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REVIEW ARTICLE

ANTI-INFLAMMATORY EFFECT OF BILBERRY (VACCINIUM MYRTILLUS L.) PREPARATION: A SYSTEMATIC REVIEW

Andjela V. Dragićević[1] **Nikola M. Stojanović**[2] **Dragana R. Pavlović**[1]

[1]Department of Pharmacy, University of Niš Faculty of Medicine, Niš, Serbia [2]Department of Physiology, University of Niš Faculty of Medicine, Niš, Serbia

Naturally-derived anti-inflammatories, such as bilberry (*Vaccinium myrtillus*), are particularly interesting due to their health benefits. Bilberries are a rich source of primary metabolites, such as sugars and organic acids, which give the fruit its taste. They also contain anthocyanins, the most abundant group of secondary metabolites, possessing various biological properties, including anti-inflammatory effects. This paper reviews current knowledge on the bioactive compounds found in *V. myrtillus* (bilberry) berries and summarizes clinical studies investigating the anti-inflammatory effects of various bilberry preparations.

The literature review was conducted using the databases PubMed, Scopus, Web of Science, Science Direct, NCBI, Google Scholar, and ClinicalTrials.gov.

V. myrtillus was found to be a significant source of bioactive molecules since components of bilberry fruits, such as polyphenols, anthocyanins, and flavonoids, are well known for their ability to modify cellular pathways implicated in the pathophysiology of many diseases. A brief review of the clinical study data related to the bilberry application highlighted its health-promoting effects, specifically its anti-inflammatory effect.

Bilberry fruit is a rich source of phenolic compounds with high biological potential. These compounds can be used commercially in pharmaceutical, cosmetic, and natural products industries. However, due to the wide variety of constituents in the fruit, their potential interactions, and the complexity of their metabolism, further in-depth studies are needed to better define and characterize the contribution of each active component and possible synergies between different compounds. Numerous clinical studies have highlighted the anti-inflammatory properties of *V. myrtillus* fruits, which could help prevent various diseases.

Keywords: berry, cyanidin 3-O-glucoside chloride, inflammation, Myrtilli fructus

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Correspondence to:
Andjela V. Dragićević
Department of Pharmacy
University of Niš Faculty of Medicine
Bulevar dr Zorana Đinđića 81, Niš, Serbia
E-mail: dragicevic.andjela@gmail.com

INTRODUCTION

According to the Food and Agriculture Organization of the United Nations, fruit production has risen continuously in recent decades (1). However, given the growing world population and current consumer preferences for organic, high-quality food and tendency towards healthy lifestyles, current fruit production may not be sufficient to meet global demand (2). As fruit production continues to increase, a significant number of by-products will inevitably be generated (3). To address this issue and improve the nutritional value of various foods, it is important to investigate the use of these by-products in functional foods as a sustainable approach to reducing waste production (3-5).

The Vaccinium species, a genus with great morphological diversity, belonging to the Ericaceae family, are widespread in Europe, Asia, North and Central America as well as in Southeast and Central Africa (3, 6-8). Due to their numerous health benefits, the use of Vaccinium species is steadily increasing. They have been shown to be effective in various diseases such as diabetes (9, 10), obesity (10), rheumatoid arthritis (11), cardiovascular and neurological disorders (12-14), atherosclerosis (8), and cancer (15). Humans consume the fruits of different species including cranberries, blueberries, bilberries, and lingonberries, some of which are of commercial importance. These species, including their flowers, leaves and fruits, are also widely used in traditional medicine in many countries (16).

The European blueberry, also known as the bilberry (*Vaccinium myrtillus L., Ericaceae*), is a small, dark blue fruit that grows on shrubs of the genus *Vaccinium* (6-8). The berries usually ripen between July and September, and this process is influenced by factors such as site conditions, altitude, and habitat type (6). Due to their limited shelf life, the fruits are best eaten fresh, but can also be preserved by freezing, drying or processing into products such as jams, juices, wines or liqueurs (17). In the past, bilberries were used in traditional medicine and today they are mainly cultivated in Northern and Eastern Europe and North Africa (18). The appearance and taste of the fruits of the cultivated bilberry (*Vaccinium sp.*) are very similar, but as there are numerous varieties of the species, no commercial bilberry cultivars have yet been introduced to the market. Bilberry cultivation areas are spread globally, and their market share is growing rapidly. The economic importance of blueberries is evident from the expansion of global production, which in 2021 for example, amounted to 163,741 hectares and 1,113,260.6 tonnes – nearly three times the acreage and double the yield compared to 2010 (19), this trend continues today.

Bilberries are considered a rich source of carotenoids and phenolic compounds, and they also contain modest amounts of various minerals and vitamins (7). Anthocyanins, the primary class of flavonoids found in berries, are responsible for giving bilberries their distinctive blue color (7,8). Research has linked anthocyanins to various beneficial health outcomes, including potential treatment or prevention of cancer, cardiovascular disease, diabetes, obesity, aging-related illnesses, urinary tract infections, and periodontal disease (3-15). Moreover, bilberries' high flavonoid content has garnered attention as a source of coloring compounds for use in food and medicine. The growing consumer preference for meals perceived as healthier and more natural has spurred increased interest in these fruits (20). Currently, many products on the market incorporate bilberries, highlighting their beneficial effects, particularly their antioxidant potential (7). In the European Union, according to the Cosmetic Ingredients and Substances Database (CosIng), extracts from *V. myrtillus* fruit, fruit water (an aqueous solution of steam distillates from the fruit), and fruit juice are used for skin conditioning (21). Additionally, extracts of the fruit and leaves are employed as astringents, refreshers, skin conditioners, and tonics (21).

Inflammation is a natural defense mechanism of the human body, triggered by potential threats such as allergens and tissue damage (22). While it serves a protective role, uncontrolled inflammation can lead to various disorders, including cancer, autoimmune diseases, cardiovascular issues, allergies, and metabolic syndrome. These conditions not only affect individuals but also have significant societal impacts. Drugs such as steroids, non-steroidal anti-inflammatory drugs (NSAIDs), and immunosuppressants are commonly used to treat and suppress inflammation. However, it is important to recognize that these medications can have side effects. Therefore, administering the lowest effective dose while achieving optimal efficacy and minimizing side effects is crucial (22,23). To enhance pharmaceutical efficacy and reduce side effects, incorporating natural anti-inflammatory agents into therapy is essential. There is a growing need to explore herbal medicines, as they play a vital role in modern healthcare. Guidelines for herbal medicines largely stem from complementary, alternative, and traditional practices. However, these guidelines must

be scientifically validated before being integrated into modern medicine.
The present review is designed to report the current knowledge on the bioactive compounds of berries from the V. myrtillus and to summarize the clinical study of the anti-inflammatory effects of different bilberry preparations published from 2007 to 2024.

METHODS

The literature search was conducted using several electronic databases, including PubMed, Scopus, Web of Science, ScienceDirect, NCBI, Google Scholar, and ClinicalTrials.gov. The search strategy used the keywords 'anti-inflammatory', 'bilberry', and '*Vaccinium myrtillus*'. Only studies published in English were included. The search covered the period from 2007 to the present and initially identified 40 records. After screening for relevance and applying the predefined inclusion and exclusion criteria, 13 studies were excluded, resulting in a final selection of 27 studies for analysis.

Bioactive compounds

Phenolic compounds represent the primary group of phytochemicals found in *V. myrtillus* berries (3, 24-25). The concentration of these compounds can be affected by various factors, including the stage of ripeness, storage conditions, and environmental factors during growth. Research has demonstrated that berry bushes grown in cold climates with short growing seasons produce fruits with higher levels of phenolic compounds compared to those cultivated in moderate climates (26). The berries from *V. myrtillus* represent an exceptionally rich natural source of anthocyanins, containing the highest levels found in any fruit (300–700 mg/100 g of fresh fruit) (3).
Anthocyanins are present in the outer layer of fruits, along with phenolic compounds, and smaller amounts can also be found in the pulp and seeds. The *V. myrtillus* is characterized by the presence of delphinidin and cyanidin, petunidin, peonidin, and malvidin (3, 24-27). The vibrant color of *V. myrtillus* berries is attributed to the high concentration of anthocyanidins, which can constitute up to 2% of the fresh mass in the peels and pulp. Approximately 90% of the phenolic compounds in bilberry fruits are anthocyanins. In bilberries, common derivatives of delphinidin, cyanidin, peonidin, petunidin, and malvidin include mono-, di-, and trisaccharides. The principal sugars found are glucose, galactose, xylose, rhamnose, and arabinose (3,8). The cyanidin 3-O-galactoside, cyanidin 3-O-glucoside, cyanidin 3-O-arabinoside, delphinidin 3-O-galactoside, delphinidin 3-O-arabinoside, delphinidin 3-O-glucoside, malvidin 3-O-galactoside, malvidin 3-O-arabinoside, malvidin 3-O-glucoside, petunidin 3-O-galactoside, petunidin 3-O-arabinoside, petunidin 3-O-acetylglucoside, peonidin 3-O-galactoside, and peonidin 3-O-arabinoside were identified in the fruits of *V. myrtillus* (8,28). According to the 10th edition of the European Pharmacopoeia, the anthocyanins in bilberry extracts are significantly abundant, with recommended level between 32% and 40% of anthocyanidins (the aglycone form of anthocyanins) (29). The fresh bilberry fruits (*Myrtilli fructus recens*) should contain at least 0.3% anthocyanins (expressed as cyanidin 3-O-glucoside chloride) and the dried fruits (*Myrtilli fructus siccus*) should have at least 1.0% of tannins (expressed as pyrogallol), according to the European Pharmacopoeia monographs (30,31).
In bilberry fruits, the predominant flavonol is quercetin, constituting over half of the total flavonoid content (3,8). The second most abundant flavonoid is myricetin, while other flavonols such as isorhamnetin, syringetin, and laricitrin are present in lower concentrations (3,8). The two primary glycosides found in bilberry fruits are rhamnosides and glucuronides (32-34). Apigenin, chrysoeriol, myricetin, myricetin-3-xyloside, quercetin 3-O-glucuronide, quercetin 3-O-xyloside, isorhamnetin 3-O-glucoside, luteolin (35) as well as kaempferol, isorhamnetin, laricitrin, syringetin, isorhamnetin 3-O-galactoside, myricetin 3-O-glucuronide, laricitrin 3-O-glucoside, syringetin 3-O-glucoside, kaempferol 3-O-glucoside, myricetin 3-O-galactoside are also described in *V. myrtillus* (36).
The fruits of the *V. myrtillus* are also a valuable source of organic acids and their byproducts. Bilberry fruits contain citric, malic, quinic, and shikimic acids. Additionally, they are rich in phenolic and hydroxycinnamic acids such as gallic, ellagic, and syringic, as well as p-coumaric, caffeic, and ferulic acids. There is also a smaller amount of dihydroxybenzoic acid, salicylic acid, and vanillic acid in the fruits. These organic acids can exist in free, etherified, or esterified forms. During the etherification process, various saccharide fractions are typically involved, forming esters with each other or with distinct phenolic compounds (32-34).

Clinical studies of different bilberry preparations

In the traditional medicine, bilberries have been used to treat various conditions, including skin ulcers, hemorrhoids, nausea, vomiting, diarrhea, mucosal inflammation, and to improve eyesight (37). Their anti-inflammatory effects are attributed to the downregulation of pro-inflammatory molecules s, such as tumor necrosis factor alpha (TNF-*α*), interleukin-1 beta (IL-1*β*), and interleukin-6 (IL-6) and enzymes inducible nitric oxide synthase (iNOS), and cyclooxygenase-2 (COX-2). These effects involve alterations in signaling pathways, including nuclear factor-kappa beta (NF-*kβ*) and Janus kinase-signal transducer and activator of transcription (JAK-STAT), as well as a reduction in reactive oxygen species (ROS) levels in both cell cultures and experimental animals (38,39). The anti-inflammatory action of different formulations of bilberries has been confirmed in several clinical studies (40-45). In these studies, bilberries were administered in the form of juices, fresh/frozen berries, freeze-dried preparations, or anthocyanin rich extract (Medox®). The anti-inflammatory effects of bilberry extracts and anthocyanins were clearly documented, and notably, none of these formulations reported any side effects (Table 1). Daily intake of anthocyanins, which are the main group of phenolic compounds in bilberries, was administered at doses of up to 640 mg/day without adverse effects reported (46).

Medox® capsules contained purified anthocyanins isolated from bilberries (*V. myrtillus*) and blackcurrant (*Ribes nigrum*), with cyanidin and delphinidin-3-O-*β*-glucosides accounting for at least 40–50% of the total anthocyanins. In a study involving 120 participants over three weeks, these capsules were shown to reduce levels of the pro-inflammatory mediators interleukin-4 (IL-4), interleukin-8 (IL-8), interleukin-13 (IL-13), and interferon alpha (IFN*α*) (40). Supplementation with bilberry juice also reduced levels of circulating high-sensitivity C-reactive protein (hs CRP), IL-6 and interferon-gama (INF-*γ*)-induced monokine (MIG). Interestingly, this study observed an unexpected increase in plasma levels of TNF-*α*; however, there were no significant effects on clinical factors, antioxidant status, or free radical levels (41). Lehtonen and co-workers (47) reported that consumption of 100 g whole frozen bilberries for 33–35 days reduces plasma levels of TNF-*α*, circulating vascular cell adhesion molecule-1 (sVCAM-1) and adiponectin in overweight and obese women. Circulating adiponectin levels positively correlate with insulin sensitivity and inversely with body fat and inflammation. The lower adiponectin levels observed after whole frozen bilberry consumption may not have been directly caused by the bilberries, but rather due to an insufficient interval between ingestion and the measurement of circulating adiponectin levels. Widén and co-workers (42) documented that consumption of 500 g of bilberries per day decreases inflammatory cytokine levels (IL-1*β*, IL-6, and vascular endothelial growth factor (VEGF)), resulting in a reduction of gingival inflammation. Additionally, the anti-inflammatory properties of bilberry anthocyanins may expedite the healing process after physical activity. A study by Lynn and co-authors (43) showed how bilberry juice consumption affects inflammation and exercise-induced muscle injury in recreational runners training for a half-marathon.

An inflammatory reaction promotes the generation of reactive oxygen species, creating a pro-inflammatory environment. In a double-blind, placebo-controlled intervention study, the ingestion of 660 mL of bilberry/red grape juice per day for nine weeks was found to reduce biomarkers of inflammation and oxidative stress in 60 men with subjective memory impairment (48). The intervention resulted in decreased levels of macrophage inflammatory protein (Mip)1*β*, VEGF, IL-6, TNF-*α*, and other biomarkers of inflammation and tissue damage (lactate dehydrogenase, LDH). Even though these markers were decreased, the memory scores of the participants remained unchanged (48).

Reactive oxygen species are produced in the inflamed colon as a result of an excess of neutrophils and macrophages associated with ulcerative colitis. In an open pilot trial involving 13 patients with mild to moderate ulcerative colitis, the consumption of 160 g of bilberry preparation, providing 210 mg of anthocyanins per day for six weeks, significantly reduced mucosal inflammation (49). This intervention also decreased the level of calprotectin in feces and modulated inflammatory changes. Specifically, it reduced the expression of IFN-*γ*, TNF-*α*, and interferon gamma receptor 2 (IFN-*γ*R2), increased serum levels of monocyte chemoattractant protein-1 (MCP-1), and altered the expression of IL-10 (44).

Metabolic syndrome (MetS) is a global health concern associated with inflammation, dyslipidemia, obesity, and hyperglycemia. In a study, the serum levels of hsCRP, IL-6, IL-12, and lipopolysaccharides (LPS) were significantly decreased in men and women with MetS when 400 g of fresh bilberries (equivalent to 200 g of berry puree and 40 g of dried berries) were incorporated into their diets (45). Interestingly, the levels of leptin and adiponectin were

Table 1. *Summary of clinical studies on anti-inflammatory effects of different bilberry extracts published from 2007 to 2024*

Intervention (reference)	Chemical profile	Study design	Outcome measure	Results
Billberry powder 3 times daily (7)	NA	Double blinded, randomized, clinical trial, 50 patients suffering from STEMI (ST-segment elevation myocardial infarction) or non-STEMI (non-ST-segment elevation myocardial infarction) 3 months	6-minute walk test, CRP, IL-6, TNF-*α*, INF-*γ*, markers of oxidative stress: (oxidized LDL, carbonylated proteins, H_2O_2, hexanoyl L lysine, NOS), markers of myocardial damage and heart failure: BNP and troponin I, markers of endothelial function: VEGF, 8-isoprostane, E-selectin, plasma lipids; total cholesterol, LDL-cholesterol, TGA	Study results have not been submitted. This may be because the study hasn't been done, the deadline for submitting results has not passed, or this study does not require the results to be submitted.
275-mg *Medox*® capsules 2 times/day, providing a total of 300 mg anthocyanins/day (40)	*Medox*® capsules contained purified anthocyanins isolated from bilberries (*V. myrtillus*) and blackcurrant (*Ribes nigrum*); cyanidin and delphinidin 3-O-*β*-glucosides constituted at least 40–50% of the total anthocyanins	Parallel-designed, placebo controlled clinical study, 120 participants, 3 weeks	Cytokines (IL-1*β*, IL-1Ra, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12, IL-13, IL-17, TNF*α*, IFN*α*, IFN*δ*), GM-CSF, Mip-1*α*, MIP-1*β*, IP-10, MCP-1, eotaxin, RANTES, CRP	↓IL-4, ↓IL-8, ↓IL-13, ↓IFN*α*, ↓RANTES
330 ml bilberry juice/day (diluted to 1 L using tap water) (41)	NA	Parallel-designed randomized clinical study, 62 participants, 4 weeks	CRP, IL-1*β*, IL-1*α*, IL-1 IL-1Rα IL-2, IL-2R IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12, IL-13, IL-15, IL-17, TNF-*α*, IFN-*α*, IFN-*γ*, GM-CSF, MIP-1*α*, MIP-1*β*, IP-10, MCP-1, MIG, eotaxin, RANTES, WBC, fibrinogen, uric acid, γ-GT, total proteins, cholesterol, TGA, biomarkers of oxidative stress status (DHAA, D-ROM, oxidized glutathione, plasma GSH redox potential)	↓CRP, ↓IL-6, ↓IL-15, ↓MIG, ↑TNF-*α*, ↑plasma quercetin, ↑ *p*-coumaric acid, no differences were observed for clinical parameters, oxidative stress or antioxidant status
250 or 500 g bilberries daily over seven days (42)	NA	24 adult with gingivitis, 7 days	BOP, cytokines (IL-1*β*, IL-1Rα, IL-6, IL-12, IP-10, PDGF-BB, MIP-1*α*, and VEGF	↓ BOP, significant differences between baseline and study endpoint after intake of 500 g of bilberries/day for three of the cytokines studied: IL-1*β* IL-6 and VEGF
200 mL bilberry juice twice per day, each 200 ml bottle also contained 80.04 ± 3.51 mg of total anthocyanins (43)	NA	Single blind, randomized, placebo-controlled, parallel study, 8 days, 21 recreational runners	DOMS, muscle damage (CK), CRP	↑moderate CRP 24 h post-race
160 g of bilberry preparation (44)	NA	Open pilot trial, 13 patients with mild to moderate ulcerative colitis, 6 weeks	TNF-*α*, MCP-1, IL-10, IL-13, IL-17*α*, IFN-γ	↓IFN-*γ*, ↓TNF-*α*, ↓p65, ↓NF-*κβ*,↑IL-22, ↑IL-10
400 g fresh bilberries 200 g of bilberry puree and 40 g of dried bilberries (eq. 200 g of fresh bilberries) (45)	NA	Randomized controlled study, 27 participants with MetS features 4-week run-in, 8-week dietary intervention, and 4-week recovery periods	Body weight, body composition, blood pressure, inflammation score, glucose and lipid metabolism, transcriptomic gene expression analyses	No differences in body weight, glucose or lipid metabolism, ↓hsCRP, IL-6, IL-12, LPS, transcriptomic gene expression analyses
100 g frozen, whole berries (47)	NA	Randomized, cross-over study, 110 female overweight and obese women, intervention (33–35 days) and wash-out (30–39 days) periods	Body composition, plasma glucose, serum total cholesterol, HDL, LDL, triacylglycerols, *γ*-GT , glycated hemoglobin, hsCRP, insulin, sICAM-1, sVCAM-1, TNF-*α*, IL-6, adipokine, HbA1c	↓TNF-*α*, ↓sVCAM-1, ↓adiponectin
330 mL of bilberry/red grape preparation, 2 times a day (48)	NA	Double blind, placebo-controlled study, 60 men with subjective memory, 9 weeks	Neuropsychological test scores and whole blood gene regulation	↓IL6, ↓TNF-*α*, ↓EGF, ↓Mip1*β*, ↓VEGF
160 g of bilberry preparation, 210 mg anthocyanins per tray (49)	NA	Open pilot trial, 13 patients with mild to moderate ulcerative colitis, 6 weeks	Sigmoidoscopy, fecal calprotectin, CAI assessment, SIBDQ	↓Fecal calprotectin levels, ↓endoscopic Mayo score
2 x 80-mg *Medox*® capsules two times per day (morning and evening) (50)	NA	Randomized, placebo controlled trial, 55 MetS or normal, 28 days	Serum levels of lipid concentration, uric acid, hsCRP, FBG, expression of P-selectin/CD62P	↓FBG, ↓TG, ↓LDL-C, ↓hsCRP, ↓ADP-induced platelet
2x 80 mg anthocyanin capsules twice daily (30 min after breakfast and supper) for a total intake of 320 mg anthocyanins/d (51)	bilberries and blackcurrants	Randomized, double-blind, placebo controlled study, 150 hypercholesterolemic participants, 24 weeks	hsCRP, sVCAM-1, TNF-*α*, IL-1*β*, lipids and glucose	↓hsCRP, ↓sVCAM-1, ↓IL-1*β*, ↓LDL-cholesterol ↑HDL-cholesterol, no significant differences in the levels of glucose and insulin
Medox® capsules - anthocyanin capsule contained 80 mg (52)	Anthocyanins that were extracted from bilberry (blueberry) (*V. myrtillus*) and black currant (*R. nigrum*)	Double-blind, randomized placebo-controlled crossover study, 31 healthy men, 4-week with a 4-week washout	Sitting systolic BP, CVD-related parameters; total cholesterol, HDL-C, total cholesterol/HDL-C ratio, LDL-C, triglycerides, lipoprotein a, fasting glucose, HbA1c, albumin/creatinine ratio, insulin, HOMA-IR, homocysteine, hematological and liver- and kidney markers, markers of inflammation and oxidative stress and plasma polyphenols	↑HDL, ↑blood glucosevon Willebrand factor No effects were observed on inflammation or oxidative stress

Medox® capsules (53)	Anthocyanins that were extracted from bilberry (blueberry) (*V. myrtillus*) and black currant (*R. nigrum*)	Randomized, double-blinded placebo-controlled study, 169 participations, 12-weeks	Anthropometric measurement, plasma ceramide, fasting blood TC, HDL-C, TG, ApoA-I, ApoB, glucose, insulin, plasma cholesterol efflux	↓ Cer 16:0, ↓ Cer 18:0, ↓ Cer 20:0, ↓ Cer 22:0, ↓ Cer 24:0, ↓ Cer 24:1, ↑enhanced cholesterol efflux capacity
Mirtoselect® (54)	Standardised bilberry extract (36 % (w/w) anthocyanins)) which equates to about 50 g of fresh bilberries formulated in gelatin capsules	Randomised, double-blinded cross-over study, 8 males with type 2 diabetes	Oral glucose tolerance testing, glucagon-like peptide-1c, antioxidant assays	↓glucose ↓insulin no change in the gut (glucagon-like peptide-1, gastric inhibitory polypeptide), pancreatic (glucagon, amylin) or anti-inflammatory (monocyte chemotactic protein-1) peptides, antioxidant (Trolox equivalent antioxidant capacity, ferric-reducing ability of plasma)
Bilberry-blackcurrant purée, 139 g Lingonberry purée, 122 g Bilberry soup, 250 ml (55)	NA	Randomized, single-blinded crossover study, 26 participations	Postprandial glucose, insulin, free fatty acids, satiety scores assessed by using visual analogy scales, heart rate variability	↓glucose ↓ insulin, ↓ non-esterified fatty acids (NEFA)
650 mg per tablet with 150mg *V. myrtillus* L. extracts, twice a day (56)	NA	Randomized double-blind study, 80 participations, 3 months	Plasma AGEs, sRAGE levels, urinary AGEs levels, transcription levels of RAGE and AGER1, gut microbiota, skin AGEs levels, body weight, body composition (body fat mass and lean mass), plasma total cholesterol, LDL, HDL and triglycerides, CRP, IL-6 and TNF-α, SCFA	Study results have not been submitted. This may be because the study hasn't been done, the deadline for submitting results has not passed, or this study does not require the results to be submitted.
Billberry powder 3 times daily (57)	NA	Double blinded, randomized, clinical trial, 50 patients suffering from STEMI (ST-segment elevation myocardial infarction) or non-STEMI (non-ST-segment elevation myocardial infarction) 3 months	6 minutes walk test, C-reactive protein, IL-6, TNF-α, INF-γ, markers of oxidative stress (oxidized LDL, carbonylated proteins, 2-OHDG, H_2O_2, hexanoyl L lysine, nitrogen oxide synthase), markers of myocardial damage and heart failure (BNP and troponin I), markers of endothelial function (VEGF, 8-isoprostane, E-selectin, LDL, TGA)	Study results have not been submitted. This may be because the study isn't done, the deadline for submitting results has not passed, or this study isn't required to submit results.
Bilberry shakes 2 times daily (containing in total 40g of dried bilberry powder equalling 480 g of fresh berries per day) (58)	NA	Randomized, double-blind, placebo-controlled study, 900 participations, 3 months	Lipid profile (HDL, TGA, total cholesterol, LDL, apo A, apo B, Lp(a) and oxidized LDL), symptom-limited bicycle ergometer test, dynamic unilateral heel-lft and unilateral shoulder flexion tests, self-reported physical activity level, troponin, NT-proBNP, hsCRP, IL-6 and HbA1c, insulin, creatinine, Cystatin C, glucose and C-peptide, fecal samples of gut microbiota composition, left ventricular systolic function, resting heart rate, systolic and diastolic blood pressure	Study results have not been submitted. This may be because the study hasn't been done, the deadline for submitting results has not passed, or this study does not require the results to be submitted.
Bilberry capsule (59)	containing bilberry dried extracts at 23.2 mg and bilberry powder at 3.2 mg,	Randomized, double-blind placebo-controlled study, 80 participations with confirmed diagnosis of dry eye, 30days and washout period for 20 days.	Schirmer's Test, Tear Film Breakup Time, Ocular Surface Index, Intraocular Pressure	Study results have not been submitted. This may be because the study hasn't been done, the deadline for submitting results has not passed, or this study does not require the results to be submitted.
Bilberry-based probiotic beverage (60)	NA	Randomized, parallel study, 32 participations	Postprandial levels of serum insulin	Study results have not been submitted. This may be because the study hasn't been done, the deadline for submitting results has not passed, or this study does not require the results to be submitted.
Capsules containing 320 mg anthocyanins derived from bilberry fruit (delphinidin type), 320 mg anthocyanins derived from black rice (cyanidin type) and a placebo control (61)	NA	Randomized, double blind,placebo contoled cross-over study, 55 participations, eaach treatment will be ingested for 28 days with a wash-out period of 4 weeks in-between.	Total/HDL cholesterol, TGA, cholesterol efflux capacity, PON-1 activity, bile acids and derivatives, glucose, fructosamine and insulin, MicroRNA expression	Study results have not been submitted. This may be because the study hasn't been done, the deadline for submitting results has not passed, or this study does not require the results to be submitted.
Bilberry extract capsules, 160 mg (25% anthocyanosides) (62)	NA	Double-blind, randomized, placebo controlled, cross-over trial 2 x 28 days	Change of the dark adaption of the pupil using the method of the dark adaption Goggles (DAG), Changes of the dark adaption using dark flashes, Changes of the weakest, correctly recognised contrast	Study results have not been submitted. This may be because the study hasn't been done, the deadline for submitting

			level, Assessment of subjective efficacy based on a visual analogue scale (VAS) rating questionnaire, assessment of clinical global impression on a 5-point rating scale	results has not passed, or this study does not require the results to be submitted.
A powder product (4 g) that will be mixed into a drink and consumed immediately prior to eating a high-fat meal consists of 3 g of a mix a maltodextrins, and 1 g of anthocyanin-rich plant polyphenol blend (100 mg bilberry extract, 300 mg black currant extract, and 600 mg black rice extract). The placebo (4 g) consists of a mix of maltodextrins (3.85 g) and Red Dye No. 40 (0.125 g) and Blue Dye No. 1 (0.025 g) (63)	NA	Randomized, placebo-controlled cross-over study, 27 participations	Plasma endotoxin, IL-6, insulin, GLP-1, GLP-2, GIP, plasma adiponectin, leptin, ghrelin, TGA, plasma total cholesterol, HDL, LDL, zonulin, plasma total polyphenols, anthocyanidins, catechins, glucose, PBMC (IL-8, IL-10, IL -1β, IL -12$_P$70, TNFα, NF-kβ)	Study results have not been submitted. This may be because the study hasn't been done, the deadline for submitting results has not passed, or this study does not require the results to be submitted.
Several different berry powders including rose hip, bilberry, blackcurrant, sea buckthorn, and lingonberry, start with 6.25 g berry powder and double the dosage each week until we reach a maximum dosage of 50 g according to the Swedish National Food Agencies recommendations, placebo comparator potato starch (64)	NA	Randomized, placebo-controlled study, 18 participations, 5 weeks	Changes in the extent of gingivitis, as determined by severity of gingival bleeding, VSEGF, saliva and gingival fluid	Study results have not been submitted. This may be because the study hasn't been done, the deadline for submitting results has not passed, or this study does not require the results to be submitted. .
ACRB - softgels consisting of anthocyanin-rich blend.. Product consumed once daily, preferably with breakfast (65)	Softgel contain: 49 mg bilberry extract; 101 mg black currant extract; and 303 mg black rice extract. The product also contain olive oil, sunflower lecithin, yellow beeswax, bovine gelatin, and water	Randomized placebo controlled study, 30 participants, 8 weeks	Plasma TGA, free fatty acids, HDL, LDL, glucose, insulin, LPS, LPS binding protein, PBMC gene expression, plasma total polyphenols, plasma total anthocyanidins, Irisin, total blood gene expression, fecal microbiota, fecal short chain fatty acids, skin carotenoids	Study results have not been submitted. This may be because the study hasn't been done, the deadline for submitting results has not passed, or this study does not require the results to be submitted.
320-mg anthoycanins twice daily (66)	Total of 17 different purified anthocyanins (mostly cyanidin 3-O-β-glucosides and delphinidin 3-O-β-glucosides were extracted from bilberry (*V. myrtillus*) and black currant (*R. nigrum*)	Double-blind randomized placebo-controlled crossover study, 31 healthy men, 4-week with a 4-week washout	Sitting systolic, diastolic blood pressure (BP) and heart rate (HR), supine BP and HR, 24-h ambulatory BP and HR, Finometer BP and HR, cardiovascular and catecholamine stress reactivity, renin, aldosterone, angiotensin-converting enzyme and catecholamines in platelets	Not significantly changed sitting BP or 24-h ambulatory BP, differences in components of the rennin–angiotensin–aldosterone system, platelet catecholamines or
				differences in CV or catecholamine responses between treatment periods were not found. Resting supine BP measured by Finometer tended to be lower in the anthocyanin period.
Standardized bilberry extract (67)	Cyanidin 3-glucoside as the anthocyanin reference 35.82%	Randomized, double-blind, placebo-controlled, parallel study, 109 healthy adult men and women, 12 weeks	Ocular tests, Ocular fatigue test	Post-load HFC-1 values were significantly improved

apolipoprotein A-I–ApoA-I; apolipoprotein B—ApoB; bleeding on probing—BOP; brain natriuretic peptide—BNP; C-reactive protein—CRP; creatine kinase— CK; delayed onset muscle soreness—DOMS; granulocyte-macrophage colony-stimulating factor—factor GM-CSF; fasting blood glucose—FBG; fecal short chain fatty acids—SCFA; glycated haemoglobin—HbA1c; γ-glutamyltransferase—γ -GT; γ glutamyl transpeptidase GGT—γ-GT; granolyte/macrophage colony-stimulating lipopolysaccharides—LPS; high sensitivity C-reactive protein—hsCRP; high-density lipoprotein cholesterol—HDL; homeostasis model assessment of insulin resistance—HOMA-IR; interferon gamma-induced protein 10—IP-10; immunoprotein—IP-10; monocyte chemoattractant protein—MCP; IL-1 receptor antagonist—IL-1Ra; low-density lipoprotein cholesterol—LDL; macrophage inflammatory protein—MIP; monokine induced by IFN-γ—MIG; nitrogen oxide synthase—NOS; platelet-derived growth factor —PDGF; platelet surface marker—P-selectin/CD62P; plasma advanced glycation end products AGEs; regulated upon activation, normal T cell expressed and secreted—RANTES; soluble intercellular adhesion molecule—sICAM-1; soluble vascular cell adhesion molecule—sVCAM-1; soluble receptor for advanced glycation end products—sRAGE; triacylglycerides—TGA;vascular cell adhesion molecule-1—VCAM-1; vascular endothelial growth factor—VEGF; white blood cells—WBC.

unaffected by bilberry administration. This suggests that bilberries may help alleviate MetS through their mild anti-inflammatory potential. In another study, Aboonabi and co-workers (50) found that subjects with MetS who consumed Medox® capsules twice a day for four weeks, experienced significant reductions in inflammation and improvements in their lipid profiles. This was evidenced by the decreased levels of inflammatory biomarkers, fasting blood glucose, low-density lipoprotein (LDL) cholesterol, triglycerides, and total serum cholesterol levels.

Supplements containing anthocyanins enhance the lipid profile by increasing high-density lipoprotein cholesterol (HDL) and lowering LDL cholesterol. In a study involving 150 hypercholesterolemic participants, Zhu and co-workers (51) found that 24 weeks of supplementation with a processed anthocyanin mixture (320 mg/day bilberry and blackcurrant) decreased serum levels of hsCRP, sVCAM-1, IL-1β, and LDL cholesterol, but increased HDL cholesterol. A similar effect was observed in a study with 31 healthy men conducted by Hassellund and associates (52). Furthermore, among the 169 participants, 12 weeks of supplementation with 40, 80, or 320 mg/day of anthocyanins resulted in a dose-dependent decrease in plasma ceramide levels accompanied by an increase in cholesterol efflux capacity (53). Hoggard and co-workers (54) conducted a randomized, double-blind, crossover study involving males with type 2 diabetes. They found that treatment with a standardized bilberry extract containing 36% (w/w) anthocyanins, equivalent to about 50 g of fresh bilberries in gelatin capsules, resulted in reduced levels of glucose and insulin. Similarly, another study also reported decreased glucose and insulin levels following bilberry supplementation (55).

Some clinical studies involving subjects treated with bilberry powder, extracts, or bilberry-based shakes and probiotic drinks have not yet produced results. This may be due to various reasons, such as the studies still being ongoing, the submission deadlines for results not yet passing, or the studies not being required to submit results at all. While research on humans has indicated that bilberries possess anti-inflammatory properties, these findings come with certain limitations. Considerable variation has been observed across these studies, which can be attributed to differences in regimens, doses, durations, sites, and populations involved.

CONCLUSION

Bilberry is well-regarded for its potential health benefits and as a functional food, supported by numerous studies that have identified and quantified various bioactive phytochemicals known to benefit human health. Research findings have demonstrated the anti-inflammatory effects of different *V. myrtillus* fruit preparations, which could contribute to the prevention of several diseases. Upon ingestion, anthocyanins are metabolized into various conjugates, which further break down into phenolic acid degradation products. Accumulating evidence suggests that the combined effects of these metabolites may explain their health-promoting properties. Additionally, there is significant inter-individual and intra-individual variability in the absorption, metabolism, distribution, and excretion of anthocyanins. Current research indicates that bilberries are among the fruits with the most significant positive impact on human health.

