in 303 patients (87.3%) (Table 1). Mandible fractures were reported in 7 (2%) patients who presented to the ED, with 4 occurring in the corpus region, 2 in the condyle region, and 1 in the ramus region. A statistically significant relation was found between gender and the reasons for MFT ($p = 0.009$). The most common reason for ED visits in males was assault (24.8%), and the most common MFT type in females was falls (79.2%) ($p = 0.009$) (Table 2).

Table 1

The distribution of age groups, bone fractures, and types of trauma in children and adolescents with maxillofacial traumas

Parameters	Values
Age groups (years)	
0–3	128 (30.6)
4–6	47 (11.2)
7–9	55 (13.2)
10–12	64 (15.3)
13–15	60 (14.4)
16–18	64 (15.3)
Types of bone fracture	
mandible	7 (2)
maxilla	4 (1.1)
zygoma	2 (0.6)
orbita	14 (4)
nasal	17 (4.9)
cranial bones*	303 (87.3)
Types of trauma	
traffic accident	6 (1.4)
assault	86 (20.6)
occupational accident	1 (0.2)
fall	275 (65.9)
sport activity	24 (5.7)
not clear	24 (5.7)
firearm injuries	2 (0.4)

Values are given as numbers (percentages).
***No bone type is specified in these records.**

Table 2

Relationship between age and gender of children and adolescents and trauma types

Characteristics	Traffic accident	Assault	Occupational accident	Falls	Sport activity	Not clear	Firearm injuries	Total
Age groups (years)								
A (0–6)	0 (0)	6 (3.8)	0 (0)	138 (87.3)	2 (1.3)	12 (7.6)	0 (0)	158
B (7–12)	3 (2)	25 (16.7)	0 (0)	104 (69.3)	12 (8)	5 (4)	1 (0.7)	150
C (13–18)	3 (2.8)	55 (50.5)	1 (0.9%)	33 (30.3)	9 (8.3)	7 (6.4)	1 (0.9)	109
Gender								
male	6 (1.9)	**77 (24.8)**	1 (0.3)	191 (61.4)	18 (5.8)	18 (5.8)	1 (0.3)	312
female	0 (0)	9 (8.5)	0 (0)	**84 (79.2)**	6 (5.7)	6 (5.7)	1 (0.9)	106

Bolded values: $p = 0.009$ (Chi-Square tests) for gender and trauma types; $p < 0.001$ for trauma type "falls": A vs. B, A vs. C, and B vs. C; $p < 0.001$ for trauma type "assault": B vs. A, C vs. A, and C vs. B; $p < 0.05$ for trauma type "sport activity": B vs. A and C vs. A.

Patients in the 0–3 age group were admitted to the ED due to MFT significantly more often [128 (30.6%)] than patients from other age groups ($p < 0.05$) (Table 1). A statistically significant relation was observed between age groups and types of trauma ($p < 0.05$).

There were statistically more fall-type MFTs in the 0–6 age group (87.3%) than in the other two age groups ($p = 0.001$). In the 7–12 age group, MFTs due to falls were statistically more common than in the 13–19 age group ($p = 0.001$). MFTs due to assault or sports activity were statistically more common in the 7–12 and 13–18 age groups than in the 0–6 age group ($p < 0.05$). Statistically more assault-related MFTs were observed in the 13–18 age group than in the 7–12 age group ($p = 0.001$) (Table 2).

ED visits for MFTs peaked in August, 46 (11%) patients, followed by May, 43 (10.2%) patients. Lower admission rates were noted in February (5.9%), November (4.3%), and in winter (19%). MFTs happened predominantly on weekdays in 308 (73.6%) patients, and between 4:00 pm and midnight in 206 (49.4%) patients. A statistically significant difference was noted between age groups and ED visit times ($p = 0.001$). Compared to other age groups, the 0–6 age group primarily visited the ED between midnight and 08:00 am [24 (77.4%)], the 7–12 age group between 4:00 pm and midnight [79 (38.3%)], and the 13–18 age group between 08:00 am and 4:00 pm [61 (33.9%)].

The patients from the 0–6 age group experienced MFTs more frequently on weekends (45%), while the patients from the 13–18 age group were more affected on weekdays

(28.9%). No statistically significant difference was found between age groups and admission days ($p > 0.05$).

Consultations from other medical departments were requested for 265 (63.4%) cases. The most frequent requests were made to the Plastic Reconstructive and Aesthetic Surgery Department – 216 (51.6%) cases (especially for females – 54.7%, and due to falls – 67.4%), followed by the Ear, Nose, and Throat Department – 39 (9.3%) cases, and the Ophthalmology Department – 28 (6.7%) cases. No statistically significant relation was found between age groups and consultation requests ($p = 0.448$). However, the 0–6 age group received more consultation requests (66.5%) than other age groups. Consultations were most frequently requested for cases resulting from falls (67.4%), followed by assaults (17.4%).

Conservative treatments were applied to 255 (61%) MFT cases. A significant correlation was found between age categories and treatment types ($p = 0.001$). Patients from the 0–6 age group (58.9%) were treated more frequently in surgical and intensive care units for MFT compared to other groups, while patients from 7–12 (40%) and 13–18 (35.7%) age groups were more likely to receive conservative treatment ($p = 0.001$). Most patients treated for MFT in surgical and intensive care units (49.7%) were aged 0–3 years, while those treated conservatively were predominantly aged 13–15 (19.6%) and 16–18 years (21.6%) ($p < 0.05$).

Discussion

The results of this research revealed a significant frequency of MFT among children and the 16–18 age group visiting the ED of the university hospital. The GCS is a widely used assessment tool for comatose patients, allowing for the rapid diagnosis of changes in consciousness following head trauma [6]. Although cranial fractures were the most common type identified in this study, and MFTs were primarily caused by falls, the mean GCS score was 14.7, indicating mild head trauma. This suggests that very severe cases were infrequent in the ED focus of this study. Periodic monitoring is essential to identify and prevent early facial asymmetry or malocclusion in developing children [7]. Furthermore, the findings can provide crucial insights for defining preventive and health-promoting policies, as well as for raising community awareness.

To gain deeper insights into the causes and treatment of pediatric MFTs, the present study relies on epidemiological data. However, the crowding of EDs and staffing shortages often result in incomplete records, complicating trauma studies. For instance, only 25.8% of the patients included in this study had sufficient information in their files to meet the research criteria. In 303 cases, the specific cranial bones involved in fractures were not documented, leading to a general classification as “cranial bone fracture”.

The inclusion criteria for this study were based on the global standard for children aged 18 years and under. The mean age of the patients included in the present study was 8.5 years, and the 0–3 age group had the highest incidence of MFT, likely due to their limited protective awareness during this developmental stage. Their active engagement in daily activities contributes to the high prevalence of trauma in this age category, compounded by underdeveloped balance skills and difficulties in maintaining control.

Significant differences were noted according to age and gender in the present study, with behavioral patterns and developmental stages influencing these results. Of the total cases evaluated, 312 (74.8%) were male. Generally, the incidence of MFT is higher in males than females, and the present study found a male-to-female ratio of 2.94 : 1. This aligns with previous studies reporting a ratio of 3 : 1, with one female affected for every three males [1, 8]. Some studies have noted even higher ratios, reaching 13 : 1 [9, 10]. These trends may relate to physiological changes and increased activity levels associated with secondary sexual traits. Findings of the present study revealed that MFTs were more prevalent in the 0–3 age group. A Chilean study also indicated that 56.3% of children under 5 years of age most frequently experienced facial trauma [11], while a global study noted that facial injuries were rare below this age, with occurrences increasing from school age to puberty [12].

The mechanisms of trauma significantly influenced the extent of damage. Consistent with Ulusoy et al. [13], the present study found that the majority of MFTs resulted from falls (65.9%), followed by assaults (20.6%). However, a meta-analysis indicated that road traffic accidents were the leading cause of MFT in individuals under 18 years [14]. Similarly, Kelimu et al. [15] reported that road accidents and falls were the most common causes of pediatric maxillofacial fractures, followed by assaults. The meta-analysis by Mohammadi et al. [14] showed that 9.9% of MFTs in children and the 13–18 age group were due to violence, and violence was increasing, especially in the Americas, exceeding sports-related MFTs. The present study noted that assaults constituted the second most common medical-legal issue, which has reportedly increased over time and correlates with older age [13]. The 13–18 age group was more susceptible to interpersonal violence, with one Turkish study showing a median age of 14.0 years for hospitalized cases [13]. Interpersonal violence among children, especially in the 7–12 and 13–18 age groups, has risen, attributed to preventative measures in developed nations, while falls and vehicle accidents remain prevalent in developing countries [11, 14]. Therefore, local strategies must be tailored to address the unique challenges posed by MFTs in specific regions.

Facial bone fractures are less common than cranial fractures in children due to the frontal projection of the skull and the relative retrusion of the face. Facial bones become more prominent with age, increasing fracture risk [16]. On the other hand, Khan et al. [16] reported that 46% of patients with MFT-related fractures between the ages of 1 and 12 were younger than 4 years. Furthermore, in a retrospective study of children younger than 12 months, the most common fracture was skull fracture [17]. Similarly, cranial bones were the most frequently fractured bones in the current research (87.3%). One study identified nasal fractures as the second most prevalent maxillofacial fracture type (30.2%), similar to the findings of the present study, although at a considerably lower rate

(4.8%) [18]. Variability in nasal fracture occurrences in children is likely due to differences in anatomical features, ethnicity, and geography. Thus, preventive measures should focus on reducing incidence and minimizing injury severity. Proper education and implementation of safety standards can significantly decrease MFT morbidity among children and the 13–18 age group. Public health professionals should provide expert guidance and recommendations to parents, educators, and caregivers.

In the current study, ED visits occurred predominantly on weekdays (73.6%), similar to the results of a Brazilian study (64%) [1]. When considering a longer time frame, monthly trauma frequency was highest in May (10.2%) and August (11%). In a Pakistani study, similar to the current study, the number of pediatric patients with facial fractures was highest in June, July, and August [16]. Although some studies reported no significant variation between months [12, 19], lower admission rates were noted in February (5.9%), November (4.3%), and in winter (19%). Climatic factors are believed to contribute to the reduced prevalence of cases during winter. Additionally, summer is the time when primary, secondary, and high schools in Türkiye are closed, and therefore, recreational activities of the 7–9 and 13–18 age groups increase. Fasola et al. [20] investigated the timing of MFT occurrences, finding most incidents between 6:00 am and 6:00 pm. In the present study, most ED visits occurred between 4:00 pm and midnight, aligning with times when children and the 13–18 age group are typically at home, indicating that MFTs were more common in domestic settings for those under 18.

Previous studies indicated that surgical interventions were primarily utilized for MFTs [18, 21]. In the present study, most patients aged 7 years and older with MFT received conservative treatment (61%). However, half of the children aged 0–3 years continued treatment in surgical and intensive care units. Consultation was sought in 63.4% of cases, primarily from the Plastic Reconstructive and Aesthetic Surgery Department (51.67%). The 0–3 age group had the highest consultation requests (32.1%), possibly due to heightened caution among healthcare professionals when treating younger patients. Additionally, plastic surgery consultations were more frequently requested for females (54.7%), likely reflecting higher aesthetic expectations.

Limitations of the study

This retrospective analysis included several limitations. First, the patients' conditions could not be extensively assessed immediately following the incidents. Additionally, the study was conducted at a single location, which may affect the generalizability of the findings. Moreover, the present study did not evaluate treatment outcomes, which limits the understanding of the long-term effects. Finally, insufficient patient data in the ED records could result in inconsistent and inaccurate information regarding MFT.

Conclusion

The incidence of maxillofacial trauma in patients under 18 years was influenced by age, gender, and types of trauma. The highest incidence of maxillofacial trauma was observed in the 0–3 age group, with falls identified as the primary cause. Males experienced maxillofacial trauma more frequently, primarily resulting in cranial bone fractures. Consultations with the Plastic Reconstructive and Aesthetic Surgery Department were more common for females, and children aged 0–3 were the ones who were most likely to require consultations. Notably, the 0–6 age group tended to receive less conservative treatment. Future multicenter studies on the etiology of maxillofacial trauma in children and more detailed records kept in emergency departments may enable the design of protective measures.

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Influence of staining on the optical properties and surface topography of low-shrinkage and conventional dental composites

Uticaj bojenja na optička svojstva i topografiju površine kompozitnih materijala male kontrakcije i konvencionalnih stomatoloških kompozita

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Abstract

Background/Aim. Matching the optical properties of composite restorations and teeth is very important in esthetic dentistry. The challenge lies not only in the initial matching but also in the fact that these optical properties change significantly in the oral environment over time. The aim of the study was to examine the initial color, translucency, fluorescence, and surface topography of various composite materials, their changes after seven days of immersion in tea, as well as after repolishing. **Methods.** Two low-shrinkage nanohybrid composites (N'Durance® and Charisma® Diamond) and two conventional composites [Tetric EvoCeram® (nanohybrid) and Gradia® Direct (microhybrid)] in shade B1 were immersed in tea for seven days. Diffuse reflection, fluorescence, and surface roughness were measured before and after immersion in tea, as well as after repolishing. Color and translucency were calculated using the CIEDE2000 and CIEDTP2000 equations. **Results.** The highest initial lightness (L*) values were observed for Charisma® Diamond, while the highest red-green (a*) and yellow-blue (b*) coordinates were observed for Tetric EvoCeram®. The following trend in color change (ΔE_{00}) was observed: Tetric EvoCeram®>N'Durance®>Charisma® Diamond ≈ Gradia® Direct. The highest color change was recorded for Tetric EvoCeram® ($\Delta E_{00} = 6.0$). Additionally, the highest translucency parameter (TP_{00}) and surface roughness before and after immersion in tea were recorded for Tetric EvoCeram®, while the highest decrease in translucency intensity after staining was observed for Gradia® Direct (33%). After repolishing, color changes for Tetric EvoCeram® and N'Durance® remained higher than the clinically acceptable threshold, while the values for Charisma® Diamond and Gradia® Direct were clinically acceptable. Repolishing restored almost complete translucency, fluorescence, and surface roughness, bringing the spectral properties of the composites closer to their initial values. **Conclusion.** The initial optical characteristics and surface roughness of the composites, as well as their modifications after immersion and repolishing, depend on the type of composite material. The process of staining altered the brightness of the fluorescence, while leaving the spectral shape unaffected. The repolishing procedure almost completely restored the optical properties of all tested composite materials.

Key words:
color; composite resins; dental materials; fluorescence; materials testing; tea.

Apstrakt

Uvod/Cilj. Usklađivanje optičkih svojstava kompozita i zuba je veoma važno u estetskoj stomatologiji. Izazov ne leži samo u početnom podudaranju, već i u činjenici da se ova optička svojstva značajno menjaju u oralnoj sredini tokom vremena. Cilj rada bio je da se ispitaju početna boja, translucencija, fluorescencija i površinska topografija različitih kompozitnih materijala, njihova promena posle potapanja u čaj tokom sedam dana, kao i posle ponovnog poliranja. **Metode.** Dva nanohibridna kompozitna materijala male kontrakcije (N'Durance® i Charisma® Diamond) i dva konvencionalna kompozita [Tetric EvoCeram® (nanohibridni) i Gradia® Direct (mikrohibridni)] u nijansi B1 potopljeni su u rastvor čaja na sedam dana. Difuzna refleksija, fluorescencija i površinska hrapavost izmerene su pre i posle potapanja u čaj, kao i posle ponovnog poliranja. Boja i translucencija izračunate su prema CIEDE2000 i CIEDTP2000 formulama. **Rezultati.** Najveće inicijalne vrednosti svetloće (L*) uočene su kod kompozita Charisma® Diamond, dok su najveće vrednosti žutocrvene (a*) i žutoplave (b*) koordinate boje bile kod

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kompozita Tetric EvoCeram®. Primećen je sledeći trend promene boje (ΔE_{00}): Tetric EvoCeram®>N'Durance® >Charisma® Diamond ≈ Gradia® Direct. Najveća promena boje primećena je kod kompozita Tetric EvoCeram® (ΔE_{00} = 6,0). Takođe, najveće vrednosti translucencija parametra (TP_{00}) i površinske hrapavosti pre i nakon potapanja u čaj zabeležene su kod kompozita Tetric EvoCeram®, dok je najveće smanjenje intenziteta translucencije nakon prebojavanja zabeleženo kod Gradia® Direct (33%). Nakon ponovnog poliranja, promene boje kod Tetric EvoCeram® i N'Durance® ostale su veće od klinički prihvatljivog praga, dok su zabeležene vrednosti za Charisma® Diamond i Gradia® Direct bile u granicama kliničke prihvatljivosti. Ponovno poliranje je skoro u potpunosti vratilo vrednosti translucencije, fluorescencije i površinske hrapavosti, dovodeći spektralna svojstva kompozita bliže početnim vrednostima. **Zaključak.** Početna optička svojstva, površinska hrapavost kao i njihove promene nakon potapanja i ponovnog poliranja zavise od tipa kompozitnog materijala. Prebojavanje je promenilo intenzitet fluorescencije, dok je oblik emisionog spektra ostao nepromenjen. Proces ponovnog poliranja skoro je u potpunosti vratio optička svojstva svih testiranih kompozitnih materijala.