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Competing Interest

The authors declare no relevant conflicts of interest.

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AFMN BIOMEDICINE

REVIEW ARTICLE

EXPLORING USABILITY, SAFETY, AND PRELIMINARY EFFICACY OF NOVEL DIGITAL INTERVENTIONS IN ADOLESCENT PSYCHOTHERAPY: A NARRATIVE REVIEW

Miodrag Stanković[1,2] **Aleksandra Stojanović**[2]

[1]Department of Psychiatry, University of Niš Faculty of Medicine, Niš, Serbia [2]Center for Mental Health Protection, University Clinical Center Niš, Niš, Serbia

Artificial intelligence (AI) is increasingly recognized as a novel tool for enhancing psychotherapy, particularly for adolescents. The integration of AI-based platforms into mental health care promises improved accessibility, personalized interventions, and support for therapeutic processes. We aimed to evaluate the usability, safety, and preliminary effectiveness of a novel AI-assisted psychotherapy intervention in adolescent populations, with a focus on ethical considerations, user profiles, and limitations. We conducted a theoretical analysis of AI integration in psychotherapy, focusing on its potential application among adolescents, current ethical debates, and user patterns, particularly in post-pandemic contexts. Adolescents aged 16–25 years, who are highly immersed in digital environments, appear to be most open to using AI platforms for psychological support. The use of AI may enhance therapeutic access; however, limitations include a lack of emotional intelligence, reduced therapist involvement, and vulnerability to unethical data usage. Concerns also arise regarding the commodification of mental health through commercially driven AI applications. AI-supported psychotherapy for adolescents holds great potential but must remain adjunctive and ethically grounded. Further empirical research is necessary to ensure the safety, therapeutic efficacy, and ethical integrity of AI technologies in psychiatric care.

Keywords: artificial intelligence, digital psychotherapy, adolescents, mental health, ethics

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Correspondence to:
Miodrag Stanković
Department of Psychiatry
University of Niš Faculty of Medicine
Bulevar dr Zorana Đinđića 81, Niš, Serbia
E-mail: miodrag.stankovic@medfak.ni.ac.rs

INTRODUCTION

Psychotherapy remains a primary psychological therapy aimed at assisting individuals with a wide range of mental states and disorders. As digital health technologies evolve, artificial intelligence (AI) has introduced new avenues for enhancing the accessibility, personalization, and scalability of psychotherapeutic care. Unlike traditional methods that rely on in-person sessions, AI-based solutions have shown promise in delivering structured, evidence-based interventions through digital interfaces, including mobile apps and conversational agents (1).

Role of AI in modern psychotherapy

Recent studies have demonstrated that AI-enabled tools can be particularly effective in managing symptoms of depression and anxiety by replicating key therapeutic mechanisms, such as cognitive restructuring, behavioral activation, and emotional support, through automated digital platforms (1-3). For instance, chat agents like Woebot and Tess have yielded promising outcomes in reducing psychological distress among young adults, providing consistent, immediate, and stigma-free support without requiring therapist involvement (1,3).

Despite the broader acceptance of AI in general medicine, its integration into mental healthcare remains in its early development stages. Many systems are still undergoing evaluation for clinical efficacy, ethical compliance, and long-term engagement (4-6). A key advantage of AI in psychotherapy is its ability to provide low-barrier interventions in underserved populations, particularly in low- and middle-income countries where mental health professionals are scarce (7).

Nevertheless, significant ethical considerations must be addressed before AI systems can be fully integrated into routine clinical practice, particularly in psychotherapy. These include concerns regarding data privacy, algorithmic bias, obtaining informed consent, and the preservation of patient autonomy and dignity (8,6,9,10). Furthermore, because AI lacks human empathy and nuanced clinical judgment, current guidelines recommend using it as a supportive tool rather than a replacement for human therapists (4,11).

Key milestones in the integration of AI into psychotherapy

The necessity for digital mental health interventions became particularly evident during the COVID-19 pandemic and associated global lockdowns. Movement restrictions, combined with a surge in demand for psychotherapeutic services and a simultaneous shortage of trained professionals, resulted in a mental health emergency on a global scale (12). Even high-income countries found themselves underprepared for the rising need. The integration of AI into mental health treatment, particularly through digital psychotherapy applications, could emerge as a promising solution for low- and middle-income countries, offering a more accessible and cost-effective method to deliver psychological support (13). Digital psychotherapy using AI can be more cost-effective, accessible, and available than traditional methods, providing a significant advantage in developing countries where access to licensed psychotherapists is often limited or expensive. The use of AI in mental health could enable the automation of therapy sessions, such as those employing acceptance and commitment therapy, which has shown efficacy in reducing symptoms of depression and anxiety, particularly among adolescents (13). Despite these promising prospects, the long-term efficacy of these digital therapies remains uncertain, especially in terms of maintaining therapeutic engagement and achieving sustained therapeutic outcomes. While early studies suggest improvements in treating depression and anxiety disorders, it is important to consider that these systems cannot replace human interaction and the personal empathy of a therapist (14).

AI and the diagnosis and treatment of mental disorders

The diagnostic process in psychiatry primarily relies on clinician-patient communication and behavioral observation. Psychiatric diagnoses are inherently interpretative, based on symptom clusters and consensus-driven diagnostic criteria. AI offers the potential to significantly improve diagnostic accuracy by objectively analyzing real-time data, including verbal and non-verbal communication, affective expression, and cognitive functioning (15, 16). Furthermore, AI systems may provide more consistent assessments of suicide risk (17).

On the therapeutic front, psychiatric treatment generally involves facilitating motivation, building adaptive coping strategies, modifying maladaptive habits, fostering psychological insight, and managing dysfunctional biological patterns, often through pharmacotherapy. The therapeutic relationship remains the primary agent of change, with the clinician's empathy and judgment being central to treatment success (18). However, AI-enhanced

systems may improve treatment outcomes, particularly in depression and anxiety disorders, by simulating therapeutic engagement and facilitating meaningful interaction (19). Recent studies suggest that AI tools like Wysa show promising results in enhancing digital mental well-being through conversational empathy. Nevertheless, it remains unclear whether such effects are sustainable over time, as this area of research is still in its early stages (20).

User profiles, ethical challenges, and evidence of AI usability

The rapid and irreversible advancement of artificial intelligence (AI) in mental health care raises complex ethical considerations. Core principles such as patient autonomy, equitable access to care, protection from discrimination, preservation of dignity, and data privacy must be thoroughly addressed before AI systems are integrated into clinical practice. Warrier et al. highlight the significance of these ethical aspects, emphasizing the need for privacy, impartiality, transparency, responsibility, and the physician–patient relationship in the context of AI in mental health (21). Alfano et al. emphasize the ethical implications of AI in psychotherapy for adolescents, underscoring the delicate balance between technological intervention and the preservation of the therapeutic alliance (22). Additionally, Yan et al. discuss the challenges of accurately recognizing mental disorders through AI applications, highlighting that AI's current limitations pose significant obstacles in clinical settings (23). Ciliberti et al. further explore the ethical dilemmas surrounding AI's role in caring relationships, emphasizing the importance of maintaining empathy and trust even in digital therapy environments (24).

Preliminary and still limited data suggest that adolescents and emerging adults represent the most frequent users of AI-based mental health services, particularly those aged between 16 and 25 years (25,26). This demographic cohort, having grown up in a technologically saturated environment, naturally incorporates digital tools into nearly all forms of communication. Feelings of physical isolation among these individuals are often mitigated by superficial but readily available virtual connections via smartphones and video conferencing. Furthermore, this generation spent a significant portion of its formative years under lockdown during the COVID-19 pandemic, relying heavily on remote and digital education systems (27). As a result, a behavioral pattern has developed in which young people exhibit a normalized comfort with digital platforms, even in contexts that traditionally rely on in-person interaction, such as psychotherapy.

This shift has led to an almost intuitive acceptance of AI-based mental health tools, where therapeutic encounters occur without direct physical presence. In contrast, adults, particularly those less fluent in emerging technologies, may struggle to communicate effectively with this cohort, increasing the risk of a generational disconnect. Nevertheless, active involvement of adults and professionals in guiding adolescents toward evidence-based digital interventions could yield long-term benefits (22). Encouraging the use of clinically validated AI tools may serve as a protective measure against reliance on unregulated, potentially harmful content that is often more accessible to young people. Introducing AI-assisted psychotherapeutic tools in schools, under the supervision of school psychologists, could help educational institutions provide much-needed mental health support to a generation that is already digitally literate. This approach may increase access to care while addressing the shortage of qualified professionals (28).

Ensuring that AI-based mental health systems comply with data protection regulations and patient privacy laws is an ethical imperative. Open questions remain regarding the storage and access of sensitive data, including session recordings: Where is the data stored? Who manages it? Who has access? Can third parties utilize the data, and under what conditions? Client privacy is especially vulnerable in digital ecosystems where personal data is often stored in cloud-based environments. These circumstances raise legitimate concerns about the trustworthiness of technology providers and their third-party collaborators. Furthermore, safeguarding client confidentiality requires robust encryption protocols and transparent governance mechanisms to ensure that sensitive data is protected and cannot be misused (28,29).

Online psychotherapy and the application of AI in psychotherapeutic practice

The advent of digital psychotherapy has significantly disrupted the traditional therapeutic "setting" based on face-to-face encounters bound by physical space and scheduled sessions. In digital formats, in-person attendance is no longer a prerequisite, and therapy becomes more flexible in terms of time and location. Therapists can now accommodate clients across various time zones, ensuring continuity of care regardless of geographical distance or personal travel. One of the most significant breakthroughs is

the decoupling of patients from their immediate local mental health resources, thus removing geographic limitations and expanding access to treatment (30,31).

Digital psychotherapy platforms enable individuals, especially those in remote or underserved areas, to engage in therapy that would otherwise be inaccessible due to professional shortages or logistical barriers. These innovations contribute to the democratization of mental health care, aligning with principles of justice and equity, and providing essential services to vulnerable populations who are typically underrepresented in traditional care systems (32,33).

For adolescents, digital therapy is often perceived as less stigmatizing and more approachable than traditional in-person models. AI interfaces do not evoke concerns about hidden agendas and can help reduce shame associated with discussing sensitive topics (34). Several AI-based applications designed for adolescents with depressive symptoms incorporate algorithms capable of identifying linguistic markers of suicidality based on user interactions with chatbots. These systems can recognize risk patterns through language use, historical user data, or clinician-provided metadata (35,36).

In some instances, AI has demonstrated a superior capacity to detect high-risk scenarios compared to even the most experienced therapists, due to its ability to process and analyze data instantaneously (37). Nonetheless, therapeutic responsibility must remain within the scope of human professionals, given ethical, legal, and clinical accountability (38,39). This highlights the importance of a hybrid model in which AI assists with data processing and risk assessment, while human therapists provide nuanced clinical judgment, empathy, and ethical oversight (40).

AI can serve as an auxiliary tool, flagging urgent situations in real time and alerting the supervising therapist, who remains actively involved in treatment decisions (41). The absence of human oversight may result in misinterpretation, misdiagnosis, or clinical harm, analogous to iatrogenic consequences (42). Patients must be fully informed about the role of AI in their treatment and its implications. Informed consent and patient autonomy must be upheld throughout the therapeutic process. Ensuring transparency about the scope and limitations of AI involvement is essential to maintaining therapeutic trust (43).

Limitations and critiques of AI use in psychotherapy

The application of artificial intelligence (AI) in psychotherapy has drawn considerable and warranted criticism. Firstly, many AI-based applications are developed within commercial frameworks, raising concerns about profit-driven motives. Suppose such platforms are designed to generate misleading claims or offer false promises of mental health improvement without empirical support. In that case, they may cause significant harm to users and hinder the future credibility of AI-assisted interventions (44).

Even when platforms operate with the highest ethical standards, therapy conducted with minimal clinician oversight still poses substantial risks. At present, AI cannot independently monitor patients or make clinical decisions. While algorithms are practical in pattern recognition, they cannot replace human intelligence. One of the most significant limitations of AI is its inability to perceive and process the emotional, symbolic, relational, and anthropological dimensions of human communication. AI operates strictly within empirical frameworks and lacks the capacity for empathy, a core element of therapeutic interaction (45).

For AI, the concept of empathy holds no evoked emotional significance. Words that may appear neutral to an algorithm can evoke strong emotional responses in humans, underscoring the importance of context and emotional resonance. In psychotherapy, language often carries meanings far beyond the literal, and such nuances are not interpretable by AI in their current form (46). Additional concerns include the lack of robust evidence supporting the diagnostic and therapeutic effectiveness of AI tools, as well as their failure to embody humanistic qualities necessary for meaningful engagement, such as self-reflection, professionalism, reliability, and the ability to recognize when a patient may be withholding or distorting the truth. These are all fundamental attributes of the psychotherapist, essential for ensuring ethical and effective mental health care (44). The absence of humanistic qualities increases the risk of oversimplification and misdiagnosis, particularly when clients engage in self-diagnosis without professional input (47).

The integration of artificial intelligence into adolescent psychotherapy represents a promising yet complex advancement in mental health care. While AI-assisted tools offer potential benefits in accessibility, scalability, and support for therapeutic interventions, they also introduce significant ethical, professional, and clinical concerns. The digital format may particularly appeal to younger populations accustomed to technological interaction; however, it cannot replace the empathic, symbolic, and relational depth characteristic of human therapeutic encounters. Adolescents, as digital natives, may benefit from supervised AI applications when these are integrated into existing mental health systems and guided by qualified professionals. Nonetheless, AI systems must be transparent, ethically designed, and subject to strict data protection standards. Without sustained clinical oversight and empirical validation of efficacy, AI-driven psychotherapy remains an adjunctive, rather than a substitutive tool in mental health care. Future research must address long-term outcomes, ethical standards, and the psychological integrity of digital interventions before they are fully integrated into psychiatric practice.

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Competing Interest

The authors declare no relevant conflicts of interest.

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AFMN BIOMEDICINE

REVIEW ARTICLE

EPILEPSY IN CHILDREN WITH CENTRAL NERVOUS SYSTEM INFECTIONS: A SYSTEMATIC REVIEW AND META-ANALYSIS

Mahsa Afrand[#] Niusha Rostampur[1] Zeinab Pourhadi[2] Javad Rezanezhad[3] Pouriya Nekoueifard[4]

[1]School of Nursing and Midwifery, Iran University of Medical Sciences, Tehran, Iran [2]Division of Pediatric Intensive Care, Department of Pediatrics, Children's Medical Center, Tehran University of Medical Sciences, Tehran, Iran [3]Rasool-e Akram Hospital, Iran University of Medical Sciences, Tehran, Iran [4]Student Research Committee, Shiraz University of Medical Sciences, Shiraz, Iran #Currently unemployed

In childhood, infections of the central nervous system may lead to neurodevelopmental disorders and complications such as epilepsy. The present study aimed to evaluate epilepsy in children with central nervous system (CNS) infections.

The present systematic review and meta-analysis included five cohort studies from international databases, PubMed, Scopus, Web of Science, and Embase, from January 1, 2010, to May 10, 2025, using keywords aligned with the study objective. The statistical analysis was performed using Stata/MP v17 and a random-effect model.

The risk ratio of epilepsy in children with etiologically diagnosed CNS infections was 0.23 (ES = 0.23; 95% CI 0.01 to 0.45; I^2 = 99.52%, P < 0.05).

Based on the results of the present study, there is a high risk of epilepsy associated with brain infections.

Keywords: epilepsy, children, central nervous system, infections

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Correspondence to:
Pouriya Nekoueifard
Student Research Committee
Shiraz University of Medical Sciences, Shiraz, Iran
E-mail: pouriyanekoueifard@tutamail.com

INTRODUCTION

Central nervous system (CNS) infections in children can be fatal or result in neurological complications. The neurological prognosis varies depending on the causative agent. A better understanding of the causative agents will help predict the neurological outcome in children (1). Studies have examined intractable seizures following meningitis and encephalitis. Evidence suggests that most children who develop acute bacterial meningitis are at high risk of developing seizures. Children who develop long-term neurological impairment after infection are at high risk of developing epilepsy (2, 3). Studies have shown that seizures are also highly prevalent in children with acute viral encephalitis, increasing the risk of developing epilepsy and intractable seizures (4, 5).

Currently, the factors that cause epilepsy in children have not been precisely determined. Based on evidence, the use of certain medications by the mother during pregnancy, environmental and genetic factors, neurobiological and perinatal factors are among the factors that cause epilepsy in children. Environmental factors are one of the most important risk factors, especially CNS infections (6-9).

Studies showed that the effects of CNS infection in children are different from those in adults (1, 10). CNS infections in children can occur at different times and through different routes. It was shown that maternal infections during pregnancy are directly linked to fetal brain abnormalities (11, 12). Infections trigger inflammatory pathways that release different inflammatory cytokines and cause morphological changes. Neonates can develop encephalitis during passage through an infected birth canal. Permanent neurological deficits result from brain infections that cause neurological dysfunction during central nervous system development, often preceding cellular damage directly related to viral replication (13). Children typically do not show the symptoms of CNS infections. These neurological signs of infection can be mild and slow-acting, making them easy to miss. Any delay in diagnosing a CNS infection in children or infants can have negative consequences. According to some research, brain infections during infancy or childhood damage the developing central nervous system and may increase the risk of epilepsy in adulthood (14-16).

Given the importance of the subject, the challenges and uncertainty of pathogenesis of epilepsy in childhood, the present study aimed to investigate neurogenic epilepsy in children having infection and stroke.

METHODS

A comprehensive search was conducted between January 1, 2010, and May 25, 2025, using keywords relevant to the study objectives, in the international databases PubMed, Scopus, Web of Science, and Embase. The present article was written in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. Searches were also conducted in other databases using keywords similar to Mesh keywords.

Search strategy keywords:

(((((("Epilepsy"[Mesh] OR "Epilepsy, Benign Neonatal"[Mesh]) OR ("Epilepsy/Complications"[Mesh] OR "Epilepsy/Diagnosis"[Mesh] OR "Epilepsy/Diagnostic imaging"[Mesh] OR "Epilepsy/Epidemiology"[Mesh] OR "Epilepsy/Etiology"[Mesh] OR "Epilepsy/Genetics"[Mesh] OR "Epilepsy/Pathology"[Mesh] OR "Epilepsy/Prevention and control"[Mesh] OR "Epilepsy/Therapy"[Mesh])) AND "Neurodevelopmental disorders"[Mesh]) AND ("Child"[Mesh] OR "Only Child"[Mesh])) AND ("Central nervous system"[Mesh] OR "Central nervous system parasitic infections"[Mesh] OR "Central nervous system bacterial infections"[Mesh] OR "Central nervous system viral diseases"[Mesh])) AND "Infections"[Mesh].

Inclusion criteria: PICO was the basis for the inclusion criteria (Table 1). All human studies, English language, type of brain infections, various brain infections, epilepsy and neurodevelopmental outcomes, prospective and retrospective studies, randomized controlled trial were included.

Table 1. *PICO method for choosing research*

PICO strategy	
Population (P)	Children aged <18 years with brain infections
Exposure (E)	CNS infections
Comparison (C)	Non-brain infections
Outcomes (O)	Risks of epilepsy

Exclusion criteria involved: Case studies of specific cancers, case reports, exploring other diagnostic options, incomplete or atypical data reporting, review studies, case report studies, laboratory studies, animal studies, letters to the editor, conference papers, and studies without full text. Using a pre-made table, two independent, blinded authors extracted data from a few chosen studies. After review and discussion with the third author of any discrepancies, a summary of the collected information was created.

The columns of the table were: first author, year of publication, design of study, number of children in case and control group, gender, mean of age, pathogens, follow-up, and outcome.

Quality assessment was performed in three domains: selection, comparison, and outcome using the Newcastle-Ottawa Scale (NOS) (17). "High quality" was defined by the NOS tool as scores greater than 7.

Statistical analyses were performed with random effects models using Stata MP.v17 software. Effect sizes were calculated using 95-CI and RMEL method.

RESULTS

Three hundred eighty-one articles that met the search criteria were found after a thorough literature search in the databases. One hundred thirty-nine articles were excluded based on exclusion criteria or title irrelevance to the study aim. In total, 242 articles were reviewed for abstracts and excluded if they did not meet the inclusion criteria. The full text of 25 articles was reviewed by two separate, blinded authors and then screened for inclusion and exclusion criteria. Only five articles were selected for review of this study after meeting the inclusion criteria (Figure 1).

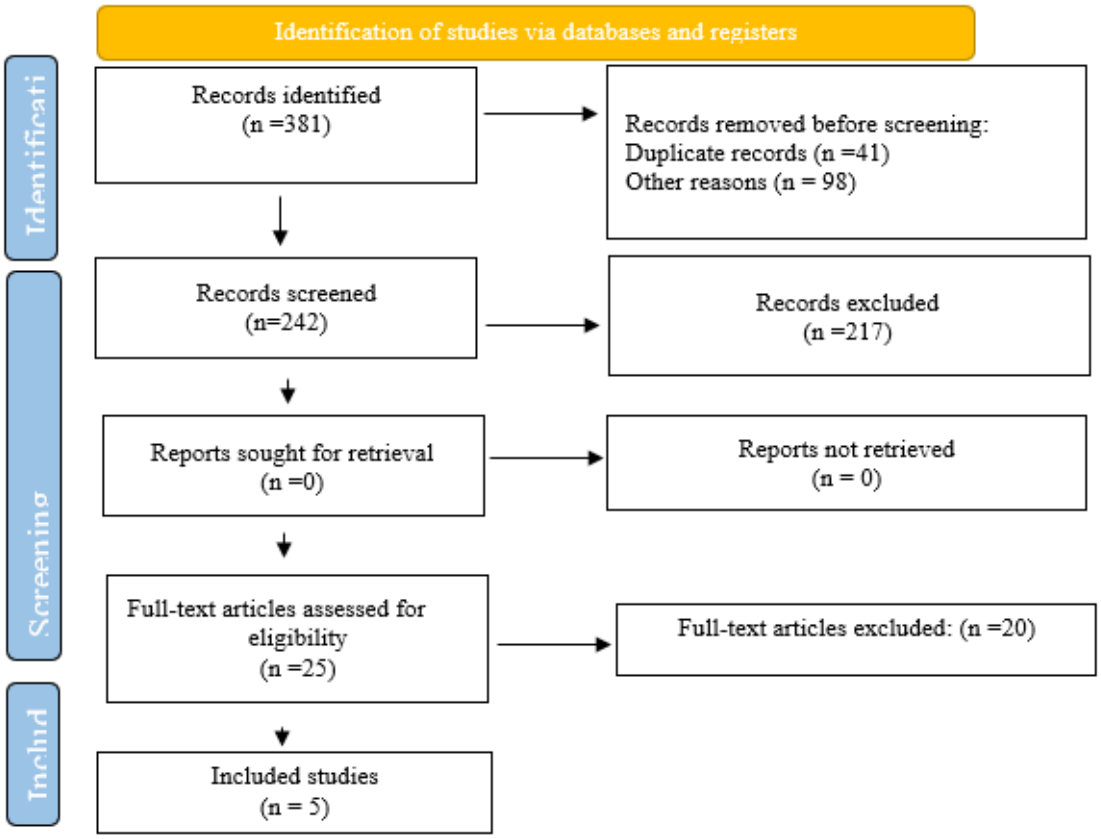

Figure 1. *Flowchart of PRISMA 2020 and selection of studies*

Characteristics of included studies
Study's characteristics were summarized in Table 2.

Table 2. *Main characteristics of the included studies*

Study	Study design	Number of participants		Gender				Mean age	Associated pathogens	Follow-up (years)	Neurodevelopmental outcomes (epilepsy)	
				Case		Control						
		Case	Control	Girl	Boy	Girl	Boy				Case	Control
Sodero et al., 2025 (18)	Prospective	53	27	25	28	9	18	7.4 years	CNS infections	5	37.8 % (8)	0
Valle et al., 2024 (19)	Retrospective	469		NR		NR		7.9	CNS infections	5	7.7% (36)	-
Bergonzini et al., 2024 (20)	Retrospective	94		31	63	-		10	CNS infections	5	62.7% (59)	-
Lykke et al., 2023 (21)	Retrospective	1432	14211	640	792	6342	7869	89 days	Group B Streptococcus	5	3.6% (52)	2.2% (312)
Lin et al., 2019 (7)	Retrospective	145	292	47	98	110	182	3.43 years	Enterovirus, *Herpes simplex* virus, Group B Streptococcus, *S. pneumoniae*	7.7	7.5% (11)	0.3 (1)

Bias assessment
Four studies received a score of 7/9 (High quality) and one study had moderate quality 6/9 (Table 3).

Table 3. *Assessment of quality of a cohort study (Newcastle Ottawa Scale)*

	Selection				Comparability	Outcome		Total
	Representativeness of exposed cohort	Selection of nonexposed cohort	Ascertainment of exposure	Outcome not present at the start of the study		Assessment outcome	Length of follow-up	
Sodero et al., 2025 (18)	*	*	*	*	**	*	-	7
Valle et al., 2024 (19)	*	*	*	*	**	*	-	7
Bergonzini et al., 2024 (20)	*	*	*	*	**	*	-	7
Lykke et al., 2023 (21)	*	*	*	*	**	*	-	7
Lin et al., 2019 (7)	*	*	*	*	*	*	-	6

*= 1 score; **= 2 score.

Risk of epilepsy in central nervous system infections in children

The risk ratio of epilepsy in children with etiologically diagnosed CNS infections was 0.23 (ES = 0.23; 95% CI 0.01 to 0.45; I^2 = 99.52%, P < 0.05) (Figure 2).

Children with brain infections were at higher risk for epilepsy for all the different pathogens tested.

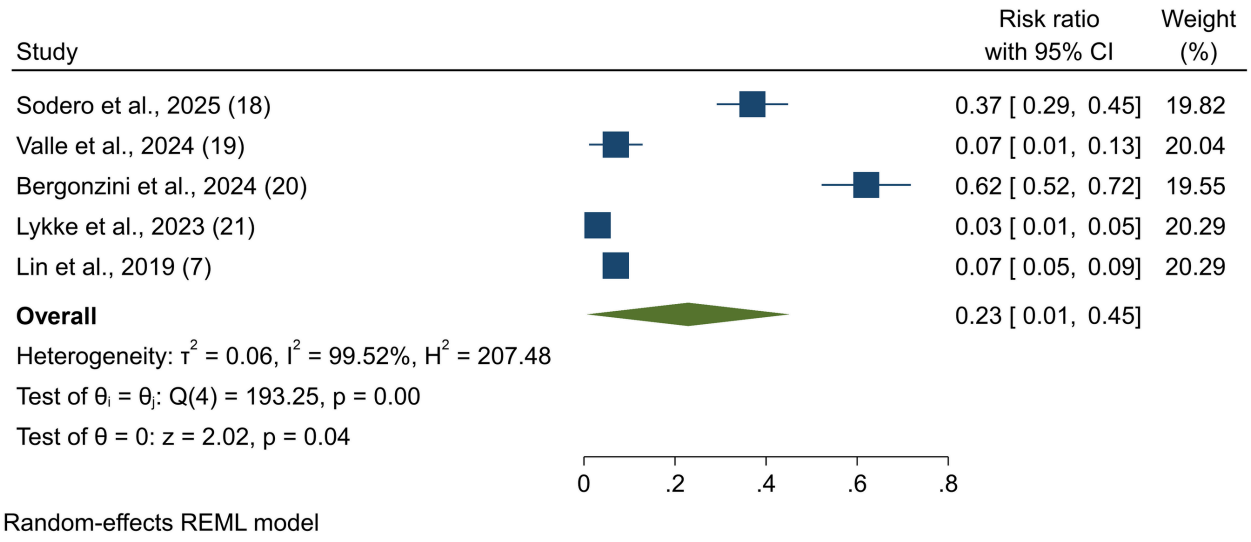

Figure 2. *Forest plot showed event rate of epilepsy in childhood brain infections*

DISCUSSION

Identifying and understanding the association between childhood brain infections and epilepsy is crucial for both treatment and neurodevelopmental prognosis (22). Studies have shown that enteroviruses are the leading cause of brain infections, followed by group B Streptococcus, S. pneumoniae, and Herpes simplex virus (22). Very few studies have addressed the association between epilepsy and infections caused by specific neurotropic pathogens in children. In the present study, five studies were eligible for inclusion and high heterogeneity was observed between studies in terms of methodology. The study population was small in most studies; therefore, the results of the present study should be interpreted with caution.

According to the present meta-analysis, the risk ratio of epilepsy in children involved in infection was 0.23. Brain infections in suspected children should be carefully evaluated as they can increase the risk of epilepsy in children (23). According to the results of the study, the long-term effects of bacterial meningitis can include intractable seizures and epilepsy, and Streptococcus pneumoniae was associated with a relatively higher risk of these conditions (24). Studies have shown that brain infections at this age may also impair children's social and communication skills. For this reason, brain infections may also affect neurodevelopmental disorders in addition to epilepsy (7). Examining these findings is very important due to the lack of studies. In addition to further investigation, studies should use larger samples and more rigorous experimental designs.

Epilepsy research continues to provide new medical treatments to increase the number of people who can fully control seizures and to reduce the side effects of treatments. In the present study, the risk of epilepsy in children with central nervous system infections was 0.23. Therefore, children diagnosed with brain infection should be monitored as they are at higher risk of developing epilepsy. Early assessment and identification of epilepsy and early intervention and treatment are of great importance. Based on the present meta-analysis, epilepsy has a high risk associated with brain infections.

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Competing Interest

The authors declared no relevant conflicts of interest.

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AFMN BIOMEDICINE

ORIGINAL ARTICLE

EFFECT OF A SUBANESTHETIC DOSE OF KETAMINE ON AWAKENING AND POSTOPERATIVE DEPRESSION AND ANXIETY IN PATIENTS UNDERGOING CORONARY ARTERY BYPASS GRAFT SURGERY

Rasool Ferasatkish[1] Nahid Aghdaii[1] Ali Sadeghi[1] Mohsen Ziyaeifard[1] Rasoul Azarfarin[2] Mirahmad Hendinezhad[3] Anahita Babaei[4]

[1]Department of Cardiac Anesthesiology, Rajaie Cardiovascular Medical and Research Center, Iran University of Medical Sciences, Tehran, Iran [2]Cardio-Oncology Research Centre, Rajaie Cardiovascular Medical and Research Center, Iran University of Medical Sciences, Tehran, Iran [3]Cardiac Anesthesiologist, Shafa Hospital, Sari, Iran [4]Department of Anesthesiology and Critical Care, Faculty of Medicine, Mazandaran University of Medical Sciences, Sari, Iran

The present study was performed to evaluate the effect of subanesthetic dosage of ketamine on awakening and anxiety and depression status among patients undergoing coronary bypass graft (CABG) surgery. In this quasi-experimental study, 50 patients scheduled for elective CABG in a tertiary referral hospital were enrolled. Participants were allocated to the ketamine and control groups. In the ketamine group, infusion began immediately after induction of anesthesia and before skin incision, at 0.375 µg/kg/min, and was discontinued at skin closure. The control group received the equivalent volume of normal saline over the same time frame. Hemodynamic parameters [mean arterial pressure (MAP), heart rate (HR)] were recorded at baseline (before anesthesia induction), after induction but before skin incision, and upon arrival in the intensive care unit ICU. Preoperative and postoperative anxiety and depression were assessed using the Hospital Anxiety and Depression Scale (HADS), with postoperative assessment performed 24 hours after extubation to ensure valid cognitive function. Sedation was assessed using the Ramsay Sedation Scale. The ketamine group showed a significantly higher proportion of awake patients at two hours post-operation (80% vs. 42.5%, $p = 0.034$) and faster recovery of consciousness over time ($\chi^2(3) = 11.18$, $p = 0.011$). Ketamine was associated with significantly lower anxiety (mean difference: -1.64, $p < 0.001$) and depression scores (mean difference: -1.52, $p < 0.001$) compared to the control group. A subanesthetic ketamine infusion, initiated after induction and continued until skin closure, significantly improved postoperative recovery in patients undergoing CABG surgery by promoting early awakening and reducing anxiety and depression scores 24 hours after the operation. These results highlight the potential of ketamine as an effective adjunct in perioperative care, warranting further investigation into its broader applications and long-term effects on patient outcomes.

Keywords: anxiety, awakening, cardiac surgery, depression, ketamine

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Correspondence to:
Anahita Babaei
Department of Anesthesiology and Critical Care
Bu Ali Sina Hospital
Pasdaran Boulevard, Sari, Iran
E-mail: babaeianahita@gmail.com

INTRODUCTION

Coronary artery bypass graft (CABG) surgery is associated with significant postoperative pain, anxiety, and depressive symptoms, which can negatively affect recovery even in patients without diagnosed psychiatric disorders (1,2). In cardiovascular surgery, there is a need for effective, opioid-sparing analgesia regimens that can provide maximal pain relief with minimal adverse effects (3). As an NMDA receptor antagonist with sedative, anxiolytic, and analgesic effects, ketamine represents a useful adjunct in perioperative pain management (4,5). Beyond its analgesic and mood-enhancing properties, ketamine exhibits significant anti-inflammatory and neuroprotective effects, including the modulation of pro-inflammatory cytokines, attenuation of ischemia–reperfusion injury, and reduction in the risk of postoperative delirium (6,7). These mechanisms are particularly relevant in the context of CABG surgery and cardiopulmonary bypass, highlighting ketamine's value as a multifaceted adjunct in perioperative care (8).

The impact of ketamine on mood and postoperative depression among surgical patients remains unclear, with conflicting findings in the literature. Some studies have reported improved mood and lower depression scores in patients receiving ketamine (9), while others have not found a significant difference between ketamine and control groups (10). These inconsistent results may be due to differences in surgical populations, dosing regimens, and outcome measures.

Several mechanisms have been proposed to explain the analgesic effects of ketamine, including modulation of pain signal transduction and interactions with muscarinic, serotonergic, opioid, and sodium channel receptors (11,12). Ketamine is often used in combination with opioids and benzodiazepines to provide a comprehensive approach to perioperative pain management (13,14). Additionally, ketamine demonstrated protective effects against local and generalized cerebral ischemia, trauma, postoperative cognitive dysfunction, and apoptosis in preclinical and clinical studies (15-18).

Despite these potential benefits, the widespread use of ketamine as an anesthetic and sedative agent was limited by concerns over the emergence of delirium and unpleasant psychotropic effects (19,20). The present study aimed to evaluate the impact of a subanesthetic dose of ketamine on awakening, postoperative depression, and postoperative anxiety in patients undergoing CABG surgery.

METHODS

In a quasi-experimental study, a total of 50 patients who were scheduled for elective coronary artery bypass graft (CABG) surgery in a referral hospital were evaluated. The inclusion criteria were age between 18 and 70 years and an ejection fraction greater than 35%. Patients were excluded if they had pulmonary, renal, or hepatic abnormalities, a history of neurological disorders, stroke, preoperative cognitive disorders, severe opioid addiction, uncontrolled diabetes, or uncontrolled hypertension.