Ključne reči:
boje; smole, kompozitne; stomatološki materijali; fluorescencija; materijali, testiranje; čaj.

Introduction

Ensuring that the optical properties of composite restorations match those of natural teeth is a crucial step in the field of esthetic dentistry. While color is commonly regarded as the primary esthetic characteristic, the significance of fluorescence should not be overlooked. Natural teeth exhibit fluorescence under ultraviolet (UV) radiation, which is particularly strong in intense daylight, especially during summer, and in certain artificial lighting environments, such as those in dance clubs and cinemas. Despite its relevance, the fluorescence of natural teeth and dental restorations remains underexplored in esthetic dentistry [1, 2].

Shade mismatching in esthetic restorations can be attributed to a variety of factors, the most significant of which is the use of inaccurate shade guides that fail to represent the true color and translucency of the restorative materials. Additional contributing factors include the surrounding environment, inadequate color rendering index of ambient lighting, physiological and psychological responses, metamerism, observation angle, the size of the visual field, mood, age, eye fatigue, and even gender. Furthermore, patients often have restorations from different brands and material types in the mouth [3, 4]. The challenge lies not only in achieving an initial match in color and fluorescence between natural teeth and restorations, but also in maintaining this match over a long time. The optical properties of restorative materials significantly change when a material is exposed to commonly consumed beverages and foods. These changes are a direct consequence of the adsorption and absorption of various colorants present in foods and beverages. Often, as time passes, these restorations become aesthetically unacceptable because of the intense change of color.

The initial color of the composite material depends on its structure, the monomer composition, filler content, and photoinitiator type [5]. One of the parameters that affects initial esthetic properties and color stability is the surface roughness of the restoration, which is related to the combination of factors, such as polishing and finishing procedures, but also to the composition of the monomer and the percentage, type, and size of filler particles [6, 7]. According to literature data, surface roughness has a direct influence on the susceptibility to extrinsic staining [8]. Different repolishing procedures can partially remove the stains and help restore the optical properties of the materials [9].

Low-shrinkage composites represent a new generation of composites used to reduce the shrinkage during polymerization and improve marginal adaptation. In addition, it would be expected that these composites have esthetic properties comparable to conventional composites. Only a few studies have been performed on the optical properties of low-shrinkage composites. Some of these composites showed similar or smaller color changes compared to the conventional ones [10, 11].

Using dental shade guides is the most common method for color communication in dentistry. Literature data indicate that restorative materials labeled with the same shade designation can vary significantly in color depending on the brand and type of material [12, 13]. Lee et al. [14] reported that color coordinates, translucency, changes in color, and translucency after curing, polishing, and thermal cycling varied among brands of the composite even though the shade designation was the same (A2).

Previous studies have revealed that exposure to various discolorations present in different foods and beverages affects the optical properties of dental composites and alters them to varying degrees [15–17]. Among the most frequently consumed beverages, tea has shown a high potential for discoloring teeth and restorations because it contains a significant amount of tannins. It can considerably change the color of composites over time.

The aim of this study was to examine the initial color, translucency, fluorescence, and surface topography of various commercial composites of the same shade designation and their changes after seven days of immersion in tea and after repolishing.

Methods

Forty samples of four commercial composites – two low-shrinkage nanohybrid (N'Durance® and Charisma® Diamond) and two conventional [Tetric EvoCeram® (nanohybrid) and Gradia® Direct (microhybrid)] (n = 10 *per* group) – were prepared according to the procedure described

by Manojlovic et al. [15]. All composites were of B1 shade. The samples were first placed in distilled water at a temperature of 37 °C for 24 hrs. After that, half of the samples in each group were immersed in tea (specifically, black tea – English Breakfast, Sir Winston company LTD, London, UK) at the same temperature of 37 °C for seven days. The tea was prepared by immersing a pre-packaged tea bag in 150 mL of boiling water for 5 minutes, following the manufacturer's instructions. The staining solution was replaced regularly to prevent bacterial contamination. The other half of the samples were immersed in distilled water, which served as the control. Prior to taking measurements, the composite samples were rinsed under tap water for ten seconds and then dried by blotting them with paper towels. Subsequently, all specimens underwent repolishing, and additional measurements were conducted.

Diffuse reflection measurements

Reflection and translucency were calculated from diffuse reflection spectra obtained using a Shimadzu UV–Visible UV-2600 spectrophotometer (Shimadzu Corporation, Tokyo, Japan) equipped with an integrating sphere (ISR-2600), over the 360–830 nanometer (nm) range with a 1 nm step. Measurements were performed before and after immersion, as well as after polishing of the specimen. The sample color was calculated from the diffuse reflection spectrum using the CIELAB color system of the International Commission on Illumination (*Commission Internationale de l'Éclairage* – CIE), which includes the lightness (L*), the red-green coordinate (a*), the yellow-blue coordinate (b*), under standard illumination (D65) source against both white and black backgrounds [18].

The total color change (ΔE_{00}) was calculated according to the following CIEDE2000 equation [18, 19]:

$$\Delta E_{00} = \sqrt{\left(\frac{\Delta L'}{k_L S_L}\right)^2 + \left(\frac{\Delta C'}{k_C S_C}\right)^2 + \left(\frac{\Delta H'}{k_H S_H}\right)^2 + R_T\left(\frac{\Delta C'}{k_C S_C}\right)\left(\frac{\Delta H'}{k_H S_H}\right)}$$

where $\Delta L'$, $\Delta C'$, and $\Delta H'$ are the adjusted values of the metric CIELAB differences in lightness, chroma, and hue, C'_s and C'_r are the adjusted chroma values for the sample and reference, calculated using the S_L, S_C, and S_H weighting functions, the k_L, k_C, and k_H parametric factors, and the chroma-hue interaction coefficient R_T [18]. The color changes (ΔE_{00}) smaller than 1.8 were considered clinically acceptable [19].

The translucency was determined from measurement results performed against a black (B) and white (W) background. The translucency parameter (TP_{00}) was evaluated from the following CIEDTP2000 equation:

$$TP_{00} = \sqrt{\left(\frac{L'_B - L'_W}{k_L S_L}\right)^2 + \left(\frac{C'_B - C'_W}{k_C S_C}\right)^2 + \left(\frac{H'_B - H'_W}{k_H S_H}\right)^2 + R_T\left(\frac{C'_B - C'_W}{k_C S_C}\right)\left(\frac{H'_B - H'_W}{k_H S_H}\right)}.$$

Fluorescence measurements

Excitation-emission matrices (EEM) were obtained from a Fluorolog®-3 Model FL3-221 spectrofluorometer (Horiba JobinYvon), which uses a 450-W xenon lamp excitation source and Hamamatsu R928 PMT detector. Measurements were performed in the front-face configuration on a 270 to 550 nm excitation range and 300 to 650 nm emission range, with 5 nm and 1 nm steps, respectively.

Total fluorescence (TF) emission is represented as a two-dimensional sum of emission intensities over the excitation-emission plane:

$$TF = \sum_{\lambda_{EX}=270nm}^{550nm} \sum_{\lambda_{EM}=300nm}^{650nm} I(\lambda_{EX}, \lambda_{EM}).$$

The TF change between sample and reference in percentage is taken as a measure of differences in fluorescence:

$$\Delta TF\ (\%) = \frac{TF_{sample}}{TF_{reference}} \cdot 100\%.$$

The contour plots were selected to represent the resulting surface in two dimensions.

Topography measurements

Surface characteristics of composite specimens were evaluated on Quesant® atomic force microscope (Agoura Hills, CA) operating in tapping mode in air with standard silicone tips (NanoAndMore Gmbh, Wetzlar, Germany), on the 15 × 15 micrometre (μm) sample area and with the persistent force of 40 Newton/meters (N/m).

Statistical analysis

The obtained data were analyzed and correlated using the statistical program SPSS, version 22 (SPSS Inc., Chicago, USA). Mean values and standard deviation were used to describe numerical data. Two-way and one-way analyses of variance (ANOVA) with Tukey's *post-hoc* test were conducted to assess significant differences in TP_{00}, ΔE_{00}, and surface roughness between the tested groups. All analyses were performed at a 95% significance level ($\alpha = 0.05$), with statistical significance set at $p < 0.05$. G*Power 3.1.9.4 software for Windows (Heinrich Heine University, Düsseldorf, Germany) was used to calculate sample sizes for the three outcomes. The difference between the two means was estimated using data from a pilot study for the discoloration test. The alpha error was set at 0.05, and the study had 80% beta power ($dz = 2.381$). Five samples *per* material group were required to observe significant differences.

Results

Despite all composites being assigned the same shade designation (B1), their CIE L*a*b* mean values (Table 1) and diffuse reflectance spectra (Figure 1 A–D) exhibited differences on both white and black backgrounds, both before and after immersion in tea. After repolishing, the spectra returned to the initial shape for all sample groups except for the Gradia® Direct on the white background.

The obtained mean values and standard deviations of ΔE_{00} after immersion in water and tea, as well as after

Table 1

Mean values of the International Commission on Illumination L*, a*, b* color system for the tested composite materials before immersion in tea

Composite brand	L*	a*	b*
N'Durance®	77.4	-0.7	4.7
Charisma® Diamond	78.5	-1.1	7.2
Tetric EvoCeram®	76.3	0.7	7.6
Gradia® Direct	77.5	-0.1	6.5

L* – lightness; a* – red-green coordinate; b* – yellow-blue coordinate.

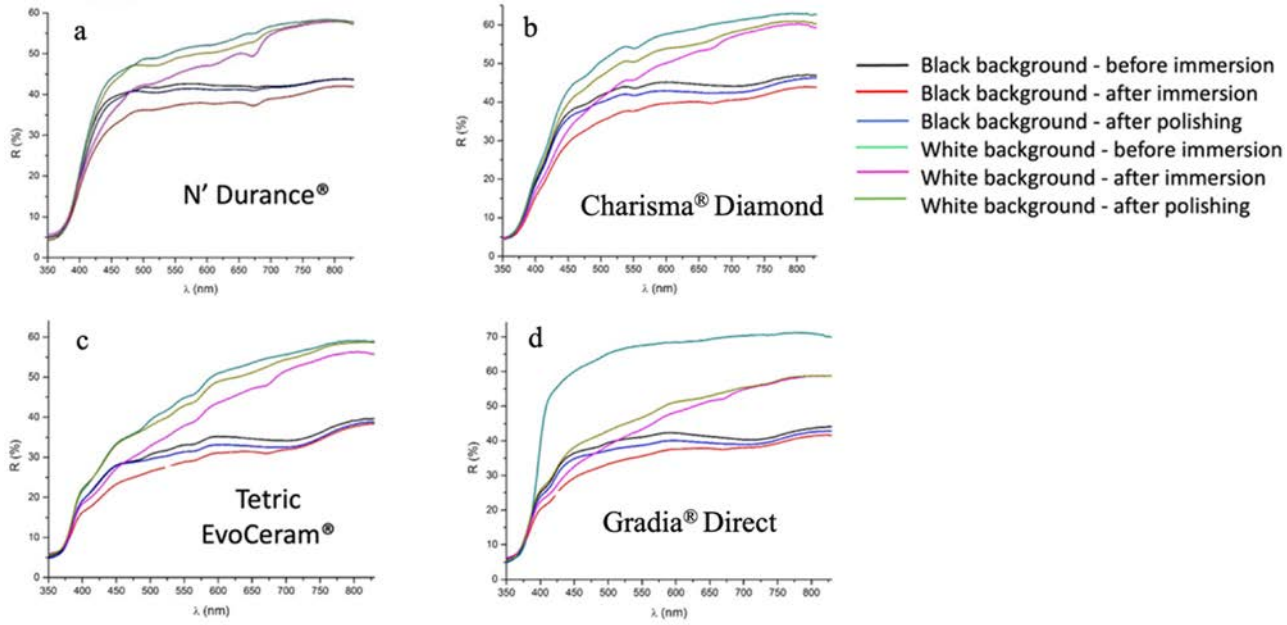

Fig. 1 – Diffuse reflectance spectra of composite samples before and after exposure to tea and after repolishing, on white and black backgrounds: a) N'Durance®; b) Charisma® Diamond; c) Tetric EvoCeram®; d) Gradia® Direct. R – reflectance.

***Note*: After repolishing, all spectra for all sample groups were returned to their initial shape, except for the Gradia® Direct group on a white background.**

subsequent repolishing, are presented in Figure 2. A statistically significant difference ($p < 0.05$) in color change was observed between samples immersed in water (control), tea, and repolished samples, and also between some of the materials, except between Gradia® Direct and Charisma® Diamond ($p > 0.05$). After repolishing, the color changes for Tetric EvoCeram® and N'Durance® remained above the clinically acceptable threshold ($\Delta E_{00} = 1.8$). The highest color change was recorded for Tetric EvoCeram® ($\Delta E_{00} = 6.0$), while ΔE_{00} values for Charisma® Diamond and Gradia® Direct were clinically acceptable.

Figure 3 presents TP_{00} values of the tested composites before and after immersion, as well as after repolishing. Initial translucency was significantly different ($p < 0.05$) for the tested composite brands, except between Gradia® Direct and Charisma® Diamond ($p > 0.05$). For all tested composites, TP_{00} values significantly increased ($p < 0.05$) after immersion in tea and were almost completely restored after repolishing, except for Tetric EvoCeram® ($p > 0.05$).

Figure 4 (a–d) shows EEM fluorescence 3D spectra of composites before and after immersion in tea, as well as after repolishing. In order to quantify the decrease, the volume under the fluorescent region was calculated. Tea staining caused different changes among the same shade-designated composites. Although the shape of the spectra remained the same, the intensity of their emission varied between groups. Gradia® Direct exhibited the highest initial fluorescence and the highest decrease in fluorescence intensity after staining (33%), while the lowest decrease (12%) was observed for the Charisma® Diamond composite. The reduction in fluorescence intensity occurred mainly in the 380–450 nm emission spectral region, where tea absorption is strongest. Repolishing reduced the staining effect on fluorescence, but not completely (Table 2).

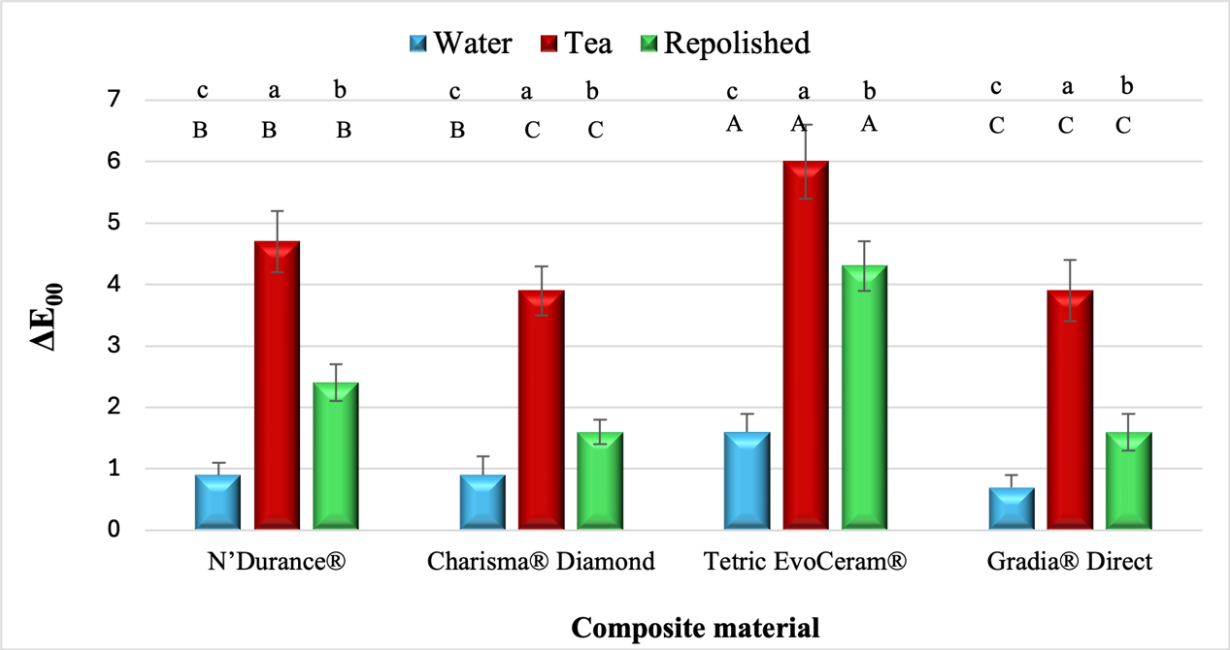

Fig. 2 – Color changes (ΔE_{00}) of composite materials after immersion in water and tea and subsequent repolishing. ΔE_{00} parameter values on the ordinate are given as numbers.

***Note*: Same letters indicate no significant differences ($p > 0.05$) between different materials (uppercase letters) and no significant differences for each material immersed in water, tea, or repolished (lowercase letters). The labels "A", "B", and "C" are assigned based on the values of the parameter. "A" is assigned to the material with the highest value. The next material is assigned label "B" if the difference is statistically significant; otherwise, it is labeled "A". The following material is compared with the previous one using the same principle. In-group assignments are presented with lowercase letters in the same way.**

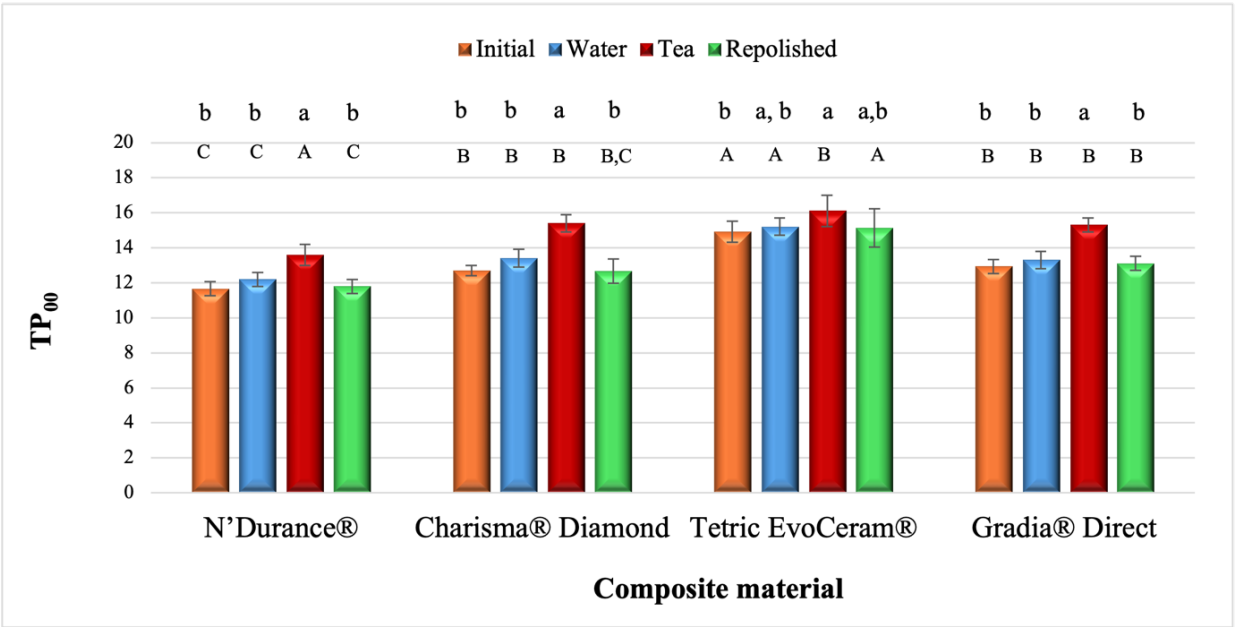

Fig. 3 – Translucency (TP_{00}) of the tested composite materials (initial, immersed in water and tea, and repolished). TP_{00} parameter values on the ordinate are given as numbers.

***Note*: Same letters indicate no significant differences ($p > 0.05$) between different materials (uppercase letters) and no significant differences for each material immersed in water, tea, or repolished (lowercase letters). The labels "A", "B", and "C" are assigned based on the values of the parameter. "A" is assigned to the material with the highest value. The next material is assigned label "B" if the difference is statistically significant; otherwise, it is labeled "A". The following material is compared with the previous one using the same principle. "B,C" mark indicates that the parameter value of the material shows no statistically significant difference from values "B" and "C", but these two show statistically different values. Other composite marks are assigned according to the same principles. In-group assignments are presented with lowercase letters in the same way.**

There was a statistically significant difference ($p < 0.05$) in the initial surface roughness between the materials, except between N'Durance® and Charisma® Diamond ($p = 0.139$). Tea staining significantly altered the surface of all tested samples ($p < 0.05$), except Tetric EvoCeram® ($p = 0.074$). No statistically significant difference was observed in the roughness of the initial and repolished samples ($p > 0.05$) for all tested composite materials except for Charisma® Diamond ($p < 0.05$) (Figure 5).

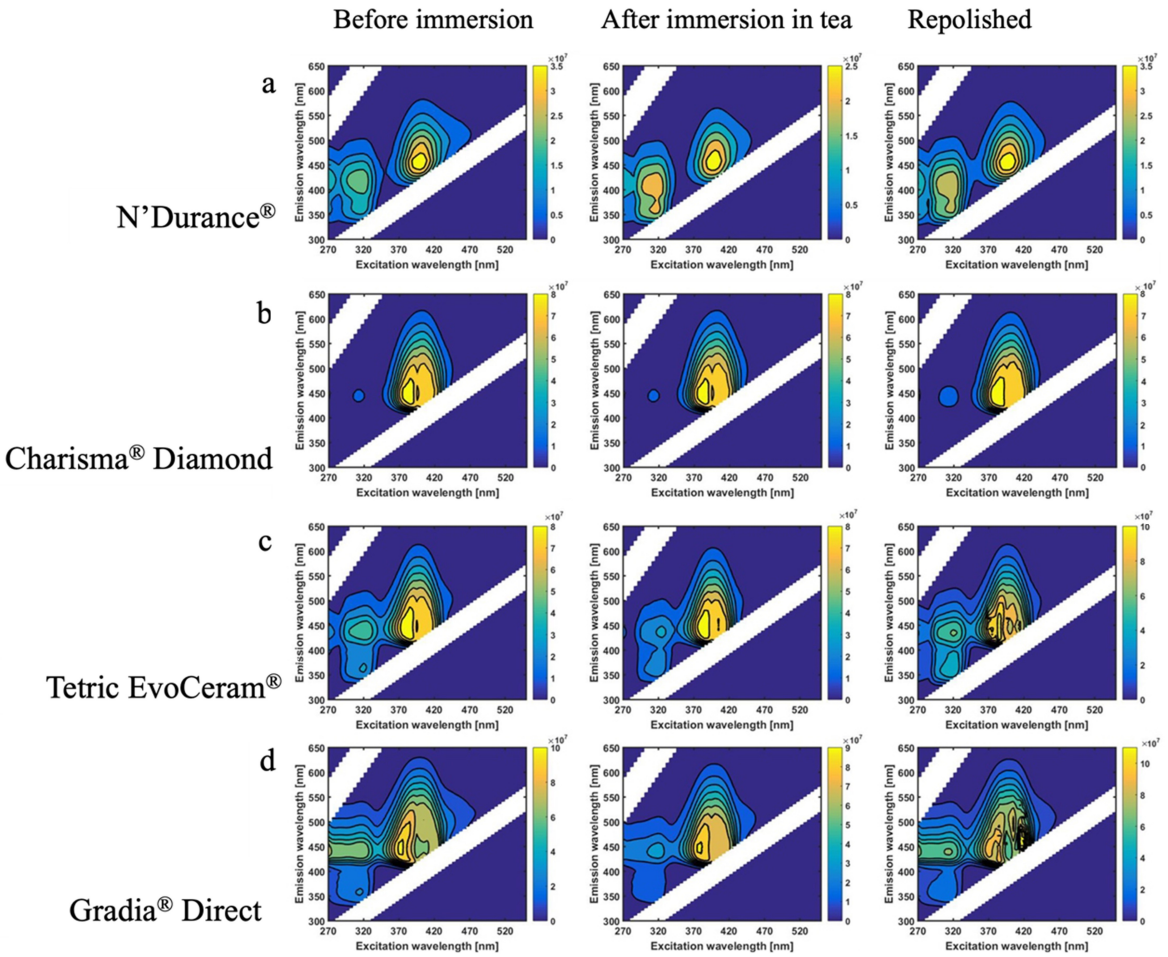

Fig. 4 – Fluorescence excitation-emission matrices (EEM) spectra of composite samples, before and after immersion in tea and after the subsequent repolishing: a) N'Durance® before immersion, after immersion and after polishing; b) Charisma® Diamond before immersion, after immersion, and after polishing; c) Tetric EvoCeram® before immersion, after immersion, and after polishing; d) Gradia® Direct before immersion, after immersion, and after polishing.

***Note*: Gradia® Direct showed the highest initial fluorescence and the highest decrease in fluorescence intensity after staining.**

Table 2

Changes in the total fluorescence after staining and after repolishing

Composite brand	after staining	after repolishing
N'Durance®	-20	2
Charisma® Diamond	-12	1
Tetric EvoCeram®	-18	3
Gradia® Direct	-33	-3

Values are given as percentages.

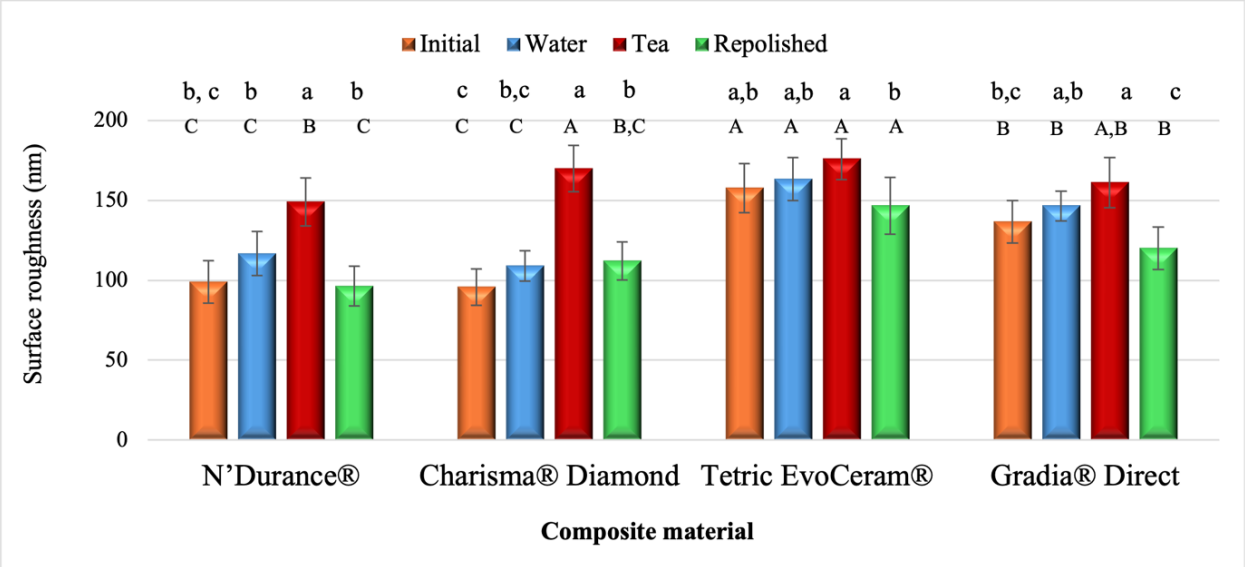

Fig. 5 – The surface roughness of composites before and after immersion in tea and after repolishing. nm – nanometer.

***Note*: Same letters indicate no significant differences ($p > 0.05$) between different materials (uppercase letters) and no significant differences for each material before immersion, immersed in water, tea, or repolished (lowercase letters). The labels "A", "B", and "C" are assigned based on the values of the parameter. "A" is assigned to the material with the highest value. The next material is assigned label "B" if the difference is statistically significant; otherwise, it is labeled "A". The following material is compared with the previous one using the same principle. "A,B" mark indicates that the parameter value of the material shows no statistically significant difference from values "A" and "B", but these two show statistically different values. Other composite marks are assigned according to the same principles. In-group assignments are presented with lowercase letters in the same way.**

Discussion

The initial optical properties of composites and their changes after staining depend on material characteristics such as filler type and concentration, particle size, type, and amount of organic matrix [20, 21], and surface quality. In this study, two low-shrinkage and two different conventional composites [based on bisphenol A-glycidyl methacrylate (BisGMA) and urethane dimethacrylate (UDMA)] were tested. The B1 shade of the composite was chosen as the lightest shade because of the assumption that lighter composites will change color more significantly under staining [22].

Despite having the same shade designation, composites from different manufacturers have different chemical constituents, and, therefore, their optical properties vary among them. Shade determination is typically conducted in clinical practice using the Vita shade guide. Nevertheless, prior research has demonstrated that the colors of composites do not align effectively with the Vita shade guide tabs, even when employing the layering technique [4, 23–26]. When comparing the shade tabs of the Vitapan classical shade guide with corresponding tabs composed of direct restorative composites, it was found that none of the materials or shade combinations achieved a satisfactory match [27]. Paravina et al. [28] discovered a lack of color compatibility among shade pairs that had the same shade identification. The A2 shade pairs have demonstrated the most optimal color matching, with C2 and B2 following closely behind.

In this study, the initial color parameters L*, a*, and b* differ among the tested B1 composite materials. Charisma® Diamond showed the highest lightness (L*) before immersion in tea, but the lowest a* value (indicating a shift toward green color). In contrast, Tetric EvoCeram® showed the lowest L* value and the highest a* and b* values (indicating a greater shift toward red and yellow).

The diffuse reflectance spectra (on both white and black backgrounds) of all examined B1 composites exhibited a consistent pattern: the reflectance of the specimens decreased after being immersed in tea, and then increased again following the repolishing process, returning to a level similar to the reflectance before immersion, except for the Gradia® Direct on the white background.

Numerous studies have shown color changes in composites after immersion in different staining solutions [15, 16, 29, 30]. According to Paravina et al. [19], ΔE_{00} larger than 1.8 is considered clinically unacceptable, and this threshold was used in this study to define clinical acceptability. The present results showed color changes higher than the clinically acceptable threshold for all tested materials. The highest total color change ($\Delta E_{00} = 6.0$) was observed for conventional, BisGMA-based nanohybrid (Tetric EvoCeram®) composites compared to the low-shrinkage nanohybrid (Charisma® Diamond and N'Durance®) and UDMA-based microhybrid (Gradia Direct®) composites. The present results indicate that the type of composite significantly affects the extent of optical changes. This result is in line with the previous report by Arocha et al. [11], who also found smaller color changes for the UDMA-based composite. They explained this by the fact

that the hydrophilic hydroxyl group of BisGMA monomer induces more water sorption than the UDMA aliphatic chain. Contrary to this result, Manojlovic et al. [10] observed lower color changes for Tetric EvoCeram® than for the low-shrinkage, BisGMA-free composites, which were exposed to tea for two days. There is no apparent explanation for these differences, but this finding may indicate that BisGMA-based composite absorbs more water-soluble pigments during a longer exposure period. Observed differences between studies can also be explained by the fact that the color stability of the composite was affected by several factors, not only by the monomer type.

The translucency of all tested composites was significantly higher after immersion in tea, but was almost completely restored after repolishing. The highest initial TP_{00} value was recorded for BisGMA-based composite Tetric EvoCeram®, compared to low-shrinkage and conventional UDMA-based composites. This finding is consistent with other studies [31, 32] and may be associated with the refractive index of BisGMA monomer compared to urethane-based monomers.

Meller and Klein [33] found that all analyzed composite brands and shade types reached their maximum fluorescence at about the same excitation and emission wavelengths, but with distinctively varying fluorescence intensities. The results obtained in this study confirm these findings. Contour plots showed no variations in the shape, but their intensities decreased differently after staining. The highest decrease of 33% was observed for the Gradia® Direct composite, while the lowest decrease of 12% was found for the Charisma® Diamond composite. Differences in the extent of fluorescence reduction after staining can be attributed to different amounts of excited and emitted light reaching and escaping the samples. The intrinsic composite's fluorescent components keep their fluorescent potential, i.e., it is not affected by the adsorbed surface barrier. However, the surface barrier absorbs incident and emitted light and reduces fluorescence intensity. The effect vanishes when the surface barrier is removed by polishing the stained composite.

Repolishing and whitening are often performed in daily practice to restore the natural appearance of restorations. In this study, we used repolishing of composites to remove adsorbed layers on sample surfaces formed by staining. After repolishing, Gradia® Direct and Charisma® Diamond showed clinically acceptable ΔE_{00} values. The translucency of all composites regains values similar to those before staining. This indicates that the main process responsible for optical changes is the adsorption of staining pigments on the surface of composites and that these pigments can be removed by polishing. These results are consistent with the results previously reported by Türkün et al. [9]. In contrast, Tetric EvoCeram® and N'Durance® composites exhibited color changes higher than the clinically acceptable threshold even after repolishing, indicating that the deeper layers of these composites were affected by staining, which suggests that the discoloration in these composites may be irreversible.