At the beginning of the study, participants underwent neurological tests and completed the Hospital Anxiety and Depression Scale (HADS) to assess their preoperative anxiety and depression levels. The HADS is a well-validated, 14-item questionnaire comprising seven questions each for anxiety (HADS-A) and depression (HADS-D), and has been used in various clinical settings. Higher scores indicate greater levels of anxiety or depression (21-23). Preoperative hemodynamic parameters, including heart rate (HR), systolic blood pressure, diastolic blood pressure, and mean arterial pressure (MAP), were also measured using standard techniques. Anesthesia induction was performed using midazolam (0.05 - 0.15 mg/kg), sufentanil (0.25 - 2 µg/kg), and propofol (1 - 2.5 mg/kg), followed by atracurium (0.15 - 0.2 mg/kg). Anesthesia was maintained with atracurium (1 - 2 µg/kg/min), propofol (50 - 150 µg/kg/min), and sufentanil (0.5 - 1.5 µg/kg/hour). Participants were allocated to the ketamine group or the control group. In the ketamine group, infusion began immediately after induction of anesthesia and before skin incision, at 0.375 µg/kg/min, and was discontinued at skin closure. The control group received the equivalent volume of normal saline over the same time frame. Bispectral index (BIS) monitoring was performed during the anesthesia maintenance phase until patients were transferred to the Intensive Care Unit (ICU).

Hemodynamic parameters (MAP, HR), arterial blood gas analysis, electrocardiogram (ECG), central venous pressure, and pulse oximetry were monitored at regular intervals, but MAP and HR were recorded at baseline (before anesthesia induction), after induction but before skin incision, and upon arrival in the ICU. The primary outcomes were awaking status (assessed using the RAMSAY sedation scale), postoperative anxiety, and depression (evaluated using the HADS). Preoperative HADS was evaluated during the pre-anesthesia clinic visit. Postoperative HADS was evaluated 24 hours postoperatively, when patients were fully awake and

oriented, to ensure validity. Also, operation time, intubation time, and length of hospital stay were evaluated.

Ethical considerations

The study was conducted in accordance with the ethical principles outlined in the Declaration of Helsinki. Prior to the initiation of the study, the protocol received approval from the Research Ethics Committee of Iran University of Medical Sciences (Ethical Code: IR.RHC.REC.1400.039). All participants were fully informed about the nature, purpose, and potential risks of the study. They provided written informed consent, ensuring that participation was voluntary and that they had the right to withdraw at any time without any effect on their medical care. Additionally, subanesthetic doses of ketamine were administered with careful attention to minimize potential adverse effects. Continuous monitoring of participants was implemented to ensure their safety and well-being during the study period. The research team remained committed to ethical standards throughout the study, with a focus on minimizing harm and maximizing benefits for all participants involved.

Statistical analysis

The study data were entered into SPSS version 24.0 for analysis. Quantitative variables were presented as mean ± standard deviation, and qualitative variables were presented as frequency and percentage. Independent sample t-test and Chi-square tests were used to analyze the differences in quantitative and qualitative variables between the ketamine and control groups, respectively. Primary outcomes, including awaking status, anxiety, and depression scores, were analyzed with a mixed-effects model to account for repeated measures. Mean differences and 95% confidence intervals (CIs) were reported. One-way analysis of variance (ANOVA) confirmed group differences in anxiety and depression scores, with significance set at $p < 0.05$. Awakening status was evaluated using ANOVA to compare the proportions of awake patients at 2 and 4 hours, and a Chi-square test for trend was used to analyze awakening patterns over time. Mediation analysis assessed the indirect effects of ketamine on anxiety and depression, utilizing bootstrapping to derive 95% confidence intervals (CIs). Where applicable, post hoc Bonferroni tests were conducted. All tests were two-tailed, with a significance threshold set at $p < 0.05$.

RESULTS

A total of 50 patients (32 male, 18 female) were included in the final analysis, with an equal male-to-female ratio (16/9) in both the ketamine and control groups. The mean age was similar between the ketamine and control groups (58.60 ± 4.78 vs. 58.32 ± 5.30 years, respectively; $p = 0.85$). Table 1 compares hemodynamic and operation-related parameters between the groups. The results indicate that there were no significant differences in intubation time, operation time, or hospitalization stay. HR during the first and second measurements was similar between groups. However, the third HR measurement showed a significant difference, with the ketamine group exhibiting lower values. Regarding MAP, there were no significant differences in the first and third measurements. However, a significant difference was observed in the second MAP measurement, with the ketamine group showing higher values.

Table 1. *Comparing hemodynamic and operation-related parameters between the ketamine and control groups*

Variables	Control group (n = 25)	Ketamine group (n = 25)	p-value
Intubation time (hours)	13.16 ± 2.44	12.92 ± 2.86	0.75
Operation time (min)	188.80 ± 31.67	202.80 ± 33.01	0.31
Hospitalization Stay	55.60 ± 6.26	53.80 ± 6.27	0.31
First HR	89.56 ± 10.66	83.84 ± 14.65	0.12
Second HR	69.0 ± 9.22	66.16 ± 8.18	0.26
Third HR	82.60 ± 7.05	77.48 ± 9.15	0.03
First MAP	98.28 ± 10.61	92.64 ± 13.32	0.11
Second MAP	60.16 ± 6.75	75.40 ± 12.99	< 0.00
Third MAP	73.76 ± 8.07	77.04 ± 13.40	0.2

As presented in Table 2, initial assessments (pre-intervention) revealed no statistically significant differences in the mean scores for anxiety (HADS-A) or depression (HADS-D) between the control group and the ketamine group ($p > 0.05$), confirming baseline homogenity between the groups. Following the intervention, statistically significant differences were observed between the two groups. Anxiety (HADS-A) and depression (HADS-D) scores were significantly reduced in the ketamine group compared to the control group ($p < 0.001$).

Table 2. *HADS scores for anxiety and depression in the ketamine and control groups*

Variables	Time-point	Control Group (Mean ± SD)	Ketamine Group (Mean ± SD)	p-value*
Anxiety (HADS-A)	Pre-intervention	8.6 ± 1.2	8.4 ± 1.3	0.412
	Post-intervention	8.2 ± 1.3	6.8 ± 1.1	< 0.001
Depression (HADS-D)	Pre-intervention	7.9 ± 1.4	7.8 ± 1.5	0.776
	Post-intervention	7.3 ± 1.5	6.1 ± 1.2	< 0.001

As presented in Table 3, patients in the ketamine group were more likely to be awake at the 2-hour assessment compared to the control group (80% vs. 42.5%, respectively; $p = 0.034$). However, by the 4-hour assessment, the difference in the proportion of awake patients between the two groups was no longer statistically significant (72% in the ketamine group vs. 52% in the control group; $p = 0.145$). At the 6-hour and 8-hour assessments, the proportion of awake patients was similar between the ketamine and control groups (88% awake in both groups). To further analyze the temporal pattern of patient awakening, we conducted a Chi-square test for trend. This analysis revealed a significant difference in the proportion of awake patients over time between the ketamine and control groups ($\chi^2(3) = 11.18$, $p = 0.011$), indicating that the ketamine group had a faster recovery of consciousness compared to the control group.

Table 3. *Comparing frequency of awaking status of patients among ketamine and control groups*

Variables	Awaking status	Control group (n = 25)	Ketamine group (n = 25)	p-value
Two hours after the operation	Awake	17 (68%)	23 (92%)	0.034
	Sleep	8 (32%)	2 (8%)	
Four hours after the operation	Awake	13 (52%)	18 (72%)	0.145
	Sleep	12 (48%)	7 (28%)	
Six hours after the operation	Awake	22 (88%)	22 (88%)	-
	Sleep	3 (12%)	3 (12%)	
Eight hours after the operation	Awake	25 (100%)	25 (100%)	-
	Sleep	-	-	

The mixed-effects model analysis revealed that patients in the ketamine group had significantly lower anxiety scores compared to the control group (mean difference: -1.64, 95% CI: -2.05 to -1.23; $p < 0.001$). Similarly, patients in the ketamine group had significantly lower depression scores compared to the control group (mean difference: -1.52, 95% CI: -1.90 to -1.14; $p < 0.001$). These effects were consistent across the subgroup analyses, and there were no significant interactions between group and the potential effect modifiers (age, sex, or surgical complexity). To further confirm these findings, we performed ANOVA tests for awakening status, anxiety, and depression outcomes (Table 4). The between-group ANOVA for postoperative anxiety (HADS) showed a statistically significant difference between the ketamine and control groups, $F(1, 48) = 31.79$, $p < 0.001$. Similarly, the between-group ANOVA for postoperative depression (HADS) revealed a statistically significant difference between the

two groups, F(1, 48) = 24.83, p < 0.001. Additionally, the between-group ANOVA for the 2-hour assessment of awakening status (Ramsay Sedation Scale) indicated a statistically significant difference in the proportion of awake patients, F(1, 48) = 4.63, p = 0.026, while the difference was no longer significant at the 4-hour assessment, F(1, 48) = 2.17, p = 0.174.

The mediation analysis showed that the effect of ketamine on postoperative anxiety (indirect effect: -0.58, 95% CI: -1.06 to -0.19) and depression (indirect effect: -0.47, 95% CI: -0.90 to -0.15) was partially mediated by the differences in awakening status between the two groups.

Table 4. *ANOVA results for awakening status, anxiety, and depression scores*

Outcome	Time point	F-value	df	p-value	Post hoc Results (Bonferroni)
Awakening status	2 hours	4.63	1, 48	0.026	Ketamine > Control
	4 hours	2.17	1, 48	0.174	No significant difference
	6 hours	-	-	-	No significant difference (88% awake in both groups)
	8 hours	-	-	-	No significant difference (all patients awake)
Postoperative anxiety	24 hours post-op	31.79	1, 48	< 0.001	Ketamine < Control (Mean difference: -1.64)
Postoperative depression	24 hours post-op	24.83	1, 48	< 0.001	Ketamine < Control (Mean difference: -1.52)

DISCUSSION

In our study, we assessed the impact of subanesthetic dosage of ketamine on hemodynamic parameters, anxiety/depression status, and awakening of patients undergoing CABG. Study findings showed that hemodynamic parameters were similar between the two groups, and on the other hand, advising subanesthetic dosage of ketamine did not have adverse impacts on the hemodynamic situation of patients undergoing CABG. Patients in the ketamine group had experienced significantly lower anxiety and depression in comparison with patients in the control group. Moreover, subanesthetic dosage of ketamine had no adverse impacts on sedation and awakening of patients, and all of the study patients were awake eight hours after the operation.

Although anesthetics tried to provide a better situation for pain control during the postoperative period, more than two-thirds of patients reported that their pain control regimen was not adequate and they felt more pain during the postoperative period (24). More than anxiety, pain in the postoperative period can cause some complications, such as nausea, vomiting, delayed surgery recovery, and extended hospitalization (25). According to this challenge, anesthetists try to provide well-planned pain management protocols to reduce the pain experiences of patients and control other related complications such as postoperative anxiety and depression. Ketamine, as one of the NMDA agents was used for its analgesic properties during the postoperative period (22). Ketamine decreases pain by inhibiting glutamate, a neurotransmitter that binds to NMDA receptors and prevents pain transmission (26). Ketamine in subanesthetic dosage (less than 0.3 mg/kg) had analgesic and opioid-sparing effects and controlled hyperalgesia due to opioid consumption during the postoperative period (8, 26). Similar studies reported that subanesthetic dosage of ketamine is not associated with significant complications of high dosage of ketamine, such as an increase in the secretion of the respiratory system, delirium, and double vision (27).

Numerous studies have reported that, regardless of the treatment results, many patients still suffer from postoperative pain (28). Lahtinen et al. in their study reported that 49% of patients undergoing CABG experienced severe pain at rest, 78% while coughing, and 62% of patients experienced pain during movement (29). In another study, patients undergoing CABG had activity-related pain until the sixth day after the operation, and most of the pain was experienced while coughing and deep breathing (30).

For many years, ketamine has been used as a general anesthetic, and in recent years, its subanesthetic dosage has been used as analgesia in the postoperative period.

Ketamine, as a non-competitive antagonist of NMDA receptors, has been shown to inhibit opioid-induced analgesic tolerance and hyperalgesia (31,32). We have found few studies that use ketamine in cardiac surgery. In Nesher et al. study, patients who received ketamine had lower pain scores and morphine consumption (28). Other studies indicate that, beyond its analgesic properties, ketamine can reduce delirium after cardiopulmonary bypass and exert antidepressant effects. (33). Depression has been known as one of the main contributors of post-operative morbidity, and patients with underlying depression at baseline may be at an increased risk of postoperative complications (34). In a retrospective study by Elsamadicy, patients who had preoperative depression had twice the chance of developing postoperative delirium (35). Blumenthal et al., in their study, concluded that post-operative depression may occur in as high as 40% of patients undergoing coronary artery bypass grafting (CABG) procedures (36). In contrast, Aguayo et al. in their study reported that only 5.1% of patients may be at risk of developing post-operative depression following the CABG procedure (37). Regardless of the discrepancies in incidence, most of the similar studies focused on the implications of post-operative depression on patient outcomes (34-37).

While much of the literature emphasizes ketamine's analgesic properties, it is important to acknowledge that its clinical effects are likely multifactorial and extend beyond pain modulation. Ketamine has well-documented anti-inflammatory properties, which may contribute to its beneficial effects in the postoperative period. Ketamine reduces the release of pro-inflammatory cytokines, such as interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α), while promoting the anti-inflammatory cytokines, such as interleukin-10 (IL-10) (38,39). These effects may mitigate the systemic inflammatory response commonly associated with surgery and anesthesia, potentially improving recovery outcomes. Reduced inflammation may also play a role in the observed improvements in mood, as inflammation is increasingly recognized as a contributor to depression and anxiety (6, 40). In addition, ketamine's neuroprotective properties may help explain its effects on postoperative recovery. Ketamine has been shown to modulate ischemia-reperfusion injury, a condition that can occur during surgery due to transient reductions in blood flow to tissues. By acting as an NMDA receptor antagonist, ketamine reduces excitotoxicity and calcium overload in neurons, thereby preventing cellular damage (7, 41). These neuroprotective effects may further contribute to improved cognitive recovery and mood stabilization in the early postoperative period.

According to the findings of our study, advice on subanesthetic dosage of ketamine can control anxiety and depression among study participants, so hopefully, the frequency of CABG postoperative complications will decrease. While ketamine led to higher arousal scores at the 2-hour postoperative assessment, the clinical significance of this finding may be limited as it did not result in measurable benefits such as shorter mechanical ventilation duration or reduced ICU stay. This suggests that the early awakening advantage observed might not independently influence long-term recovery metrics or overall clinical outcomes. Also, one of the most promising clinical implications of ketamine use in the perioperative setting is its potential to facilitate early extubation and expedite ICU discharge, contributing to fast-tracking protocols. However, this aspect was not evaluated in the present study. Future research should prioritize outcomes such as time to extubation, readiness for ICU transfer, hospital length of stay, or patient-reported functional recovery, and their impact on overall resource utilization and patient recovery. Including these endpoints in future protocols could provide critical insights into the broader applicability of ketamine in enhancing perioperative care efficiency.

In conclusion, it seems that sub-anesthetic dose of ketamine administration significantly enhances postoperative recovery by promoting a faster return to consciousness and reducing anxiety and depression in patients undergoing CABG surgery. The findings suggest that ketamine not only enhances early awakening status but also has a beneficial effect on psychological well-being following surgery. These results highlight the potential of ketamine as an effective adjunct in perioperative care, warranting further investigation into its broader applications and long-term effects on patient outcomes.

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Statement of Ethics

This study protocol was reviewed and approved by the

Research Ethics Committee of Rajaie Cardiovascular, Medical and Research Institute, approval number: IR.RHC.REC.1400.039, issued on 2021-07-31

Competing Interest

The authors declare no relevant conflicts of interest.

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AFMN BIOMEDICINE

ORIGINAL ARTICLE

PROTECTIVE EFFECTS OF GREEN TEA AND BILBERRY AGAINST SECONDARY HYPERLIPIDEMIA IN GENTAMICIN–INDUCED NEPHROTOXICITY IN RATS

Milica Veljković[1] Nikola M. Stojanović[1] Tanja Džopalić[2] Dragana R. Pavlović[3] Dušan Sokolović[4] Milan Petković[1]

[1]Department of Physiology, University of Niš Faculty of Medicine, Niš, Serbia [2]Department of Immunology, University of Niš Faculty of Medicine, Niš, Serbia [3]Department of Pharmacy, University of Niš Faculty of Medicine, Niš, Serbia [4]Department of Biochemistry, University of Niš Faculty of Medicine, Niš, Serbia

The aim of this study was to examine whether green tea and bilberry have beneficial effects on secondary hyperlipidemia that developed due to gentamicin-induced renal impairment.

The GM group of rats was treated with gentamicin only, the GT group was given green tea only, the B group received bilberry only, while the C group was given saline only. The GT+GM group received green tea simultaneously with gentamicin, whereas the B+GM group was given bilberry with gentamicin.

The results showed that gentamicin significantly increased total cholesterol, LDL, and triglycerides, while it decreased HDL compared with the control group. However, when either green tea or bilberry was applied together with gentamicin, this secondary hyperlipidemia was significantly ameliorated, as evidenced by a significant increase in HDL and a decrease in LDL, cholesterol, and triglycerides in comparison to the GM group.

The beneficial effects of both green tea and bilberry on secondary hyperlipidemia in gentamicin-induced nephrotoxicity occurred due to their powerful antioxidant properties. They are both functional foods, widely available in nature, and can be used as cost-effective additional therapy together with gentamicin, without affecting its activity in killing bacteria.

Keywords: gentamicin, nephrotoxicity, green tea, bilberry, lipids

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Correspondence to:
Milica Veljković
Department of Physiology
University of Niš Faculty of Medicine
Bulevar dr Zorana Đinđića 81, Niš, Serbia
E-mail: milica.veljkovic@medfak.ni.ac.rs

INTRODUCTION

Gentamicin (GM) is an aminoglycoside antibiotic widely used in the treatment of life-threatening bacterial infections. It has a wide spectrum of activity against both Gram-positive and Gram-negative bacteria, including *P.aeruginosa* and other Gram-negative enteric bacilli. It is still a crucial antibiotic, although dose-dependent ototoxicity and nephrotoxicity limit its use. Both adverse effects are found to be caused by oxidative stress (1,2).

There is increasing concern about secondary hyperlipidemia, which occurs in gentamicin-induced nephrotoxicity. It is documented that even other aminoglycosides, such as puromycin, cause critical hyperlipidemia with an elevation in key lipoproteins (3).

Polyphenols are secondary metabolites mainly present in fruits and vegetables that are considered to be functional foods. Those are green tea, coffee, cocoa, red wine, dark chocolate, cherries, and berry fruits (such as bilberry). Nowadays, functional foods have been the focus of research due to increasing interest in healthy diets and lifestyles (4). It is documented that they have a therapeutic effect in the prevention and amelioration of chronic and lifestyle diseases induced by oxidative stress due to their antioxidant and anti-inflammatory effects (2). In our study, we used green tea (GT) and bilberry (B) as functional foods and investigated their possible protective effect in secondary hyperlipidemia caused by GM-induced nephrotoxicity that was established in our previous experiments (5,6).

METHODS

Animals (48 adult Wistar rats, both male and female, weighing 200-250g) were acclimatized for 14 days, then divided into six groups of eight animals each. They were given a standard rat chew diet and had free access to water. Green tea and bilberry extracts were prepared as described in our earlier papers (5,6).

The control (C) group was injected intraperitoneally with saline for 15 days. The green tea (GT) group was treated with green tea extract orally (150 mg/kg/day) for 15 days. The bilberry (B) group was orally given bilberry extract (100 mg/kg/day) for 15 days. Kidney damage in the GM group was caused by intraperitoneal injection of GM (100 mg/kg/day) during the last eight days of the experiment. The last two groups (GT+GM and B+GM) were treated with the same dose of GT and B extracts, respectively, over the course of all 15 days of the experiment, and were simultaneously given GM during the last eight days.

Twenty-four hours after the last treatment, the animals were sacrificed using ketamine as an anesthetic. Blood was collected from the aorta, and its samples were sent to the Clinic of Nephrology, University Clinical Center Niš, for biochemical estimation of lipid parameters. All experimental steps were done in accordance with the principles of the local Ethical Committee, by which it was approved (number 01-2625-8).

Biochemical analysis

For biochemical analysis, lipid parameters in all animals (total cholesterol, LDL, HDL, and triglycerides) were determined using standard biochemical methods on automatic analyzers in the laboratory of the Clinic of Nephrology, University Clinical Center Niš.

Statistical analysis

All data were reported as mean ± standard deviation. Statistical significance of differences between groups was computed by one-way analysis of variance (ANOVA) followed by Tukey post hoc test for multiple comparisons (Graphpad Prism version 5.03, San Diego, CA, USA). P values less than 0.05 were considered significant.

RESULTS

Rats in the GM group showed a significant increase in total cholesterol (Figure 1), LDL (Figure 2), and triglycerides (Figure 3), as well as a decrease in HDL cholesterol (Figure 4), when compared to the C group. This is a clear indication of secondary hyperlipidemia associated with rat nephrotoxicity caused by gentamicin, which was proved in our earlier experiments (5,6). Our experimental doses of both GT (150 mg/kg/day) and B (100 mg/kg/day) ameliorated and counteracted renal impairment and secondary hyperlipidemia induced by GM, making it less toxic. In groups where both GT and B were simultaneously given with GM (GT+GM and B+GM), total cholesterol, LDL, and triglyceride levels (Figures 1, 2, and 3) were significantly lower in comparison to the GM group, while HDL levels were significantly higher (Figure 4). Nevertheless, when compared to the C group, total cholesterol, LDL, and triglyceride levels (Figures 1, 2, and 3) were higher, and HDL levels (Figure 4) were lower, but without statistical significance. In addition, there was no statistical significance among the lipid parameter values mentioned above in groups that were only given plant extracts (GT and B groups) when compared to the control group.

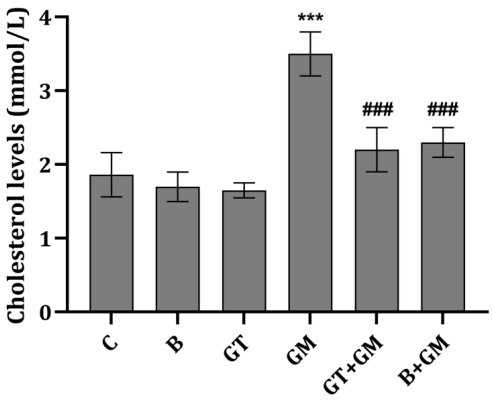

Figure 1. *Total cholesterol levels in rats*

C—control; B—bilberry; GT—green tea; GM—gentamicin
*** p < 0.001 vs C; ### p < 0.001 vs GM

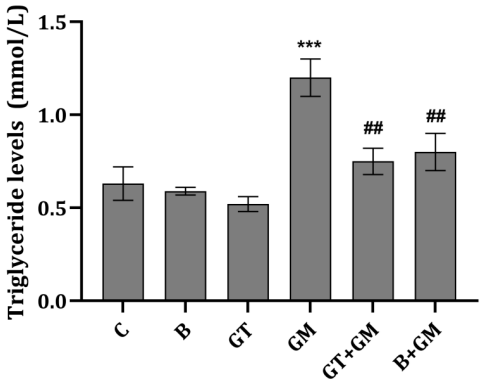

Figure 3. *Triglyceride levels in rats*

C—control; B—bilberry; GT—green tea; GM—gentamicin
*** p < 0.001 vs C; ## p < 0.01 vs GM

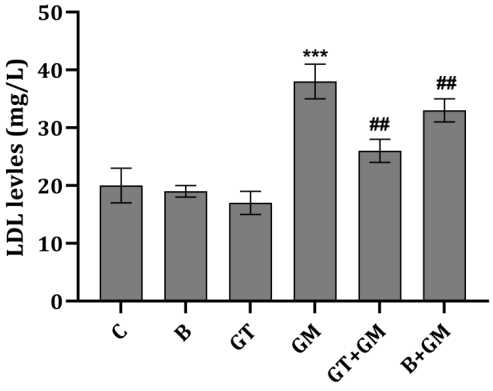

Figure 2. *LDL levels in rats*

C—control; B—bilberry; GT—green tea; GM—gentamicin
*** p < 0.001 vs C; ## p < 0.01 vs GM

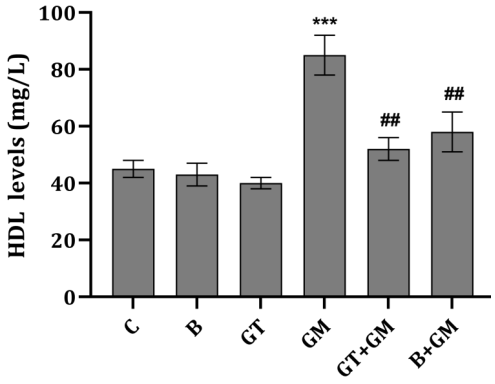

Figure 4. *HDL levels in rats*

C—control; B—bilberry; GT—green tea; GM—gentamicin
*** p < 0.001 vs C; ## p < 0.01 vs GM

DISCUSSION

The administered doses and length of treatment periods with GM, GT and B were determined according to the available literature and our earlier experiments (5,6).

Aminoglycoside antibiotics, including GM, are widely used as therapy for Gram-negative infections. One of the main adverse effects during its use is nephrotoxicity, which is responsible for 10-15% of all cases of acute renal failure. The main site for its toxicity is the proximal tubules, as GM is predominantly accumulated there (7).

In our previous studies (5,6), GM nephrotoxicity was confirmed by serum increase in urea and creatinine in rats and by histopathological changes in the kidneys, mostly in proximal tubules. The documented nephrotoxicity occurs together with secondary hyperlipidemia. The induced increase in total cholesterol that was recorded in our study was similar to the data related to hypercholesterolemia linked with puromycin-induced nephrotoxicity in rats (8).

Data found in the literature point out that the main way GM causes nephrotoxicity is through oxidative stress, by the generation of reactive oxygen species (ROS) (5,6). These oxygen metabolites cause ischemic, toxic, and inflammatory tissue impairment during GM use. The hydrogen peroxide production inside the mitochondria was boosted in a dose-dependent manner by GM treatment. It also stimulates the production of superoxide anion and hydroxyl radical in the renal cortical mitochondria. Another mechanism of its toxicity

is the release of iron from mitochondria initiated by the generation of hydrogen peroxide. Since these are the ways GM induces its toxicity, research was conducted to try to use antioxidants and iron chelators as a means to reduce GM nephrotoxicity, without affecting bactericidal activity (8).

Functional foods are rich in polyphenols and are the focus of research due to increasing interest in healthy nutrition. Its daily intake has been linked with reduced risk of metabolic, cardiovascular, and lifestyle diseases, such as obesity, type II diabetes mellitus, and hypertension. Polyphenols found in green tea are catechin, epicatechin, and epigallocatechin, whereas anthocyanidins (pelargonidin, cyanidin, delphinidin, peonidin, petunidin, and malvidin) can be found in bilberry. These polyphenols are strong antioxidants and can increase the intrinsic antioxidant defenses of an organism, thus decreasing oxidative stress (4). Applying 200 or 400 mg/kg bw/d of polyphenol extract of *A. grossedentata* via gastric gavage documented antioxidant effects with the depletion of TNF-α, IL-6, and NF-kB levels in LDLr-/- mice fed with a cholesterol-free diet (HFD) after 12 weeks, with the stronger dosage of 400mg/kg being more effective (9).

In our previous work (5,6), we proved that GM caused nephrotoxicity via oxidative stress by significantly increasing malondialdehyde (MDA) and decreasing catalase values. In this paper, we are studying the possible role of secondary hyperlipidemia in the development of renal impairment in rats induced by GM. Gentamicin caused a significant increase in total cholesterol, LDL, and triglycerides, and a significant decrease in HDL cholesterol fraction when compared to control. However, when given together with GM, both functional foods (B and GT) showed a significant hypolipemic effect, increasing HDL, but decreasing total cholesterol, LDL, and triglycerides when compared to the GM group. The antihyperlipidemic activity of both bilberry and green tea extracts is through the antioxidant effect that inhibits lipid peroxidation induced by GM. Antioxidant activity of the extracts occurs in two ways. The first mechanism is by donating a hydrogen atom. The second way is by inhibiting the lipase enzyme via the activity of the extracts, hence preventing the breakdown of lipids. Polyphenols found in functional foods are also known to decrease lipoprotein secretion through the liver and intestines and to enhance the secretion of bile acids that increase the rate of lipid excretion. By these mechanisms, they are performing their hypolipemic activity (10).

Other authors have reported that the main reason for the increase in total cholesterol of uremic patients and experimental animals is due to increased production of cholesterol in the liver and increased production of lipoproteins caused by the developed hypoalbuminemia. The hypertriglyceridemia that occurred secondary to renal damage was thought to develop because of delayed removal of triglyceride-rich lipoproteins from the circulation due to a decrease caused by lipoprotein lipase activity (11).

In our previous studies (5,6), we documented that GM caused nephrotoxicity due to oxidative stress and that both GT and B ameliorated renal impairment through their antioxidant activity. In this experiment, we tried to analyze if these same substances would have a beneficial effect in secondary hyperlipidemia caused by GM-induced nephrotoxicity. Both are considered to be functional foods.

It has been demonstrated that interest in functional food has significantly increased. Polyphenols, the strong antioxidants abundantly found in functional foods, including GT and B, are documented to have beneficial effects on hyperlipidemia through various mechanisms. Both GT and B, as sources of polyphenols, are widely available in nature, part of everyday nutrition, and are generally safe. They may offer safe and cost-effective therapy that can ameliorate not only GM-induced nephrotoxicity, but also secondary hyperlipidemia that comes with it, without affecting its antibacterial activity.

Acknowledgements

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Statement of Ethics

This study protocol was reviewed and approved by the Faculty of Medicine Ethical Committee, approval number 01-2625-8, issued on April 8, 2014.

Competing Interest

The authors declare no relevant conflicts of interest.

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AFMN BIOMEDICINE

ORIGINAL ARTICLE

PARAMETERS OF OXIDATIVE STRESS IN PATIENTS WITH BENIGN PROSTATE HYPERPLASIA, CHRONIC PROSTATITIS, AND PROSTATE CANCER

Andrej Veljković[1] Jovan Hadži-Djokić[2] Dragoslav Bašić[3,1] Ljubomir Dinić[3,1] Aleksandar Skakić[3,1] Nina Medojević[3] Ognjen Radović[4] Stefanos Roumeliotis[5] Konstantinos Leivaditis[5] Gordana Kocić[2]

[1]Department of Biochemistry, University of Niš, Faculty of Medicine, Niš, Serbia [2]Serbian Academy of Sciences and Arts, Belgrade, Serbia [3]University Clinical Center Niš, Clinic of Urology, Niš, Serbia [4]University of Niš, Faculty of Economics, Niš, Serbia [5]2nd Department of Nephrology, AHEPA Hospital, School of Medicine, Aristotle University of Thessaloniki, Thessaloniki, Greece

Benign prostatic hyperplasia (BPH), chronic prostatitis (CP), and prostate cancer (PC) are frequently occurring conditions that affect the prostate gland, with overlapping clinical features and potentially shared pathogenetic mechanisms. A growing body of research indicates that oxidative stress (OS) is a critical factor in both the onset and advancement of these disorders. Xanthine oxidase (XO) is a known enzymatic source of reactive oxygen species (ROS); however, its involvement in prostate disease pathogenesis remains underexplored.

The study included 17 patients with CP, 10 with BPH, and 15 with PC. Ten healthy individuals served as controls. Serum samples were collected for the BPH and CP groups, while PC samples were obtained from surgical tissues. OS was assessed by measuring thiobarbituric acid-reactive substances (TBARS) and advanced oxidation protein products (AOPP). XO activity was determined spectrophotometrically in plasma and tissue homogenates.

Serum concentrations of TBARS and AOPP were markedly higher in individuals diagnosed with BPH and CP relative to those in the healthy control group ($p < 0.001$). Similarly, XO activity was markedly increased in these groups. In PC tissue, both TBARS and AOPP concentrations, as well as XO activity, were significantly higher than in non-tumor prostate tissue ($p < 0.001$), indicating local OS and enzymatic ROS production.

These findings confirm that systemic and tissue-level OS is elevated in BPH, CP, and PC. XO may represent a shared mechanism linking inflammation and carcinogenesis. The study supports further investigation into the therapeutic potential of antioxidants and XO inhibitors as adjunct strategies in prostate disease management.

Keywords: benign prostate hyperplasia, chronic prostatitis, prostate cancer, oxidative stress

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Correspondence to:
Andrej Veljković
Department of Biochemistry
University of Niš Faculty of Medicine
Bulevar dr Zorana Đinđića 81, Niš, Serbia
E-mail: andrej.veljkovic@medfak.ni.ac.rs

INTRODUCTION

Prostatic disorders rank among the most prevalent health issues observed in older male populations. The onset of these diseases typically begins around the age of 40. Starting at approximately 50% in 60-year-olds, the occurrence increases substantially, reaching up to 90% by the age of 85 (1). Benign prostatic hyperplasia (BPH) is a condition associated with high morbidity but a very low mortality rate (2). In recent years, chronic prostatitis (CP) has emerged as a major issue in urology (3). Around 90% of men exhibiting symptoms of prostatitis are classified as having chronic prostatitis/chronic pelvic pain syndrome (CP/CPPS), or category III, characterized by a symptom duration of at least three months and the lack of a detectable urinary tract infection (4).

Prostate cancer (PC) represents the second most frequently diagnosed malignancy in the male population (5). Although the exact mechanisms underlying prostate tumor development are not fully understood, a strong association has been documented between oxidative stress (OS) and increased cancer risk. A growing body of evidence indicates that OS contributes significantly to both the initiation and advancement of PC (6). The imbalance caused by excessive reactive oxygen species (ROS) and insufficient antioxidant protection is recognized as a major contributor to the pathogenesis of several prostate-related conditions. Chronic inflammatory processes in the prostate have been linked to age-associated hormonal fluctuations and infections (7). Such sustained inflammation within prostatic tissue facilitates the formation of free radicals, contributing to OS. At sites of inflammation, injury, or infection, immune cells are known to stimulate the production of free radicals. Accordingly, OS in these patients may significantly contribute to disease progression. OS may stem from bacterial infections and/or the body's inflammatory reaction to these pathogens (8).

The inflammatory process can initiate proliferation and induce DNA modifications in prostate tissue caused by OS. Repeated injury to tissue, along with the presence of OS, can initiate compensatory mechanisms that drive increased cellular division, thereby elevating the likelihood of abnormal tissue growth or the formation of neoplastic lesions (9). Damage to DNA in this context may disrupt normal transcriptional and replication processes, activating multiple intracellular signalling pathways and ultimately contributing to genomic instability, a hallmark feature in the development of cancer (10).

Among the harmful effects exerted by ROS, lipid peroxidation is particularly damaging, resulting in permanent impairment of cellular membrane structure and function. Thiobarbituric acid-reactive substances (TBARS) are recognized as terminal products and dependable biomarkers of lipid peroxidation activity (11).

Proteins are also susceptible to oxidative modifications caused by free radicals. Research indicates that plasma levels of advanced oxidation protein products (AOPP) are markedly higher in individuals with urinary tract disorders (12). The release of these oxidative markers may be associated with inflammatory processes or a reduction in the body's antioxidant capacity. OS contributes to urinary tract injury through direct cellular toxicity, potentially by disrupting vascular function or acting as a key regulator of various pathological mechanisms.

One recognized pathway for ROS generation involves the enzyme xanthine oxidase (XO). XO produces uric acid, which is the end product of adenine nucleotide breakdown (13). This enzyme is widely present in various tissues across multiple animal species, where it facilitates the oxidation of both endogenous and exogenous compounds. In mammals, XO exists in two interchangeable forms: xanthine dehydrogenase and XO. Specifically, XO is the isoform responsible for generating oxidative radicals. Under certain pathological conditions, xanthine oxidase becomes a major contributor to the production of superoxide anion radicals (O_2^-) (14,15).

An important question is whether XO activity may represent a key factor contributing to the onset and progression of the BPH, CP, and prostate carcinogenesis.

The purpose of this research was to evaluate the extent of OS in individuals diagnosed with BPH, chronic prostatitis, and PC, as indicated by the concentrations of TBARS and AOPP. Furthermore, our study sought to assess XO activity as a possible contributor to ROS generation in the plasma of men with prostate disorders, compared to healthy controls. The overarching objective was to explore the therapeutic potential of XO inhibitors and antioxidants in managing these diseases.