A similar reasoning can be applied to discuss changes in composites' fluorescence. Fluorescence is also restored almost completely after repolishing stained composites, which indicates that the adsorbed surface layer affects the fluorescent properties to different extents, depending on the type of adsorbed pigments. Affected by the adsorbed pigments interface, the photons scatter inside the composites, and the fluorescent beam that tries to leave the matrix needs to penetrate this adsorbed pigment obstacle for the second time. By removing the obstacle, in this study, we showed the complete restoration of the initial fluorescence values. This might indicate that the interior of the composite's structure does not affect the fluorescence intensity of the manufactured composite and that the fluorophores inside the composite remain unaffected by staining. On the contrary, literature data show a different point of view. It is assumed that fluorescence is highly affected by the absorption coefficient of the composite itself, regarding the differences among the size of filler particles and their distribution among the tested composite groups [34]. Ameer and Mualla [35] have also found that composites with different filler particles show different changes in fluorescence intensity during the discoloration, even among different shades of the same manufacturer. In their investigation, fluorescence of composites with nanofillers decreased more than that of the microhybrids. This research suggests a possible connection between the composite's chemical composition and the stability of its fluorescence. Many authors explain this fluorescence intensity decrease as a result of the degradation of the organic composite matrix and the subsequent deactivation of fluorophores [36, 37].

It is known that surface polishing plays an important role in color determination. The unpolished material is more susceptible to staining from food and drinks [38]. The polishing degree depends on the material type and its chemical constituents. Although for Tetric EvoCeram® the difference is not significant, all materials showed a unique trend of higher roughness after immersion and lower after repolishing samples. In general, a higher roughness after immersion could be attributed to chemical erosion from tea due to its slightly acidic nature and adsorption of stains on the sample surfaces [39]. Initially, higher surface roughness was recorded for conventional compared to low-shrinkage composites. Following immersion in tea, comparable values were observed for all tested composites, and subsequent repolishing significantly restored the surface roughness values. Although Tetric EvoCeram® showed initially the highest roughness and also the highest values of ΔE_{00} after staining, the results of this study indicate that the susceptibility to staining did not necessarily influence the initial roughness. N'Durance showed significantly lower initial roughness but higher ΔE_{00} compared to Gradia® Direct. This finding is consistent with the research conducted by Reis et al. [8], which also concluded that the surfaces with the highest level of polish were not always the least prone to stains. The composition of the material, including the specific monomers and fillers used, influences its propensity to undergo color changes. Smaller filler particles do not consistently result in a superior polished surface and reduced susceptibility to discoloration.

The results indicate that external discoloration and the adsorption of pigments onto material surfaces are significant

factors in the staining of composites. Repolishing materials after staining partially eliminates adsorbed stains and pigments from the composite surface, hence restoring the optical characteristics and roughness of the materials. Further studies on optical properties and surface topography are needed to clarify the correlation between the type of composite, surface roughness, time of exposure, and color changes.

Conclusion

According to the obtained results and considering restrictions of the study, one can conclude the following: even though the shades of all specimens were the same (B1), their initial color, translucency, fluorescence, and surface roughness varied among composites from different manufacturers. Immersion in tea significantly changed the optical properties of all tested materials. These changes were reliant upon the specific material used. The BisGMA-based composite exhibited a greater color change when immersed in tea compared to BisGMA-free composites. Staining changed the intensity of the composites' fluorescence but not the shape of the fluorescence spectra. Repolishing nearly restored translucency, fluorescence, and surface roughness, while partially eliminating discoloration and returning the spectral properties of composites to levels closer to their initial state.

Acknowledgement

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Assessment of the effectiveness of Tactical Combat Casualty Care training provided to non-medical military personnel in Türkiye

Procena efikasnosti obuke za Taktičko zbrinjavanje ranjenika u borbi za nemedicinsko vojno osoblje u Turskoj

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Abstract

Background/Aim. Tactical Combat Casualty Care (TCCC) protocols are trauma protocols used on the battlefield aimed at preventing death in the first hours of injury. In Türkiye, a regulation requires security and safety personnel to undergo TCCC training. This training is provided to non-medical military personnel (MPs) by specialized military and civilian healthcare professionals at various hospitals. The TCCC protocols include standardized interventions through protocols such as Massive hemorrhage, Airway, Respiration, Circulation, Hypothermia/Head injury (MARCH) protocol and Military Acute Concussion Evaluation (MACE) protocol to ensure consistency in trauma care. The aim of the study was to evaluate the effects of TCCC on MPs who received the training. **Methods.** The study included MPs who received TCCC training at Istanbul's Sancaktepe Hospital between March 7 and May 13, 2022, and between October 3 and December 2, 2022. Volunteers who consented to participate were asked survey questions to evaluate their knowledge before and after the training on various medical conditions to assess changes in knowledge and skills. Their opinions about the training were solicited both before and after the training. In addition to the survey questions, participants were asked to provide their views and suggestions about the training through open-ended questions. **Results.** The study included 49 volunteers aged between 23 and 35. There was a statistically significant increase in knowledge and skill levels regarding medical conditions before and after the training ($p < 0.001$). Participants concluded that the training was beneficial and that the knowledge and skills acquired could be applied in the field. **Conclusion.** Providing TCCC training will help MPs perform life-saving interventions under fire, thereby reducing fatalities and disabilities. Additionally, TCCC training will increase health literacy awareness among civilians, enabling trained personnel to not only save lives on the battlefield but also provide first aid in civilian emergencies.

Key words:
armed conflicts; emergency medicine; military personnel; models, theoretical; surveys and questionnaires.

Apstrakt

Uvod/Cilj. Protokoli Taktičkog zbrinjavanja ranjenika u borbi (*Tactical Combat Casualty Care* – TCCC) su protokoli za zbrinjavanje osoba sa povredama koji se koriste na bojnom polju sa ciljem sprečavanja smrti u prvim satima nakon povrede. U Turskoj, propis zahteva da pripadnici obezbeđenja i bezbednosnih snaga prođu obuku iz TCCC. Ovu obuku nemedicinskom vojnom osoblju (VO) pružaju specijalizovani vojni i civilni zdravstveni radnici u raznim bolnicama. Protokoli TCCC uključuju standardizovane intervencije kroz protokole kao što su protokol za kontrolu disanja, cirkulacije i prevenciju i zbrinjavanje masivnog krvarenje i povreda glave/hipotermiju (*Massive hemorrhage, Airway, Respiration, Circulation, Hypothermia/Head injury* – MARCH) i vojni protokol za akutnu procenu potresa mozga (*Military Acute Concussion Evaluation* – MACE) da bi se osigurala doslednost u zbrinjavanju trauma. Cilj rada bio je da se proceni efekat TCCC na VO koje je prošlo obuku. **Metode.** U studiju je bilo uključeno VO koje je prošlo TCCC obuku u bolnici *Sancaktepe* u Istanbulu u dva vremenska perioda, i to od 7. marta do 13. maja 2022. i od 3. oktobra do 2. decembra 2022. godine. Dobrovoljcima koji su pristali da učestvuju u istraživanju postavljena su pitanja iz upitnika, u vezi sa različitim medicinskim stanjima, u cilju procene promena u njihovom znanju i veštinama pre

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i posle obuke. Mišljenje učesnika o obuci prikupljeno je i pre i posle završene obuke. Pored pitanja iz ankete, učesnici su zamoljeni da iznesu svoje stavove i sugestije o obuci putem pitanja otvorenog tipa. **Rezultati.** U studiju je bilo uključeno ukupno 49 dobrovoljaca životnog doba između 23 i 35 godina. Utvrđeno je statistički značajno povećanje nivoa znanja i veština nakon obuke u odnosu na stanje pre obuke ($p < 0,001$). Učesnici su zaključili da je obuka bila korisna i istakli da bi se stečena znanja i veštine mogli primeniti u realnim situacijama na terenu. **Zaključak.** Sprovođenje TCCC obuke pomoći će VO da efikasno primeni spasonosnu pomoć u borbenim uslovima čime će se smanjiti smrtnost i invaliditet među povređenima. Pored toga, TCCC obuka će povećati nivo zdravstvene pismenosti među civilima, omogućavajući obučenom VO ne samo da spasava živote na bojnom polju, već i da pruža prvu pomoć u civilnom okruženju u vanrednim situacijama.

Ključne reči:
sukob, oružani; medicina, urgentna; vojno osoblje; modeli, teorijski; ankete i upitnici.

Introduction

Tactical Combat Casualty Care (TCCC) encompasses trauma protocols utilized in combat scenarios, aiming to prevent fatalities within the crucial first hours following injury. These protocols commence with care under fire, proceed with casualty care on-site, and conclude with casualty evacuation [1]. Interventions that are particularly emphasized in the TCCC guidelines are tourniquet application (TA), control of external hemorrhage, administration of tranexamic acid, surgical airway management, needle decompression for tension pneumothorax, spinal protection in penetrating injuries, establishment of vascular access, fluid resuscitation in shock and hypotension, intraosseous vascular access, provision of analgesia, prevention of coagulopathy, administration of antibiotics, simulation-based scenario training, and the use of plasma in trauma resuscitation [2].

Regulations in Türkiye concerning the TCCC training of law enforcement and security personnel were published on March 22, 2016. In accordance with this regulation, military and civilian healthcare professionals specializing in emergency medicine (EM) provide TCCC training for military personnel (MP) who are non-medical professionals at various hospitals [3]. This training program includes theoretical and practical instruction, covering basic knowledge of human anatomy and physiology, physical examination, basic and advanced life support, airway management, vascular access techniques, trauma management, interventions for environmental emergencies, and actions to be taken when confronted with environmental emergencies. Through theoretical instruction and practical exercises, MPs are prepared to provide initial medical assistance in the absence of healthcare professionals. Following this training, MPs are expected to be able to perform life-saving interventions for injured personnel in field conditions or potential conflict zones until they reach medical facilities or until patients are transferred to healthcare personnel.

The aim of this study was to evaluate the opinions of personnel who underwent TCCC training provided by EM specialists, EM assistants, and auxiliary healthcare personnel serving in the EM clinic of our Hospital, as well as to assess the knowledge, skills, and behavioral changes resulting from the training.

Methods

Sample selection

The study included MPs who underwent TCCC training at our Hospital between March 7 and May 13, 2022, and also between October 3 and December 2, 2022, and agreed to participate. One MP who attended the training but declined to participate in the study was not included. The ethics approval for the study was obtained from the Ethics Committee of Sancaktepe Sehit Prof. Dr. Ilhan Varank Training and Research Hospital (No: E-46059653-050.99-215846834). The study was conducted in accordance with the Declaration of Helsinki.

Details of the training

The TCCC training provided at our Hospital consisted of both theoretical and practical sessions conducted over a period of eight weeks. EM specialists, EM assistants, emergency department nurses, and healthcare personnel from the national medical rescue team delivered this training. The theoretical component of the training, the first stage, involved presentations using Microsoft PowerPoint software by EM specialists. Models were used as needed during these presentations. Necessary medical devices and equipment were introduced, and their principles of use were explained. The second stage of the training involved practical exercises and drills based on scenarios.

Research design

Volunteers who consented to participate in the study were evaluated both before and after training to assess changes in their knowledge and skills using a five-point Likert scale (1 – no knowledge; 2 – limited knowledge; 3 – moderate knowledge; 4 – good knowledge; 5 – excellent knowledge). Survey questions were directed to measure the level of knowledge regarding fundamental concepts and definitions, respiratory physiology, respiratory system physical examination, vertebral examination, pulse examination, cardiopulmonary resuscitation and its quality, hemorrhage, bleeding control, shock, TA, Massive hemorrhage, Airway, respiration, Circulation, Hypothermia/Head injury (MARCH) protocol, on-site patient assessment, intervention

for thoracic injuries on-site, medical care under fire, explosion and gunshot injuries, intervention for patients in a hot environment, limb amputations, intervention for limb amputations, Military Acute Concussion Evaluation (MACE) protocol, assessment of consciousness status, signs of respiratory distress, airway management, airway opening maneuvers, anaphylaxis, high-altitude illnesses, epistaxis, hypothermia/frostbite, eye irrigation, wound care, and application of dressings (Table 1).

Prior to training, participants' opinions regarding the potential benefits of the training were assessed using a three-point Likert scale (1 – no opinion; 2 – undecided; 3 – believed training would be beneficial). In the survey conducted after training, the participants' opinions concerning the effectiveness of the training and the applicability of the acquired knowledge in the field were evaluated using a three-point Likert scale (for the usefulness of training: 1 – I do not think it was beneficial, 2 – I am undecided, and 3 – I think it was beneficial; for the applicability of the acquired knowledge in the field: 1 – I cannot apply it, 2 – I am undecided, and 3 – I can apply it). In addition to the survey questions, the participants were asked to express their views and suggestions regarding the training through open-ended questions.

The volunteers were asked survey questions in their native language. Care was taken to ensure that the questions were clear, understandable, free of medical terminology, and as close to daily language as possible. After analyzing the responses to the survey questions using statistical methods, the questions were subsequently translated into English.

Data collection tool

A survey consisting of questions prepared through Google Forms was administered to the MPs participating in the two training groups, comprising a total of 63 individuals, *via* the WhatsApp application. Prior to accessing the survey questions, the participants were required to read the informed consent form and confirm if they were willing to participate. The completion of the informed consent form was mandatory for accessing the survey questions.

Statistical analysis

The mean age of the volunteers and the percentages of responses to the survey questions were recorded. Mean values and standard deviations of scores obtained from the Likert scales were calculated, and statistical analysis was performed using the Wilcoxon signed-rank test. Significance

Table 1

Main topics and subheadings covered in the tactical combat casualty care training

Main topics	Subheadings
Basic definitions/anatomy and physiology	stress definition
	functions of the skin
	respiratory rate of a normal adult
Physical examination	thoracic examination
	vertebral examination
	pulse check
Cardiopulmonary resuscitation	high-quality chest compressions
	cardiopulmonary resuscitation
	components of basic life support
Shock and bleeding control	hemorrhage
	tourniquet application
	bleeding control
	shock
MARCH protocol	MARCH protocol and its components
Patient intervention and care under fire and in the field	on-site patient assessment
	intervention for thoracic injuries on-site
	medical care under fire
	explosion and gunshot injuries
	medical care for patients in the hot zone
	intervention for head trauma in the hot zone
Limb amputations	limb amputations
	intervention for limb amputations
Altered consciousness	MACE protocol
	assessment of consciousness status
Airway assessment and management	signs of respiratory distress
	airway management
	airway opening maneuvers
Various clinical situations	anaphylaxis
	high-altitude illnesses
	epistaxis
	hypothermia/frostbite
Wound care and interventional procedures	eye irrigation
	wound care/dressing applications

MARCH – Massive hemorrhage, Airway, Respiration, Circulation, Hypothermia/Head injury; MACE – Military Acute Concussion Evaluation.

was set at $p < 0.001$. The Statistical Package for the Social Sciences (SPSS) for Windows v. 26 (IBM Corporation, Chicago, Illinois) was utilized for statistical analysis.

Results

Forty-nine volunteers consented to participate in the study and answered the survey questions. The mean age of the volunteers was 29.47 (min 23, max 35) years.

Table 2 shows the percentages of the participants' response scores on the Likert scale before and after training.

The statistical comparison of the score averages of the questions and sections before and after training revealed a statistically significant difference ($p < 0.001$). However, there was no statistically significant difference between the pre- and post-training scores concerning the perceived usefulness of the training. The participants expressed their belief in the training's potential benefits before training and confirmed its effectiveness after training ($p = 0.189$) (Table 3). Through open-ended questions, the participants were asked about the skills acquired after training and the perceived benefits of the training. The acquired skills emphasized post-training included triage, providing initial care under fire, medication use, TA, and basic and advanced life support. Regarding the perceived benefits of the training, the participants highlighted the importance of gaining the ability to intervene and provide care under fire.

Table 2

Responses to the Likert scale questions directed to the participants before and after training

Training topic	Before training					After training				
	1	2	3	4	5	1	2	3	4	5
Basic definitions/anatomy and physiology	22.40	38.90	25.20	12.2	1.30	1.30	2.80	4.10	57.00	34.80
Physical examination	38.10	41.30	15.30	3.70	1.60	1.33	2.00	6.13	43.56	46.98
Cardiopulmonary resuscitation	44.96	32.27	17.47	5.30	0	1.33	2.73	5.46	44.22	46.26
Shock and bleeding control	26.60	43.00	11.50	17.00	1.90	0	2.52	2.06	39.30	56.12
MARCH protocol	70.60	19.85	4.75	3.20	1.60	1.00	3.05	3.05	51.00	41.90
Patient intervention and care under fire and in the field	32.42	38.80	20.40	7.70	0.68	0.85	1.80	2.90	45.47	48.98
Altered consciousness	56.35	29.35	6.35	7.95	0	2.00	1.00	6.10	50.00	40.90
Airway assessment and management	37.56	35.43	15.38	10.03	1.60	0.70	2.70	1.37	44.23	51.00
Various clinical situations	45.65	33.75	13.50	6.70	0.40	2.00	2.58	6.12	38.80	50.50
Wound care and interventional procedures	30.67	31.73	27.00	9.53	1.07	0.70	2.00	4.76	37.42	55.12
Opinions about training										
usefulness of training	11.10	9.50	79.40	-	-	4.10	0	95.90	-	-
applicability of acquired knowledge in the field	-	-	-	-	-	0	4.1	95.90	-	-

Values are given as percentages.

***Note*: for "Opinions about training", participants' opinions were assessed using the first three points of the Likert scale; for the applicability of acquired knowledge, it was impossible to give answers before the training was conducted.**

For the 5-point Likert scale response options, see the Methods section.

Table 3

Statistical analysis of responses before and after training

Training topic	Before training	After training	*p-values
Basic definitions/anatomy and physiology	2.31 ± 0.92	4.21 ± 0.73	< 0.001
Physical examination	1.75 ± 0.64	4.42 ± 0.64	< 0.001
Cardiopulmonary resuscitation	2.08 ± 0.74	4.31 ± 0.71	< 0.001
Shock and bleeding control	1.95 ± 0.72	4.43 ± 0.59	< 0.001
MARCH protocol	1.39 ± 0.73	4.53 ± 0.54	< 0.001
Patient intervention and care under fire and in the field	1.93 ± 0.70	4.35 ± 0.65	< 0.001
Altered consciousness	1.58 ± 0.42	4.32 ± 0.73	< 0.001
Airway assessment and management	2.09 ± 0.81	4.40 ± 0.64	< 0.001
Various clinical situations	2.04 ± 0.75	4.33 ± 0.79	< 0.001
Wound care and interventional procedures	2.10 ± 0.73	4.44 ± 0.70	< 0.001
Usefulness of training	2.68 ± 0.67	2.92 ± 0.40	0.189

MARCH – Massive hemorrhage, Airway, Respiration, Circulation, Hypothermia/Head injury.

Values are given as mean values ± standard deviation. *Wilcoxon signed-ranks test; significance: $p < 0.001$.

Discussion

In our study, when assessing the knowledge of MPs who received tactical field training, it was observed that there was a significant increase in their level of knowledge after training compared to before training. The participants believed the training would be beneficial before training, and kept the opinion that it was indeed beneficial after training.

Wars result in both material and moral losses for all parties involved. Deaths or injuries sustained by soldiers during wars lead to a loss of strength on the battlefield. Therefore, providing life-saving interventions to wounded soldiers on the battlefield and preventing possible disabilities are crucial. Rapid and accurate administration of such interventions on the battlefield ensures that injured soldiers not only survive and continue fighting but can also return to civilian life without significant disabilities after the war. Furthermore, receiving TCCC training will enable soldiers to intervene in medical emergencies they may encounter after returning to civilian life.

The first hour following trauma and the interventions that can be performed during this time are crucial determinants of survival and are often referred to as the "golden hour" [4]. In war, many deaths occur before reaching centers where professional medical care can be provided. In a study investigating deaths on the battlefield in the USA from 2001 to 2011, it was found that 87.3% of deaths occurred before reaching any medical facility. Furthermore, 90.9% of mortality was attributed to hemorrhage, with 33.7% of hemorrhages originating from extremities [5]. Extremity hemorrhage has been identified as the most common cause of preventable deaths during the Vietnam War [6]. Hence, TA emerges as a key aspect of battlefield care. While tourniquet use, a primary component of TCCC training, can be life-saving in hemorrhage-related deaths on the battlefield, incorrect or delayed application may lead to various neurovascular deficits and limb losses. In a study examining 69 injured individuals who underwent TA in a hospital treating Ukrainian military personnel, the accuracy rate of applied tourniquets was found to be 24.6%. It was observed that tourniquets were applied late in 8 cases with over 1 L of blood loss and were incorrectly applied in 12 cases [7]. In a study conducted by Schreckengaust et al. [8], volunteers who received training on TA as part of TCCC training and who were subsequently requested to apply tourniquets in a battlefield simulation demonstrated a correct application rate of 87% on the first day, which increased to 94% on the fourth day, with TA times reduced from 43 s to 38 s. It was found that the training provided shortened the time for TA and effectively stopped arterial flow [8]. In our study, we found a statistically significant increase in the participants' level of knowledge regarding TA after training. Additionally, 95.9% of participating MPs indicated that they could apply the knowledge and skills acquired during training on the battlefield. Correct application of tourniquets is crucial in the care of injured individuals under fire and in hot zones. TCCC training emphasizes the importance of tourniquet use. Efforts should be made to improve the accuracy of the TA through training and repeated training sessions.

In a study conducted with the Chinese army, participants were divided into three groups: reserve officers who had received military training (MT) but not medical education; those who had received medical education in addition to MT and had undergone 40 hrs of first aid training *per* year; fresh officers who had undergone 10 hrs of first aid training *per* year. A new curriculum was developed for officers who had received MT but not medical education, and the performance of these three groups on the battlefield was evaluated from a medical perspective. It was observed that the officers who had received training under the new curriculum achieved similar scores to those who had received medical education in addition to MT and had undergone 40 hrs of first aid training *per* year. In addition, both of these groups outperformed the fresh officers who had undergone 10 hrs of first aid training *per* year [9]. This study demonstrates that personnel without medical education can attain the necessary skills to perform interventions on the battlefield through TCCC training. Similarly, our study revealed an increase in the knowledge levels of MPs regarding the topics covered in the training. Furthermore, it was observed that the participants acquired the ability to perform interventional procedures through practical exercises.

TCCC training should be repeated at regular intervals for personnel who have received it. During the recertification of nine US National Guard soldiers who had previously undergone TCCC training, six questions related to TCCC guidelines were posed, and their performance in applying tourniquets and stopping bleeding was evaluated. On average, 2.2 of the questions were answered correctly, with a success rate of 44% for TA and 22.2% for bleeding control. The authors emphasized the necessity of providing TCCC training at regular intervals and conducting recertification [10]. Another study undertaken by Oury et al. [1] examined the effect of trauma center-based education on procedural skills, medical knowledge, and confidence in application among special operations medical personnel. The study included a total of 108 students from 18 courses conducted over a period of two years. The results showed a 2% increase between the pre- and post-test scores; this increase was not statistically significant. Nevertheless, there was a significant increase in the participants' confidence levels in the application of procedural skills, such as cricothyrotomy, chest tube insertion, TA, FAST examination, needle thoracostomy, and airway management, as well as general patient care skills. Similarly, in our study, it was observed that 95.9% of the MPs who participated in the TCCC training developed confidence in their ability to apply the knowledge and skills they acquired in the field.

Suresh et al. [11] evaluated the pre-deployment training, confidence, and preparedness feelings of military health personnel deployed to war zones through an online survey. According to the participants, the most important skills were related to controlling bleeding, administering

treatment/medication, providing care during combat, and managing burns. Of the 254 participants, 74.8% expressed confidence in their combat casualty care skills. The study found that confidence increased with the number of deployments and the duration of pre-deployment training. Similarly, in our study, the participants highlighted the importance of skills acquired in triage, providing initial care under fire, medication administration, TA, and basic and advanced life support after training.

TCCC training primarily aims to enable MPs to perform life-saving interventions on the battlefield. Furthermore, a study that provided law enforcement personnel with training based on TCCC guidelines and examined cases where they intervened from a medical perspective found that those who received the training were able to perform life-saving interventions. Notably, successful results were achieved in bleeding control [12]. The study demonstrated that the training provided could be utilized not only on the battlefield but also in civilian life.

Conclusion

Providing TCCC training to military personnel and repeating it regularly will enable them to perform life-saving interventions under fire, resulting in reduced fatalities and disabilities. Periodic repetition of training will facilitate the refreshing of knowledge, the learning of new intervention techniques, if applicable, the transfer of experience in military medicine, and the acquisition of different perspectives. Therefore, enhancing and sustaining TCCC training *via* new methods and viewpoints is essential. In addition, TCCC training will foster health literacy among civilians, equipping trained individuals to not only save lives on the battlefield but also provide immediate emergency assistance in civilian life. This further underscores the growing importance of TCCC training with each passing day.

Conflict of interest

The authors declare no conflict of interest.

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CASE REPORT

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Unrecognized severe obstructive sleep apnea as a dominant risk factor for non-arteritic anterior ischemic optic neuropathy in an apparently healthy patient

Neotkrivena teška opstruktivna apneja u snu kao dominantan faktor rizika od nearteritične prednje ishemijske optičke neuropatije kod naizgled zdravog bolesnika

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Abstract

Introduction. Non-arteritic anterior ischemic optic neuropathy (NAION) is typically manifested by sudden, painless vision loss in one eye, often immediately upon waking up. The visual loss remains fairly stable over time but can be followed by similar manifestations in the fellow eye. One of the main causes for NAION is impaired hemodynamic regulation, followed by hypoperfusion of the optic nerve head. Recognized risk factors include arterial hypertension, hyperlipidemia, diabetes mellitus, smoking, etc. **Case report.** A 43-year-old man with a sudden painless right eye vision loss and typical symptoms and signs of NAION is presented. The ophthalmological examination, followed by fluorescein angiography and visual field testing, confirmed the diagnosis, while medical history had not revealed any of the standard risk factors. During hospitalization, the medical staff noted that the patient was snoring, waking up during the night while feeling short of breath, followed by excessive daytime sleepiness, which was later confirmed by family members as symptoms that had been present for several months. The patient was referred to a somnologist, who, after diagnostics, confirmed the presence of severe, untreated obstructive sleep apnea (OSA) and indicated treatment with a continuous positive air pressure (CPAP) device. The patient responded well to treatment, showing subjective and objective improvements in sleep quantity and quality. No progression of right eye NAION or vision impairments in the left eye has been noted after 6 months from diagnosis and CPAP introduction. **Conclusion.** OSA was probably the main underlying cause of NAION in this patient. We point out that OSA screening in high-risk patients can contribute to the early diagnosis and prevention of NAION.

Key words:
diagnosis; optic neuropathy, ischemic; risk factors; sleep apnea, obstructive.

Apstrakt

Uvod. Nearteritična prednja ishemijska optička neuropatija (*non-arteritic anterior ischemic optic neuropathy* – NAION) obično se manifestuje iznenadnim, bezbolnim gubitkom vida na jednom oku, često odmah po buđenju. Gubitak vida ostaje prilično stabilan tokom vremena, ali može biti praćen sličnim manifestacijama na drugom oku. Jedan od glavnih uzroka NAION-a je poremećena hemodinamska regulacija, praćena hipoperfuzijom prednjeg dela optičkog nerva. Prepoznati faktori rizika su arterijska hipertenzija, hiperlipidemija, dijabetes melitus, pušenje, itd. **Prikaz bolesnika**. Prikazan je 43-godišnji muškarac sa iznenadnim, bezbolnim gubitkom vida na desnom oku i tipičnim simptomima i znacima NAION-a. Pregled oftalmologa, praćen fluoresceinskom angiografijom i ispitivanjem vidnog polja, potvrdili su dijagnozu, dok u anamnezi nije otkriven nijedan od standardnih faktora rizika. Tokom hospitalizacije, medicinsko osoblje je primetilo da bolesnik hrče, budi se tokom noći sa osećajem nedostatka vazduha i da pokazuje dnevnu pospanost, što su kasnije članovi porodice potvrdili kao simptome prisutne već nekoliko meseci. Bolesnik je upućen specijalisti za poremećaje spavanja, koji je nakon dijagnostike konstatovao

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tešku nelečenu opstruktivnu apneju u snu (*obstructive sleep apnea* – OSA) i indikovao lečenje aparatom za kontinuirani pozitivni vazdušni pritisak (*continuous positive air pressure* – CPAP). Bolesnik je dobro reagovao na lečenje, pokazujući subjektivno i objektivno poboljšanje u dužini i kvalitetu sna. Nije zabeležena progresija NAION-a desnog oka ili oštećenja vida na levom oku posle 6 meseci od dijagnoze i uvođenja CPAP-a. **Zaključak**. Glavni i osnovni uzrok NAION-a kod prikazanog bolesnika je najverovatnije bila OSA. Ukazujemo na to da utvrđivanje OSA-e kod bolesnika sa visokim rizikom može doprineti ranoj dijagnozi i prevenciji NAION-a.

Ključne reči:
dijagnoza; neuropatija, optička, ishemička; faktori rizika; apneja u snu, opstruktivna.

Introduction

Non-arteritic anterior ischemic optic neuropathy (NAION) is a major cause of blindness or severely impaired vision in adults. It is the most common cause of acute optic neuropathy in patients older than 50 years and the second most frequent form of optic neuropathy after glaucoma [1]. The typical clinical presentation of NAION includes a sudden, unilateral, and painless loss of vision. The rapid development indicates that NAION is caused by the sudden disruption of blood flow to the optic nerve, further supported by the fact that it is almost always unilateral. The main pathogenic mechanism is believed to be ischemic damage of the laminar and retrolaminar regions in the anterior part of the optic nerve, mostly due to hypoperfusion of short posterior ciliary arteries (SPCAs). Several risk factors have been linked to NAION, such as diabetes mellitus (DM), atherosclerosis, arterial hypertension, hyperlipidemia, and the use of some medications such as phosphodiesterase type 5 inhibitors. NAION is common in older individuals, given the increasing prevalence of a number of risk factors in this population [2]. NAION is a naturally progressive disease, and the contralateral eye involvement rate is 15–20% in the following 5 years [3].

Exposure to brief hypoxic episodes has been anecdotally linked to NAION in young and healthy people. Combat airplane pilots have reported visual field defects while performing high-G-force maneuvers [4, 5]. Given that neurons cannot rely on glycolysis as a source of energy, the nervous system, including the optic nerve and retina, is critically dependent on an uninterrupted oxygen supply for energy production *via* oxidative phosphorylation. Prolonged hypoxia or repeated hypoxic episodes have been shown to exhaust the nervous system's capacity for repair, resulting in more permanent structural damage.

Obstructive sleep apnea (OSA) is a chronic progressive disease with repetitive interruptions in ventilation during sleep due to complete or partial collapse of the pharyngeal part of the airway. This cessation of breathing is followed by a drop in oxygen saturation and/or waking [6]. OSA is the most common disorder in the spectrum of sleep-related breathing disorders, affecting more than 900 million people worldwide [7]. OSA is characterized by specific symptoms and signs, including excessive daytime sleepiness, pronounced snoring, and acknowledged episodes of breathing interruptions during sleep or upon waking. The diagnosis requires the occurrence of a minimum of five obstructive respiratory events, such as apneas, hypopneas, or waking associated with respiratory effort *per* hour of sleep, as measured by the apnea-hypopnea index (AHI) [8]. OSA is considered mild if AHI is 5–15 events *per* hour of sleep (5–15/hr), moderate if AHI is 15–30/hr, and severe if AHI is ≥ 30/hr [9].

Screening for OSA includes a detailed medical history of daily and nocturnal symptoms, physical examination and the use of standardized questionnaires, such as Epworth Sleepiness Scale (ESS) [10] and STOP-BANG scoring model [acronym stands for: snoring, tiredness, observed apnea, high blood pressure, body mass index (BMI), age, neck circumference, and gender] [11]. Definitive diagnosis is established by performing a full night polysomnography with monitoring of at least the respiratory flow, respiratory effort, pulse oximetry, snoring, and body position [9].

OSA has been associated with several ocular disorders, such as floppy eyelid syndrome, keratoconus, glaucoma, central serous chorioretinopathy, and retinal vein occlusion [12]. Recent studies have indicated that OSA can play an important role in NAION pathogenesis due to recurring hypoxia and hypoxemia, as well as endothelial dysfunction [13, 14]. Patients with NION have been found to have a greater prevalence of OSA and *vice versa*, and some studies showed that untreated OSA can lead to unilateral NAION progression or damage of the temporal peripapillary retinal nerve fiber layer in the contralateral optic nerve [15, 16].

Case report

A 43-year-old man presented to the ophthalmology outpatient clinic with unilateral (right) vision loss without pain or other symptoms. Ophthalmological examination showed that the patient had visual acuity (VA) of 1/60 in the right eye and 1.0 in the left eye. Intraocular pressure for both eyes was 14 mm Hg. A dilated fundus examination demonstrated optic disc edema in the right eye, and initial edema in the left eye was detected (Figure 1). In the left optic disc, a small cup-to-disc ratio of 0.3 was observed, consistent with the so-called 'disk-at-risk' (Figure 1). Fluorescein angiography was performed, confirming the suspicion of right NAION. Octopus (Haag-Strait) G Standard top visual field testing showed a right inferior altitudinal defect and an initial inferior altitudinal visual field defect in the left eye (Figure 2). He had a normal peripheral retinal examination. Ocular motility, alignment, and cranial nerve function testing appeared normal. The patient used corrective lenses for myopia (-6.0 Dsph).