METHODS

Patients

The study included 17 patients with CP treated at the Clinical Center Kragujevac and the Health Center Knić, 10 patients with BPH and 15 patients with PC treated at the University Clinical Center Niš. Serum samples were

collected from patients with BPH and CP, while tissue samples were obtained for the examination of PC.
All patients underwent routine diagnostic procedures. CP was diagnosed through microbiological and biochemical analysis of prostatic fluid. Carcinomas were diagnosed via biopsy and confirmed pathohistologically following radical prostatectomy.
Participants were categorized into three clinical groups according to their specific disease diagnosis.
Group I—17 patients with CP;
Group II—10 patients with BPH;
Group III—15 patients with PC.
The control group consisted of 10 healthy individuals, selected in relation to the clinical groups, where the absence of disease was confirmed by the concentration of prostate-specific antigen (PSA). Control subjects had no acute or chronic illnesses or hypertension. The participants were matched based on age and gender.
Blood samples from patients with BPH, CP, and the control group were collected, then centrifuged at 3000 rpm to isolate plasma, which was subsequently stored at -20°C until further analysis. After radical prostatectomy, tissue specimens from PC patients were obtained by a pathologist, after which they were homogenized and used for analysis. The control tissue was the prostate tissue most distant from the cancerous region.
Informed consent was obtained from all participants prior to their inclusion in the study. The research adhered to the principles of the Declaration of Helsinki and received approval from the Ethics Committee of the Medical Faculty in Niš (Decision No. 12-8818-2/8) on September 23, 2020.

Lipid peroxidation products quantification
Lipid peroxidation products in plasma and tissue were quantified by a slightly adapted version of the method described by Nabavi et al. (16). The levels of TBA-reactive lipid peroxidation products were measured spectrophotometrically at 532 nm against a blank, with results expressed in µmol/L and µmol/mg.

AOPP concentration quantification
Plasma and tissue concentrations of advanced oxidation protein products (AOPP) were assessed spectrophotometrically following the procedure outlined by Vitko et al. (12). AOPP levels were reported as µmol/L chloramine T equivalents.

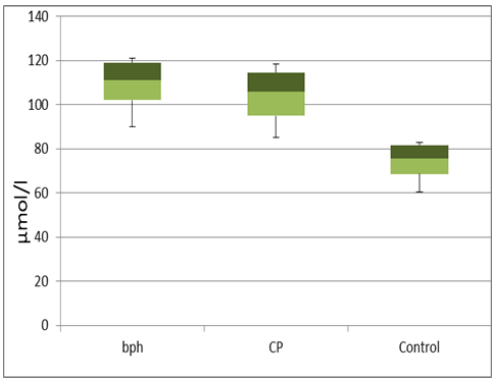
XO activity assessment
XO activity was determined spectrophotometrically using xanthine as a substrate. The assay measured uric acid production over a set time in the absence of NADH, where molecular oxygen acted as the sole electron acceptor. Uric acid formation was monitored at 293 nm, and XO activity was expressed as units per mg of tissue protein in the homogenate (17).

Determination of protein concentration
The amount of protein in cancer and healthy tissue was measured following the procedure outlined by Popović et al. (18), with results expressed as mg of protein per liter.

Statistical analysis
Data were presented as mean ± standard deviation (SD). A p-value less than 0.05 was considered statistically significant. For comparisons between two independent groups, a Student's t-test was used, and the following parameters were reported: t-value and degrees of freedom. For more than two groups, a one-way analysis of variance (ANOVA) was performed, including the presentation of the F-value, between-group and within-group degrees of freedom and corresponding p-values. Statistical analyses were conducted using the Statistical Package for the Social Sciences (SPSS) for Windows, version 11.0 (Chicago, Illinois, USA).

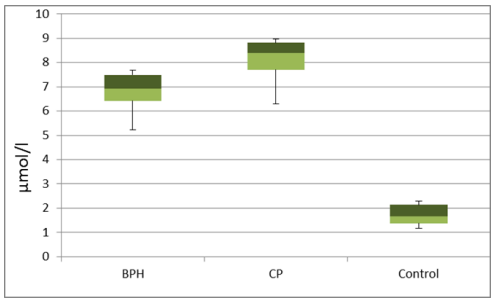
RESULTS

In the serum of the patients with BPH, CP, and healthy controls, we determined the level of lipid peroxides in the form of TBARS (Figure 1). Serum TBARS levels were significantly elevated in patients with BPH and CP compared to the control group ($p < 0.001$). However, no statistically significant differences were observed between the patient groups.
Next, we measured the level of oxidation of proteins, via AOPP concentration (Figure 2). Serum AOPP levels were significantly higher in patients with BPH and CP compared to the control group ($p < 0.001$), with no notable differences observed between the patient groups.
Then, we assessed XO enzyme activity (Figure 3) as a potential contributor to OS in patients with BPH and CP. The plasma XO activity was significantly elevated in both patient groups compared to the controls ($p < 0.001$). In the subsequent phase of our study, we quantified OS markers in PC tissue. As the control, we used healthy tissue located outside of the tumor site.



Group	Mean	St.dev	P value
BPH	111.42	12.46	< 0.001 vs control
CP	105.02	19.42	< 0.001 vs control
Control	73.96	12.73	

Figure 1. *Vertical boxplot for TBARS values in patients with BPH, CP, and controls*



Group	Mean	St.dev	P value
BPH	6.82	1.46	< 0.001 vs control
CP	8.39	4.42	< 0.001 vs control
Control	1.78	0.53	

Figure 2. *Vertical boxplot for AOPP values in patients with BPH, CP and controls*

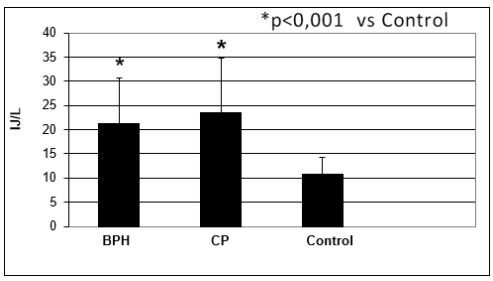
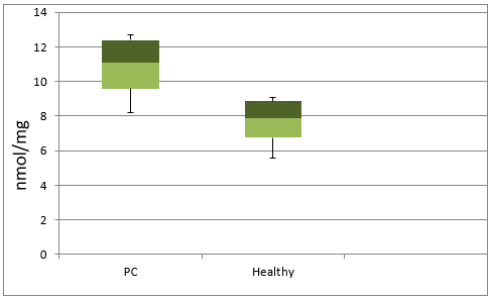


Figure 3. *Xanthine oxidase activity in patients with BPH, CP, and controls*

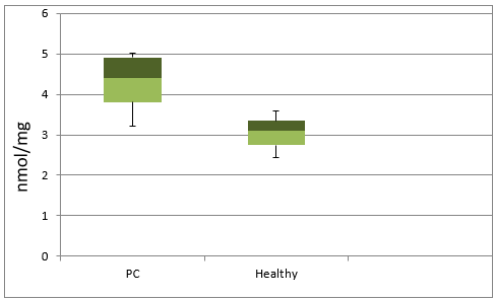
Next, we found the significantly increased level of TBARS in PC tissue when compared to control healthy tissue ($p < 0.001$) (Figure 4).



Group	Mean	St.dev	P value
PC	11.1	1.95	< 0.001 vs healthy
Healthy	7.96	0.75	

Figure 4. *Vertical boxplot for TBARS values in PC tissue and healthy prostate tissue*

Figure 5 illustrates the concentration of AOPP in PC and healthy control prostate tissue. We observed the significantly increased level of AOPP in PC tissue when compared to control tissue ($p < 0.001$). (Figure 5).



Group	Mean	St.dev	P value
PC	4.45	0.62	< 0.001 vs Healthy
Healthy	3.16	0.34	

Figure 5. *Vertical boxplot for AOPP values in PC tissue and control tissue*

Assessing XO enzyme activity as a potential contributor to OS in PC, we found that XO activity was significantly higher compared to healthy prostate tissue (Figure 6).

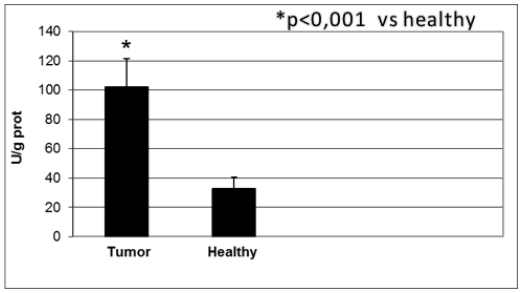


Figure 6. *XO values in tissue of the patients with PC and control tissue*

DISCUSSION

Benign prostatic hyperplasia (BPH), as well as chronic prostatitis (CP), remain diseases with an insufficiently understood etiopathogenesis, where the therapeutic approach is not yet fully defined. As one of the most common benign tumors, BPH is treated based on the severity of symptoms throughout a patient's lifetime (19). A large number of patients suffer from prostatitis, which ranks among the most common conditions encountered in urology (20). Prostatitis poses a particular diagnostic and therapeutic challenge when there is no confirmed bacterial colonization in prostatic tissue, yet inflammatory infiltrates are present. A key question is whether the pathogenetic mechanisms connect these benign conditions to PC, a tumor commonly diagnosed in men over 50 years of age (21). Is there a potential association between these three diseases and increased OS levels?

The prostate gland is very sensitive to free radicals due to the high lipid content of its cellular membranes and glandular capsule. The assessment of lipid peroxidation is commonly performed by measuring TBARS, which serve both as toxic molecules and biomarkers of OS (22). Our findings demonstrate that patients with BPH have significantly elevated TBARS levels compared to healthy controls (Figure 1), supporting the involvement of OS in BPH progression (23,24). It has been proposed that BPH might represent an immune-mediated condition characterized by pronounced inflammation (25,26). Chronic inflammation within the prostate contributes to disruptions in sex steroid hormone balance. Furthermore, infections can promote infiltration of immune cells, including macrophages and neutrophils, into prostatic tissue. These immune cells produce reactive oxygen and nitrogen species, which can overwhelm antioxidant defences and trigger OS. Chronic oxidative damage to prostatic tissue initiates compensatory cell proliferation, which eventually results in hyperplastic growth and the development of prostatic adenoma (27,28).

The most significant indicator of OS's systemic effect and prostate injury is likely the level of AOPPs. Our study demonstrated that AOPP concentration is higher in the serum of patients with BPH when compared to healthy individuals (Figure 2). Hong Yan Li et al. (29) demonstrated that in a remnant kidney model, elevated levels of AOPPs were associated with accelerated renal injury progression, as indicated by significant increases in tubular fibrosis and glomerulosclerosis. The direct toxic effect of AOPP was further supported by experiments showing that its administration raised urinary protein excretion in sham-operated rats (30). Moreover, chronic exposure to AOPP in this kidney model was found to elevate TBARS levels and diminish antioxidant defences (29). Free radicals can also lead to direct damage to DNA and accelerate apoptosis, processes that may contribute to cellular hyperplasia (31).

In our study, we also included patients with CP type III, characterized by inflammation in prostate fluid without the presence of bacteria. Disruptions in redox balance trigger numerous oxidative-reductive reactions that are fundamental to the pathophysiology of inflammation. Multiple studies have shown that OS plays a significant role in the onset and progression of chronic inflammation, contributing to the pathogenesis of various chronic conditions, including chronic prostatitis (32,33). Our findings indicated that serum levels of both TBARS and AOPP were significantly elevated in patients with chronic prostatitis (CP) compared to healthy individuals (Figure 1 and 2). ROS can also be generated by inflammatory cells in CP (34). The superoxide anion radical, released by phagocytes recruited to inflammatory sites, is considered a primary radical in the process of the cellular and tissue injury in these regions. This process can also lead to apoptosis dysregulation in many chronic inflammatory diseases (35). A possible mechanism underlying the increased OS levels in non-bacterial inflammatory prostatitis (category IIIa) is that inflammatory reactions can trigger inflammatory cells to generate and release numerous inflammatory mediators, such as inflammatory cytokines, cytochrome P450, and NADPH cytochrome P450. These factors may contribute to metabolic disturbances in the hypoxanthine-xanthine oxidase system, leading to the production of free radicals. Xanthine oxidoreductase (XOR), a pivotal enzyme in this system, can convert between its dehydrogenase and oxidase forms, catalysing the transformation of hypoxanthine and xanthine into uric acid while producing highly reactive superoxide anion radicals (O^{2-}) (36).

Therefore, XO activity is considered a significant source of free radical generation. The role of XO-induced ROS production has been implicated in the pathogenesis of ischemic injuries affecting the intestines, liver, and kidneys (37). XOR is essential for urate biosynthesis, and our previous research demonstrated that XO activity substantially contributes to ROS production in experimental renal injury (38). XO has been linked to both ischemic damage and fibrotic processes (39). In our investigation, we noticed a statistically significant elevation of XO activity in the serum of patients with CP and benign BPH when compared to healthy controls (Figure 3). Additionally, inflammatory cells can stimulate enzymes and factors such as cyclooxygenase-2 (40), nuclear factor kappa B (NF-κB) (41), inducible nitric oxide synthase, as well as various oxidants and pro-inflammatory cytokines, leading to increased generation of free radicals including superoxide anion ($^{02-}$), hydroxyl radicals (OH), nitric oxide (NO), and hydrogen peroxide (H2O2) (42). Our results are in accordance with the results of Person et al. (43), who, in a parallel double-blind controlled study, demonstrated increased XO activity in prostatic fluid. They also found that administering allopurinol to patients for 240 days led to a decrease in subjective symptoms and a reduction in prostatic secretion of xanthine and urate.

In our previous article, we reported a high level of XO activity and OS in samples of PC (44). In this investigation, the results are similar. Concentrations of TBARS and AOPP are higher when compared to healthy prostate tissue (Figure 4 and 5), and prostate tissue exhibits higher XO activity (Figure 6). We can associate enhanced XO activity with the transformation of XO from its dehydrogenase form to its oxidase form, a process driven by the reversible oxidation of thiol groups. An additional potential mechanism involves irreversible proteolytic damage triggered by increased peroxynitrite levels (45).

To explore the impact of XO activity on increased OS, we measured the levels of prooxidants and the degree of oxidative modifications in lipids and proteins. TBARS, as end products of lipid peroxidation, are highly electrophilic molecules capable of reacting with cellular nucleophiles to form DNA adducts and oligomers. They also bind to nucleic acids, creating adducts with deoxyguanosine, deoxyadenosine, and deoxycytidine (46). TBARS-DNA oxidation products have been reported to exert pro-mutagenic effects, inducing genetic changes in oncogenes and tumor suppressor genes within human tumors (47). In the present study, TBARS concentrations were significantly elevated in the serum of patients with BPH and CP, as well as in cancerous tissue compared to healthy controls. These results are consistent with findings by Yilmaz et al. (48), who observed increased lipid peroxidation. Furthermore, protein oxidation levels were higher in PC tissue relative to healthy tissue. AOPPs may arise through the oxidation of specific amino acid side chains by aldehydes generated during lipid peroxidation, serving as early and reversible markers of protein oxidation. Pande et al. (49) also reported elevated AOPP levels in PC patients compared to healthy individuals. These results suggest a strong connection between overall OS in the body and the localized prooxidant conditions within tumor tissue, reinforcing the idea that the male reproductive system preserves a redox-controlled microenvironment essential for maintaining redox balance. OS may represent a common pathogenic mechanism linking BPH, CP, and PC. Despite these results, certain limitations of the study should be recognized. The redox parameters assessed can only be deemed meaningful after excluding other conditions linked with OS. OS is undoubtedly only one of several mechanisms contributing to tumor development. Our research provides a foundation for larger clinical trials aimed at exploring the role of redox biomarkers in a wider cohort of patients with prostate disorders. Future studies should also evaluate the potential therapeutic benefits of antioxidant treatments across the spectrum of prostate diseases, from BPH to cancer.

CONCLUSION

The findings of this study demonstrate that the levels of lipid peroxides and AOPP are markedly increased in patients with BPH, CP, and PC, implying that systemic OS contributes to the development of these diseases. XO activity appears to be a potential producer of ROS in the prostate and may serve as a link between inflammation and carcinogenesis. These findings support the potential therapeutic role of XO inhibitors and antioxidants as adjuvant treatments for prostate diseases.

Acknowledgements

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Statement of Ethics

The research adhered to the principles of the Declaration of Helsinki and received approval from the Ethics Committee of the Medical Faculty in Niš (Decision No. 12-8818-2/8) on September 23, 2020.

Competing Interest

The authors declare no relevant conflicts of interest.

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AFMN BIOMEDICINE

ORIGINAL ARTICLE

CHARACTERISTICS AND MANAGEMENT OF PATIENTS WITH MULTIPLE MYELOMA: A SINGLE-CENTER EXPERIENCE

Ina Konstantinović[1] **Irena Ćojbašić**[1,2] **Miodrag Vučić**[1,2]

[1]Clinic of Hematology, Allergology and Clinical Immunology, University Clinical Center Niš, Niš, Serbia [2]Department of Internal Medicine, University of Niš Faculty of Medicine, Niš, Serbia

Multiple myeloma is the second most common hematological malignancy, in which pathological plasma cells secrete monoclonal proteins, ultimately leading to end-organ damage. Albumin, β2-microglobulin, and LDH are considered highly relevant biomarkers for the diagnosis and prognosis of this disease. Different treatment options are available depending on age and eligibility for autologous stem cell transplantation.

The aim of this study was to investigate basic characteristics, prognostic biomarkers, and the use of different treatment protocols in newly diagnosed multiple myeloma patients.

The study included 50 patients with newly diagnosed multiple myeloma. Data were collected from their medical records, and the statistical analysis was performed using the SPSS 15.0 program. The average age of patients at the time of diagnosis was 64, with a predominance of female patients. The most common type of myeloma was IgG kappa. More than half of the patients had an advanced stage of multiple myeloma and a high-risk disease, according to the prognostic score at the time of the diagnosis. The results showed that almost all patients had elevated levels of β2-microglobulin. The most commonly used protocols in younger patients eligible for transplantation were VTD and CTD, whereas patients who were not suitable for transplantation were treated with melphalan-based protocols.

The results of this study indicate that basic characteristics, prognostic biomarkers, and the treatment modalities used in newly diagnosed multiple myeloma patients are similar to those described in global clinical practice.

Keywords: multiple myeloma, prognosis, treatment

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Correspondence to:
Ina Konstantinović
Clinic of Hematology, Allergology and Clinical Immunology
University Clinical Center Niš
Bulevar dr Zorana Đinđića 48, Niš, Serbia
E-mail: inakon97@gmail.com

INTRODUCTION

Multiple myeloma (MM) is a hematological disorder characterized by the abnormal proliferation of plasma cells, which results in the pathological production of monoclonal proteins and end-organ damage (1). Multiple myeloma most commonly occurs in older people, with the median age at the time of the diagnosis being 69. It is 1.5 times more common in men than women. The highest incidence of the disease is in Australia, Western parts of Europe, and the United States. In 2018, there were more than 160,000 cases of MM worldwide. Also, in 2018, it was estimated that approximately 106,000 people died as a result of the disease (2).

Historically, end-organ damage in MM was once referred to as CRAB (hypercalcemia, renal failure, anemia, and bone lesions). More than two-thirds of patients with MM have bone pain and anemia at the time of diagnosis. Immunosuppression is a common state in patients with MM, due to the abnormal synthesis and function of immunoglobulins often accompanied by decreased complement activity. Patients usually have relative lymphocytosis, Rouleaux formation. Sometimes eosinophilia can be present as well (3). In MM patients, there are both structural and functional abnormalities of platelets which often cause bleeding, although some patients tend to develop thrombotic complications (3).

The most common type of M protein in patients with MM is IgG, followed by IgA and light chain type of the disease, and rarely other types of immunoglobulins. Only 15% of patients have MM with overproduction of light chains. This group of patients has a poorer prognosis compared to those with immunoglobulins as M protein, as well as a higher incidence of bone lesions, renal failure, and amyloidosis (4). The most commonly used staging system for MM was established by Durie-Salmon (5). It includes levels of M protein as well as CRAB criteria. At the beginning of the 21th century, a new staging system was developed. It was named the International Staging System (ISS) and it is calculated based on albumin and β2 microglobulin levels (5). One of the most important biomarkers in MM is β2-microglobulin, and its concentration in the serum correlates with the size of the tumor mass. Its values at the time of the diagnosis can predict the response to treatment and disease outcome (6). According to the ISS score, patients can be divided into three stages with different median overall survival (MOS): stage 1 (MOS—62 months), stage 2 (MOS—44 months), and stage 3 (MOS—29 months) (7). LDH is another significant biomarker and its increased levels indicate high proliferation rate, aggressiveness of the disease as well as the presence of tumor mass (7).

According to The National Multiple Myeloma Diagnostic and Therapeutic Guidelines, the first-line treatment for patients with newly diagnosed MM is largely based on whether the patient is eligible for autologous stem cell transplantation (ASCT). Key factors in deciding if a patient is eligible for ASCT are age, comorbidities, and overall performance status. Patients who are candidates for ASCT usually start treatment with four to six cycles of protocols which include a proteasome inhibitor in combination with immunomodulator or cytostatic drug. The most commonly used protocols are VTD (thalidomide, bortezomib, dexamethasone), CVD (cyclophosphamide, bortezomib, dexamethasone), VRD (lenalidomide, bortezomib, dexamethasone), PAD (bortezomib, doxorubicin, dexamethasone), CTD (thalidomide, cyclophosphamide and dexamethasone) and TAD (thalidomide, doxorubicin and dexamethasone) (8). In patients with high-risk disease (ISS score 2 or 3), it is recommended to use VTD or VRD protocol because they contain both an immunomodulator and a proteasome inhibitor (8). Older patients or those who are not eligible for ASCT are usually treated with VTD or VRD, or with protocols which contain melphalan in combination with an immunomodulator or a proteasome inhibitor such as MPT (thalidomide, melphalan and prednisone) or MPV (bortezomib, melphalan and prednisolone) (8).

The aim of this study was to determine whether basic characteristics, prognostic biomarkers and the treatment modalities used in newly diagnosed multiple myeloma coincide with those that are globally recognized.

METHODS

This research was conducted as a retrospective study. All data were obtained from the medical records of 50 newly diagnosed MM patients who were treated at the Clinic of Hematology, Allergology and Clinical Immunology, University Clinical Center Niš. The subjects were patients diagnosed with MM and treated between 2021 and 2023. The values of biomarkers used in the paper were the ones recorded at the time of the diagnosis.

Multiple myeloma was diagnosed according to the guidelines of the International Myeloma Working Group (9). The main diagnostic criteria for MM are ≥ 10% clonal plasma cells in the bone marrow and at least one of the

following myeloma-defining events: end-organ damage (hypercalcemia, renal insufficiency, anemia, osteolytic bone lesions), > 1 focal lesion detected on MRI, serum free light chain ratio ≥ 100 mg/L and ≥ 60% of bone marrow plasma cells (9).

For clinical staging and prognosis of patients with MM, Durie-Salmon and ISS staging systems were used. According to the ISS staging system, all patients were divided into three stages (8).

All types of treatment protocols used for multiple myeloma patients in this study were in accordance with The National Multiple Myeloma Diagnostic and Therapeutic Guidelines (8).

Statistical analysis was performed using SPSS version 15.0 (Statistical Package for the Social Sciences, Chicago, IL, USA). The results are shown through mean values and standard deviation (SD). The Student's t-test was used to test the statistical significance of biomarkers ($p < 0,01$). This study was approved by the Ethics Committee of the University Clinical Center Niš, Serbia (date: December 12, 2024; number: 37288/7). The study was conducted in accordance with the Declaration of Helsinki.

RESULTS

This study included 50 newly diagnosed multiple myeloma patients. There were 24 men (48%) and 26 women (52%). The average age of patients at the time of making the diagnosis was 64.22 ± 10.35 years (64.46 ± 10.78 for men and 64 ± 10.14 for women). There was no statistically significant difference in age between men and women (Table 1). The youngest patient was 39 and the oldest patient was 86 at the time of the diagnosis. There were 4 patients (8%) below the age of 50.

In 60% of patients, the levels of β2 microglobulin were higher than 5.5 mg/L, 14% had levels higher than 3.5 mg/L and lower than 5.5 mg/L, whereas in 26% of patients β2 microglobulin was below 3.5 mg/L at the time of the diagnosis (Figure 1). The mean value of β2 microglobulin was 11.8 ± 12 mg/L. The mean value of LDH at the presentation was 380.48 ± 175.41 U/L. LDH levels were elevated in 28% of patients. The mean value of albumin was 31.72 ± 5.61 g/L, and its levels were < 35 g/L in 74% of patients at the time of the diagnosis. There were no statistically significant differences in the parameters examined between genders ($p < 0.01$) (Table 1).

Table 1. *Age and biomarker concentrations at the time of establishing MM diagnosis*

Parameter	All patients	Men	Women	p
Number	50	24	26	-
Age	64.22 ± 10.35	64.46 ± 10.78	64 ± 10.14	p = 0.88
β2 microglobulin	11.8 ± 12.5	10.62 ± 10.32	12.9 ± 14.34	p = 0.52
Albumin	31.72 ± 5.61	30.96 ± 4.6	32.38 ± 6.48	p = 0.38
LDH	380.48 ± 175.41	365.13 ± 199.15	396.85 ± 152.35	p = 0.53

X±SD – mean value ± standard deviation; p – probability value

The most frequent type of myeloma was IgG kappa (42%), followed by IgG lambda(28%), IgAkappa (10%), kappa and lambda light chain MM (10%), and IgA lambda (8%). Only onepatient had the biclonal type of MM (IgG kappa and lambda) (Figure 2). According to the Durie-Salmon staging system, 34% were stagedas IIIA, 32% wereIIIB, 14% were IIA, 10% were IIB, and 10% were IA. There were no patients staged as IB (Figure 3). Most patients had ISS 3 (66%), 24% of patients had ISS 2, and 10% of patients had ISS 1 at the time of the diagnosis (Figure 4).

The treatment started in 45 patients right after establishing MM diagnosis. One patient did not meet the requirements for the beginning of the treatment. In four patients, it was decided to begin the treatment with high doses of dexamethasone due to the impaired kidney function, before making definite decision about which MM treatment protocol should be used. Out of those 45 patients who began their treatment right after establishing MM diagnosis, 42.2% of them received VTD, followed by CTD (24.4%), CVD(9%), TAD(2.2%), and Vel-Dex(2.2%). In older patients and/or those who were not candidates for ASCT, melphalan-based protocols were used, most commonly MPT (13.4%), followed by MP (4.4%) and MPV (2.2%) (Figure 5).

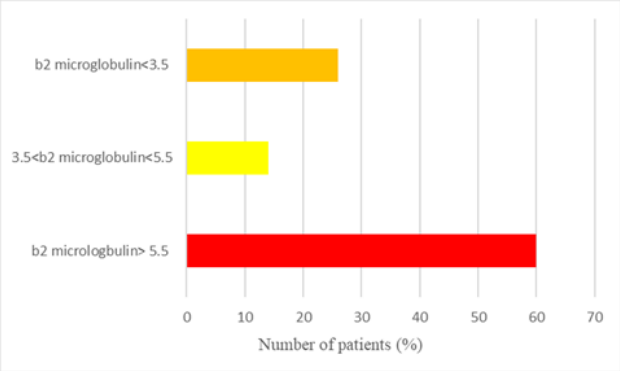

Figure 1. *Levels of β2 microglobulin (mg/L) in newly diagnosed MM patients*

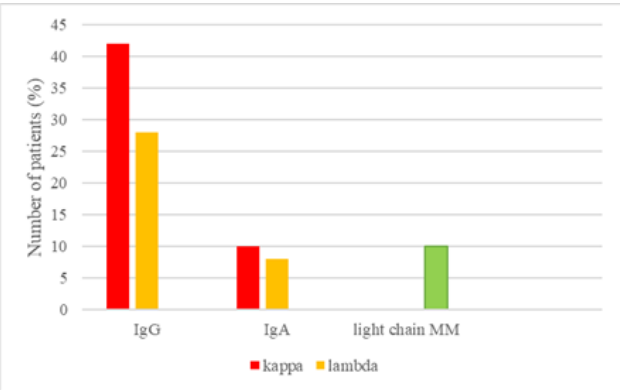

Figure 2. *Types of multiple myeloma*

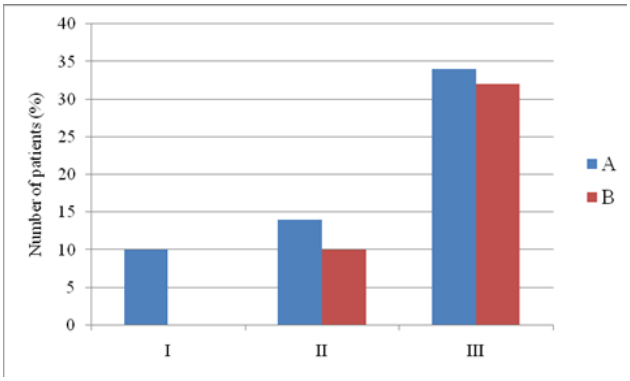

Figure 3. *Stages of MM according to Durie Salmon staging system*

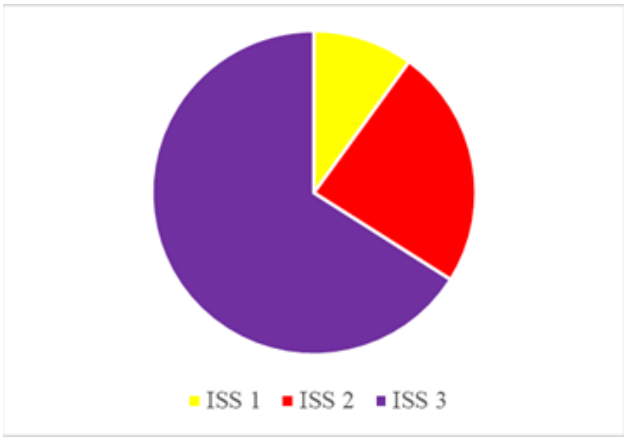

Figure 4. *ISS score at the time of the MM diagnosis*

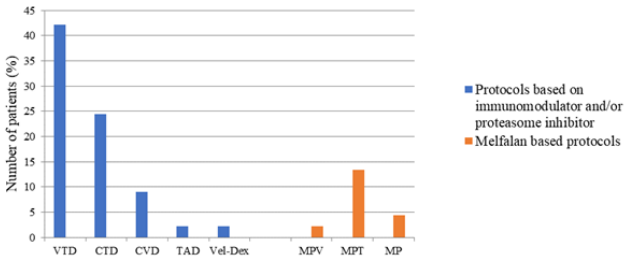

Figure 5. *Protocols used in newly diagnosed MM patients*

DISCUSSION

Multiple myeloma is the second most common blood cancer and it accounts for 1% of all malignancies (10). According to the International Agency for Research on Cancer, in 2020, there were 474 newly diagnosed MM cases in Serbia (226 men and 248 women). The results from the same year showed that there were 332 death cases (160 men and 172 women) from this disease in the whole country (11). Multiple myeloma is 1.5 times more common in males than females (2). In our study, there were 4% more women than men newly diagnosed with MM.

The average age of patients with MM diagnosis is 69 worldwide, with approximately 40% of patients being older than 75 at the time of making the diagnosis (12). In this study, the average age at the time of establishing the MM diagnosis was 64.22 ± 10.35, with 14% of patients being 75 or older. This is in correlation with the global median age for MM diagnosis. It is very unusual to diagnose MM in younger patients, and it seems to occur more frequently in males than in females (10). One study

reported that almost half of the patients diagnosed with MM younger than 40 had low-risk disease and better overall survival rate, probably due to a lower number of comorbidities and better ability to tolerate treatment protocols (13). Other studies have also confirmed that patients with MM below the age of 50 were mostly males with lower ISS score and Durie-Salmon stage, more favorable prognostic features, longer life expectancy, and better response to different lines of therapy (14). In our study, there were four patients (8%) younger than 50 at the time of the diagnosis, three males, and one female. The female patient had ISS 1 and was staged as IA. Two of the male patients had ISS 3 and were staged as IIIA and IIIB, respectively, whereas the third male patient had ISS 2 and was staged as IIA.

Multiple myeloma can be classified into several different types depending on which immunoglobulin and/or light chain is mostly present: IgG (52%), IgA (21%), light chains only (16%), biclonal (2%), and other forms (IgM, IgD and IgE which are extremely rare) (15). Our study demonstrated similar findings, showing that the most common type was IgG (70%), followed by IgA (18%) and kappa and lambda light chain MM (10%). Only one patient (2%) had the biclonal type of MM (IgG kappa and lambda). IgA type of MM is thought to have a poorer prognosis compared to IgG type. In about 15% of patients, MM cells secrete only light chains of immunoglobulins (kappa or lambda) (4). These free light chains are detectable in the serum by using the light chain assay, which has greatly replaced the quantification of Bence Jones proteins in urine (16). Light chains MM is more common in younger patients and has a worse prognosis compared to IgG and IgA types (17). Biclonal gammopathies are extremely rare and account for less than 5% of all MM patients. They are more symptomatic compared to their monoclonal counterpart. However, there are no significant differences in prognosis and how the patients respond to therapy (18).

Durie-Salmon score was the first established staging system for MM. It includes levels of M protein as well as CRAB criteria, and it is still widely used in clinical practice worldwide. Further substaging into A and B is based on the levels of serum creatinine (cut off value 177 µmol/l) (6,8). In our study, most patients were staged as IIIA (34%) and IIIB (32%). A 10-year period prospective study found that median survival for stage I was 53 months, 31 months for stage II, and 24 months for stage III. There was a significant difference in survival period between substages of stage III, with IIIA having median survival of 38 months and IIIB 18 months (19). Calculating ISS before the beginning of the treatment is very important because this score can help determine the overall prognosis of the disease. According to a major study that collected data from 11 worldwide multicenter clinical studies, out of 3,060 individuals, 38% had ISS 1, 38% had ISS 2, and 24% had ISS 3 (7). Another study found that 29% of the 1,516 newly diagnosed myeloma patients had ISS 1, 38% had ISS 2, and 33% had ISS 3 (20). However, in this study, 66% of patients had ISS 3, 24% of patients had ISS 2 and 10% of patients had ISS 1 at the time of the diagnosis. Both Durie-Salmon score and ISS are effective in differentiating patients with worse prognosis from those with better prognosis and are widely used in clinical practice (21).

One of the most significant biomarkers in both the diagnosis and prognosis of MM is β2 microglobulin. Its levels are usually elevated in MM patients at the presentation of the disease (7). Also, β2 microglobulin is closely related to kidney function. Studies reported that β2 microglobulin levels were significantly higher in patients with poor renal function and GFR lower than 60 mL/min at the time of diagnosis (22). In this study, 88% of patients had elevated levels of β2 microglobulin at the time of diagnosis. In 60% of patients, β2 microglobulin levels were higher than 5.5 mg/L, indicating a high-risk disease (ISS 3).

The levels of albumin in serum are also considered to be a relevant prognostic factor in MM. The levels of albumin lower than 35 g/L are associated with a more severe form of the disease and poorer performance status, as well as kidney failure (23). In this study, 74% of patients had serum albumin levels lower than 35 g/L at the time of the diagnosis. LDH is another important biomarker, and its high levels correlate with the aggressiveness of the tumor (8). However, its levels are rarely elevated at the time of the diagnosis (24). This is in correlation with the results obtained in this study, where only 28% of patients had elevated levels of LDH. As the disease progresses, LDH levels usually rise, which is an indicator of a poorer prognosis (24). Other studies have also confirmed the normality of LDH at the time of the diagnosis and its elevation with the progression of the disease. It is suggested that LDH levels in combination with ISS score could be very useful in determining the overall survival of MM patients (25).