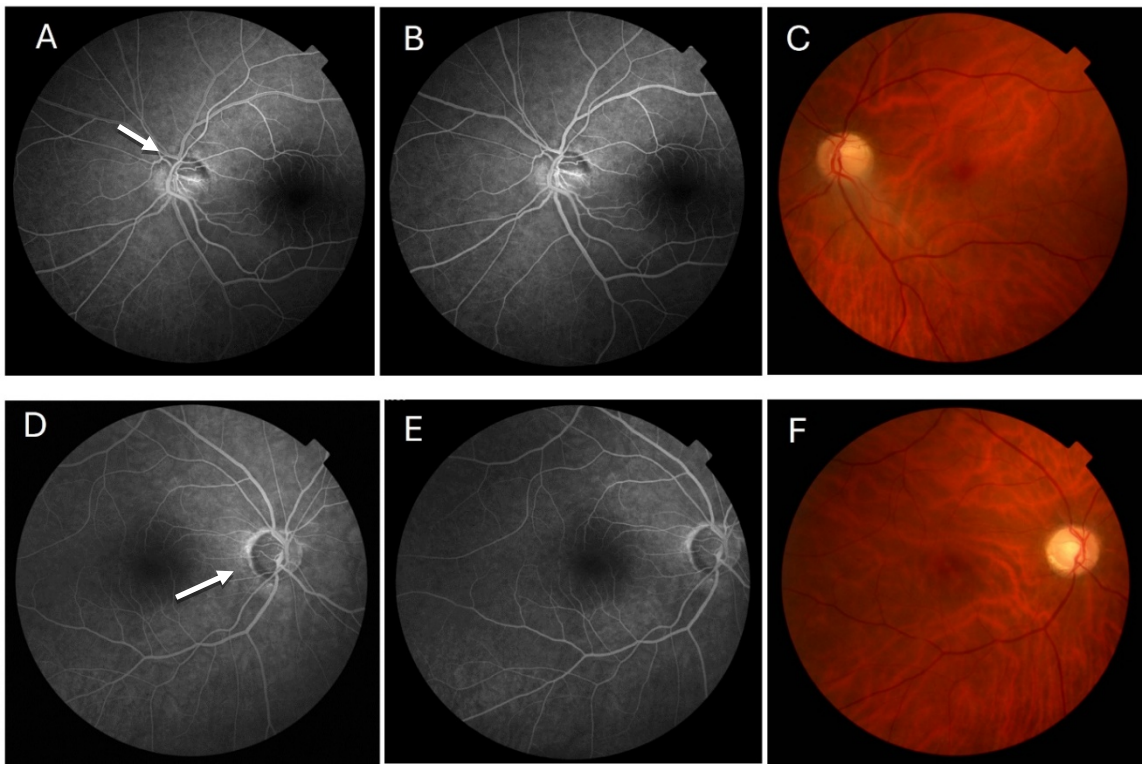

Fig. 1 – Fluorescence angiography (FA) in a 43-year-old patient with non-arteritic ischemic optic neuropathy (NAION) one month after the onset of symptoms. A, B) Partial NAION in the left eye. In the early A-V phase (0:23.7), on the optic nerve head, segmental (clockwise from 10.30 to 14.30 hrs) lesion of the papilla affected by an infarction, with extravasation of the contrast seen at the end of examination. The arrows in images A and D indicate optic disc involvement due to infarction. C) Left eye fundus. D, E) The first image of the right eye, taken at the beginning of the second minute, shows the optic disc as hypofluorescent temporally, becoming relatively fluorescent by the 11th minute, and fully fluorescent at the end of the FA – a consequence of NAION on the right eye. F) Right eye fundus.

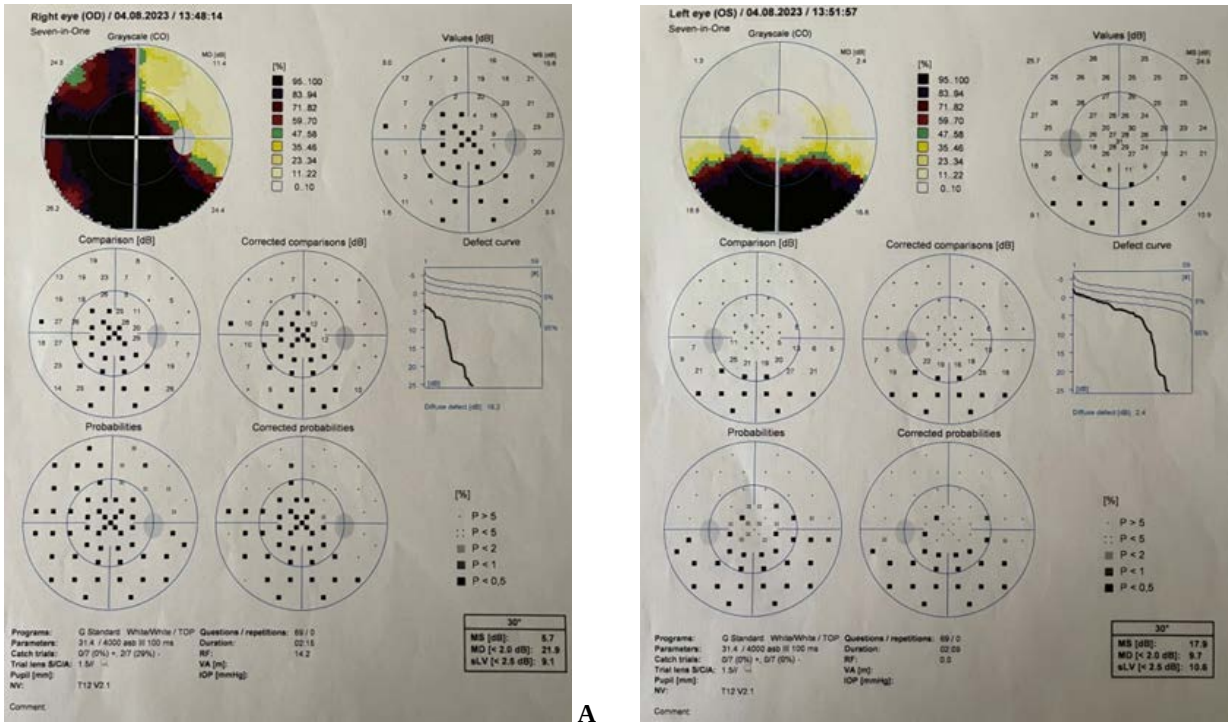

Fig. 2 – A) Right inferior altitudinal defect. B) Left eye initial inferior altitudinal visual field defect.

Testing for the three primary mutations associated with Leber's hereditary optic neuropathy (LHON) was conducted, and the results were negative. Next-generation sequencing of mitochondrial DNA was also performed and yielded negative results. Screening for the most common mutations in the *DNAJC30* gene associated with autosomal recessive LHON was also negative.

The patient was diagnosed with right NAION, based on his symptoms, visual field loss, and segmental optic disk edema typical for the condition.

The patient's medical record, examination, and autoanamnestic data did not show the presence of traditional risk factors for NAION, such as older age, arterial hypertension, DM, elevated blood lipids, or medication use, so the patient was admitted to the neurology department in order to perform further diagnostic procedures and exclude other reasons for sudden unilateral vision loss.

Brain magnetic resonance imaging, including orbits, was performed to exclude potential infiltrative processes and showed two unrelated chronic micro-ischaemic brain lesions (Figure 3).

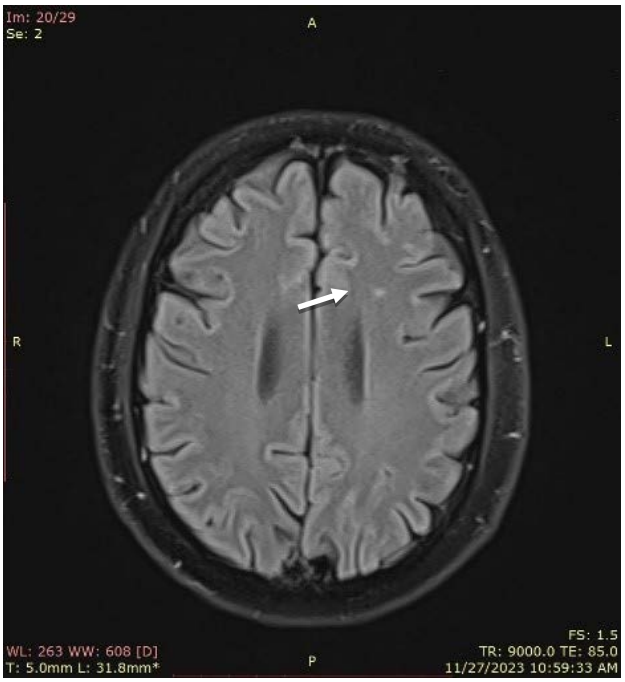

Fig. 3 – Magnetic resonance imaging shows two isolated chronic micro-ischaemic brain lesions.

During hospitalization, healthcare personnel in charge of patient care witnessed the patient's loud snoring, followed by breathing pauses, gasping for air, and waking during the night, including excessive daytime sleepiness (falling asleep while waiting for the examination, frequent napping during the day). Further interviews with the patient's family members confirmed that they had seen the patient exhibiting similar symptoms several months prior to vision loss.

Given that the patient exhibited a significant history of OSA symptoms, he was referred to a somnologist for further diagnostic procedures. Detailed anamnesis, examination, and the use of standardized questionnaires revealed a high probability of OSA (STOP-BANG score 5/8) with excessive daytime sleepiness (ESS score 14/24). Since no significant comorbidities or signs of other sleep-related disorders were present, a limited attended polysomnography was indicated and performed in hospital settings.

The patient underwent a single-night attended cardiorespiratory polysomnography using a type 3 polygraphy system (Alice Night One, Philips Respironics, the Netherlands). The analysis of all-night limited polysomnography records (Figure 4) revealed the presence of OSA syndrome with a very severe degree, with an AHI of 52.6/hr. Respiratory events were associated with a drop in peripheral arterial oxygen saturation to 79% (the lowest saturation values were just 79%). The oxygen desaturation index was 45/hr. Saturation with less than 90% of oxygen was present at 25% of the recording time. Respiratory events (apnea, hypopnea) were dominantly present when the patient was sleeping on his back.

The somnologist indicated the use of an auto-continuous positive airway pressure (CPAP) device with a nasal mask, which was very well accepted by the patient after initial titration (pressure range 4–10 cm H_2O). Further follow-ups after one week, one month, and six months of use showed adequate adherence and compliance (use more than 4 hrs for more than 70% of nights), with average AHI of less than 3/hr (normal range), and resolution of sleep related symptoms (snoring, apnea, non-restorative sleep) as well as excessive daytime sleepiness.

The patient was initially treated with corticosteroid therapy, antiplatelet therapy, and neuroprotective therapy. Subsequently, the patient was treated with a CPAP device. The follow-up ophthalmological examination after 6 months of the initial diagnosis showed no change in VA and no signs of NAION progression.

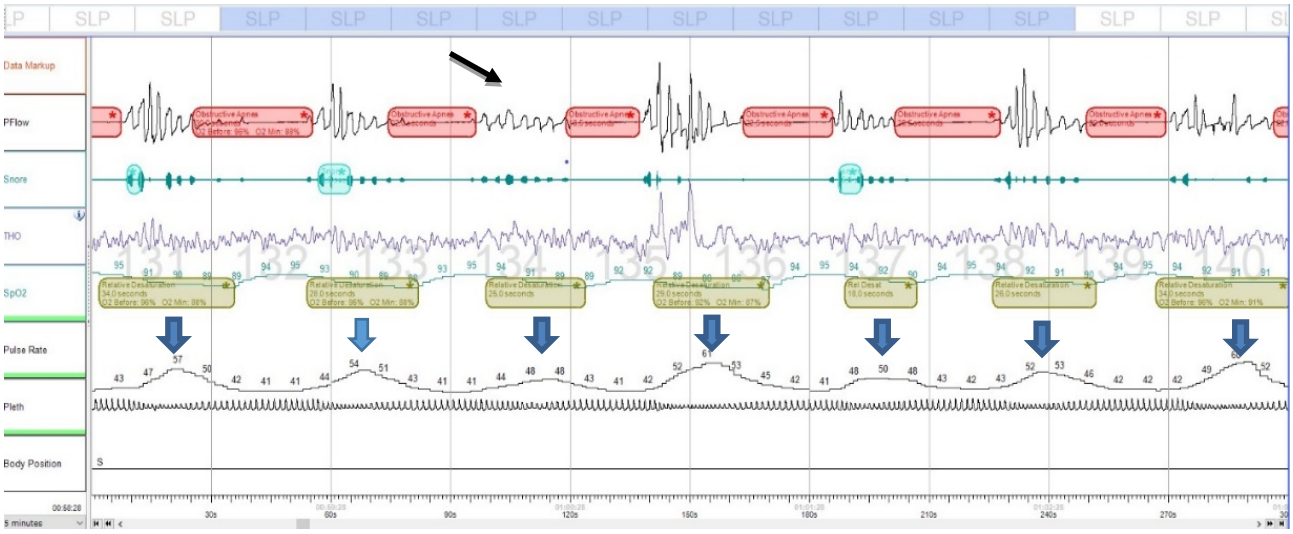

Fig. 4 – A 5-min excerpt of the limited polysomnography recording showing repetitive obstructive apneas (red field), followed by oxygen desaturation events (green field) and elevated heart rate (blue arrow).

Discusion

We presented an unusual case of NAION in a 43-year-old patient who, on admission, had no obvious associated risk factors for this disease. However, with careful observation and clinical history, followed by specialist examination and diagnostic procedures, the presence of unrecognized severe OSA was confirmed as a potential main risk factor for NAION.

NAION is a complex and multifactorial disease. Although the mechanisms underlying the pathogenesis of NAION have not been elucidated, it is generally accepted that it is intimately linked to impairments of microvascular regulation of the preliminary zone of the head of the optic nerve. The most widely cited hypothesis concerning the sequence of events that precede manifestations of NAION is that transient hypoperfusion (such as in cardiac dysfunction or pulmonary embolism) within the densely packed optic disc leads to infarction, followed by edema and consequent compression of the microvasculature in already constricted space of the optic canal, resulting in hypoperfusion. Thereby, the vicious circle infarct-edema-compression-infarct underlies the neuronal dysfunction with vision loss as its consequence [17].

Typical systemic vascular risk factors such as male gender, hypertension, DM, hyperlipidaemia, smoking, migraines, etc., are also the risk factors for NAION [18]. Most of these factors are not recognized in our patient, except for the male gender. Further diagnostic procedures have been performed to exclude other causes for sudden unilateral vision loss [19, 20]. Whole-genome sequencing has not been performed; therefore, genetic diseases cannot be excluded as the cause of vision loss in this patient.

A recent meta-analysis has found that patients with OSA have a four times higher risk of NAION [12]. Initial relations between OSA and NAION were noticed in a case report published in 1988, where the patient with severe OSA and normal visual function had bilateral optic disk edema, which resolved with the use of tracheostomy (treatment of choice for OSA before CPAP) [21]. Later studies have found that most of the NAION symptoms appeared in the morning, suggesting that some nocturnal events, such as blood pressure or sympathetic activity variations, could lead to or function as a trigger for NAION [22, 23]. OSA has been recognized as causing repetitive intermittent nocturnal hypoxia, followed by significant variations in sympathetic activity, with manifestations like cardiac arrhythmias, arterial hypertension, and hypotension episodes, so OSA gained interest in NAION research [24, 25].

Several studies have further researched the potential relationship between these two disorders, analyzing not only OSA-induced hypoxia and the effect on catecholamines but also the elevated cranial pressure on the optic nerve during apneic events, variations of ocular perfusion pressure, and the effects of OSA on other risk factors [15, 26]. Stein et al. [27] found that patients with diagnosed and untreated OSA have a 16% greater chance of NAION compared to subjects without OSA.

Although there is a strong association between OSA and NAION, OSA is rarely assumed by the ophthalmologist or other medical specialists. Even with the obvious symptoms and signs, it is estimated that 80–90% of OSA patients are still unrecognized and, therefore, untreated [14, 28].

Due to the estimated high prevalence of OSA in NAION patients, it has been suggested that patients with confirmed NAION should be screened for OSA and *vice versa* [26].

In the presented case, OSA was not previously diagnosed, but the educated clinical observation followed by extended anamnesis and heteroanamnesis led to proper diagnostics and treatment.