The most commonly used treatment protocols for a newly diagnosed MM patients eligible for ASCT are VTD, CVD, PAD and VRD, whereas melphalan-based protocols such as MPV and MPT are usually used in patients who are not eligible for ASCT (8). In this study, most patients were treated with VTD as the first-line protocol (42.2%),

followed by CTD (24.4%) and MPT (13.4%). Other first-line protocols included were CVD, MPV, TAD, MP and VEL-Dex. This choice of treatment complies with worldwide recommendations for the management of multiple myeloma. According to the EHA-ESMO clinical practice guidelines for treatment of multiple myeloma, the most frequently used first-line protocols for ASCT eligible patients are VTD, VRD and VCD. Prior to 2019, MPV and Rd (lenalidomide and dexamethasone) were the recommended treatment protocols for patients who were not candidates for ASCT. However, compared to MPV and Rd, VRD has shown a notable advantage in clinical trials, and it is currently the recommended first-line protocol for those ineligible for ASCT (26). Autologous stem cell transplantation is still considered to be the treatment of choice for young and fit older patients. The upper age limit for ASCT is usually 65, although sometimes doctors can approve transplantation in older patients if they are fit and with few comorbidities. The best induction protocols for patients who are eligible for ASCT are those based on three different medications. VTD protocol has been proven to be a treatment of choice before transplantation, with usually 4-6 cycles given before the procedure (27). The combination of proteasome inhibitors and immunomodulatory drugs is still considered the best therapy option for both ASCT eligible and ineligible patients (8). The introduction of daratumumab, an anti-CD38 monoclonal antibody, used in combination with proteasome inhibitors and immunomodulatory drugs, has significantly improved the depth of response and progression-free survival rate both in newly diagnosed and relapse/refractory MM patients (28). The combination of daratumumab and VTD is approved as the first option for the induction therapy for patients who are candidates for ASCT (26). The first-line treatment of choice for individuals ineligible for ASCT is the combination of daratumumab with MPV and Rd; VRD is given only in case that the aforementioned combinations are not available. (26). However, it should be noted that it is required to develop a specific treatment plan for each multiple myeloma patient individually, based on their diagnostic and prognostic characteristics.

The results of this retrospective study indicate that basic characteristics of the newly diagnosed multiple myeloma patients correlate with those which are described in the global clinical practice. Biomarkers measured at the clinical presentation of the disease and protocols used for the treatment of MM patients are fully consistent with the internationally accepted guidelines for the diagnosis and treatment of multiple myeloma.

Acknowledgements

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Statement of Ethics

This study was approved by the Ethics Committee of the University Clinical Center Niš, Serbia (date: December 12, 2024; number: 37288/7).

Competing Interest

The authors declare no relevant conflicts of interest.

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AFMN BIOMEDICINE

ORIGINAL ARTICLE

NUTRITIONAL KNOWLEDGE AMONG PROSPECTIVE HEALTHCARE PROFESSIONALS

Bojana Miladinović[1] Milica Milutinović[1] Dušanka Kitić[1] Milica Randjelović[1] Nikolina Savić[1] Katarina Simonović[2] Maja Nikolić[3,4]

[1]Department of Pharmacy, University of Niš Faculty of Medicine, Niš, Serbia [2] Clinic of Dermatovenereology, University Clinical Center Niš, Niš, Serbia [3] Department of Social Medicine and Hygiene with Medical Ecology, University of Niš Faculty of Medicine, Niš, Serbia [4] Public Health Institute Niš, Niš, Serbia

Unhealthy eating habits and a lack of physical activity are well-known risk factors for chronic illnesses, and many countries in transition have struggled to make progress in addressing diet-related non-communicable diseases. Education on diet and nutrition plays a crucial role in public health, and healthcare professionals are essential in promoting healthy eating habits. However, many healthcare providers feel insufficiently prepared to offer such guidance due to inadequate nutrition education and training during medical studies.

This research aimed to assess nutritional knowledge among pharmacy and medical students in south-eastern Serbia. A cross-sectional survey was conducted among undergraduate pharmacy and medicine students in the fall of 2022. The instrument for data collection was a questionnaire composed of 16 questions with multiple-choice questions about the role of specific nutrients, with only one correct answer per question. Nutritional knowledge was assessed based on the percentage of correct answers and classified as excellent (65-100%), moderate (45-64.9%), or poor (less than 45%). A total of 379 undergraduate students of pharmacy (n = 151) and medicine (n = 228) completed the survey. The majority of participants had poor nutritional knowledge, with an average score of 44.86%. The knowledge level varied significantly by study group ($p = 0.039$) and year of study ($p < 0.001$). Only 1.85% of students reported having quite good nutritional knowledge.

These findings indicate multiple deficiencies in the nutrition knowledge of medical and pharmacy students. As students' nutritional knowledge requires improvement, the development of specific programs that promote healthy lifestyle behaviors among students is strongly recommended. Additionally, medical students should receive more comprehensive, curriculum-based instruction in nutrition.

Keywords: nutrition, students, higher education, health sciences, questionnaire

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Correspondence to:
Maja Nikolić
Department of Social Medicine and Hygiene with Medical Ecology
University of Niš Faculty of Medicine
Bulevar dr Zorana Đinđića 81, Niš, Serbia
E-mail: maja.nikolic@medfak.ni.ac.rs

INTRODUCTION

Inadequate eating habits and physical inactivity are well-established and important risk factors contributing to chronic diseases (1). Unfortunately, Serbia, a country in transition, has shown limited progress toward achieving the diet-related non-communicable disease targets (2-4). Most people in Serbia do not have healthy eating habits due to excessive animal fat and red meat consumption, and low intake of fish and seafood, whole grains, vegetables, and fruit. At present, Serbia is in a stage of transition where nutrition-related non-communicable diseases, including coronary heart disease, stroke, cancer, obesity, type 2 diabetes, and hypertension, are dominating adult morbidity and mortality and are very high or growing rapidly in prevalence. Among other measures, nutrition education could help reduce and better manage these health problems in the Serbian population. (5).

Healthcare workers play the most significant role in promoting healthy dietary patterns (6,7), and patients consider physicians among the most credible sources of nutrition information (8). However, most of them feel unqualified to discuss dietary recommendations with patients, which may be attributed to inadequate education and preparedness during their training at the faculty. The barriers to providing dietary counselling are a lack of time and competence, and a lack of knowledge and resources (9). Several studies suggest that well-educated medical students feel prepared to discuss specific dietary recommendations with patients (10,11), but globally, nutrition has been underrepresented in the curriculum of many medical schools (12-14).

The COVID-19 pandemic is expected to increase the risk of all forms of malnutrition, so it is very important to know and understand the recommendations for food intake. The role of competent health workers in providing nutrition advice is crucial (15).

Identifying weaknesses in nutrition knowledge among medical students may provide guidance to improve their nutrition practice in the future. Therefore, the aim of the present study was to assess the nutritional knowledge of pharmacy and medicine students in south-eastern Serbia.

METHODS

Research Design

A cross-sectional study was conducted on randomly selected medicine and pharmacy students recruited from the University of Niš Faculty of Medicine between November 1 and December 15, 2022. The sample size for the study was calculated assuming that we would receive a large effect size for two independent study groups, d = 0.08, α = 0.05, and a study power of 95.18%. Based on those parameters, each of the groups required at least 42 subjects. The sample size calculation was performed in G*Power 3.1.9.2.

Study population

A total of 379 undergraduate pharmacy (n = 151) and medicine (n = 228) students in the 2nd to 4th years completed the survey. The criteria for inclusion in the study included a willingness to participate voluntarily in the study, being 2nd-4th year students, and not having passed the advanced nutrition course in the teaching curriculum. The exclusion criterion was the failure to complete the questionnaire anonymously. Before data collection, the participants received information about the objective of the study and signed the written consent form. The informed consent document explicitly informed potential participants that their responses had no bearing on academic performance.

The sample was stratified by study group, year of study, and sex.

Research instruments and data collection

The authors followed the latest version of the Declaration of Helsinki given by the World Medical Association, and the study protocol was approved by the Ethical Committee of the Public Health Institute Niš (No. 12-3785/5).

The study used a self-administered nutrition knowledge questionnaire, designed based on the report of Allaffi et al. (2012) and adjusted appropriately (16). The questionnaire was distributed during breaks from regular theoretical/practical lectures.

The questionnaire was pre-tested on ten students (5 males and 5 females) to assess comprehension of questions. The questionnaire (Supplementary data) was hand-delivered and contained 16 short questions about basic nutrition knowledge.

The first part of the questionnaire consisted of a short demographic form, and respondents were asked for their gender, height, weight, study group, and year of studies. Body mass index (BMI) was calculated using the values of weight (in kg) and height (in m) according to the student's statements.

The second section of the questionnaire consisted of 16 questions with multiple-choice answers, each of four possible answers (one correct), about the role of certain nutrients (Supplementary data). The answers were systemized according to the study program— Pharmacy or Medicine, and according to the year of study. All questions were equally weighted (incorrect or missing answer/correct answer).
Supplementary material is available at AFMN Biomedicine online.

Data analysis

The obtained data were systematized and processed in SPSS Statistical Software Package version 25.0. The basic sociodemographic characteristics of participants were estimated using descriptive statistics. Descriptive statistics are presented as frequencies, percentages, means, and standard deviations. Scores of the correct answers were calculated by summing the number of correct responses of all questions divided by the total questions multiplied by 100 to yield a percentage score. Nutritional knowledge was calculated as a percentage of correct responses and ranked as excellent 65-100%, moderate 45-64.9%, and poor < 45%. Differences in scores between study programs were examined using independent-samples t-tests, whereas differences across years of study were analyzed using one-way ANOVA followed by Duncan's post-hoc test. Statistical significance was defined as $p < 0.05$.

RESULTS

A total of 400 undergraduate medical students were recruited, and 379 valid questionnaires were received (response rate 94.75%).
The demographic data of the study participants are presented in Table 1.
Among 379 respondents, 151 were pharmacy students, and 228 were medicine students. A total of 99 were males (26.1%), aged between 20 to 23 years. Most students had a BMI within a normal range (18.5 to 24.99), and only 6 students were obese.
Among the 151 pharmacy students, 76 were in their 2nd year of study, and 75 were in their 3rd year of study. Of the 228 respondents, 35 were students of medicine in their 2nd year of study, 77 were in 3rd year of study, and 116 were in 4th year of study.
The average score for correctly answered questions in the overall sample was 44.86% (Table 2).

Table 1. *Socio-demographic data of students (N = 379)*

	Pharmacy students n (%)	Medicine students n (%)	Total N (%)
Age			
20	76 (50.3)	35 (15.4)	111 (29.3)
21	75(49.7)	77 (33.8)	152 (40.1)
22	-	116 (50.8)	116 (30.6)
Sex			
Female	121 (80.1)	159 (69.7)	280 (73.9)
Male	30 (19.9)	69 (30.3)	99 (26.1)
Body mass index (BMI)			
Less than 18.5	9 (6.0)	21 (9.2)	30 (7.9)
18.5 to 24.99	118 (78.2)	181(79.4)	299 (78.9)
25 to 29.99	23 (15.2)	21 (9.2)	44 (11.6)
30 and more	1 (0.7)	5 (2.2)	6 (1.6)

There were significant statistical differences in the average score between pharmacy students and students of medicine (averaging 44.77% vs. 44.11%, respectively, $p < 0.001$). Most students (more than 50%) gave the correct answer to eight questions, while poor knowledge was shown in the other eight questions. A statistically significant difference in the knowledge of Pharmacy and Medicine students in individual questions is also shown in Table 2.
Pharmacy students showed good knowledge of nutrients that can help prevent thrombosis, the relationship between protein intake and calcium metabolism, the usefulness of dietary fiber in lowering blood cholesterol levels, hydrogenated fats, and roles and sources of vitamins and minerals. Medicine students answered correctly a high percentage of the questions about the number of fat calories, deficiency of vitamin B1, and the preventive effects of fruits and vegetables on different types of cancer.
Regarding the percent of correct answers (Table 3), significant differences were found among students of different study years ($p < 0.001$) related to eight questions. The students of higher study years have been shown to have more nutritional knowledge.
The obtained results and statistical analysis showed the differences in nutrition knowledge between students of the second, third, and fourth years of Pharmacy and Medicine. The biggest differences exist among the fourth-year students, where pharmacy students showed better

nutrition knowledge than medicine students.

Differences in nutrition knowledge scores by different years of study and study programs are shown in Table 4. All groups had an almost equal mean percentage of correctly answered questions (the second year of study about 38%; the third year about 45% and the fourth year about 50%), but with statistically significant difference between pharmacy and medicine students in all three years ($p < 0.001$).

Most participants had poor nutritional knowledge—54.35% (23.79% of males, 76.21% of females). The proportion of participants with moderate nutritional knowledge was higher among males (68.07%) compared with females (31.93%). Only seven students (all female) were rated as excellent in their nutritional knowledge (Table 5).

Table 2. *Percentages of correct answers among pharmacy and medicine students (N = 379)*

	No. (%)	No. (%)		
	Overall	**Pharmacy students**	**Medicine students**	**p-value**
Q1.	243 (64.1)	83 (55)	160 (70.2)	**p < 0.001**
Q2.	159 (42)	70 (46.4)	89 (39)	**p = 0.019**
Q3.	105 (27.7)	46 (30.5)	59 (25.9)	p > 0.05
Q4.	132 (34.8)	54 (35.8)	78 (21.1)	p > 0.05
Q5.	75 (19.8)	27 (17.9)	48 (21.1)	p > 0.05
Q6.	206 (54.4)	94 (62.6)	112 (49.1)	**p < 0.001**
Q7.	216 (57)	94 (62.3)	122 (53.5)	**p = 0.001**
Q8.	116 (30.6)	40 (26.5)	76 (33.3)	**p = 0.004**
Q9.	152 (40.1)	68 (45)	84 (36.8)	**p = 0.007**
Q10.	38 (10)	12 (7.9)	26 (11.4)	**p = 0.027**
Q11.	191 (50.4)	72 (47.7)	119 (52.2)	p > 0.05
Q12.	182 (48)	69 (45.7)	113 (49.6)	p > 0.05
Q13.	198 (52.2)	70 (46.4)	128 (56.1)	p > 0.05
Q14.	221 (58.3)	103 (68.2)	118 (51.8)	**p < 0.001**
Q15.	208 (54.9)	69 (45.7)	139 (61)	**p = 0.027**
Q16.	278 (73.4)	110 (72.8)	168 (73.7)	p > 0.05
Average score for correctly answered questions (%)	44.86	44.77	44.11	**p < 0.001**

Based on the independent-samples t-test. Q1 – Q16: questions from the nutrition knowledge questionnaire.

DISCUSSION

To the best of our knowledge, this was the first study on the nutritional knowledge of medical students in Serbia. The response rate in the current study (94.75%) was much higher than reported by authors in similar studies (17,18) where the response rates were 36% and 40%, respectively.

We found that medical students had generally poor nutrition knowledge and the average score for correctly answered questions in the present study (44.86%) was slightly lower than observed in another survey of nutrition knowledge (49.6-57.14%) (18). Nutritional KAP (Knowledge Attitude and Practices) scores of Chinese students were significantly higher ($p < 0.05$) than international students (19). Medical students from Lisboa, Portugal, have a good baseline nutrition knowledge, which is evident in the fact that for each question at least 50% of the students gave the right answer (20).

Table 3. *Percentages (%) of correct answers among 2nd, 3rd and 4th year students (N = 379)*

	Overall	2nd Year Students	3rd Year Students	4th Year Students	p value
Q1.	243 (64.1)	54 (48.6)[a]	105 (69.1)[b]	84 (72.4)[b]	**p < 0.001**
Q2.	159 (42)	46 (41.1)[a]	59 (38.8)[a]	54 (46.6)[a]	p > 0.05
Q3.	105 (27.7)	41 (36.9)[a]	40 (26.3)[a,b]	24 (20.7)[b]	**p = 0.021**
Q4.	132 (34.8)	34 (30.6)[a]	55 (36.2)[a]	43 (37.1)[a]	p > 0.05
Q5.	75 (19.8)	15 (13.5)[a]	44 (28.9)[b]	16 (13.8)[a]	**p = 0.001**
Q6.	206 (54.4)	51 (45.9)[a]	79 (52)[a,b]	76 (65.5)[b]	**p = 0.009**
Q7.	216 (57)	62 (55.9)[a]	82 (53.9)[a]	72 (62.1)[a]	p > 0.05
Q8.	116 (30.6)	22 (19.8)[a]	49 (32.2)[a,b]	45 (38.8)[b]	**p = 0.007**
Q9.	152 (40.1)	45 (40.5)[a]	55 (36.2)[a]	52 (44.8)[a]	p > 0.05
Q10.	38 (10)	6 (5.4)[a]	15 (9.9)[a]	17 (14.7)[a]	p > 0.05
Q11.	191 (50.4)	49 (44.1)[a]	83 (54.6)[a]	59 (50.9)[a]	p > 0.05
Q12.	182 (48)	53 (47.7)[a]	69 (45.4)[a]	60 (51.7)[a]	p > 0.05
Q13.	198 (52.2)	39 (35.1)[a]	93 (61.2)[b]	66 (56.9)[b]	**p < 0.001**
Q14.	221 (58.3)	59 (53.2)[a]	90 (59.2)[a]	72 (62.1)[a]	p > 0.05
Q15.	208 (54.9)	44 (39.6)[a]	93 (61.2)[b]	71 (61.2)[b]	**p = 0.001**
Q16.	278 (73.4)	71 (64)[a]	110 (72.4)[a,b]	97 (83.6)[b]	**p = 0.003**

[a,b] Different letters indicate a significant difference in the knowledge of students in the second, third and fourth year of study, based on ANOVA test (Duncan post-hoc) ($p < 0.05$). Q1 – Q16: questions from the nutrition knowledge questionnaire.

Table 4. *Percentages (%) of correct answers to the nutrition knowledge questions among pharmacy and medicine students according to different year of study (N = 379)*

	2nd Year			3rd Year			4th Year		
	PS	MS	p-value	PS	MS	p-value	PS	MS	p-value
Average score for correctly answered questions (%)	38.88	38.96	**p < 0.001**	45.49	46.35	**p < 0.001**	51.48	47.21	**p < 0.001**

Based on the independent-samples t-test. Q1 – Q16: questions from the nutrition knowledge questionnaire. PS - Pharmacy Students, MS – Medicine Students

Table 5. *Average score for correctly answered questions*

Group	Gender	Poor (%)	Moderate (%)	Excellent (%)	Total (%)
Medical students	M F	36 88	36 65	0 4	72 157
Pharmacy students	M F	13 69	17 48	0 3	30 120
Total	379 (100%)	206 (54.35%)[a]	166 (43.8%)[a]	7 (1.85%)[b]	379 (100%) **p = 0.039**

[a,b] Different letters denote significant difference among students knowledge based on the ANOVA test (Duncan post-hoc) ($p < 0.05$)

The results of the survey conducted in 2017 at one University in Croatia showed that the students of pharmacy possessed greater knowledge regarding dietary supplementations than the students of medicine and dentistry ($p < 0.001$). The survey included 506 students that take the survey anonymously and thus showed their points of view and knowledge on dietary supplements. Pharmacists are those who mainly recommend supplements to patients and the patients themselves often address them for advice (21).

The research taken in California supports the recommendations that increased education of health workers on diet, in general, has a significant role in preventing and treating chronic diseases (22).

Trabucco and associates conducted a comparative study on the nutritional knowledge of medical students who practice sports across two European medical faculties. The questionnaire used in their research differed from the one employed in our study, and the statistically significant difference was observed between the groups regarding the sources from which students obtained their nutritional information (23).

The studies about nutrition knowledge and attitude mainly examine physicians and pharmacists (24-26). There is also a need to improve their nutritional knowledge in many countries, and the story should start from the beginning of education.

The results showed statistical significance in the knowledge of medicine and pharmacy students ($p < 0.001$). Pharmacy students had better knowledge about antioxidants ($p < 0.001$). Only 48% of students were familiar with the connection between cancer development and nutrition. As the students of both study groups will work with patients who need proper dietary advice and who need to know about the importance of certain nutrients for health and the prevention of many diseases, it is necessary to work on increasing the level of their knowledge. Good knowledge in these areas will provide greater security and confidence to future doctors and pharmacists when advising patients.

Despite the benefits of training, aware and informed healthcare workers for the delivery of evidence-based nutrition, the new curriculum will need to overcome some challenges in this field and the motivation for change.

A review of the literature on nutrition knowledge and attitudes among medical students produced many findings; however, existing studies considered specific and different types of questions (dietary supplements are most prevalent, whole grain consumption, surgery, aging, dietary sodium intake, sports nutrition, breastfeeding, and many more). The medical student population is considered one of the finest student populations, which are highly valued due to the position they take in society and the well-being of the people they counsel and treat (27-29).

This study has some limitations that must be noted. The generalizability of these results should be used carefully, i.e., results represent the perspectives of students from only one university in Serbia. Our further research could be focused on expanding the study to include a greater number of universities.

This study showed that most of the pharmacy and medicine students lacked nutritional knowledge. Nutrition and its outcomes should have more attention in the medical and pharmacy program curriculum in Serbia to provide better patient prevention and health care to future physicians and pharmacists.

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Statement of Ethics

This study protocol was reviewed and approved by the Ethical Committee of the Public Health Institute, Niš, approval number 12-3785/5-8.

Competing Interest

The authors declare no relevant conflicts of interest.

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AFMN BIOMEDICINE

ORIGINAL ARTICLE

DIAGNOSTIC ACCURACY OF IMMATURE PLATELET FRACTION PARAMETERS IN DISTINGUISHING IMMUNE THROMBOCYTOPENIC PURPURA FROM HYPOPRODUCTIVE THROMBOCYTOPENIA IN PEDIATRIC PATIENTS: A CROSS-SECTIONAL STUDY

Shahla Ansari **Zahra Soheilirad**

Department of Pediatric Hematology-Oncology, Ali Asghar Children's Hospital, School of Medicine, Iran University of Medical Sciences, Tehran, Iran

Immune thrombocytopenic purpura (ITP) is a common cause of thrombocytopenia in pediatric patients, often requiring differentiation from hypoproductive thrombocytopenia. This study assesses the diagnostic accuracy of immature platelet fraction (IPF) parameters in distinguishing ITP from hypoproductive thrombocytopenia.

A cross-sectional study was conducted at Hazrat Ali Asghar Hospital, Tehran, enrolling 165 children under 18 years with confirmed thrombocytopenia (platelet count <150 × 10^9/L). Participants were selected based on specific inclusion and exclusion criteria. Data were collected using a pre-designed checklist, and complete blood counts with a particular focus on IPF measurements were performed using the BC-6800 automated hematology analyzer. Clinical diagnoses of ITP and hypoproductive thrombocytopenia were confirmed via bone marrow examination and immunophenotyping. Statistical analyses included receiver operating characteristic (ROC) curve analysis to evaluate the diagnostic performance of IPF.

The mean IPF for patients with ITP was significantly higher than for those without (30.5 ± 12.9 vs. 7.4 ± 3.4, $P < 0.001$). The ROC curve analysis yielded an area under the curve (AUC) of 0.96, indicating excellent discriminative ability of IPF. The optimal cutoff value for IPF was determined to be 11.20%, with a sensitivity of 0.97 and specificity of 0.94. Multivariate analysis confirmed an independent association between higher IPF levels and ITP diagnosis (adjusted odds ratio = 1.25, 95% CI: 1.10 - 1.43, $P < 0.001$).

The IPF parameter is a reliable and sensitive diagnostic tool for differentiating ITP from hypoproductive thrombocytopenia in pediatric patients. This study supports the integration of IPF measurement into clinical practice to enhance diagnostic accuracy in children with thrombocytopenia.

Keywords: thrombocytopenia, immature platelet fraction, immune thrombocytopenic purpura, pediatrics

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Correspondence to:
Zahra Soheilirad
Department of Pediatric Hematology-Oncology
Ali Asghar Children's Hospital, School of Medicine
Iran University of Medical Sciences, Tehran, Iran
E-mail: drsoheilirad@hotmail.com

INTRODUCTION

Pediatric hematology addresses a range of blood disorders, with thrombocytopenia being one of the most prevalent conditions encountered in clinical practice. Among the various etiologies, immune thrombocytopenic purpura (ITP) and hypoproductive thrombocytopenia stand out due to their distinct pathophysiological mechanisms and implications for management (1). ITP is characterized by an autoimmune process leading to increased platelet destruction, while hypoproductive thrombocytopenia results from insufficient platelet production, often linked to bone marrow disorders or systemic diseases (2). The differentiation between these two forms of thrombocytopenia is crucial as it directly impacts treatment strategies and clinical outcomes (3, 4). While bone marrow examination remains the gold standard for distinguishing between these two causes, recent literature indicates that immature platelet fraction (IPF) parameters could be valuable biomarkers in this context (5). IPF is a parameter that reflects the proportion of young, newly released platelets in circulation, measured using fluorescence flow cytometry. This parameter quantifies the percentage of immature platelets out of the total platelet population, which can provide insight into the bone marrow's response to thrombocytopenia. Elevated IPF levels have been associated with increased platelet production, indicating a compensatory response in conditions like ITP, while lower levels may suggest inadequate production in hypoproductive thrombocytopenia (6, 7).

Despite these advances, the majority of existing research has focused on adult populations, leaving a significant gap in our understanding of IPF dynamics in children. Pediatric studies are limited, and the applicability of adult findings to the pediatric population remains uncertain (6, 8). This underrepresentation necessitates a focused investigation into IPF parameters in children, particularly to understand their role in distinguishing between ITP and hypoproductive thrombocytopenia (7, 9). The present study aims to address this gap by comparing IPF parameters in children diagnosed with ITP to those with hypoproductive thrombocytopenia. We hypothesize that distinct differences in IPF profiles will emerge between these groups, reflecting their underlying pathophysiologies. By elucidating these differences, our research seeks to enhance diagnostic accuracy and inform clinical decision-making in pediatric hematology. This study not only aims to validate the utility of IPF as a diagnostic tool but also aspires to contribute novel insights that may lead to improved management strategies for children suffering from thrombocytopenia.

METHODS

Study design and subjects

This diagnostic accuracy cross-sectional study was conducted at Hazrat Ali Asghar Hospital in Tehran, Iran, involving children under 18 years of age, with thrombocytopenia (defined as a platelet count of less than 150 × 10^9/L). The inclusion criteria were being under 18 years of age and having thrombocytopenia confirmed by two independent samples. Children who had received platelet transfusions in the previous five days, as well as those with conditions that could affect IPF values—such as sepsis, other inflammatory diseases, or the use of antiplatelet medications—were excluded from the study.

Data collection and measurements

Data were collected using census sampling from October 2020 to August 2022 via a pre-designed checklist that included demographic and clinical information, such as age, sex, and the underlying cause of thrombocytopenia.

Blood samples were collected to perform complete blood counts (CBC) for all patients. A volume of 3 cc of venous blood was drawn from each patient into CBC tubes containing EDTA as an anticoagulant. The samples were gently inverted five times to ensure proper mixing. All samples were stored at room temperature and analyzed within eight hours of collection. The CBC was analyzed using the BC-6800 auto hematology analyzer, employing fluorescence flow cytometry with the So Cube technology. In this process, the reticulocyte mode of the device was activated, allowing for the staining of mRNA within the cytoplasm of platelets. Fluorescence intensity was assessed using a laser, with higher fluorescence indicating a greater presence of immature platelets. Approximately 1 ml of peripheral blood was specifically allocated for measuring the IPF. The IPF was calculated based on the proportion of immature platelets and expressed as a percentage of the total platelets (10). Additionally, CBC indices such as WBC, Hb, platelet count, and RDW were measured for all patients.

The clinical diagnoses of ITP and hypo-productive thrombocytopenia were confirmed based on bone marrow examination and immunophenotyping, in accordance with the standard criteria outlined by international guidelines (11-13).

Sample size estimation

Sample size estimation was based on a presumed effect size of 0.3, a power of 95%, and a type I error of 5% using G*Power software version 3.1.3 with the sample size calculation formula for correlational studies. The total adequate sample size was determined to be 166 participants.

Ethical considerations

This study was conducted in accordance with the principles outlined in the Declaration of Helsinki. Ethical approval was obtained from the Institutional Review Board at Iran University of Medical Sciences (code: IR.IUMS.FMD.REC.1400.543). After providing a detailed explanation of the study's objectives and procedures, informed consent was obtained from the parents or guardians of all participating children, with assent sought from children when appropriate, in accordance with age and cognitive ability. Confidentiality of participants' data was strictly maintained throughout the study. All collected data were anonymized, and identifying information was securely stored and accessible only to authorized personnel. In this study, participants were not subjected to any additional risks beyond those associated with standard clinical practice.

Statistical analysis

Statistical analysis was performed using the SPSS software, version 16.0 (SPSS Inc., Chicago, IL, USA). Descriptive statistics were calculated for all relevant variables, including demographic data and laboratory results. Continuous variables were expressed as mean ± standard deviation (SD), while categorical variables were presented as frequencies and percentages. To assess the diagnostic accuracy of IPF parameters in distinguishing between ITP and hypoproductive thrombocytopenia, receiver operating characteristic (ROC) curve analysis was conducted. The area under the ROC curve (AUC) was calculated to evaluate the discriminative ability of IPF values between the two groups (with and without ITP). Optimal cut-off values for IPF parameters were established using Youden's index, which maximizes the sum of sensitivity and specificity. Sensitivity, specificity, positive predictive value, and negative predictive value were calculated for these cut-off values. To further validate these findings, a multivariate analysis using logistic regression was conducted to assess the impact of age, gender, and disease type on IPF. Additionally, Pearson correlation coefficients were computed to explore the relationship between IPF and other continuous variables. To assess differences in IPF means across groups (e.g., ITP, ALL, Aplastic Anemia), one-way ANOVA was utilized, followed by post-hoc Tukey's HSD test for pairwise comparisons. Statistical significance was set at a p value of < 0.05 for all tests.

RESULTS

Participants

A total of 165 children under 18 years of age diagnosed with thrombocytopenia participated in this study. Among the participants, 93.94% were aged 1 to 10 years, and 51.52% were male. The majority of participants (52.73%) presented to the hospital due to bruising, and 46.06% were diagnosed with ITP. Individual and disease characteristics of the participants are summarized in Table 1.

Table 1. *Demographic and clinical characteristics of participants (N=165)*

Characteristic		Frequency (n)	Percentage (%)
Age	<1	3	1.82
	1-10	156	94.55
	>10	6	3.64
Gender	Male	85	51.52
	Female	80	48.48
Reason for hospital visit	Bruise	87	52.73
	Common cold	1	0.61
	Epistaxis	23	13.94
	Fever	34	20.61
	Pain	4	2.42
	Weakness and lethargy	18	10.91
Type of disease	ITP	76	46.06
	ALL	66	40.00
	Aplastic anemia	23	13.94

IPF in the diagnosis of ITP

As indicated in Table 2, the mean IPF for patients with ITP was significantly higher than that for patients without ITP (30.5 ± 12.9 vs. 7.4 ± 3.4, $P < 0.001$). Receiver operating characteristic (ROC) curve analysis was performed to evaluate the diagnostic accuracy of IPF in distinguishing between ITP and hypoproductive thrombocytopenia. The area under the ROC curve (AUC) was calculated to be 0.96, indicating excellent discriminative ability. The optimal cutoff value for IPF, determined using the Youden's index, was 11.20%, with a sensitivity of 0.97 and specificity of 0.94 (Table 3, Figure 1).

Table 2. *IPF Mean values in patients with and without ITP*

Group	Mean IPF (SD)	P-value
Without ITP	7.4 (3.4)	<0.001
With ITP	30.5 (12.9)	

Table 3. *Diagnostic accuracy of IPF for ITP diagnosis*

Parameter	Value
Optimal cut-off value	11.20%
Sensitivity	0.97
Specificity	0.94
Positive predictive value	0.85
Negative predictive value	0.98
Area under ROC curve (AUC)	0.96

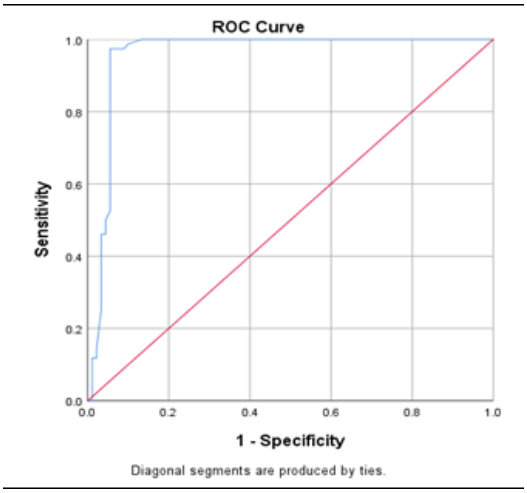

Figure 1. *Receiver operating characteristic (ROC) curve for distinguishing ITP from other causes of hypoproductive thrombocytopenia*

Patient characteristics and their relationship with IPF

Table 4 summarizes the relationship between patient characteristics and mean IPF values. A significant difference in mean IPF was observed across different disease types ($P < 0.001$). Additionally, significant correlations were identified between IPF and key laboratory parameters, including WBC, Hb, and platelet count. A negative correlation was observed between IPF and WBC ($R = -0.239$, $P = 0.002$), with patients exhibiting low WBC levels (<4 x 10^9/L) showing a higher mean IPF (25.00 ± 10.00) compared to those with high WBC levels (>11 x 10^9/L) (12.00 ± 8.00). Similarly, a negative correlation was found between IPF and platelet count ($R = -0.290$, $P < 0.001$). Patients with low platelet counts (<50 x 10^9/L) demonstrated a significantly higher mean IPF (27.00 ± 12.00) than those with high platelet counts (>100 x 10^9/L) (8.5 ± 5.00). In contrast, a positive correlation was identified between Hb and IPF ($R = 0.288$, $P < 0.001$). Patients with low Hb levels (<8 g/dL) had a lower mean IPF (10.50 ± 7.50), while those with high Hb levels (>12 g/dL) exhibited a higher mean IPF (28.00 ± 11.50). These findings highlight that IPF levels increase with rising Hb levels, suggesting a distinct relationship between bone marrow activity and hemoglobin concentration.

To further validate these findings, a multivariate analysis using logistic regression was conducted to assess the impact of age, gender, and disease type on IPF. After adjusting for these variables, the association between IPF and ITP remained significant (adjusted odds ratio = 1.25, 95% CI: 1.10 - 1.43, $P < 0.001$), confirming that higher IPF levels are independently associated with the diagnosis of ITP.

Table 4. *IPF by individual and disease characteristics*

Characteristic		Frequency (n)	IPF Mean (SD)	P-value
Age	<1	3	16.00 (20.00)	0.380
	1-10	156	18.20 (16.20)	
	>10	6	9.50 (13.40)	
Sex	Male	85	19.20 (15.60)	0.274
	Female	80	16.40 (16.60)	
Type of Disease	ITP	76	30.50 (12.90)	<0.001
	ALL	66	5.60 (3.30)	

IPF: Immature platelet fraction; ITP: Immune thrombocytopenia; ALL: Acute lymphoblastic leukemia.