In their recent study, Li et al. [16] have proven that untreated OSA has the potential to cause subclinical nerve damage in the contralateral optic nerve in patients with NAION, followed by temporal quadrant thinning of the peri-

papillary retinal nerve fiber layer, probably because the papillomacular bundle located there has the highest oxygen deficit sensitivity.

Chang et al. [29] have shown that one of the main risk factors for second eye involvement in NAION patients with moderate to severe OSA was noncompliance with CPAP treatment, raising the chances by more than four times. In our case, the patient showed continuous adherence and compliance to CPAP treatment, which led to the elimination of his OSA signs and symptoms. He also had no signs of NAION progression in his right eye, nor signs of NAION in his left eye after 6 months of follow-up.

Conclusion

The case we present hereby supports the inclusion of OSA in the risk factors for NAION. Moreover, this case emphasizes the importance of screening for OSA in all patients in the high-risk groups for NAION and patients already diagnosed with NAION. Given the reported association between NAION and OSA, a thorough history should be screened for OSA when NAION is diagnosed. Furthermore, perhaps most importantly, screening for and diagnosing OSA should alert clinicians to conduct an ophthalmological examination. Such screening could easily be implemented in primary care, reducing the time from presentation to diagnosis of NAION. Importantly, OSA is a modifiable risk factor and can be successfully treated with CPAP. Implementation of this simple and effective algorithm has the potential to significantly reduce the risk of optic disk lesions, as well as other microvascular complications precipitated by hypoxic damage. Together, this will result in early diagnosis and prevention of irreversible degenerative changes of the optic nerve.

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One century of the Pharmacological Institute at the Faculty of Medicine in Belgrade – Arnold Holste, Radivoje Pavlović, and Ilija Dimitrijević

Jedan vek Farmakološkog instituta Medicinskog fakulteta u Beogradu – Arnold Holste, Radivoje Pavlović i Ilija Dimitrijević

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history, 19th century; history, 20th century; history of medicine; pharmacology; serbia.

Ključne reči:
istorija, 19. vek; istorija, 20. vek; istorija medicine; farmakologija; srbija.

Introduction

The earliest written document on medicine in Serbia is found in the Hilandar Typikon, created through the translation and adaptation of the typikon from the Greek Monastery of the Virgin Evergetide in Constantinople, likely in 1199. These ecclesiastical-legal writings were prepared by Saint Sava. Another important document is the Studenica Typikon, created in a similar manner—probably in 1207—based on the Hilandar Typikon. Chapter 40 of both typikons addresses the organization of hospitals in the Hilandar and Studenica monasteries. For this reason, Saint Sava is considered, among other things, the founder of medicine and legislation among Serbs [1].

The most studied document in the field of pharmacology is the Hilandar Medical Codex, written in the vernacular language using Cyrillic script. The creation of the first manuscript is believed to date back to the late 14th century based on the writings of Western European medical schools and Byzantine, Arab, and African medicine. A significant decline in medical practice occurred during the period of Ottoman rule, which lasted approximately 4 to 4.5 centuries [2].

In modern Serbia, pharmacology as a scientific discipline was founded after the establishment of the Faculty of Medicine in Belgrade in 1920, which was significantly delayed by World War I. The Pharmacological Institute began its work in 1924, under the leadership of its founder, Professor Arnold Holste, who also served as its director. Professor Holste studied experimental pharmacology and toxicology in prominent scientific centers across Europe and beyond. He was also a professor of pharmacology and toxicology at the University of Jena in Germany [3].

Collaborators on the stabilization and improvement of the Institute were doctors who studied in Pest and Geneva, Dr. Radivoje Pavlović, and a little later, Dr. Ilija Dimitrijević. They were our first trained pharmacologists, privileged to work alongside Professor Holste. These three scientists formed a highly motivated core for teaching and research. They wrote textbooks and manuals for medical students in the Serbian language. The first generation of students could study pharmacology [4, 5].

A century after the establishment of the Pharmacological Institute at the Faculty of Medicine in Belgrade, Serbia, conditions were created for pharmacology in Serbia to develop into a serious and independent scientific field.

Professor Arnold Holste

Biography and education

Professor Arnold Holste (Figure 1) [6] was born in Hanover, Germany, to father Ferdinand, a senior civil servant, and mother Dorothea Maria Sophia, a housewife [3]. Some sources state that he was born on April 28, 1865, and others on August 28, and even the years 1863 and 1869 are mentioned. The question of his exact birth date was first initiated by Pro-

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fessor M. Mikuličić from the University of Zagreb in 1932. It has since been reliably established that the correct date of his birth is August 28, 1863. This confusion, to which Professor Holste personally contributed, soon brought him a lot of problems in his professional life [7].

Fig. 1 – Arnold Holste [3].

He completed both elementary and high school in his hometown. He enrolled in medicine in 1882 in Göttingen, continued his studies in Berlin, and later again returned to Göttingen, where he was promoted to doctor on March 8, 1888, with the experimental work "Ligation of the urinary ducts of rabbits", which he finished while working during his studies [3].

Professional and teaching-scientific activities

Professor Holste worked for two years as a physician's assistant at the Medical Polyclinic Epstein in Göttingen, Germany, volunteered for three months at the Regional Midwifery School, and was a practicing physician for six years in the Construction Office in Hanover, Germany. In the attachment for his habilitation, Professor Holste wrote: "Having gotten to know the work of medical institutes on numerous trips abroad in Europe and North America, I returned to Göttingen, where I spent four years with Professor Max Vervorn, with the main intention of studying new methods in physiology, so that I could devote myself to experimental pharmacology". After that, he worked in the pharmaceutical industry for four years. This was followed by two years (1911–1913) of experimental work at the Pharmacological Institute of the University of Strasbourg in Strasbourg (then Germany, today Strasbourg, France), as a volunteer with Professor Oswald Schmiedeberg (1838–1921). Schmiedeberg came in 1872 from Dorpat (now Estonia, at that time Russia), where he studied pharmacology with Professor Rudolf Buchheim (1820–1879), who first began to conduct experimental research in pharmacology [8].

In 1913, on the recommendation of Professor *Schmiedeberg*, Professor Holste became an honorary assistant at the Institute of Pharmacology of the Faculty of Medicine of the University of Jena, Germany, under the directorship of Professor Heinrich Kionka. There, he continued to engage in scientific work, but now completely independently. After his habilitation thesis "On peony alkaloid" (1915), he was elected to the position of private assistant professor with the introductory lecture "Physiology of the heart". Four years later, he was promoted to the title of associate professor at the suggestion of Professor Kionka. During World War I, Professor Holste managed the Pharmacological Institute independently, since Professor Kionka was mobilized [3].

Professor Arnold Holste ended his academic career in Germany in May 1924, following his appointment as a full-time contractual professor of pharmacology at the Faculty of Medicine in Belgrade. This appointment was made by decree of King Aleksandar Karađorđević on December 28, 1923, upon the recommendation of the Minister of Education. The contract lasted three years, with the commitment of Professor Holste to extend it for at least another two years. To end his safe and well-paid job in Jena, Professor Holste stated several reasons, some of which were: "to start teaching experimental pharmacology and toxicology", "to acquire the right to a pension by receiving Yugoslav citizenship", and "to take care of his old age". Future events cruelly denied his understanding of the terms offered [7].

The main task of Professor Holste was the founding of the Pharmacological Institute (not the Institute of Pharmacology), which he headed for eight years, from its foundation in 1924 to 1933. His wife, Irmgard Seifert (born in 1891, married Holste, somewhere written as Holete), was the first laboratory technician. She worked until 1933, when the contract of Professor Holste as a regular professor expired [3].

The establishment of the Pharmacological Institute included the following: 1) physical organization of the Institute – organizing classrooms for conducting theoretical classes, organizing laboratories for practical teaching and scientific research, organizing the library; 2) formation of teaching staff; 3) writing textbooks and manuals for students; 4) participation in expert bodies in the field of pharmacology and toxicology; 5) participation in national and international scientific and professional meetings [9].

Professor Holste taught in the Serbian language, which he learned within three years, and used the Serbian Cyrillic script. With the assistance of Milija Magarašević, a future physician, he authored the textbook *Experimental Pharmacology* (1929) [10]. In it, he stated: "Empirical pharmacotherapy was achieved through patient observation, which is, of course, unreliable. It becomes valid only when such experience receives experimental confirmation".

Professor Holste is the first toxicologist in modern Serbia and is recognized as the founder of toxicology as a scientific discipline in our country. In his textbook *Basics of Toxicology* (1930), he explained why toxicology should be studied as a separate field [11]: "The need for independent toxicology teaching grew more and more, as the doctor was required to make an early diagnosis of poisoning and take over their treatment". Toxicology is not only intended for medical professionals, but also holds importance for other professions.

Together with collaborators Dr. Radivoje Pavlović and Dr. Ilija Dimitrijević, Professor Holste contributed to the

writing of the *Lexicon: Doctor in the House*, edited by Aleksandar Kostić, a book that had ambitions to become a "true folk medical encyclopedia". They wrote in the field of pharmacology and pharmacognosy, totaling 186 references [12].

"Professor Holste was very popular among students, not only for his instructive lectures but also for his helpfulness, tact, and love for students". He published more than 50 articles. Most of them were published in German, French, and Italian, with the majority published prior to his arrival in Belgrade [6].

Professor Holste published more than 20 articles in Serbian, thus contributing to domestic journals and popular pharmacological and toxicological texts in newspapers. He also delivered a great number of free lectures in courses for graduate medical students from all over the country. Such a great contribution to Serbian science shows the great support and loyalty of Professor Holste to the country he chose to live and work in. Professor Holste passed on valuable research methods and techniques, already proven in his own scientific work, to his collaborators Dr. Radivoje Pavlović and Dr. Ilija Dimitrijević [6].

More than others, Professor Holste dealt with the following areas: cardiovascular pharmacology and pharmacology of cardiotonic glycosides on the experimental model of isolated frog heart, physiology and pharmacology of the uterus, diuretics, and biochemical isolation of active principles from plants. "As an experimenter, he was very meticulous; he only liked clean and precise work, which is why he gave his own modification of the experimental technique in some of his studies" [6].

Professor Holste is involved in the work of many organizations that were responsible for the advancement of the pharmacology profession and various legal regulations in the medical field. He was a member of several important institutions and scientific societies. Among them were: the Main Health Council at the Ministry of Social Policy and Public Health of the Kingdom of Serbs, Croats, and Slovenes, which is the highest expert advisory body of the Ministry of Health; the Permanent Expert Council for Drug Testing, established in 1926, whose work aims to evaluate drugs submitted by the Ministry of Health; the Commission for the Control of Drugs of Biological Origin; the Commission for the Development of the State Pharmacopoeia; the Directorate for the Protection of Industrial Property at the Ministry of Trade and Industry. He also served as the Delegate of the Kingdom of Serbs, Croats, and Slovenes at the International Conference for the Unification of Maximum Doses of Heroic Medicines. Additionally, he was a member of several scientific societies, including the German Pharmacological Society, the Society of German Naturalists and Doctors, the German Society for Internal Medicine, and the Biological Society in Belgrade [6].

Due to legal and administrative regulations that required university professors to retire at the age of 70, Professor Holste resigned from his position as director of the Institute in 1933 upon reaching that age. However, he never officially retired, neither in 1933 nor, according to the University of Jena, in 1934. Due to his poor financial situation, he had to continue working as a part-time professor, though with a steadily decreasing income. His final contract extension was granted on March 16, 1935, and it expired on April 5, 1938. Death, unfortunately, was faster. Professor Holste passed away in Belgrade on April 12, 1937, at the age of 74 [3].

Professor Holste was buried at the New Cemetery in Belgrade on April 14 by his wife, Irmgard (not "Nirgord", as mistakenly recorded in some documents). He was buried under the name "Holete", according to certain official records. The grave was excavated between 2002 and 2004, and the current location of his remains is unknown, as well as the whereabouts of his tombstone, although a photograph of the tombstone exists in the cemetery archive. His complete legacy, described as "laboratory and office items and books", was donated to the University of Belgrade by Irmgard Seifert, who later returned to Germany [3]. As Dr. Radivoje Pavlović wrote: "The entire staff of the Pharmacological Institute in Belgrade will always remember him with gratitude for all the good, beautiful, and useful things that the founder and first director of the Pharmacological Institute gave them during his lifetime, both in word and deed". He added: "...the activity of Professor Holste was very abundant and varied. In addition to his professional achievements, he was very interested in archaeology, ancient history, and classical languages; he traveled a lot and knew not only the whole of Central and Western Europe but also visited America and Africa. Personal contact and the conversation with him were always very interesting" [6].

Professor Radivoje A. Pavlović

Biography and education

Professor Radivoje Pavlović was born on October 5, 1893, in the town of Szigetcsép (Serbian name *Čip*) on Csepel Island (former Austro-Hungary), about 50 km south of Budapest, to father Alexander, an Orthodox priest, and mother Zorka (born Petrović), a housewife. In secondary publications, mistakes are made about his place of birth [4].

Dr. Pavlović finished elementary school in Serbian Kovin, 15 km south of *Čip*, where his father was a parish priest (before that, he served in *Čip*). After his father's death at the age of 34, his mother moved to Novi Sad with him and his sister Melania, who later graduated from high school for girls. There, he attended the Serbian Orthodox High School, one of the best and most influential Serbian high schools at the time, as a scholarship holder of the "Athanaseum" endowment. He graduated in 1912. There he acquired a broad general culture, knowledge of classical languages, classical and modern literature, fine arts, and he developed his musical talent under the influence of music professor Isidor Bajić [13].

Dr. Pavlović studied medicine in Budapest as a student of the Tekelijanum institution, an endowment founded in 1838 by doctor of law Sava Popović Tekelija. He entered the faculty in 1912 and was promoted to doctor of medicine on May 25, 1918 (Figure 2) [13].

Fig. 2 – Radivoje Pavlović (taken with permission from the Pavlović family archives).

Dr. Pavlović's colleagues said he "completely lost his sight very early, almost at the beginning of his university career. Although the blow was ferocious, everyone who knew Dr. Pavlović knows very well that he endured it with admirable courage and that he did not give in for a moment" (Simo Milošević) [13]. He had a wife, Stanija (born in 1901 in Đurđevo, Kragujevac, Serbia, died in 1988 in Belgrade), a pediatrician at the Institute for the Protection of Mothers and Children. They had two daughters: Miroslava (Pavlović-Hournac), a biologist and world-renowned scientist, and Zorka (Zorica Stević), a primary care physician, who died in Belgrade in 1990. Zorica is survived by her son, Đorđe, and daughter, Jasna, who both live and work with their families in Belgrade [4].

Professor Radivoje Pavlović died suddenly on August 20, 1938, in Zlatibor, Serbia. He was buried at the New Cemetery in Belgrade [13].

Professional and teaching-scientific activities

He first worked as a corps doctor with the Danube Howitzer Division, stationed in Petrovaradin and Subotica, Serbia, holding the rank of reserve medical captain, second class. After this, he worked as a specialist in internal and nervous diseases in Novi Sad, Serbia, serving as an assistant physician at the state City Hospital and in the children's home and hospital "Institute Marije Trandafil for Serbian Orthodox Orphans", commonly known as Trandafil's Orphanage. There were a lot of war orphans after World War I. For three months, he worked in the recruitment commission of the District Command in Veliki Bečkerek, Serbia. Later, he worked in Belgrade as a specialist in internal medicine until April 1930. He went to Berlin (1920–1922), where he studied biochemistry under Professor Peter Rona, a physician and biochemist. He specialized in internal medicine under Professor Alfred Goldscheider, internist, military doctor, and neurologist with great clinical achievements. Dr. Pavlović stated: "As a role model of a doctor, I always had the late Professor Jendrassik (Pest), and as a scientist, Mr. Professor Rona" [14].

In 1922, he became an honorary assistant at the First Internal Medicine Clinic in Belgrade, where the director was Professor Aleksandar Josifovich Ignjatovski, a Russian emigrant, the first professor of internal medicine. In October 1924, Dr. Pavlović was assigned to Professor Arnold Holste as a translator from German to Serbian. At the beginning of 1926, he was transferred to the Institute of Pharmacology as an assistant. Dr. Pavlović was the first pharmacology assistant in modern Serbia. In March 1927, he was elected to the position of assistant professor and held a very notable introductory lecture, "Subjectivity in therapy", where he presented his understanding of the thinking side of the doctor in his therapeutic activity [14]. He was re-elected to the same title in 1930 and was promoted to associate professor in 1933. After the resignation of Professor Holste in 1933 and at his suggestion, he became the director of the Institute until the end of his life [4].