DISCUSSION

Differentiating ITP from hypoproductive thrombocytopenia in pediatric patients is crucial for effective management and treatment outcomes. Our study adds to this important area by demonstrating the diagnostic accuracy of IPF as a reliable biomarker. Our findings indicate that the mean IPF for patients with ITP was significantly higher than for those without ITP. This result aligns with recent findings by Asghar et al. (14), who reported that IPF was significantly elevated in hyperdestructive thrombocytopenia compared to hypoproductive conditions, with a median IPF of 21% in

the hyperdestructive group versus 6.5% in the hypoproductive group, emphasizing IPF's role in reflecting the underlying pathophysiology associated with increased platelet destruction. Moreover, our ROC curve analysis revealed an AUC of 0.96, indicating excellent discriminative ability for IPF in differentiating ITP from hypoproductive thrombocytopenia. This finding is consistent with Adly et al. (15), who suggested that IPF could serve as a rapid and inexpensive automated marker for distinguishing thrombocytopenia due to destruction versus production issues. They identified an optimal cut-off value for IPF at 9.4%, achieving a sensitivity of 88% and specificity of 85.7%, which supports our findings that higher IPF values correlate with ITP. Supporting our results, Goel et al. (5) found that the mean IPF was significantly higher in patients with increased peripheral destruction of platelets (13.4%) compared to those with decreased production (4.6%). Their study established an optimal cutoff of 5.95% for differentiating the two conditions, with a sensitivity of 88% and specificity of 75.9%. This further underscores the utility of IPF as a diagnostic tool in clinical settings. Additionally, our study aligns with findings from a recent study conducted by Shetageri et al. (16), which evaluated the utility of platelet indices in differentiating hyperdestructive from hypoproductive thrombocytopenia. Their prospective analysis involving 315 cases of hyperdestructive thrombocytopenia and 54 cases of hypoproductive thrombocytopenia revealed that mean platelet indices were significantly higher in the hyperdestructive group, reinforcing the importance of distinguishing these conditions.

Another significant contribution to this discussion is the work by McDonnell et al. (9), which highlighted the utility of IPF in differentiating ITP from bone marrow failure (BMF) and predicting bleeding risk. Their retrospective study involving 272 patients found that an IPF greater than 5.2% effectively distinguished ITP from BMF with 93% sensitivity and 91% specificity. They also noted that lower absolute immature platelet numbers correlated with severe bleeding, indicating that IPF measurement not only aids in diagnosis but also in identifying patients at increased risk of hemorrhage. This finding emphasizes the potential clinical utility of IPF in managing pediatric ITP patients. Negash et al. (17) corroborate our findings by showing that platelet indices, including IPF, were significantly higher in ITP patients compared to those with hypoproductive thrombocytopenia. Their study demonstrated that IPF exhibited strong predictive capacities, suggesting that this parameter may enhance diagnostic accuracy and reduce the need for invasive procedures like bone marrow aspiration.

In our study, we observed significant negative correlations between IPF and laboratory parameters such as WBC, Hb, and platelet count. This relationship suggests that IPF may reflect the overall hematologic status of the patient, which is consistent with the observations by Strauss et al. (10), who noted elevated IPF in acute ITP cases, indicating accelerated platelet turnover. Furthermore, Ali et al. (18) demonstrated that the IPF% was significantly higher in cases of increased platelet consumption, reinforcing the diagnostic value of IPF in diverse clinical scenarios. The multivariate analysis in our study confirmed that higher IPF levels were independently associated with the diagnosis of ITP, highlighting its practical utility in clinical settings. Jeon et al. (7) also found that IPF could effectively distinguish ITP from other causes of thrombocytopenia, reporting a median IPF of 8.7% in the ITP group versus 5.1% in non-ITP cases. They proposed a diagnostic predictive scoring model that considers IPF as a critical parameter, further emphasizing the importance of IPF in clinical practice. A recent systematic review and meta-analysis by Walle et al. (19) further supports our findings, revealing that the pooled mean value of IPF significantly increased in ITP patients compared to those with hypoproductive thrombocytopenia. The pooled sensitivity and specificity of IPF in differentiating the conditions were notable, reinforcing its role as a reliable, non-invasive diagnostic tool that can enhance clinical decision-making.

Despite the promising findings of our diagnostic accuracy cross-sectional study, several limitations should be acknowledged. First, the cross-sectional design limits the ability to establish causality, as it captures data at a single point in time. Second, while our sample size was adequate for the analysis, the distribution of cases between hyperdestructive and hypoproductive thrombocytopenia was unequal, which may affect the robustness of comparisons. Additionally, variations in laboratory techniques and equipment across different institutions may impact the consistency of IPF measurements. Lastly, the study focused primarily on IPF; thus, other relevant clinical factors and platelet parameters that may influence the diagnosis were not extensively analyzed.

In conclusion, our findings validate the utility of IPF as a non-invasive diagnostic tool in pediatric patients with thrombocytopenia. By elucidating distinct differences in IPF profiles between ITP and hypoproductive thrombocytopenia, we aim to enhance diagnostic

accuracy and inform clinical decision-making. Future research should focus on larger, multicenter studies to further confirm these findings and explore the implications of IPF in guiding treatment strategies for children with thrombocytopenia. This approach will not only improve patient outcomes but also pave the way for standardized diagnostic protocols in thrombocytopenia management.

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Statement of Ethics

This study protocol was reviewed and approved by the Institutional Review Board at Iran University of Medical Sciences, approval number IR.IUMS.FMD.REC.1400.543, issued on December 14, 2021.

Competing Interest

The authors declare no relevant conflicts of interest.

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AFMN BIOMEDICINE

ORIGINAL ARTICLE

VITILIGO-LIKE DEPIGMENTATION AS A PREDICTOR OF PROLONGED RESPONSE TO IMMUNE CHECKPOINT INHIBITORS IN PATIENTS WITH ADVANCED MELANOMA: A SINGLE-CENTER EXPERIENCE

Aleksandar Popović[1] **Ana Stojković**[1] **Ana Cvetanović**[1,2] **Andrija Jović**[3] **Milica Radić**[1,2] **Irena Conić**[1,2] **Ivan Petković**[1,2]

[1]Clinic of Oncology, University Clinical Center Niš, Niš, Serbia [2]Department of Oncology, University of Niš Faculty of Medicine, Niš, Serbia [3]Clinic of Dermatovenerology, University Clinical Center Niš, Niš, Serbia

Immune checkpoint inhibitors (ICIs) have significantly improved outcomes in melanoma patients. Vitiligo-like depigmentation (VLD) is a well-documented adverse event of ICIs, which is generally well-tolerated and often associated with prolonged response to treatment. The aim of this study was to assess the effect of VLD occurrence on survival in melanoma patients treated with ICIs.

We conducted a retrospective analysis of unresectable stage III and stage IV melanoma patients treated with pembrolizumab or nivolumab at the University Clinical Center Niš from May 2017 to February 2024. The Chi-square or Fisher's exact test was used to evaluate categorical variables. Progression-free survival (PFS) and overall survival (OS) were estimated using the Kaplan–Meier method (95% CI; $p < 0.05$). Survival rates between patients with and without VLD were compared using a log-rank test ($p < 0.05$).

A total of 109 patients were included, of whom 22 patients developed VLD (20.2%). Median follow-up in the VLD group was 39.4 months, with 59% still alive and progression-free three years after treatment initiation. The overall response rate (ORR) was 72.7% vs. 29.9%, with 31.8% vs. 13.8% of complete responses in favor of the VLD subgroup. The occurrence of VLD was associated with significantly longer median PFS (8.148 vs. 52.862 months; $p = 0.0001$) and median OS (12.715 vs. not reached (NR) months; $p = 0.0001$).

Prediction of VLD occurrence is not currently possible; therefore, it cannot be considered a predictive parameter per se, but it can be associated with prolonged response to ICI treatment in patients who develop this adverse event.

Keywords: melanoma, vitiligo-like dermatitis, survival, immunotherapy

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Correspondence to:
Aleksandar Popović
Clinic of Oncology
University Clinical Center Niš
Bulevar dr Zorana Đinđića 48, Niš, Serbia
E-mail: popovic992@yahoo.com

INTRODUCTION

Metastatic melanoma is the most aggressive form of skin cancer with a poor prognosis, and its overall incidence is increasing rapidly, with the highest rates in northern countries in Europe, such as the Netherlands and Ireland (1). During the last 20 years, multiple approaches have resulted in a better understanding of tumor immunology and the genomic characteristics of melanoma. With the introduction of immune checkpoint inhibitors (ICIs) as new therapeutic options, survival for melanoma patients has immensely improved (2). Cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) and programmed cell death protein 1 (PD-1) are the most widely used ICIs (3). They both regulate the T-cell response. Although CTLA-4 muffles T-lymphocyte activation in the early phase of the immune response, PD1-mediated immune response plays a role in the later effector phase (4). Their T cell-mediated antitumor effect can also trigger a series of immune-related adverse events (irAEs), with skin toxicity being the most frequent. Cutaneous irAEs are heterogeneous and tend to be low-grade and well-tolerated. Vitiligo-like dermatitis (VLD) is a site-specific, well-tolerated irAE that affects melanoma patients predominantly treated with anti-PD-1 antibodies.

Despite a clear benefit for patients with advanced melanoma and the existence of long responders, a vast number of them (30-50%) fail to respond or develop resistance to treatment. Potential biomarkers associated with response to ICIs therapy in melanoma are widely researched, but only LDH has been introduced into everyday clinical practice (5-7).

Several irAEs have been associated with prolonged response to ICS, with VLD being one of them. The aim of this study was to assess the effect of VLD occurrence on progression-free survival (PFS) and overall survival (OS) in advanced melanoma patients treated with ICIs.

METHODS

We conducted a retrospective analysis among 109 unresectable stage III and stage IV melanoma patients treated with pembrolizumab or nivolumab at the University Clinical Center Niš from May 2017 to February 2024. The included patients had unresectable stage III and stage IV (metastatic) melanoma with Eastern Cooperative Oncology Group performance status (ECOG PS) 0-1 and at least one radiological assessment by Response Evaluation Criteria in Solid Tumors (RECIST 1.1) after treatment initiation. The Chi-square or Fisher's exact test were used to evaluate the categorical variables, as appropriate for the category size. PFS and OS were estimated using the Kaplan–Meier method (CI 95%; $p < 0.05$). Survival between the patients with or without VLD was compared using a log-rank ($p < 0.05$). Survival analyses were performed in SPSS v 26 (Chicago, IL, USA). Ethical approval for this study was obtained from the Institutional Ethics Committee (approval no. 13826, May 29, 2020).

RESULTS

A total of 109 patients were included, comprising 22 patients who developed VLD (20.2%). The median time of VLD onset was 10.44 months (range 3.94-31.15). There was no statistically significant difference in the occurrence of VLD based on gender ($p = 0.192$), mean age ($p = 0.99$), BRAF mutation status ($p = 0.206$), elevated LDH ($p = 0.582$), or ECOG PS ($p = 0.166$). Median follow-up in the VLD group was 39.4 months, with 59% still alive and progression-free three years after treatment initiation.

The overall response rate (ORR) was 72.7% vs 29.9%, with 31.8% vs. 13.8% of complete responses in favor of the VLD subgroup. Patient characteristics and VLD occurrence are summarized in Table 1. The occurrence of VLD was associated with significantly longer median PFS (8.148 vs. 52.862 months; $p = 0.0001$) (Figure 1), and median OS (12.715 vs. NR months; $p = 0.0001$) (Figure 2).

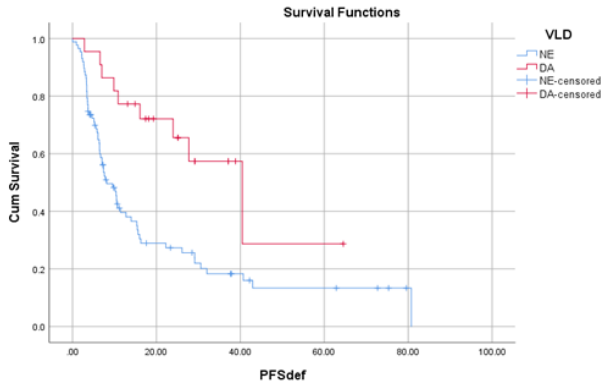
DISCUSSION

Immune checkpoint pathways keep the immune system in balance through the inhibition of T-cells. They adjust the duration and level of the immune response through downregulation of T cell response, therefore reducing unnecessary tissue damage (8). These pathways are used

Table 1. *Patient characteristics and VLD occurrence*

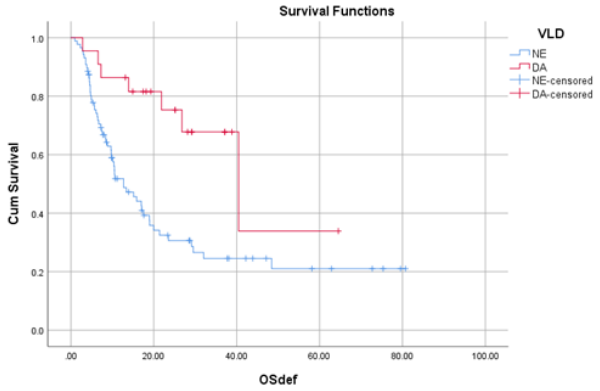
	VLD (n=22) n (%)	Non-VLD (n=87) n (%)	P value
Age (years, mean ± SD)	63.18 ± 12.316	65.6 ± 12.294	0.99
Sex			0.498
Male	16	52	
female	6	34	
ECOG PS			0.568
0	16	51	
1	6	36	
Elevated LDH			0.568
	31	8	
BRAF mutation status			0.206
BRAF mutant	26	4	
BRAF wild type	61	18	

VLD: Vitiligo-like dermatitis; SD: standard deviation; ECOG PS: Eastern Cooperative Oncology Group performance status; LDH: lactate dehydrogenase



VLD: Vitiligo-like dermatitis; PFS: Progression-free survival;

Figure 1. *Progression-free survival and VLD occurrence*



VLD: Vitiligo-like dermatitis; OS: Overall survival;

Figure 2. *Overall survival and VLD occurrence*

by malignant cells to evade normal immune responses by expressing ligands that overactivate them (9). Immune checkpoint inhibitors (ICIs) overcome this tumour-induced inhibition, creating a proinflammatory microenvironment potentially leading to an antitumor effect.

Initially, they were introduced in the treatment of advanced melanoma, and later in adjuvant and perioperative settings. Their mechanism of action led to the usage of nine, currently FDA-approved ICIs, in eighty-five treatment settings in many different cancers (10).

Currently, the most widely used ICIs in melanoma treatment are PD-1 antibodies pembrolizumab and nivolumab, CTLA-4 antibody ipilimumab, and lately, LAG-3 inhibitor relatlimab.

Due to their unique mechanism of action, new immune-related adverse events were observed, which differed from well-known and dose-dependent adverse events of conventional chemotherapeutic agents. Also, patients with preexisting autoimmune disorders should be approached in a precautionary manner (11). This is likely due to prolonged T-cell activation mostly, which can affect many organs. IrAEs involving skin can develop in 30–50% of the patients treated with ICIs, therefore making them the most frequent adverse event (12).

They can manifest a plethora of dermatological conditions ranging from mild, such as psoriasis, vitiligo, lichenoid dermatitis, and maculopapular rash, but also severe and life-threatening, such as Drug Reaction with Eosinophilia and Systemic Symptoms (DRESS), Stevens-Johnson and Acute generalised exanthematous pustulosis (AGEP) (13). An abundance of lymphocytes in the skin may be the reason why irAEs occur so often on the skin and can develop so early. Even though the median time to onset of skin toxicity is 4-6 weeks, they can occur more than a year after treatment initiation (14).

Although similar to vitiligo, which affects the general population, ICI-induced VLD shows different clinical and biological patterns (15). VLD has a progressive nature and tends to occur at an older age with bilateral distribution and confetti-like presentation (16).

Although VLD may have a certain psychosocial effect on patients, it is considered a benign adverse event of ICI treatment that is well tolerated. Prevalence of vitiligo in the general population ranges from 0.5-2%. On the other hand, VLD can occur in 2-25 % of melanoma patients treated with ICIs and is rarely described in other solid cancers (17). In addition, the occurrence of VLD is more often observed in patients treated with anti-PD-1 compared with CTLA-4 antibodies (13,18). In our analysis, VLD was observed in 20.2% of the patients treated with ICIs. The high percentage among our patients may be due to the inclusion of patients solely treated with anti-PD-1 agents and the fact that our analysis included only patients who had at least one RECIST evaluation and were, therefore, on treatment for at least three months.

Compared with other skin irAEs, VLD tends to develop later with a median time to onset of 6-9 months, which is sooner than reported in our paper (10.44 months), although this may be due to less frequent dermatological assessment and consequently later reporting of adverse events (19,20).

The occurrence of dermatological irAEs was more often observed in older populations treated with anti-PD-1 drugs. Still, in our case, there was no statistically significant difference in age when VLD and non-VLD groups were compared (21,22). Also, skin toxicity tends to be less

observed in the female population (23,24). In our study, there was a numerical but not a statistical significance, which is most likely due to a small number of patients included. As for ECOG PS, no impact was observed regarding the occurrence of irAEs, which is in correlation with our results (25). Regarding the BRAF mutational status, opposing results were observed. One analysis showed less frequent occurrence of VLD in patients bearing BRAF mutation, while no significant difference was observed in others (24,25). In our analysis, no difference in the occurrence of VLD regarding the BRAF mutational status was observed.

It is assumed that VLD develops due to CD8+ activations which target both melanoma cells and melanocytes due to their shared expression of melanocyte differentiation antigens. Therefore, it is hypothesized that VLD development can be a sign of immunogenic response and may predict response to ICI treatment.

The VLD occurrence was associated with better response in melanoma patients treated with anti-PD-1treatment (19). This was also observed in our analysis with almost two-and-a-half-fold higher ORR and a doubling of the number of complete responses to treatment.

Significant prolongation of PFS and OS was observed in several meta-analyses, which was also observed in our analysis with a very high statistical significance (26). This indicates that vitiligo, a relatively benign adverse event, can be a good clinical sign of a prolonged response to ICI treatment.

Interestingly, regression of ICI-induced VLD may be associated with disease progression; therefore regular dermatological assessments are of utmost importance (20).

Potential biomarkers for the prediction and early detection of irAEs are currently under investigation, and they can be both site-specific and non-specific. As for the skin-specific adverse events, there are few promising biomarkers. High levels of rheumatoid factor (RF) before treatment initiation showed a higher possibility of developing skin-specific irAEs (27). Also, circulating blood cell counts represent a potential and easily accessible biomarker for both adverse event prediction and response to treatment, but further studies are needed (23).

Although being an adverse event, VLD also presents a potential sign of a favorable outcome. Certain vitiligo-specific soluble biomarkers, such as CD25 and CXCL9 levels, and also the levels of regulatory T cells, were being assessed before and during ICI treatment, showing their possible usage in predicting the response. However, furthers studies are needed (28).

Although our study included a modest number of patients, it still presents an addition to real-world data regarding the ICI-treated patients.

The usage of ICI in everyday practice is growing each day, with their inclusion in the treatment of numerous malignancies, making them one of the most exploited and explored medicaments. Even though there is still a large proportion of patients who have an inadequate response to treatment, predictive biomarkers for treatment response are widely explored, with several of them showing promising results. Aside from predictive biomarkers of response, prediction of the duration of response is also clinically significant and could help us tailor treatment to the individual; therefore, avoiding overexposure to treatment and potential late toxicity. The occurrence of VLD is an easily observed adverse event and is associated with a favorable outcome in melanoma patients treated with ICIs, making it a very convenient surrogate biomarker of prolonged response to ICI treatment.

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Statement of Ethics

Ethical approval for this study was obtained from the Institutional Ethics Committee (approval no. 13826, May 29, 2020).

Competing Interest

The authors declare no relevant conflicts of interest.

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AFMN BIOMEDICINE

ORIGINAL ARTICLE

CRUCIAL ROLE OF PARENTS' ENGAGEMENT IN SCHOOL-BASED HEALTH EDUCATION

Roberta Marković

Public Health Institute Niš, University of Niš Faculty of Medicine, Niš, Serbia

Health education programs implemented in schools in Serbia should be improved methodologically, with special emphasis on intersectoral action and inter-organizational partnerships at all levels, which implies identifying the roles of children, teachers, parents, and all community representatives.

The aim of our study was to define and analyze the role of parent engagement in school-based health education in Serbia in order to clarify and propose possible improvements.

The focus group gathered nine health and non-health professionals in 2022 to interactively discuss previously defined topics related to health education in schools. The focus group was recorded, transcribed verbatim, coded, and analyzed by three research team members. In this qualitative study, a thematic analysis approach was implemented for data analysis.

Focus group participants stressed the advantages of the analyzed programs, as well as their weaknesses, regarding parental engagement in school-based health education. Parents are not involved in designing health education programs planned for their children, and are only sporadically involved in program activities. The evaluation of "Health-promoting schools' programs" showed that parental role was recognized theoretically, but there seems to be a lack of practical application, i.e., active involvement of parents, which is also supported by the results of our research.

Activities aimed at strengthening school-based health education should be directed towards advocating a parental role in all phases of health education program development and implementation.

Keywords: health education programs, schools, parental role

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Correspondence to:
Roberta Marković
Public Health Institute Niš, Serbia
University of Niš Faculty of Medicine
Bulevar dr Zorana Đinđića 50, Niš, Serbia
E-mail: robertarim@yahoo.com

INTRODUCTION

The parental role is crucial in the health education of children and development of desired behavior patterns that will lead to good and healthy life. Creating values, standards, and behavior in a basic social unit, such as the family, can generate longer-lasting important habits for health. The process started in the family continues in the school where, beside education, children should be enabled to acquire knowledge and skills that are required for good health (1). This can be achieved through well-designed, modern health policies and health education programs implemented in schools (2) and communities. Many European countries are implementing the recommendations of the "European Network of Health Promoting Schools" [ENHPS] project (3) with varying achievements (4); nevertheless, Serbia was not recruited for the program. In Serbia, health education in schools, for the last fifteen years, has been organized through two programs established throughout the country (program of health education in primary schools and program of health education in secondary schools). The general objectives of these programs refer to: improving school children's knowledge on healthy life styles, establishing attitudes and behavior in accordance with a healthy lifestyle, improving health education in schools, and achieving cooperation between the school, family, and community with the aim of improving children's' health. The content of health education programs includes the following topics: nutrition, physical activity, body care, health and the environment, development of skills to resist social pressure (smoking, alcohol, drugs), proper use of health services, reproductive health and AIDS, communication and relationships with others, environmental protection. Previous study indicated that the existing programs are only partially related to the health, educational needs and challenges that school children in Serbia are faced with. An extremely important need to improve the methodology of these programs' implementation, with special emphasis on intersectional action and inter-organizational partnerships at all levels was also pointed out (5). This implies identifying the role of children, teachers, parents and all community representatives in the health education process. The aim of our study was to define and analyze the role of parent engagement in school-based health education in Serbia in order to clarify and propose possible improvements.

METHODS

Methodology approach was based on the Focus group that was organized in December 2022 as a part of health education programs' evaluation. The evaluation covered the programs held for elementary and high-school children in the Nišava district in the period 2010-2020. The Focus group theme was "Health education for children and youth —vision of the future", and it was structured in order to facilitate an open dialogue regarding health education in schools, as well to promote possible improvements in a current and evidence-based manner. The focus group participants were nine professionals (six women and three men), from the Public Health sector, educational sector, NGOs, media, and local municipality sectors, with more than 15 years of professional experience in the field of interest, who were selected according to formerly defined criteria. The moderator of the Focus group was the Head of the Center for Health Promotion of Districts in the Public Health Institute. The Focus group was led in such a way to initiate productive interaction between all participants.
Since COVID-19 epidemic instructions were still in force, the Focus group was organized as a Zoom meeting lasting three hours. In advance, the supplementary material (6,7) as well as the Focus group agenda were sent to the Focus group participants. According to the existing scientific and specialized data, available health indicators, and good practice patterns from working with children and youth, the Focus group topics were defined in line with targeting a few fields; one of them related to stakeholder network and community role, encompassing the parental role. This study was aimed at the analysis of the Focus group findings related to parent's engagement in school-based health education.

Data analysis

After recording and being transcribed verbatim, an analysis was done according to a thematic analysis approach (8,9). When the preliminary categorization phase was done, three research team members read and coded each transcript in detail. A coding template grew through the analysis, expanding the key concepts. Themes were extracted, encoded, and selected as the smallest units, and afterwards, an interpretive analysis was done. Each of three researchers independently read the verbatim transcripts and noted keywords and important observations. Finally, the researchers joined, compared, and analyzed their independent remarks.

RESULTS

Concerning the identification of persons responsible for health education, the Focus group participants suggested, beside others, parents as crucial for children's health education. Regarding the role of parents for the analyzed health education programs, they defined the following:

The representative of the Public Health institute stated: "*Health education in primary and secondary schools, carried out in accordance with the defined methodology and reports on school-based health education, is delivered regularly. Reports submitted to the Public Health Institute do not contain data on parental participation in health education activities*".

The educational advisor from educational sector added: "*Parents are not part of any program activities. Organized educations for parents are very rare, and topics of interest are not systematically included*".

The pedagogist from the Municipality office stated: "*Parents participate in the work of the school through parent councils and through initiatives by teaching staff, but their role in health education at school is not recognized. Sometimes, parents do not participate due to pressure from children who feel uncomfortable and ashamed about their parents' involvement*". The pedagogist pointed out: "*Parents are deeply interested in their children's health and well-being, but often they do not have enough awareness and knowledge about risk factors, i.e., about protective factors, regarding health. Parents are not completely clear about the role of the school doctor or the role of the pediatrician in health education or developing good lifestyles*".

Representative of the Youth NGO remarked: "*There is a shift of responsibility from children to parents, from parents to teachers and educational institutions, and vice versa. There is an unspoken question of responsibility for health education*".

The educational advisor from the educational sector quoted: "*It is evidence-based that the support and involvement of parents in health education activities at school is a prerequisite for the success of health education programs. When parents are involved in the design of school health programs, the result is programs that are more tailored to the real needs of students. In our health education programs at school, parental participation is rare; there are individual activities, on voluntary individual basis, but we need parents systematically involved in needed activities*". She added: "*Due to previous experience, parental involvement is far more meaningful when achieved through partnerships with teachers. Also, students whose parents are involved in health promotion programs tend to have better academic achievements*".

The second representative from the Municipality noted: "*Parents' understanding of their role is a prerequisite for their willingness to engage in supportive parenting. Here, parental aspirations regarding their children's future careers are present, but not their self-awareness regarding active participation in health promotion*".

Representative of NGO that gathers media in the city pointed out: "*Transparency and effectiveness are also increased when parents help shape programs that foster the relation between the school and community, which should be supported by media activities*".

DISCUSSION

The results of the international "Health Behavior in School-Aged Children Survey", conducted in the Republic of Serbia in 2017/2018, which the Focus group participants received as preparation for discussion, indicate a high exposure of school children in Serbia to risk factors that can be prevented. The influence of many of the recognized risk factors depends on the capacity and role of parents in the health education of children, which begins in the family, at home, and continues in school. Those risk factors could be protective factors if parents manage them in an appropriate way (10). Parental role in this process is complex and multidimensional. In order to respond to a variety of children's needs, and specially children's health needs, they should have an adequate level of awareness, knowledge and skills (11), since parental self-efficacy was found to impact parenting capacities in health education.

One of our study findings is that parents are neither systematically involved in designing health education programs planned for their children nor involved in the implemented activities. Experience gained through Health Promoting Schools [HPS] emphasized that schools should organize health events based on healthy practices promoted in community and at home for families. Due to these HPS findings, after community surveys/health assessments, parents should be involved in: health education workshops, health checks and sports activities for parents and children, health festivals and cooking competitions (12). Parents should be informed about children's needs, consulted in the choice of methods, invited to participate in activities; a sense of belonging to

the program should be built, with understanding why it is good for their children and how to support them in the process of developing new healthy habits. Our study found these aspects completely missing in our programs. In analyses from Hong Kong HPS family role, parental role is just mentioned through recognized need to strengthen the relation between family and school and the community (13). It seems that this relation was still scarce in spite of defined HPS methodology. The study from Netherland pointed that parents observe health behavior promotion as a significant feature of their parenting role. Even when parents do not consider health behavior as a primary goal, they recognize health promotion issues as a routine in family life. In addition, parents identify the need to be supported by school and school teachers since teachers are recognized as key persons in developing healthy life styles. Some parents consider physical activity, cooking, food tasting, and sports activities a common concern between parents and teachers. Parents' insights of their influence on child's behavior are diverse, and the study proposes networking with healthcare professionals who could collaborate with parents and teachers in order to empower and support them in the process of health education (14).

Results of our study indicate that in spite of participation in parent councils, parents are not involved or recognized in health education programs. One of the pillars in HPS in Europe is a tendency to parental engagement as a tool to develop the sense of ownership, which is crucial for health education outcomes. This different cultural atmosphere is lacking in HPS Europe-wide, but the good example from Nederland showed a possible way: children have their lunch at home or bring lunch to eat at school; parents are involved in lunch preparation; the lunch succeeded in being a catalyst for wider school health promotion (15). Our Focus group participants highlighted that understanding the parental role is a precondition for their willingness to engage in supportive parenting and this is one of the pillars we should consider in the future. It is necessary to raise the level of parent's awareness regarding their role in the health education of school children, to present the importance of inclusion in school programs and activities, to consult them on topics and methods of work, and to invite them actively to participate, organize, and even lead activities and campaigns within the program. Ambitious goals relate to organizing the education for parents on healthy parenting, developing relationships with children throughout their upbringing, recognizing risks, and recognizing good, healthy choices for children (16). Parental role in health education in schools was recognized theoretically, but it seems that practical implementation was missing, and this important segment of health education strategies has been developed recently. A guide "Parents for Healthy Schools" from 2019 stressed the involvement of parents as "important strategy for getting schools to provide healthy school nutrition environments and services, opportunities for physical activity throughout the school day, tobacco-free environments, and health services and support for students with chronic health conditions". These guides outlined a frame and key materials, needed for school groups working with parent. They might be used to involve parents in "supporting a healthy school environment" (17).

Our Focus group participants pointed out the importance of parental involvement in the design of school-based health education. A precondition is the improvement of health literacy capacity. Similar to ours, a previous study recognized the importance of offering the parents possibilities to engage in school-based health literacy programs (18). The overall success of children in school, as well as their development, is better when parents are involved, but in an adequate way. Work on health literacy contributes to the involvement of parents so that they can cover various contents of health education in the necessary methodological way (19,20).

Parental activities and involvement should be supported by wider community. The Ottawa Charter, from the first Health Promotion Conference in 1986, calls for joint action by individuals, communities, and whole society (21). The Ottawa Charter recognizes the importance of social-ecological preconditions for health promotion: peace, place to live, education, food, income, a stable ecosystem, sustainable resources, social justice, and equity. It emphasizes the importance of citizen participation and empowerment, which requires advocacy and mediation skills. Lessons learned from research on interventions based on the Ottawa Charter's health promotion strategies include evidence that investing in healthy public policies is a key strategy, and supportive environment needs to be created at the individual, social and structural levels, and personal skills need to be enthusiastically combined with other strategies to be effective.

Similar to our study, results of systematic review on school-based physical activity intervention programs, including parental participation from 2022, pointed out that response and types of parental engagement still represent a knowledge gap. Education and psychology theoretically

recognize parental role in adopting healthy habits, however, parental role in health education in schools is insufficient. The mutual connection between teachers, parents, and children in this area is also only at the level of theory, with rare exceptions (22), and further strategic activities are required.

CONCLUSION

Parental engagement in school-based health education programs is seen as a supportive factor in the adoption of healthy lifestyles in children, that will lead to healthy development and good life. Despite the existing guidelines of good practice and the experiences of the HPS program worldwide, the parental engagement in school-based health education is recognized mostly at theory level in Serbia, though globally as well. Therefore, future activities should be directed on advocating parental involvement as crucial for successful school-based health education outcomes.

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Statement of Ethics

Ethics approval was not required as no biomedical intervention was performed, experimentation on animals, humans, and clinical trials were included.

Competing Interest

The authors declare no relevant conflicts of interest.

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AFMN BIOMEDICINE

ORIGINAL ARTICLE

IMPACT OF HEALTH LITERACY AND SEXUAL BEHAVIOR ON UNPLANNED PREGNANCY AND ABORTION

Milena Maričić[1] **Vanja Pažun**[1] **Verica Trbović**[1] **Katarina Pavlović-Jugović**[1] **Danijela Jezdimirovicić**[1] **Jovana Milosavljević**[2] **Miloš Stepović**[2]

[1]The College of Health Sciences, Academy of Applied Studies Belgrade, Serbia [2]University of Kragujevac, Faculty of Medical Sciences, Department of Anatomy, Kragujevac, Serbia

It has been proven that health education is directly related to numerous decisions of young people regarding their reproductive health and sexual behavior. The aim of this paper is to evaluate the impact of health literacy and sexual behavior on the occurrence of unplanned pregnancies and abortions among young girls.

We used STOFHLA to assess the health literacy level of young females and a questionnaire to collect socio-demographic characteristics of respondents and their behavior in the area of reproductive health. Descriptive statistics were presented in percentages, and a chi-square test was performed to assess the existence of correlation between categorical variables using SPSS. A total of 220 female respondents were included. The levels of health literacy were as follows: adequate literacy was observed in 78.2% of participants, marginal literacy in 15.9%, and inadequate literacy in 5.9%. Unplanned pregnancies occurred in 7.3%, and all respondents had an abortion. The chi-square test confirmed the existence of a connection between health literacy and unplanned pregnancies, abortions, and the age of the respondents.

The frequency of sexual intercourse and the use of contraceptives showed a statistical association with unplanned pregnancy/abortion.

Education of young people about sexual behavior needs to be an imperative of health institutions, and it is necessary to carry out more effective education at elementary school and high school. Good health literacy is a prerequisite for good reproductive knowledge.

Keywords: health literacy, reproductive health, young women, prevention, abortion

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Correspondence to:
Miloš Stepović
Department of Anatomy
University of Kragujevac Faculty of Medical Sciences
Svetozara Markovica 69, Kragujevac, Serbia
E-mail: stepovicmilos@yahoo.com

INTRODUCTION

Health literacy plays a crucial role in shaping individuals' understanding and making decisions regarding various aspects of healthcare, including reproductive health (1). Adequate education on reproductive health significantly influences the well-being and life outcomes of young girls. (2). Knowledge and prevention of unplanned pregnancy or abortion are in close relation to the sexual education of young minds, which should start before puberty. For young girls, the level of health literacy can significantly influence their awareness, access, and attitudes toward abortion; moreover, it ensures that individuals are well informed before becoming sexually active. (3, 4). Health literacy encompasses the ability to obtain, comprehend, and apply health-related information to make informed decisions about one's well-being (5). When it comes to abortion, a complex and sensitive issue, young girls with higher levels of health literacy are more likely to make informed choices, seek appropriate medical advice, and navigate the legal, emotional, and physical dimensions of the decision-making process effectively (6). Many unplanned pregnancies among young people are followed by abortion. Such interventions can influence not only outcomes that may manifest later in adult life, such as infertility or difficulties in maintaining a pregnancy, but, in many cases, mental health may be significantly compromised shortly after the abortion procedure (7). There is a high risk of developing mental health disorders, including anxiety and depression, and in some cases even suicidal behavior, often as a consequence of stigmatization and feelings of being judged.

Conversely, limited health literacy can lead to confusion, misinformation, and poor decision-making, which may increase the risks associated with unsafe abortions, inadequate post-abortion care, and emotional distress (8). Understanding how health literacy impacts the reproductive choices of young girls, particularly in relation to abortion, is essential for developing targeted educational and healthcare interventions (9). This knowledge can empower young individuals to make well-informed choices, ensuring their safety, well-being, and access to appropriate healthcare resources (10).

Sexual behaviour can be defined as the process through which humans and other species demonstrate or express aspects of sexuality, encompassing a broad range of activities related to reproduction, social bonding, and emotional expression (11). The influence of sexual activity and contraceptive use on accidental pregnancies among young girls is a crucial issue in public health as well as in social science. Adolescent pregnancy rates remain a significant concern globally, particularly in areas where access to comprehensive sexual education and contraceptive methods is limited (12). Young girls, often in their teens, may face physical, emotional, and social challenges if they experience unintended pregnancies, which can alter their life trajectories (13). Sexual activity among adolescents is often coupled with limited awareness of reproductive health and contraceptive options, leading to higher risks of unplanned pregnancies. Additionally, despite the availability of contraception, inconsistent or incorrect use, along with barriers such as stigma, misconceptions, and limited access, can contribute to contraceptive failure (14). This issue requires a nuanced understanding of how sexual behaviours and contraceptive practices intersect to shape outcomes for young girls (15). Understanding these factors is essential for developing effective policies, educational programs, and health interventions to reduce the incidence of accidental pregnancies and promote healthier reproductive choices (16).