Professor Ilija Dimitrijević wrote: "As a teacher, he was one of the most popular among students; his lectures were at an enviable level both in terms of conscientiousness of processing and clarity of presentation, and they always corresponded to modern understandings. His speech was suggestive and was always listened to with the greatest attention". The harmonious atmosphere within the institute, initially established by Professor Holste, was maintained by his associates. "He was a very responsible man. When he accepted a new person into his institute, from that moment on, he took responsibility for their future." A good example for any time [13].

In 1928, Dr. Pavlović wrote the textbook *Recipe book* (in Serbian *Receptura*), the first of its kind in our country. The second edition was revised and supplemented in 1933. The *Recipe book* covers a wide range of medications, primarily magistral preparations, custom-made drugs important for clinical practice. It discusses their dosage forms, methods of prescription, and the components of a proper prescription. The book begins with Rp., an abbreviation of the Latin word "recipe", which means "to take", presented without any graphic additions. Such precision is not always present in today's professional literature and clinical practice [15].

The first textbook for the study of drugs, called *Materia medica with pharmacodynamic data and recipes*, was written by Dr. Radivoje Pavlović and Dr. Ilija Dimitrijević at the initiative of Professor Holste, published in 1929 [16]. In the preface, the authors state: "...the book should help students understand theoretical lectures in pharmacology and clinical subjects, demonstrations of drugs and medicines, and practical exercises from recipes. It should make it easier for doctors to navigate medications and prescribe medications in daily practice." At the end of the book are 172 recipes as well as a very well-made and organized glossary (register).

Dr. Simo Milošević wrote: "Radivoje Pavlović had another characteristic of a true scientist and head of a scientific institution: scientific selflessness. For him, it was not important whether he signed the work, only that it was done. And finally, he constantly worried about those who have to

take over the job after him, for the heirs, which is a rare case in our environment" [13].

He published a large number of papers in international journals, which shows the quality of the presented results and knowledge of foreign languages. He spoke Hungarian, German, French, and English, then Latin and Greek from the classical languages, and used Russian and Italian. About his publications, Dr. Ilija Dimitrijević wrote: "They bore the imprint of his personality as a scientist, showed comprehensive knowledge of the subject, testified to the clear presentation of scientific problems, the precision of the tests performed, the critical judgment of the results, and the caution in drawing conclusions" [13].

In addition to 25–26 published works in which he was mostly the sole author, and occasionally one of the two co-authors, a large number of publications were created under his leadership.

One of Professor Pavlović's works refers to medical terminology, whose topicality has not yet been surpassed. Discussions on this subject began as early as 1842 in the Society of Serbian Literature, and continued in the Serbian Medical Society shortly after its founding in 1872. Unfortunately, despite the very clear guidelines given by Professor Pavlović, this area has still not been worked on nor been organized. He believes that "medical terminology in any language will never and can never be fully elaborated, settled, or definitive in all details, as long as the language itself is alive. But in the main issues, certain directives must be established, there must be some support". There are two possibilities for creating medical terminology. One is finding a Serbian equivalent for each foreign medical term, in which there are folkloristic and neologistic approaches, and the other is the transcription of foreign medical terms directly into the language [17].

He believes that mistakes occur because "semi-literate intellectuals, so numerous in their professions, do not study their language enough during their schooling, and become resistant to the influence of grammar, syntax, and stylistics of foreign languages, especially if they are still engaged in those environments for a long time" [17].

Medical Review journal

Professor Pavlović was one of the founders, editor-in-chief, and the owner of the journal called *Medical Review* (*Medicinski pregled* in Serbian). It served as a joint publication for Serbian, Bulgarian, Croatian, and Slovenian doctors and was published in each of these respective languages. The editorial board included representatives from the medical faculties in Belgrade, Zagreb, Ljubljana, and Sofia. Over the course of its 14.5-year run, the journal published 178 issues in 170 volumes. Thanks to the dedication of his wife, publication continued for approximately two and a half years after Professor Pavlović's death. The November 1938 issue of *Medical Review* was entirely dedicated to the life and work of Professor Pavlović, whom we often mention in this text [13].

Extracurricular activities

Professor Pavlović had numerous professional obligations outside the faculty. He was, for instance, a member of the Permanent Expert Council for Drug Testing, the Biological Society in Belgrade, the Serbian Medical Society, and the German Pharmacological Society [13].

Dr. Simo Milošević said: "He was a Greek by the fineness of his spirit, a Roman by the height of his morals, a Slav by the kindness, breadth, and warmth of his soul" [13].

Professor Ilija N. Dimitrijević

Biography and education

Professor Ilija N. Dimitrijević was born on March 29, 1896, in Belgrade, to father Nikola, a café owner and rentier, and mother Donka, a housewife. He had three sisters – Zorka, Pava (died at childbirth), and Frosina. Frosina's two daughters, Miroslava and Dragoslava, along with Dragoslava's son and daughter, are his surviving descendants. Although of Cincar origin (his grandfather Zaharija came from Kruševo, present-day North Macedonia), Professor Ilija Dimitrijević identified as a Serb and celebrated the family patron saint, Saint Nicholas. During World War I, Professor Dimitrijević, his parents, and his three sisters lived in Geneva, Switzerland. He was married to Zora (born in Pirot, Serbia, in 1897, died in Belgrade in 1976), the daughter of Pera Aranđelović, a pharmacist from Niš, Serbia, and Mara, a housewife. They had no children. Following World War II, Professor Dimitrijević lost his family house at Studentski trg 4 in Belgrade and was given an apartment in exchange [5].

Dr. Ilija Dimitrijević completed elementary school and attended high school in Belgrade until the seventh grade, when his education was interrupted by World War I. He finished the eighth grade in 1916 at the Serbian high school in Nice, France. That same year, he began studying medicine in Geneva, where he obtained the title doctor of medicine in 1921 (Figure 3) [18].

He died on January 1, 1968, and was buried at the New Cemetery in Belgrade [5].

Fig. 3 – Ilija Dimitrijević (taken with permission from the Dimitrijevć family archives).

Professional and teaching-scientific activities

After graduating, Dr. Dimitrijević completed a one-year military service in Belgrade and held the rank of reserve medical captain, first class. In 1923, he began working as a volunteer at the First Internal Medicine Clinic, and in 1924, he became an assistant. He specialized in internal medicine in Belgrade and received the general license to practice private medicine in 1923 and the local license in 1924. The first time he practiced medicine was until 1930, and the second was during World War II (the first time he retired), from the summer of 1943 to 1945. Dr. Dimitrijević spent a year (1925–1926) in Paris, France, and Berlin, Germany, where he studied and completed his specialization in biochemistry. In Paris, he worked in the laboratory of Professor Chauffard at the Internal Clinic, and in Berlin, in the laboratory of Professor Pincussen. Upon returning to Paris, he completed a course in microbiology at the Pasteur Institute. The academic paths of Dr. Pavlović and Dr. Dimitrijević are very closely connected. In fact, the promotion of Dr. Pavlović to a higher position within the Institute allowed Dr. Dimitrijević to apply for the vacant position. In March 1927, he was transferred from the Internal Medicine Clinic to the Institute as an assistant professor following Dr. Pavlović's advancement. He was appointed assistant professor in 1931, after which he began teaching both practical and theoretical classes, alongside Professor Holste and Professor Pavlović [5].

After the death of Professor Pavlović, Professor Dimitrijević was appointed director of the Pharmacological Institute in September 1938. He held this position until 1953 (except during World War II when he did not work at the Faculty), after which he was "removed" from the Faculty. This position cannot be denied, as can be seen in recent secondary publications [5].

He became an associate professor in 1939 and a full professor in 1950. At the same time, he was a member of the Council of the Faculty of Medicine. In November 1948, the Council appointed him the head of the Department of Therapy (pharmacology, balneology, and physical therapy) and elected him to be a member of the Forensic Medicine Board. His work at the Faculty of Medicine stopped for the first time due to his retirement on April 3, 1943, during the Nazi occupation. He was succeeded by Dr. Vojislav Ristić, a pharmacology teacher at the Faculty of Veterinary Medicine in Belgrade [19].

As a professor and scientific researcher, Dr. Ilija Dimitrijević is perhaps most accurately described by the so-called "Characteristics", formulated shortly after World War II. They included biography, expertise, political and ideological commitment. In those "Characteristics" from 1949 and 1950, it is written: "A very good expert knows his subject well and follows science. He conducted scientific work and published scientific papers. A good organizer, conscientious and meticulous in his work. He does all professional work entrusted to him diligently and conscientiously. A very good lecturer and makes sure to give students as much practical knowledge as possible. He is fair to the students. According to colleagues, tactful. He is working on a textbook". It has been added: "as a lecturer, one of the best at the university...his lectures are always attended... he has a very good attitude towards the students during the exam...very well-read...a man of quite a wide culture and education", but is "comfy by nature...a weak experimenter" [20].

As a teacher and pedagogue, Professor Dimitrijević is fondly remembered by his post-war students. One student recalled: "It was a pleasure to study with Professor Ika Dimitrijević, as we students called him. He respected every candidate and listened carefully to their answers. Through the exam questions, he patiently, skilfully, and benevolently guided students through the entire material. With a noble appearance, always impeccably dressed, he gave lectures in a packed amphitheater, where students immediately absorbed every word he said" [5].

Together with Professor Radivoje Pavlović, Professor Ilija Dimitrijević wrote a textbook on pharmacology titled *Materia medica: with pharmacodynamic data and recipes* [16]. In 1949, another textbook, *Pharmacology*, was published based on the lectures of Professor Ilija Dimitrijević [21]. There are 12 chapters in the book based on the effects/mechanisms of drug action and on the organ systems they act on.

By the time Dr. Dimitrijević was elected full professor, he had published 31 papers. Some of them, based on their focus and approach, would be classified within the domain of today's clinical pharmacology. His knowledge of foreign languages (French and German, including English, Russian, and Italian) enabled him to present his scientific results both to the domestic and European scientific public [5].

Professor Dimitrijević is the sole author of most of his published works. From 1932 to 1934, he worked at the Physiological Institute of the Faculty of Philosophy in Belgrade with Professor Ivan Đaja. Together, they published several papers on thermoregulation and thermogenesis under experimental conditions. The Biological Society in Belgrade was established as a branch of the Biological Society in Paris. The Society had its own journal, *Comptes rendus des séances de la Société de biologie et de ses filiales*, which published the oral presentations of its members. Among the contributors were Professor Pavlović and Professor Dimitrijević [22].

In both his scientific work and his teaching, Professor Dimitrijević, like Professor Pavlović, adhered to the principles of Professor Holste, mentioned in the preface of the textbook *Materia medica*: "Today we must strictly distinguish the description of drugs (*Materia medica*) from experimental pharmacology, which in recent years has again found its way to practical medicine...The triad *Materia medica*, experimental pharmacology, and pharmacotherapy, together constitutes modern drug science as a whole. These three modern disciplines developed from each other in the order mentioned and are constantly in the closest possible relationship" [16].

Among Professor Dimitrijević's most significant contributions outside the Pharmacological Institute was teaching pharmacology at other faculties. This began in 1939 with the establishment of the Pharmacy Department within the Faculty of Medicine. That same year, by the decision of the Faculty Council, he was appointed part-time professor of pharma-

cology and became a member of the first Council of this Department. In the fall of 1948, he also became involved with the Faculty of Dentistry in Belgrade, an integral part of the Great School of Medicine. He was appointed part-time associate professor of pharmacology. He created a teaching program, announced a vacancy for an assistant, hired demonstrators, and started lectures. Some secondary sources have claimed that Professor Dimitrijević was the first pharmacology teacher at the Faculty of Veterinary Medicine in Belgrade. However, as suggested by some sources, Dr. Vojislav Ristić was actually in this position, who succeeded the briefly appointed part-time assistant Siniša Bogdanović [5].

Professor Dimitrijević was a member of many professional bodies throughout his career. From 1938, he served on the Permanent Expert Council for drug testing, first as a member and later as its president. He was an extraordinary member of the Main Sanitary Council and a member of several commissions, including the Commission for Medicines of Biological Origin and the Commission for the Preparation of the Pharmacopoeia, serving first as a member and then as chairman of the Opium Commission at the Ministry of Trade and Industry. He was also a member of the Directorate for the Protection of Industrial Property at the Ministry of Trade and Industry, the Financial and Construction Committee for the Building Project of the Faculty of Medicine in October 1939, the Forensic Medical Committee, and the Commission for the Selection of Land for the Construction of the Pharmacological Institute. In addition, he was a member of the Editorial Board of two prominent medical journals – the *Serbian Archive for All Medicine* and *Medical Review* [5].

During World War II, immediately following the bombing of Belgrade in 1941, at the onset of the short-lived April war against fascist Germany, Professor Dimitrijević along with Associate Professor Siniša Bogdanović and approximately thirty other university professors, was arrested and detained at the Banjica concentration camp, where Professor Dimitrijević was held for three weeks [5].

In the early 1950s, Professor Dimitrijević was "removed" from the Faculty, despite never having been involved in politics. The term "removed" was used instead of "dismissed/transferred to another workplace" and was typically applied only after the academic and administrative staff had been restructured to align with the needs of the new state. Files of teachers and associates, in which "Characteristics" had a special place, were created based on attitudes, opinions, findings of the party representatives, faculty committees, and state authorities [20].

In "Characteristics" from 1949, it is stated: "According to the testimony of his assistant (Atanacković), he shows a tendency to fail advanced students in the exam" [20]. However, this claim was later denied. Dr. Dimitrije Atanacković had been an assistant at the Pharmacological Institute since 1940. In 1947, the Faculty of Medicine was founded in Skopje, where he served as the director and assistant professor at the Pharmacological Institute [23].

The action taken against Professor Dimitrijević was supported by both the professional and student press. In August 1953, Miroje Perović [24], a medical student and organizational secretary of the Communist League Committee of the Faculty of Medicine, wrote: "...he does not provide help or support to his colleagues" and "he has been promising a textbook on pharmacology for years, but to this day nothing has come of it". These statements ignored the fact that Professor Dimitrijević's pharmacology textbook had already been published by the state institution, Science Book [21]. Among the employees at the Institute, the only teacher was Professor Siniša Bogdanović, who held the position of part-time professor in 1949 and became a full-time professor. In 1953, assistants were among others, Dr. Milenko Milošević, Dr. Mulenko Medaković, and Dr. Vladislav Varagić [23].

According to the decision of the Council of the Faculty of Medicine, adopted by the Council for Education and Culture of the Republic of Serbia on October 21, 1953, Professor ime Ilija Dimitrijević was "immediately dismissed from all duties and functions at the Medical High School" [25], which included the Faculty of Medicine, Pharmacy, and Dentistry.

On the initiative of the Serbian Medical Association from 1993, the Teaching and Scientific Council of the Faculty of Medicine in Belgrade, in 2001, annulled all legal acts of the Faculty of Medicine related to the "removal" of 24 teachers and associates. Professor Dimitrijević himself never requested this during his lifetime [26].

After being "removed" from Medical High School, Professor Dimitrijević was briefly employed at the Council for Public Health and Social Policy of the Republic of Serbia. In March 1954, he was transferred to the Institute for Testing and Control of Medicines of the Republic of Serbia in Belgrade. He worked there until his final retirement in 1964 [5].

Conclusion

The Pharmacological Institute was founded in 1924 by Professor Arnold Holste, a professor of pharmacology and toxicology at the University of Jena, Germany, who was also its first director. Professor Radivoje Pavlović and Professor Ilija Dimitrijević, Serbia's first trained pharmacologists and toxicologists, were the first associates of Professor Holste. All three possessed exceptional medical education, fluency in foreign languages, and broad general knowledge. Together, they represented an excellent and powerful nucleus for the foundation, development, and advancement of pharmacology and toxicology in modern Serbia.

Acknowledgement

We are deeply and sincerely grateful for the valuable oral and written biographical information, as well as the photographs provided, to Miroslava Pavlović Hournac, daughter of Professor Radivoje A. Pavlović, who passed away on November 1, 2019, in Antibes, France, to Miroslava Jovanović, niece of Professor Ilija N. Dimitrijević, who passed away in Belgrade on September 8, 2020, and to Jasna Majerle, granddaughter of Professor Radivoje A. Pavlović from the National Library of Serbia, who, among other things, generously assisted in the acquisition of literature and other materials.

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ARTICLE RETRACTION NOTICE

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The article titled “Factors associated with physician burnout syndrome: a comparative analysis” by Marko Stojanović, Nataša Rančić, Miodrag Stojanović, published in *Vojnosanitetski pregled* 2025; vol. 82, issue 3, pp. 146–155, is withdrawn at the author’s request because the license (permission) for the use of the questionnaire in their research, which was the subject of that article, had expired, making its use unauthorized without the consent of the owner of the questionnaire. The authors’ request was considered and approved at the journal’s Editorial Board meeting on June 25, 2025.

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

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

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

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

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

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

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Examples of references:

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Primeri referenci:

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Tabele

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

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