This work aimed to assess the influence of health literacy and sexual activities of young girls on the occurrence of accidental pregnancy and abortions in the Serbian population.

METHODS

This research was conducted as a cross-sectional observational study among female students of the College of Health in Belgrade from November 2023 to February 2024. After the lectures, female students had the opportunity to complete the survey with voluntary consent, with the assistance of the teacher (surveyor) as needed, for a duration of 12 minutes for STOFHLA and approximately 10 minutes for the questionnaire about general information and sexual behavior.

As instruments of research, the following questionnaires were used:

1. STOFHLA population - Short Test of Functional Health Literacy in Adults - test of functional health literacy among the adults (an abbreviated version of the questionnaire TOFHLA (STOFHLA) that consists of 36 parts, which assesses the ability to read and understand information from the health care environment)
2. General information questionnaire of respondents who referred to the demographic, social, and economic characteristics of respondents, health knowledge, and

sexual behaviour.

Respondents were introduced to the research objectives and procedures before beginning. Ethical standards were aligned with the International Declaration of Helsinki (Declaration of Helsinki). To ensure the privacy of research subjects and the confidentiality of information collected about them, all necessary steps were taken in accordance with the General Data Protection Regulation (GDPR). Signed informed consent for participation in the research was obtained from each respondent. The study protocol was reviewed and approved by the Ethics Committee of the Academy of Applied Studies Belgrade (Approval No. 01-577/6).

The research included respondents aged 18 to 30. Only fully completed questionnaires were considered valid; incomplete and/or partially filled questionnaires were excluded. The questionnaires were completed anonymously under the supervision of a teacher from the institution, and no additional interventions were required for data collection. The study focused on a group of young people, aiming to assess sexual behavior and activity in relation to health literacy levels and their impact on unplanned pregnancy and/or abortion.

TOFHLA has already culturally adapted to the Serbian language and shows good internal consistency (Cronbach's alpha = 0.94) (17). A perfect score on the literacy test was 36 points, and it took 7 minutes to administer.

The definition of health literacy levels and associated scores were as follows:

1. Inadequate health literacy implied the impossibility of reading and understanding the text related to health, with points between 0 and 16.
2. Marginal health literacy refers to the difficulty in reading and understanding the health-related text with points between 17 and 22.
3. Adequate health literacy is the ability to read and understand most of the health-related text, with points between 23 and 36.

Questions about sexual behaviour consisted of the following:

1. Are you sexually active?
2. How often do you have sex?
3. What type of contraception do you use most frequently?
4. Did you or your partner have experience with an unwanted pregnancy?
5. Did you or your partner have experience with abortion?

The study employed a purposive sampling approach targeting female students, who were invited to voluntarily complete the survey after being informed about the study's objectives and procedures. The minimum required sample size was determined using the G*Power software for a chi-square test, with parameters set at $\alpha = 0.05$, statistical power $(1–\beta) = 0.95$, and effect size $w = 0.3$, yielding a calculated sample size of 220 respondents.

The following statistical analyses were performed: measures of central tendency, including the mean and median, and measures of variability The following statistical analyses were conducted: measures of central tendency, including the mean and median, and measures of variability, specifically the standard deviation, were calculated for continuous variables. For categorical variables, the results were presented as proportions or percentages. A chi-square test was performed to assess the correlation between categorical variables. Significance was noted whenever the p-value was less than 0.05. Statistical Package for the Social Sciences (SPSS), version 23, was used to perform these tests.

RESULTS

In this research, 220 female respondents participated, the majority of them 20 years old or younger (72.7%). The average age of respondents in this study was 20.2 ± 1.5. It was assessed that health literacy was adequate in 78.2%, marginal in 15.9% and inadequate in 5.9% (Figure 1).

We wanted to determine the impact of health literacy on sexual behaviour and the occurrence of unplanned pregnancies and abortion. Health literacy was found to be significantly different in the two examined age groups ($p = 0.03$). Younger respondents had more inadequate and marginal health literacy compared to the older group, and a lower level of health literacy. Considering the frequency of sexual intercourse, no significant difference was found in different levels of health literacy ($p = 0.213$). Most respondents reported having sexual intercourse several times per week (81.5%), and the majority of them demonstrated an adequate level of health literacy. Health literacy levels did not have a significant impact on the choice of contraceptive methods ($p = 0.783$); however, the most commonly used contraceptive method was the condom (64.6%). A concerning finding was that 17.5% of respondents reported using the withdrawal method of contraception (coitus interruption), although 86.1% of these individuals had adequate health literacy. Additionally, 8.3% of respondents reported not using any contraceptive method. The influence of health literacy on unplanned pregnancy and abortion showed a statistically

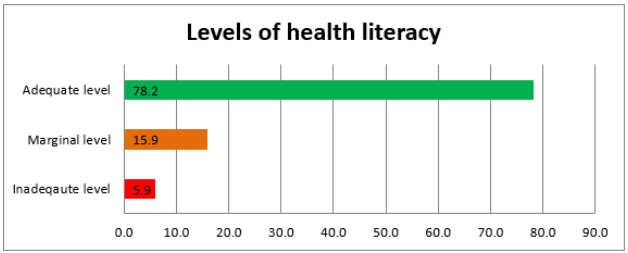

Figure 1. *Levels of health literacy among young female respondents*

significant correlation (p = 0.047), with 7.3% of respondents reporting having experienced this situation. All respondents within this group reported having undergone an abortion, and 56.3% of them had an adequate level of health literacy (Table 1).

As all respondents (100%) with an unplanned pregnancy had an abortion, we wanted to examine the connection with sexual behaviour. It was found that the frequency of sexual intercourse had a significant impact on unplanned pregnancy, and the most unwanted pregnancies occurred in the respondents having sexual intercourse multiple times per week or once a week (p = 0.021). Having sexual intercourse more regularly increased the chance of having an unplanned pregnancy and vice versa, whereas respondents who had sexual intercourse once a month, a couple of times a year, or once a year never had an unplanned pregnancy. Unplanned pregnancy also had a significant correlation with contraception choice (p = 0.014). The highest number of unplanned pregnancies occurred in the respondents using an inadequate type of contraception, the withdrawal method, or no contraception (Table 2).

DISCUSSION

Contemporary research on health literacy demonstrates trends similar to those observed in the present study, particularly with regard to differences in health literacy levels between younger and older populations. For example, in studies conducted in Portugal, researchers found that the level of health literacy in Portugal was generally high, but it was associated with an individual's socioeconomic status (18, 19).

A study from Italy showed that students performed better in health literacy compared to the public sector employees (20). Additionally, data from a study conducted in Italy indicated that students had lower levels of health literacy compared with those in other countries, highlighting the need to improve health education within school settings (21). The findings further suggest that older individuals who often have lower socioeconomic status and lower educational attainment tend to demonstrate lower levels of health literacy. This may result from limited exposure to health education programs and insufficient digital literacy, which is frequently associated with lower levels of general literacy. A similar study conducted in Hungary in 2021 showed that older generations have a weaker ability to make informed decisions about their health, which was attributed to their lower educational status, emphasizing the need for programs that focus on prevention and public health (22).

On the other hand, older people, who are more often faced with health problems, show better results when it comes to understanding and using health information. This trend was confirmed by a 2022 study in Slovenia, which showed that older people, compared to younger individuals, have better health literacy, likely due to longer exposure to health services and information, as well as greater interest in health-related topics (23).

Recent research on health literacy and adolescent sexual behavior provides a clearer understanding of the complex relationship between these factors. A systematic review from 2017 indicates a significant association between health literacy and health behaviors in adolescents, indicating that a higher level of health literacy can contribute to healthier decisions regarding sexual behavior (24).

Our results showed that the level of health literacy did not have a significant influence on the choice of contraceptive methods among the respondents. The most frequently used method was the condom, while approximately 18% of respondents with an adequate level of health literacy relied on the withdrawal method as a form of contraception. These findings are consistent with a study conducted among students at the University of Zagreb, which showed that while students had good knowledge of the preventive effects of regular contraceptive use, their understanding of emergency contraception and the non-contraceptive benefits of contraceptives was more limited. The research also showed that informing students through comprehensible professional literature significantly affects the regular use of contraception (25).

Although our research did not show a significant association between health literacy and the choice of contraception, it is worrying that a high percentage of respondents with adequate health literacy use less reliable methods, such as the withdrawal method (coitus

Table 1. *Descriptive statistics of observed variables and chi-square test of influence of health literacy on each individual variable*

	Descriptive statistics		Health literacy levels			Chi-square test	
	Number	Percentage	Inadequate	Marginal	Adequate	Pearson Chi square value	Significance
Age Group							
To 20 years	160	72.7	12 (7.5%)	30 (18.8%)	118 (73.8%)	6.963	0.031*
Above 20 years	60	27.3	1 (1.7%)	5 (8.3%)	54 (90.0%)		
Frequency of sexual intercourse							
everyday	8	4.0	0 (0%)	2 (25%)	6 (75%)	20.155	0.213
more times per week	65	32.5	5 (7.7%)	7 (10.8%)	53 (81.5%)		
once a week	21	10.5	2 (9.5%)	1 (4.8%)	18 (85.7%)		
more times per month	48	24.0	0 (0%)	6 (12.5%)	42 (87.5%)		
once a month	31	15.5	2 (18.2%)	5 (16.1%)	24 (77.4%)		
more times per year	11	5.5	2 (18.2%)	0 (0%)	9 (81.8%)		
once per year	3	1.5	0 (0%)	2 (66.7%)	1 (33.3%)		
once in couple years	2	1.0	0 (0%)	0 (0%)	2 (100%)		
never	11	5.5	1 (9.1%)	2 (18.2)	8 (72.7)		
The most used contraceptives							
stopped ejaculation	36	17.5	2 (5.6%)	3 (8.3%)	31 (86.1)	4.764	0.783
condom	133	64.6	7 (5.3%)	17 (12.8%)	109 (82.0%)		
anti-baby pill	17	8.3	1 (5.9%)	4 (23.5%)	12 (70.6%)		
pill for the next morning	3	1.5	0 (0%)	1 (33.3%)	2 (66.7%)		
don't use contraception	17	8.3	2 (11.8%)	2 (11.8%)	13 (76.5%)		
Unplanned pregnancy							
Yes	16	7.3	2 (12.5%)	5 (31.3%)	9 (56.3%)	6.333	0.047*
No	204	92.7	11 (5.4%)	30 (14.7%)	163 (79.9%)		
Abortion							
Yes	16	7.3	2 (12.5%)	5 (31.3%)	9 (56.3%)	6.333	0.047*
No	204	92.7	11 (5.4%)	30 (14.7%)	163 (79.9%)		

*Statistically significant value, less than 0.05

Table 2. *Influence of frequency of sexual intercourse and contraceptives on unplanned pregnancy*

	Unplanned pregnancy		Chi-square test	
	Yes	No	Pearson Chi square value	Significance
Frequency of sexual intercourse				
everyday	0 (0%)	8 (100%)	18.005	0.021*
more times per week	3 (4.6%)	62 (95.4%)		
once a week	3 (14.3%)	18 (85.7%)		
more times per month	1 (2.1%)	47 (97.9%)		
once a month	0 (0%)	31 (100%)		
more times per year	0 (0%)	11 (100%)		
once per year	0 (0%)	3 (100%)		
once in couple years	1 (50%)	1 (50%)		
never	0 (0%)	11 (100%)		
The most used contraceptives				
stopped ejaculation	4 (11.1%)	32 (88.9%)	12.500	0.014*
condom	2 (1.5%)	131 (98.5%)		
anti-baby pill	1 (5.9%)	16 (94.1%)		
pill for the next morning	0 (0%)	3 (100%)		
don't use contraception	3 (17.6%)	14 (82.4%)		

*Statistically significant value, less than 0.05

interruption), or do not employ any form of contraception. A study conducted in South Africa analyzing the most commonly used contraceptive methods showed that condoms, injectable contraceptives, and pills were the most frequently used methods, along with periodic abstinence. The main reasons for not using contraception were being unmarried, having irregular sexual activity, or not being sexually active (26). A systematic review, partly focused on analyzing the most commonly used contraceptive methods, showed that women most frequently use intrauterine devices (IUDs), contraceptive patches, implants, pills, condoms, and intercourse on non-menstrual days (27).

Our results showed a significant correlation between unplanned pregnancy and the choice of contraception, with the highest number of unplanned pregnancies recorded among respondents who used inadequate methods of contraception, such as coitus interruptus or did not use contraception at all. These findings are consistent with research conducted in Iran, where it was found that inadequate health literacy can lead to improper use of contraceptive methods and an increased risk of unplanned pregnancy (28). Unwanted pregnancies most often occur due to non-use, incorrect use, or inconsistent use of contraception, as indicated by the results of our study. The most commonly used reversible methods of contraception are oral contraceptive pills and condoms. Condoms have an annual failure rate of 9% and 18%, while pill failures are twice as high among women under 21, contributing significantly to the risk of teenage pregnancy. The use of intrauterine methods (IUDs) and implants has the lowest reported rate of unplanned pregnancy, less than 1% (29).

In a survey of 21 countries, with a complete insight into the rate of termination of pregnancy, it was shown that pregnancies in the period of 15-19 years are most common in America, least common in Switzerland, and relatively high in the former Soviet states. The percentage of unwanted pregnancies that ended in abortion varies from 17% in Slovenia to 69% in Sweden (30). Given the population data of the countries examined, it can be concluded that a large percentage of unwanted pregnancies result in abortion, which aligns with the findings of our study.

Since the process of terminating a pregnancy can be a very traumatic experience for young people, it is crucial that they have an adequate support system from family and their environment. Such experiences can have long-term consequences, particularly by lowering self-esteem, affecting mental health, and increasing the risk of anxiety and depression (31). Prevention of unwanted pregnancies through adequate information, destigmatization, and educational programs on contraceptive methods can help reduce the occurrence of unwanted pregnancies, abortions, and potential complications affecting reproductive and overall health.

Limitations

This study has several limitations. Its cross-sectional design precludes establishing causal relationships

between health literacy, sexual behavior, and reproductive outcomes. The sample was restricted to female students from a single health college in Belgrade, limiting the generalizability of the findings to broader populations. Data were self-reported, which may introduce recall bias and social desirability bias, particularly given the sensitivity of topics such as sexual activity, contraception, and abortion.

CONCLUSION

The results of our research, in accordance with studies conducted in the world, indicate that health literacy is not always a decisive factor in sexual behavior and the choice of contraception used among young people. Although the condom was most commonly used, it is worrying that a significant percentage of respondents used inadequate contraceptive methods, such as the withdrawal method (coitus interruption), which was associated with a higher rate of unplanned pregnancies. Similar trends have been observed in international studies, which emphasize the need for more comprehensive health education programs. These interventions should focus on improving young people's knowledge and skills in using health information, especially in the context of sexual and reproductive health.

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Statement of Ethics

The study protocol was reviewed and approved by the Ethics Committee of the Academy of Applied Studies Belgrade (Approval No. 01-577/6). Complete written informed consent was obtained from the respondents for the publication of this study.

Competing Interest

The authors declare no relevant conflicts of interest.

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AFMN BIOMEDICINE

CASE REPORT

SEAT BELT SYNDROME: THYROID CARTILAGE FRACTURE

Sladjana Andjelić[1] **Eleftheria Papadopoulou**[2] **Tanja Nikolić**[3] **Goran Čolaković**[1] **Milena Popović**[1]

[1]Institute for Emergency Medicine, Belgrade, Serbia [2]Tzaneio General Hospital of Piraeus, Piraeus, Greece [3]Ars Medica, Belgrade, Serbia

Seat belt syndrome (SBS) is a new pattern of injuries in road traffic accidents (RTAs). It refers to injuries caused by the interaction of the human body and the safety belt and consists of a wide spectrum of injuries (musculoskeletal and visceral).

In this paper, we present a case of a front-seat passenger with polytrauma sustained in an RTA, who, as a literature rarity, also suffered a thyroid cartilage fracture (TCF) caused by a seat belt.

Thyroid cartilage fracture should be kept on the list of possible injuries with a high index of suspicion for this syndrome, based on the mechanism of injury and hyperflexion of the neck, even without signs of direct neck trauma.

Keywords: seat belt syndrome, traffic accident, thyroid cartilage fracture

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Correspondence to:
Sladjana Andjelić
Institute for Emergency Medicine Belgrade
Franse d'Eperea 5, Belgrade, Serbia
E-mail: sandjelic94@gmail.com

INTRODUCTION

Motor vehicle accidents are one of the most significant causes of morbidity and mortality worldwide (1). In 2021, in Serbia, 482 road traffic accidents (RTA) with fatalities were recorded, in which a total of 521 people lost their lives: 229 in passenger cars (44%), 148 pedestrians (28%), and 48 cyclists (9%), while more than 13.000 were injured (2).
The use of seat belts (SB) as protective systems for drivers and passengers in the car is one of the indicators of traffic safety (3). Properly fastened, seat belts reduce the deceleration force on passengers in the moment of impact, extend the stopping distance for the passengers' bodies, distribute the impact force over a larger surface area, and reduce the effect of the impact force with their elasticity. They also prevent secondary collisions between passengers and the vehicle's internal structures, as well as ejection from the vehicle during or after the crash (3). The protective effect of the seat belt is enhanced when combined with airbags. Serbian research shows that 37% of the passengers who die in RTAs die as a result of improperly fastened seat belts (2). Seat belts are responsible for distinctive injury patterns known as the "seat belt syndrome" (SBS) (4). SBS consists of the following triad: superficial skin bruises, visceral injuries, and musculoskeletal injuries (5).
Herein, we present the case of a front-seat passenger (FSP) with polytraumatic injuries sustained in an RTA, who, in addition to the usual SBS injuries, also suffered from a thyroid cartilage fracture (TCF), which is a rarity in the literature.

CASE REPORT

The car veered out of control in an RTA involving a married couple in a car on a highway, with the husband as the driver and the wife as the front seat passenger. The car struck a concrete wall with the front passenger side of the vehicle. The wife, aged 58, was injured. The car was moving at a speed of 60 km/h, and at the moment of the collision, the wife (the front seat passenger) noticed that the steering wheel was unresponsive and the car was moving on its own accord. Attempting to regain control of the vehicle, she made a motion of hyperflexion and lateral extension of the spine. She was in that position when the car collided with the concrete wall.
The Emergency Medical Services (EMS) and firefighters were called to extract the woman from the automatically locked car and release her from the seatbelt and deployed airbag (Figure 1).

Figure 1. *The appearance of the impacted vehicle with the patient inside*

The EMS team found the front seat passenger conscious and actively mobile inside the vehicle. She denied having lost consciousness or suffering from chronic disease. She complained of dysphagia and odynophagia.
She was afebrile, slightly cyanotic, anicteric, hoarse, dysphonic, and dyspnoeic. Her vital signs were: a pulse of 78 beats/min; blood pressure of 110/80 mm Hg; respiratory rate of 28 breaths/min, blood oxygen saturation by pulse oximetry on ambient air at 79%, and blood glucose level at 7 mmol/l. There were no visible head injuries. She was noted to have an anterior midline transverse neck bruise in zone 2 (Figure 2).

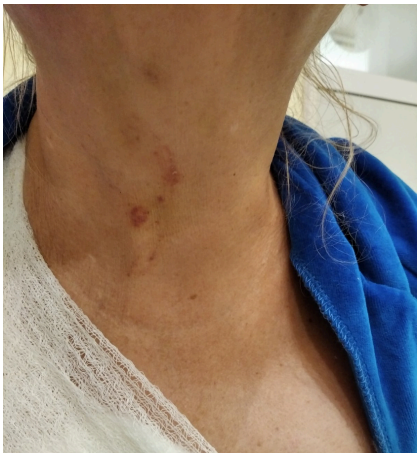

Figure 2. *External neck sign (anterior midline transverse neck bruise in zone 2)*

Her neck showed slight oedema with crepitations and subcutaneous emphysema, and the trachea was positioned midline. The right arm was in a forced position, edematous, deformed, and painful, with a palpable peripheral pulse. Other physical findings were normal.
She was extracted from the vehicle using a cervical collar and vest-type extrication device. Her right arm was immobilised, oxygen was administered by mask at a flow rate of 4 L/min (resulting in SaO2 of 85%), and intravenous (IV) opioid derivates and lactated Ringer's solution were administered. She was unable to tolerate the supine position and was transported to the hospital in a seated position with vital signs continuously monitored.

Upon admission, a comprehensive diagnostic workup was performed: laboratory tests, chest X-ray, radiographs of the right humerus and forearm, multidetector computed tomography (MDCT) scan of the neck, thorax, and abdomen (plain and after IV contrast administration), Doppler of the blood vessels of the neck, and echocardiography. The following diagnoses were established: fractures of the proximal humerus and the wrist, the second and third right rib fractures, and a larynx injury to the glottis and subglottis, with a TCF at the level of the anterior commissure (Figure 3).

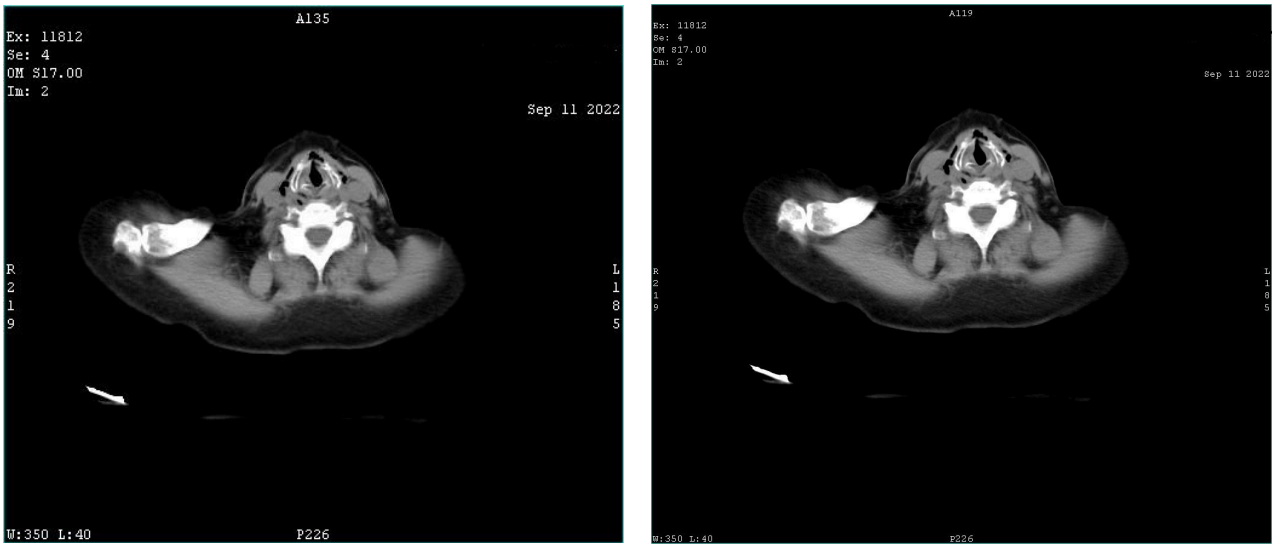

Figure 3. *Multidetector computed tomography scan of the neck: a thyroid cartilage fracture at the level of the anterior commissure with laryngeal injury of both the glottis and subglottis, and signs of pronounced emphysema in the soft tissues of the neck around the larynx and the retropharyngeal space*

There were signs of pronounced soft tissue emphysema in the laryngeal and retropharyngeal regions of the neck. The vocal cords were slightly voluminous. There were signs of pneumomediastinum–a small amount of free air along the esophagus. There were no fluid collections in the mediastinum. The trachea and main bronchi showed no obstruction, and the tracheal wall was intact. There were no signs of pneumothorax or pleural effusions. Pulmonary parenchyma showed no infiltrative-nodular changes, collapse zones, or consolidations. There were no traumatic lesions to the parenchymal organs and no free air or fluid in the abdomen.
A manual repositioning of the right arm was performed. Intravenous antibiotics, corticosteroids, and H2-receptor antagonists were administered. Hospitalisation and emergency tracheotomy (ET) were suggested, which the patient refused, and insisted on conservative management.
One year later, the patient recovered, but the thyroid cartilage healed irregularly with residual hoarseness as a permanent disability.

DISCUSSION

Our patient met all the criteria for SBS: an anterior midline transverse neck bruise, a laryngeal injury of the glottis and subglottis with TCF, fractures of the second and third ribs, signs of neck emphysema, voluminous vocal cords, and

signs of pneumomediastinum. According to Clement & Kelechukwu's classification (5) of SBS (overt and covert), our patient is classified as type one.

The presence of a seat belt sign increases the likelihood of chest injury by nearly four times and is a sign of some form of internal injury in 30% of cases (5), while its absence does not exclude the presence of other components of the SBS (6).

Although sporadic, occurring in less than 5% of all neck injuries, laryngeal injury (LI) can be life-threatening for the injured patient. Sittitrai et al. (6) concluded in a retrospective study that 86% of blunt LI occurred in motor vehicle collisions. Typically, LI is associated with other injuries in polytrauma, as was the case with our patient.

TCF is a rare but life-threatening injury because the thyroid cartilage is the only laryngeal cartilage necessary for the stability and integrity of the airway (7). The TCF occurs when the force of impact causes hyperflexion of the neck that exceeds the maximum flexibility of the thyroid cartilage. This mechanism of injury was present in our case, where the seat belt limited the neck hyperflexion.

During the initial approach to an RTA-related injury, it is important to note the characteristics of the injured and the mechanism of injury (the type of vehicle, speed, collision type, vehicle deformation, and body kinematics). In our case, the car was travelling at a speed of 60 km/h, it hit a wall on the front passenger's side, and the force of impact activated the airbag and automatically locked the vehicle door. A high index of suspicion for TCF in our patient's case should be considered because of the deceleration of the body, the hyperflexion of the neck during the collision, and the presence of the external neck sign. Taiwanese authors report an unrestrained car passenger with an isolated TCF caused by head impact on the windshield and neck hyperflexion during an RTA (8).

Three clinical findings indicate the presence of laryngeal fractures: hoarseness, subcutaneous emphysema, and a palpable fracture (6). Even though our patient was wearing a seat belt, she suffered polytraumatic injuries, with an emphasis on a potentially life-threatening airway obstruction (LAO) due to TCF, neck emphysema, and pneumomediastinum. Timely diagnosis and treatment are often crucial for the survival of severely injured patients with SBS. Computed tomography is considered the gold standard for diagnosing LI (9), and ET is the treatment of choice for LAO.

According to Fuhrman et al. (10), ET is justified even without a prior laryngoscopic examination if the patient cannot tolerate being in the supine position. According to Schaeffer's classification (Table 1) (11), our patient also suffered from a type II injury (oedema, hematoma, minor mucosal disruption without exposed cartilage, and non-displaced fractures on CT scan) which indicated an ET to provide respiratory clarity. Our patient, however, refused to be admitted to the hospital, as well as undergoing ET and insisted on conservative treatment.

Seat belt syndrome is a new pattern of injuries in RTAs, even when the seat belt is properly fastened. Thyroid cartilage fracture should be kept on the list of possible injuries with a high index of suspicion in cases with SBS, based on the mechanism of injury and external signs of laryngeal trauma.

Table 1. *Schaefer–Fuhrman Classification of laryngeal injuries (11)*

Group	Symptoms	Sings	Management
I	Minor airway symptoms	Minor endolaryngeal hematoma, edema or laceration without detectable fracture	Observation, humidified O2
II	Airway compromise	Edema or hematoma, minor mucosal disruption without exposed cartilage and non-displaced fractures noted on CT	Tracheostomy, direct laryngoscopy, esophagoscopy
III	Airway compromise	Massive edema, mucosal disruption, displaced fractures, exposed cartilage and/or cord immobility	Tracheostomy, exploration/repair
IV	Airway compromise	Group III + two or more fracture lines, skeletal instability or significant anterior commissure trauma	Tracheostomy, exploration/repair, stent required

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Statement of Ethics

Complete written informed consent was obtained from the involved patient for the publication of the study and accompanying images.

Competing Interest

The authors declared no relevant conflicts of interest.

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AFMN BIOMEDICINE

CASE REPORT

BEHCET'S DISEASE WITH RECURRENT APHTHOUS STOMATITIS, POLYARTHRALGIA, AND SUPERFICIAL VEINS THROMBOSIS

Vita Ruzhanska[1,3] Iuliia Pashkova[2] Dmytro Grebeniuk[1] Andrii Sidorov[1]
Svitozar Prevar[4] Oleksandr Smiiukha[5] Yuriy Hnatiyk[6] Arina Ivanitsa[7]

[1]Department of Endoscopic and Cardiovascular Surgery, Vinnytsia, National Pirogov Memorial Medical University, Vinnytsya, Ukraine [2]Department of Internal Medicine, Medical Faculty No. 2, National Pirogov Memorial Medical University, Vinnytsya, Ukraine [3]Cardiology Department of the Cardiology Clinic, Military Medical Clinical Center of the Central Region, Vinnytsya, Ukraine [4]Department of Therapeutical Stomatology, National Pirogov Memorial Medical University, Vinnytsya, Ukraine [5]Department of Oncology, X-ray Diagnostics and Therapy, National Pirogov Memorial Medical University, Vinnytsya, Ukraine [6]Department of Surgery of Medical Faculty Nr. 2, Vinnytsia, National Pirogov Memorial Medical University, Vinnytsya, Ukraine [7]Department of Pathological Physiology, Vinnytsia, National Pirogov Memorial Medical University, Vinnytsya, Ukraine

Behçet's disease is a systemic, chronic, idiopathic inflammatory illness of unknown etiology with a recurrent course, manifested by a characteristic triad: recurrent aphthous stomatitis, ulcerative changes in the mucous membranes and skin of the genital organs, and inflammatory changes in the eyes. In addition, the musculoskeletal system, nervous system, gastrointestinal tract, vascular system, genitourinary tract, and cardiopulmonary system can be affected, leading to significant morbidity and mortality. A prolonged period typically elapses between the onset of symptoms and the diagnosis of Behçet's disease due to variable and sometimes intermittent symptoms, the need to rule out clinical mimics of disease onset, the absence of a specific blood test or disease marker, and, unfortunately, a general lack of awareness about the condition.

We present a case of a 35-year-old male patient with a clinical diagnosis of Behçet's disease featuring mucous membrane involvement (recurrent aphthous stomatitis), joint involvement (polyarthralgia) of the knees and elbows, and polyneuropathy of the lower extremities manifesting as reduced sensitivity without functional impairment, of moderate severity; subacute phlebothrombosis of the superficial veins of the left lower extremity in the recanalization stage, and chronic venous insufficiency stage I without circulatory impairment.

A low incidence of Behçet's disease, combined with its involvement of various systems and organs, complicates diagnosis and precludes early treatment. The collection, systematization, and detailed analysis of available clinical cases will facilitate the development of improved diagnostic and treatment algorithms.

Keywords: Behçet's disease, recurrent aphthous stomatitis, polyarthralgia, superficial veins thrombosis

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Correspondence to:
Dmytro Grebeniuk
Department of Endoscopic and Cardiovascular Surgery
National Pirogov Memorial Medical University
Vinnytsya, Ukraine
E-mail: grebeniukdi@vnmu.edu.ua

INTRODUCTION

Behçet's disease (Adamantiades-Behçet's disease, Silk Road disease) is a systemic chronic idiopathic inflammatory disease of unknown etiology with a relapsing course, characterized by a distinctive triad: recurrent aphthous stomatitis, ulcerative changes of the mucous membranes and skin of the genital organs, and inflammatory changes of the eyes (1). In addition, the targets of involvement may include the musculoskeletal system, nervous system, gastrointestinal tract, vascular system, urogenital tract, and cardiopulmonary system, leading to significant morbidity and mortality (2). There is typically a prolonged interval between the onset of symptoms and the establishment of a diagnosis of Behçet's disease (BD), caused by variable and sometimes intermittent symptoms, the need to rule out clinical mimics of the disease's onset, the absence of a specific blood test or disease marker, and, unfortunately, a general lack of awareness about this condition. In the absence of pathognomonic laboratory tests, the diagnosis of this disease relies on clinical criteria (3).

Adamantiades-Behçet's disease is recorded worldwide, but its prevalence is particularly high in the Middle East, Far East, and Mediterranean regions. This condition is also referred to as the "Silk Road disease," acknowledging the fact that the highest incidence of this pathology has been recorded in countries located along the Silk Road, stretching from East Asia to the Mediterranean. Turkey has the highest prevalence of BD (approximately 80–370 cases per 100,000 population), followed by Iran, Saudi Arabia, Iraq, Israel, northern China, and Korea (4, 5). In the United Kingdom, the estimated prevalence of BD is 0.64 cases per 100,000 population. The classic age group affected by this condition is individuals aged 20–40 years, although BD is also observed in children and older patients. The incidence is higher among men in regions with high prevalence, such as Turkey and the Middle East, but the gender distribution varies in other countries. The disease typically progresses more severely in young men (6).

BD is predominantly sporadic but can occur in familial clusters. The etiology remains unknown to date, but a combination of genetic factors, particularly a strong correlation with human leukocyte antigens, specifically HLA-B51, and environmental factors may play a role. The genetic marker HLA-B51 is detected in approximately 60% of patients with BD. Genome-wide association studies (GWAS) have identified HLA-B51 and HLA ERAP1 (endoplasmic reticulum aminopeptidase 1, also known as a regulator of tumor necrosis factor receptor type 1 shedding) as genes predisposing to the development of BD. In susceptible individuals, as-yet-unidentified environmental factors play a pathogenic role, potentially including microbial exposure, as well as cellular and humoral immunity. The proinflammatory cytokine cascade, inflammatory responses, relapsing-remitting course, and responses to immunosuppressive treatment in BD suggest that the disease has an autoinflammatory-autoimmune nature. The action of infectious agents, particularly heightened sensitivity to *Streptococcus sanguinis* antigens, indicates their possible pathological influence. Although many other infectious agents, including *Staphylococcus aureus*, *Herpes simplex* virus type 1, and *Prevotella species*, have been identified as potential culprits, their direct link to the development of BD has not been confirmed. The current understanding is that exposure to infectious or external agents somehow triggers an autoinflammatory response in genetically predisposed individuals. Despite numerous studies dedicated to the mechanisms underlying BD, a long road lies ahead to fully comprehend the complexity of this pathology (7).

In BD, a characteristic triad of clinical symptoms is observed, involving lesions of the oral mucosa in the form of aphthae, ulcers of the mucous membranes and skin of the genital organs, and ocular involvement in the form of uveitis or iridocyclitis. The formation of oral ulcers is noted in all patients and is considered one of the early symptoms, often preceding the development of systemic manifestations by months or even years. The disease typically begins with the appearance of aphthae with cloudy contents on the gums, tongue, and mucous membranes of the cheeks and lips, which then transform into ulcers 2–12 mm in diameter, bright pink in color, round in shape, with an erythematous border. The surface of the ulcers may be covered with yellow pseudomembranes. These ulcerative defects tend to merge, and the affected area may become a continuous ulcerated surface. On the mucous membranes of the glans penis, vagina, or scrotum, painful aphthae appear, which develop into ulcers resembling those in the oral cavity but are typically larger, deeper, and irregularly shaped. In approximately 10% of patients, ocular involvement is the first symptom of the disease, usually developing after ulcerative stomatitis. Patients with ocular involvement report a variety of complaints, including blurred vision, eye pain, photophobia, tearing, and periorbital hyperemia.

Skin lesions are also frequently observed in the form of erythema nodosum, papules, folliculitis, and rashes resembling erythema multiforme. Subungual abscesses and ulcers are not uncommon. Musculoskeletal involvement occurs in approximately half of the patients and is characterized predominantly by mono- or oligoarthritis of large joints, with polyarthritis developing rarely. Gastrointestinal involvement manifests as abdominal pain and diarrhea. Intestinal bleeding and perforation may occur. The ileocecal region is most commonly affected, with the esophagus, transverse colon, and ascending colon involved less frequently. Chronic progressive central nervous system involvement is noted in 10–20% of patients and is more common in men who develop the disease at a younger age. In the early stages of the disease, during the acute phase, aseptic meningitis or meningoencephalitis may develop, presenting with headache, fever, and neck stiffness. According to various studies, pulmonary involvement in BD ranges from 1% to 7%. Possible manifestations of lung involvement include pulmonary artery aneurysms, arterial and venous thromboses, pulmonary infarction, recurrent pneumonia, obliterative bronchiolitis, and pleuritis. Renal involvement in BD is significantly less common than in other vasculitis cases and is less severe. Proteinuria, hematuria, and mild renal insufficiency are occasionally observed. Cardiac involvement is rare and may present as pericarditis, myocarditis, coronaritis, endocarditis, mitral valve prolapse, or other conditions. Typical symptoms include superficial and deep vein thromboses (8, 9).

Although BD is a relatively young disease (described in 1937), it already has 16 sets of diagnostic/classification criteria. The first of these was proposed by H. Curth in 1946, less than a decade after the disease was described (10). Currently, the criteria of the International Study Group for Behçet's Disease (ISG, 1990), the International Criteria for Behçet's Disease (ICBD, 2006), and the International Team for the Revision of the International Criteria for Behçet's Disease (ITR-ICBD, 2014) are used. The ISG criteria are less sensitive but more specific than the ITR-ICBD criteria.

The ISG criteria utilize five elements: recurrent oral ulcers—small and/or large aphthae or herpetiform ulcerations recurring at least three times per year, observed by a physician and patient; recurrent genital ulcers—aphthous or scarring ulcerations observed by a physician or patient; ocular lesions—anterior uveitis, posterior uveitis, cells in the vitreous on slit-lamp examination, or retinal vasculitis, observed by an ophthalmologist; skin lesions–erythema nodosum, pseudofolliculitis, papulopustular eruptions, or acneiform nodules observed by a physician in postpubertal patients not receiving corticosteroids; and a positive pathergy test evaluated by a physician within 24–48 hours. In the ISG criteria, the presence of oral aphthae is mandatory. Two additional items from the remaining four are required for a reliable BD diagnosis (11).

For the International Criteria for Behçet's Disease (ICBD), vascular manifestations were added to the five elements defined in the ISG criteria, as they are a characteristic feature of BD and were used in many criteria prior to the ISG. Vascular involvement manifests as superficial phlebitis, deep vein thrombosis, large vein thrombosis, arterial thrombosis, or aneurysms. Thus, the ICBD employs six items: aphthous stomatitis, genital aphthae, skin manifestations in the form of pseudovasculitis and erythema nodosum, ocular involvement in the form of anterior or posterior uveitis and retinal vasculitis, vascular manifestations, and a positive pathergy test. In the ICBD, genital mucosal aphthae and ocular involvement carry greater diagnostic weight than others and are thus assigned two points each. The other four elements (oral mucosal aphthae, skin lesions, vascular manifestations, and pathergy test) are assigned one point each. A patient must score three or more points for a reliable BD diagnosis (7).

The International Team for the Revision of the International Criteria for Behçet's Disease (ITR-ICBD), due to the low sensitivity of the ISG clinical diagnostic criteria, prompted their revision and reassessment. The ITR-ICBD presented data from 27 countries, including results from 2,556 patients with clinically diagnosed BD and 1,163 controls with diseases mimicking BD or exhibiting at least one major BD feature. These criteria include seven elements: ocular lesions, oral mucosal aphthae, and genital mucosal aphthae, each assigned two points, while skin lesions, neurological manifestations, and vascular manifestations are assigned one point each. A positive skin pathergy test is also assigned one point. A patient scoring four or more points is classified as having BD (12).

The primary goal of BD treatment is to suppress inflammation and reduce the frequency and severity of disease relapses. For treatment to be effective, it must be initiated as early as possible. The location and extent of involvement, as well as the severity of the disease, are key factors in determining the choice of medications. The European League Against Rheumatism (EULAR) recommendations for BD management, developed in 2008 and updated in 2018, assist in addressing various

aspects of this pathology. Systemic treatment for BD is considered when topical medications are ineffective and begins with the prescription of colchicine, which effectively manages recurrent oral and genital ulcers and may also reduce joint swelling. For moderate-to-severe forms of the disease, corticosteroids (prednisolone, methylprednisolone) are prescribed to control inflammation caused by BD. The most common side effects of corticosteroids faced by BD patients include weight gain, persistent heartburn, high blood pressure, and bone thinning (osteoporosis). Immunosuppressants (azathioprine, cyclosporine A, cyclophosphamide) are often prescribed in combination with corticosteroids to suppress the immune system (level of evidence III, strength of recommendation C). Medications that modify the immune response, such as interferon alpha-2b, are used to regulate the immune system and the intensity of inflammation. It can be used alone or in combination with other drugs to help control the progression of skin ulcers, joint pain, and ocular inflammation. Medications that block tumor necrosis factor-alpha (TNF-α), such as infliximab and adalimumab, are effective in treating certain manifestations and symptoms of BD, particularly in patients with more severe or refractory symptoms, such as refractory thrombosis, providing there is a low risk of bleeding and pulmonary artery aneurysms are ruled out (level of evidence III, strength of recommendation C) (13–16). The use of anticoagulant therapy for the treatment and prevention of thromboembolic complications in BD remains an open question (17–19). Results from a study by E. Seyahi demonstrated the ineffectiveness of anticoagulant therapy without immunosuppressants in preventing recurrent venous thrombosis, leading researchers to conclude that venous thrombosis should be treated with immunosuppressive agents (20).

This study is a fragment of the research work “Development and implementation of innovative technologies in the treatment and prevention of violations of the integrity and patency of blood vessels in wartime conditions”, state registration number–0123U100204.

CASE REPORT

Patient M., born in 1987, was admitted to the therapeutic department on September 7, 2022, for inpatient treatment. At the time of examination, the patient reported complaints of pain in the knee and elbow joints, which had been bothering him since 2015, the presence of painful ulcers (aphthae) on the mucous membrane of the oral cavity and tongue, and a sensation of numbness in the lower extremities that emerged in 2021.

During the collection of the medical history, it was established that the patient had been repeatedly treated in various specialized medical institutions with a diagnosis of reactive arthritis, but without sustained improvement. On objective examination, the patient’s general condition was satisfactory, with clear consciousness. He was oriented in space and time. The patient had a normosthenic body build. BMI was 26.5 kg/m^2. The skin and visible mucous membranes were of normal flesh color. Peripheral lymph nodes were not enlarged. The spinal axis was intact, with full range of motion and no pain. Respiratory rate was 18 breaths per minute. Body temperature was 36.5°C. The thyroid gland was not enlarged on palpation. The spinal axis was intact, with full range of motion. Percussion over the lungs revealed a clear pulmonary sound, and auscultation indicated vesicular breathing with no wheezing detected. Blood pressure was 120/80 mmHg. Pulse was 74 beats per minute, rhythmic, tense, and symmetrical. Cardiac borders were unchanged. Heart sounds were clear and rhythmic, with no accents or murmurs detected. The tongue was moist, with a canker sore 1 cm in size present at the tip of the tongue and another canker sore 5 mm in size on the right lateral surface of the tongue, both covered with a fibrinous coating and painful. On the mucous membrane of the inner surface of the lower lip, three painful aphthae, each 5 mm in size, covered with a fibrinous coating, were identified (Figure 1).

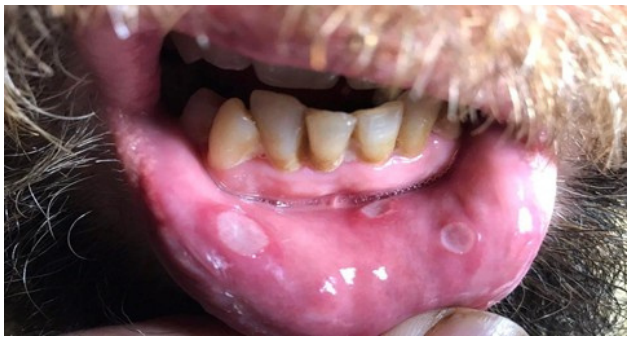

Figure 1. *Canker sore on the mucous membrane of the lower lip*

On objective examination, the following was established: the abdomen was soft and painless on palpation. The liver was not enlarged, palpable along the edge of the right costal arch; the liver edge was soft, elastic, and painless.

The spleen was not palpable. The lumbar thrust symptom was negative on both sides. The kidneys were not palpable. Neurological status: tendon reflexes from the arms and legs were moderately reduced, D=S. Hypesthesia in the lower extremities in a "high socks" pattern. Cranial nerves showed no focal changes. According to otoscopy data, palpation, and percussion of the paranasal sinus projections were unremarkable. The mucous membranes of the upper respiratory tract were pale pink, clean, and nasal breathing is unobstructed. The external auditory canals were clean and patent, the tympanic membranes were gray-pearly, and identifying landmarks were visible. Whispered speech was audible at 6 meters in both ears. Color perception was intact. Visual acuity of the right eye = 1.0, visual acuity of the left eye = 1.0. The ocular adnexa were normal. Normal trichromat. Fundoscopy: optic discs were pale pink with clear borders, A:V ratio = 2:3, maculae showed no changes. Rectal examination: at 3, 7, and 11 o'clock, external and internal nodes up to 0.8 cm in diameter were present without signs of inflammation; sphincter tone was preserved, no blood observed on the glove during palpation, and the coccyx was painless.

Data from laboratory and instrumental examinations: complete blood count: erythrocytes 5.1×10^{12}/L, Hb 156 g/L, leukocytes 6.3×10^{9}/L, platelets 239×10^{9}/L, ESR 3 mm/h; urinalysis–yellow color, complete transparency, specific gravity 1030, acidic pH, glucose and protein not detected, leukocytes 1–3 per field of view, erythrocytes absent, salts absent, flat epithelium 0–1 per field of view, mucus ++++. Biochemical blood analysis: creatinine 86 µmol/L, blood glucose 4.9 mmol/L, ALT 22 U/L, AST 13 U/L, CRP 1.7 mg/L, uric acid 266 µmol/L, rheumatoid factor 9.63 IU/mL, Anti-CCP < 1.5, CIC 172 arbitrary units, antinuclear antibodies IgG 0.97 CP. Blood tests for HIV antibodies–not detected, HBsAg–negative, HCV–negative. Blood analysis for HLA-B51 separated from HLA-B5, EDTA blood– negative, HLA-B52 separated from HLA-B5, EDTA blood–negative.

ECG: sinus rhythm, regular, horizontal position of the heart's electrical axis, heart rate 53 beats per minute. The chest X-ray: the lungs show no visible focal or infiltrative changes. Heart and aorta are within normal limits. The chest X-ray of the knee joints shows no visible bony or traumatic changes. The X-ray of the elbow joints shows no visible bony or traumatic changes.

During the examination, the patient developed complaints of pain in the left lower extremity. An ultrasound examination of the lower extremity vessels was performed, and the patient was examined by a vascular surgeon.

The results of Doppler ultrasonography of the arteries of both lower extremities were as follows. Right: along the course of the common femoral artery (CFA) and superficial femoral artery (SFA), localized single heterogeneous atherosclerotic plaques were identified, predominantly consisting of dense zones, some exhibiting a clear echo-shadow phenomenon. These plaques stenose the lumen of the CFA by up to 20–25% in diameter and the SFA by up to 45–50% in diameter. The intima-media complex (IMC) in the areas accessible for visualization measures 0.9 mm, with normal echogenicity and preserved differentiation into layers. No occlusions were detected. Left: localized single heterogeneous atherosclerotic plaques were observed along the course of the CFA, predominantly consisting of dense zones, some with a clear echo-shadow phenomenon, stenosing the lumen of the CFA by up to 30–35% in diameter. The IMC in the areas accessible for visualization measures 0.9 mm, with normal echogenicity and preserved differentiation into layers. No occlusions were detected. Along the course of the CFA, additional localized single heterogeneous atherosclerotic plaques with predominant dense zones and some exhibiting a clear echo-shadow phenomenon were noted, stenosing the lumen of the CFA by up to 30–35% in diameter.

The results of Doppler ultrasonography of the veins of both lower extremities were as follows. Right: within normal limits. Left: the common femoral vein (CFV), deep femoral vein (DFV), and superficial femoral vein (SFV) were patent, with complete lumen compression and no pathological blood reflux detected. In the area of the ostial valve of the great saphenous vein (GSV), no pathological reflux was detected. The popliteal vein was patent and responsive to compression, with no pathological reflux detected. The posterior tibial veins were patent and responsive to compression. The peroneal veins were patent and responsive to compression. The GSV, at the point of departure from the ostial valve, had a main trunk diameter of 3.3 mm, not dilated, with the lumen decreasing to 2.0 mm without deviating from the main bed. At the level of the upper third of the thigh, the GSV was visualized in the main bed, dilated to 4.8 mm, with the lumen filled up to 80% with isoechoic thrombotic masses (Figure 2A), exhibiting localized parietal blood flow along the posterior wall of the vessel (Figure 2B) and no compression during compression testing. One partially thrombosed perforating vein was identified in the middle third of the thigh (Figure 3). At the level of the knee joint, the GSV lumen measured 2.8 mm and was fully

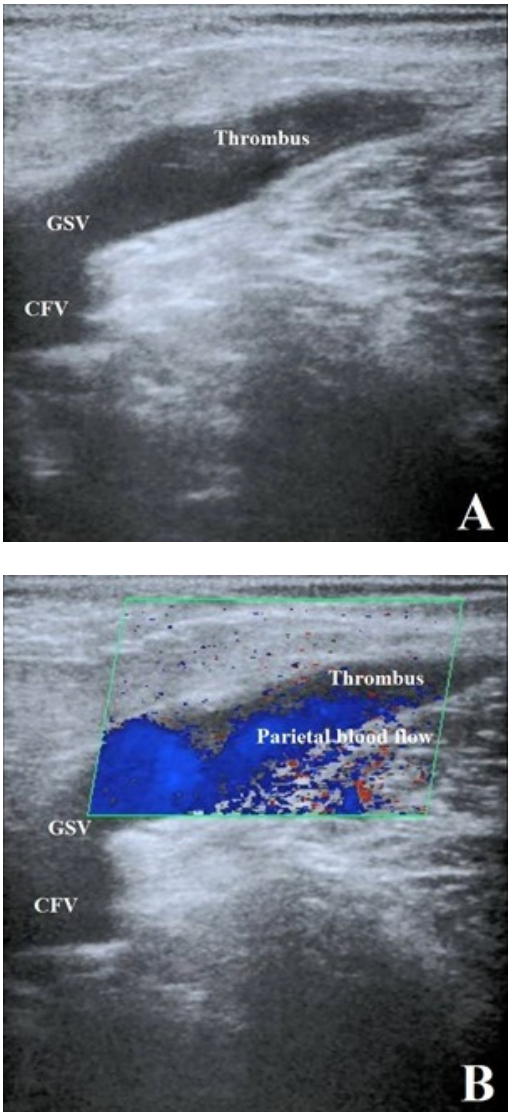

Figure 2. *GSV thrombosis (A) with localized parietal blood flow along the posterior wall (B)*

compressible during compression. On the lower leg, the GSV measured 2.3 mm, not dilated, with a perforating vein in the proximal part from which an enlarged thrombosed branch with up to 20% recanalization was noted. The small saphenous vein (SSV) measured 1.4 mm, not dilated, and was patent; in the middle third of the lower leg, it was dilated to 3.2 mm. In the SSV basin, two dilated insufficient perforating veins were detected in the lower leg. The examination revealed dilated sural veins of the gastrocnemius muscle, as well as the medial and lateral heads of the gastrocnemius muscle, which were patent.

Vascular diagnosis: Consequences of a gunshot through-and-through wound to the right thigh (2015) with damage to the right femoral artery; autovenous prosthetic replacement of the femoral artery using a segment of the great saphenous vein (GSV) from the left lower extremity, manifesting as obliterative atherosclerosis of the lower extremity arteries. Chronic arterial insufficiency stage I of the right lower extremity. Chronic venous insufficiency stage I of the left lower extremity without impairment of circulatory function.

The patient underwent a skin pathergy test, which was negative (Figure 4).

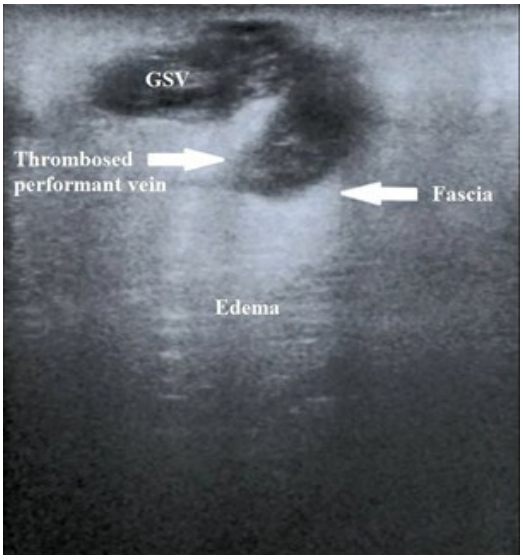

Figure 3. *Partially thrombosed performant vein in the middle third of the thigh*

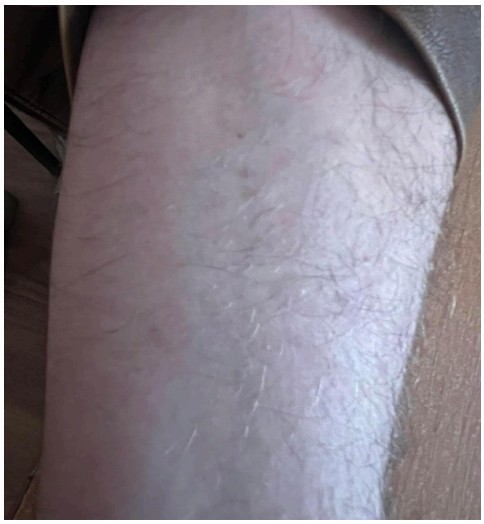

Figure 4. *Skin pathergy test on the inner surface of the left forearm*

According to the criteria of the International Team for the Revision of the International Criteria for Behçet's Disease (ITR-ICBD, 2014), the patient scored four points (two points for oral mucosal aphthae, one point for neurological manifestations, and one point for vascular manifestations).
The following diagnosis was made to the patient–Behçet's disease with involvement of the mucous membranes (recurrent aphthous stomatitis), joint involvement (polyarthralgia) of the knees and elbows, polyneuropathy of the lower extremities manifesting as reduced sensitivity without functional impairment, of moderate severity. Subacute superficial vein phlebothrombosis of the left lower extremity in the recanalization stage, chronic venous insufficiency stage I without circulatory impairment.
Subsequently, the treatment was prescribed to the patient. It included Azathioprine at a dose of 100 mg/day (under monitoring of complete blood count, quantitative CRP, urea, creatinine, and total bilirubin after one month), Methylprednisolone at a dose of 4 mg/day, Colchicine 0.5 mg 1 tablet once daily, Meloxicam 15 mg 1 tablet once daily after meals for 2 weeks, Pantoprazole 40 mg 1 tablet once daily 30 minutes before meals for 6 weeks.
Additionally, a vascular surgeon added a combined Diosmin/Hesperidin preparation at a dose of 900/100 mg/day for 2 months, Acetylsalicylic acid at a dose of 100 mg/day after dinner for 3 months, and Class II compression stockings for the left lower extremity to be worn during the day.
During further observation, upon reducing the dose of Methylprednisolone, the patient experienced a recurrence of canker sores on the mucous membrane of the inner surface of the lower lip, leading to the decision to maintain the dose at 4 mg/day.
Considering the relatively rare occurrence of Behçet's disease and the absence of specific diagnostic tests, establishing a diagnosis can take a prolonged period, as observed in this particular patient.

DISCUSSION

Adamantiades-Behçet's disease is an extremely rare condition, yet it is distributed worldwide. The prevalence of the disease, frequency of clinical manifestations, and genetic predisposition may vary across different regions. The diagnostic process can involve numerous challenges, delaying early diagnosis of the disease, which is critically important for the timely initiation of baseline treatment. The evolution of diagnostic criteria over the past two decades has been made possible only through extensive international collaboration, leading to the development of the International Criteria for Behçet's Disease (ITR-ICBD, 2014), which are currently the most sensitive.
As noted above, BD can affect multiple organs and systems of the body. The most common initial manifestation of this disease is oral cavity involvement, as observed in our patient, while the most frequent manifestation overall is the involvement of the genital mucosa. A similar presentation with initial clinical manifestations was reported in a 29-year-old woman from the Balkan Peninsula in Southern Europe, who experienced recurrent oral and genital ulcers. The mentioned symptoms involving the skin and mucous membranes were characterized by recurrent painful erosions and ulcers. Other dermatological manifestations included erythema nodosum on the skin of the lower extremities (21). Oral aphthae in BD must be differentiated from common aphthae, recurrent aphthous stomatitis, pemphigus vulgaris, herpes simplex, fungal infections, syphilis, and traumatic injuries. Acne-like rashes in BD on the trunk, thighs, or lower extremities typically differ somewhat from classic acne, as they present as sterile pustules, with comedones usually absent (22).
In the literature, a case of BD is described in a 26-year-old man from Poland, who, in addition to painful erosions on the oral mucosa recurring approximately 3 to 7 times per year and on the scrotal skin, exhibited skin involvement of the right thigh in the form of a large erosion. Additionally, acne-like lesions were present on the skin of his face and back (23). In BD, skin ulcers should be distinguished from pyoderma gangrenosum—arguably the most well-known condition associated with the pathergy phenomenon (24).
In contrast to the previous clinical cases, other instances of BD are reported in a 31-year-old dark-skinned man from Tanzania and a 21-year-old man from India, in whom the pathology manifested simultaneously with oral mucosal involvement in the form of painful ulcers recurring at least three or more times per year over several years, recurrent genital aphthae, and posterior uveitis combined with conjunctivitis (25–26). Genital ulcers in BD should be differentiated from syphilis, herpes simplex, chancroid, lymphogranuloma venereum, and genital trauma (27).
Musculoskeletal involvement in BD manifests as painful inflammation of the knee, ankle, elbow, or wrist joints, but without the development of erosive defects. Ocular complications typically appear several years after cutaneous symptoms and can vary, even leading to blindness. BD can also affect the gastrointestinal, cardiovascular, and nervous systems. There may be

erosions of the intestinal mucosa, as well as vomiting and diarrhea. A similar clinical case was reported by scientists from Buenos Aires (the capital of Argentina), who described a 34-year-old patient with an 8-year history of flares of oral and genital mucosal ulcers and intestinal perforation in BD (28). However, such gastrointestinal manifestations were not observed in our patient.

Cardiovascular manifestations in BD may include superficial or deep thrombophlebitis, cardiomyopathy, or pericarditis. Neurological manifestations in this condition arise due to inflammation of the central nervous system, either from localized vascular damage or peripheral polyneuropathy. Neurological symptoms are present in less than 10% of cases and typically develop an average of 5–6 years after the initial non-neurological manifestations. The most common forms result from meningoencephalitis caused not by infection but by inflammation. Neurological deficits are characterized by sensory disturbances, pyramidal syndrome, seizures, cerebellar syndrome, vestibular syndrome, and oculomotor paralysis. Thrombosis of small cerebral vessels or large venous sinuses manifests as intracranial hypertension. Polyneuritis is rare, but when it occurs, it presents with confusion, psychiatric disorders, and dementia (29). A case of BD with neurological manifestations was described by scientists from the University of Malaya in Malaysia (Southeast Asia). In a 47-year-old man, BD manifested with typical clinical features, including oral and genital mucosal ulcers and severe panuveitis. However, two years into the course of the underlying disease, the patient developed complaints of difficulty walking over the past year. Neurological examination revealed pyramidal weakness in both upper and lower extremities, with involvement of cranial nerves IX, X, and XII. The patient also reported blurred vision in both eyes and was examined by an ophthalmologist. Fundoscopy revealed bilateral optic nerve atrophy with attenuated retinal vessels and pigmentary retinal changes. Magnetic resonance imaging of the brain showed small, round, multiple hyperintense foci in both frontal lobes on T2-weighted images. These lesions were absent on T1-weighted images, indicating acute or subacute brain involvement and enabling the diagnosis of “Neuro-Behçet” (30). Neurological manifestations of BD were not observed in our patient.

The pathergy phenomenon in BD patients occurs with varying frequency depending on the patient’s ethnicity. It is typically observed in individuals from regions with high BD prevalence and is estimated to be positive in approximately 70% of cases; however, in our patient, the skin pathergy test was negative (31).

CONCLUSIONS

The main findings of this study can be summarized as follows:

1. Behçet’s disease is a chronic, relapsing systemic vasculitis of unknown etiology, with a prevalence in Europe ranging from approximately 1 case per 15,000 to 500,000 population.
2. Men are more prone to a severe disease course, with the development of pulmonary aneurysms, ocular involvement, thrombophlebitis, and neurological manifestations.
3. The clinical presentation in the typical form of the disease manifests as aphthous stomatitis, uveitis or iridocyclitis, and ulcers of the mucous membranes and skin of the genital organs. Generalization of the pathological process most often presents with involvement of large joints and small-caliber vessels, the central nervous system, lungs, and much less frequently—the heart, gastrointestinal tract, and kidneys.
4. Among narrowly specialized physicians, there is a lack of diagnostic awareness regarding BD, leading them to typically diagnose localized pathology, which in turn may falsely delay the preliminary stage of disease diagnosis.
5. The diagnosis of BD is based on the analysis of the disease’s clinical manifestations, as there are no pathognomonic laboratory tests to verify the diagnosis of BD, while timely establishment of the diagnosis is essential for optimizing the management of such patients and preventing disease progression.

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Statement of Ethics

Complete written informed consent was obtained from the involved patient for the publication of the study and accompanying images.

Competing Interest

The authors declared no relevant conflicts of interest.

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AFMN BIOMEDICINE

CASE REPORT

ISOLATED RIGHT FALLOPIAN TUBE TORSION IN A 13-YEAR-OLD ADOLESCENT

Behnaz Souizi[1] **Mehrnaz Mehrdoost**[1] **Marzie Torkmannejad Sabzevari**[2] **Narjes Forouhar**[3]

[1]Department of Obstetrics and Gynecology, School of Medicine, Mobini Maternity Hospital, Sabzevar University of Medical Sciences, Sabzevar, Iran [2]Department of Midwifery, School of Nursing and Midwifery, Sabzevar University of Medical Sciences, Sabzevar, Iran [3]Department of Obstetrics and Gynecology, School of Medicine, Mobini Maternity Hospital, Non-communicable Disease Research Center, Sabzevar University of Medical Sciences, Sabzevar, Iran

Isolated fallopian tube torsion is a rare condition, especially in adolescents, and can often be misdiagnosed due to nonspecific symptoms.

We report the case of a 13-year-old sexually inactive girl who was admitted to Shahid Mobini Hospital in Sabzevar, Iran, with worsening pain in the right lower abdomen and hypogastrium over the past four days. Transabdominal ultrasound revealed normal ovaries and a hemorrhagic solid cystic mass measuring 60 x 35 mm in the left adnexa. However, an urgent laparotomy subsequently revealed isolated torsion of the right fallopian tube.

This case highlights the importance of considering isolated fallopian tube torsion in adolescents presenting with lower abdominal pain. Prompt diagnosis and surgical intervention are crucial for preserving reproductive health.

Keywords: salpingectomy, fallopian tube, torsion, laparoscopy, laparotomy, oophorectomy

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Correspondence to:
Narjes Forouhar
Department of Obstetrics and Gynecology
School of Medicine Mobini Maternity Hospita
Sabzevar University of Medical Sciences, Sabzevar, Iran
E-mail: drnforouhar@hotmail.com

INTRODUCTION

Fallopian tube torsion is a rare gynecological emergency, accounting for only 1 in 1.5 million women per year (1). It can occur in two forms: adnexal torsion, which involves the twisting of both the fallopian tube and the ovary, and isolated torsion of the fallopian tube, which is an even rarer occurrence (2). The exact etiology of fallopian tube torsion is not always clear, but there are several potential contributing factors. Congenital anatomical variations in the structure and support of the fallopian tubes can predispose some women to torsion (2). Pelvic inflammatory disease (PID) and associated adhesions may also distort the normal pelvic anatomy, increasing the risk of torsion (3). Prior pelvic or abdominal surgeries, such as tubal ligation or ectopic pregnancy treatment, can alter the surrounding anatomy and predispose the fallopian tube to twisting (4). The presence of ovarian cysts or tumors can pull on the fallopian tube, leading to torsion (5). Pregnancy, particularly in the first trimester, is another risk factor due to the increased size and mobility of the uterus and adnexa (6). Sudden movements or changes in position may also trigger the twisting of a Fallopian tube.

Isolated Fallopian tube torsion (IFTT), in the absence of an associated adnexal mass or cyst, is an even rarer occurrence (4, 6). The etiology of IFTT is not fully understood, but proposed mechanisms include congenital anomalies, tubal spasm, pelvic adhesions, and tubal peristalsis (3-4). The clinical presentation is often non-specific, with abdominal pain being the most common symptom. This can lead to a delay in diagnosis, which is especially problematic given the risk of irreversible tubal ischemia and necrosis if left untreated. Laparoscopy is considered the gold standard for both the accurate diagnosis and management of IFTT (7). Early recognition and prompt surgical intervention are critical for preserving tubal function and avoiding salpingectomy and also preventing life-threatening complications such as peritonitis, sepsis, and death. However, the rarity of IFTT makes it a challenging diagnosis to consider, particularly in the absence of an associated adnexal mass (4-7). This case report presents a rare instance of isolated Fallopian tube torsion in a young woman, with a focus on the importance of maintaining a high index of suspicion for this condition in the appropriate clinical setting.

CASE REPORT

A 13-year-old sexually inactive girl was admitted to Shahid Mobini Hospital in Sabzevar, Iran, with a chief complaint of right lower abdominal and hypogastric pain that had worsened over the past four days. She had previously visited another medical facility and received painkillers, which did not alleviate her symptoms. The patient noted that the pain improved when lying down and was not related to eating, movement, urination, or defecation. Physical examination revealed tenderness in the lower abdomen without guarding. A pelvic examination indicated normal external genitalia, and there were no complaints related to urinary or bowel function. Laboratory evaluations, including blood tests, showed normal results. A transabdominal ultrasound revealed normal ovaries and a hemorrhagic solid cystic mass measuring 60 x 35 mm in the left adnexa and 20 cc of free fluid around it (Figure 1). No transvaginal ultrasound or digital examination was performed due to the age of the patient and the patient's declaration of virginity.

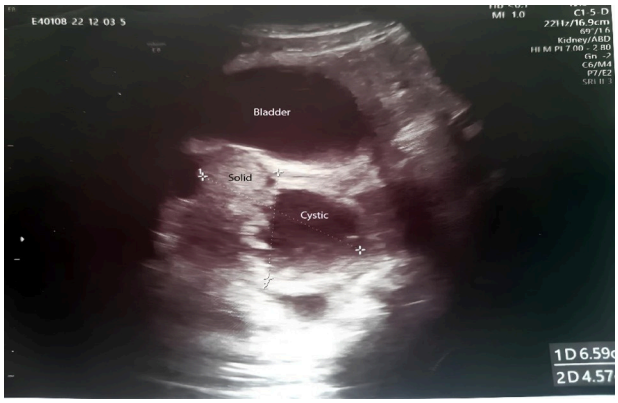

Figure 1. *Ultrasound representation of a hemorrhagic solid cystic mass with a size of about 60 x 35 mm in the left adnexa*

The preliminary diagnosis was a hemorrhagic cyst, and emergency laparotomy was performed. Intraoperatively, the diagnosis was identified as isolated torsion of the right fallopian tube. Upon opening the fascia and peritoneum, 50 cc of serous fluid was evacuated from the abdominal cavity. The uterus, both ovaries, and the left fallopian tube appeared normal. The right fallopian tube, however, was found to be twisted multiple times, exhibiting signs of congestion, necrosis, and deformation (Figure 2).

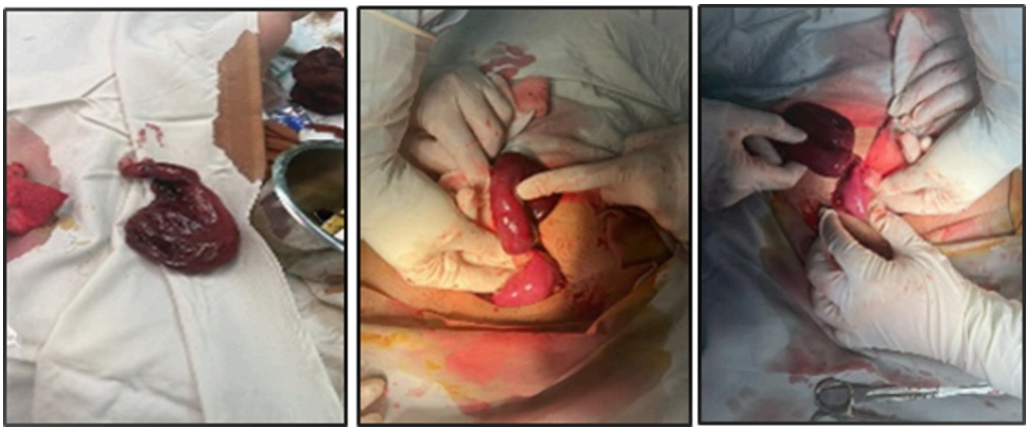

Figure 2. *Isolated torsion of the right fallopian tube*

The twisted tube was positioned on the left side, which had led to the misinterpretation of a cyst on the ultrasound. A tissue sample was sent for pathological evaluation. Histopathology revealed the right fallopian tube wall to be extensively congested, hemorrhagic, and ischemic, which was consistent with the clinical diagnosis of fallopian tube torsion. The postoperative course was uneventful, and the patient was discharged after three days.

DISCUSSION

Isolated torsion of the fallopian tube is extremely rare, with an estimated incidence of approximately one in 1,500,000 women annually. About 80% of cases occur in women of reproductive age, and approximately 12% during pregnancy (8). In our study, as in most reported cases, right fallopian tube torsion was observed. Contributing factors include the position of the sigmoid colon, slower blood flow in the right ovarian vein, and quicker surgical interventions on the right side. Torsion of the fallopian tube is a significant but uncommon cause of lower abdominal pain in adolescents, often difficult to diagnose before surgery. Immediate recognition and timely intervention are crucial for preserving tubal function (9). Diagnosis typically occurs during laparoscopy or laparotomy. Tubal and ovarian pathologies are rare in childhood, accounting for about 0.2% of pediatric surgeries, with 18-33% of adnexal surgeries related to torsion. Research indicates that oophorectomy or salpingo-oophorectomy is performed in 91% of torsion cases, with successful detorsion occurring in only 9% (10).

Ovarian torsion is often suspected due to ultrasound findings, such as an enlarged ovary or specific signs like the vortex sign. In contrast, isolated fallopian tube torsion frequently presents without these indicators, complicating diagnosis in acute abdominal cases lacking typical signs of ovarian torsion. Furthermore, if ovarian torsion is associated with a cyst requiring cystectomy, surgery should be performed by an experienced surgeon to minimize tissue damage (11). Identifying factors indicative of isolated fallopian tube torsion is essential before surgical intervention. While cervical motion tenderness can suggest pelvic inflammatory disease, it may also indicate adnexal torsion. This tenderness might reflect an inflammatory process due to twisting of the fallopian tube, as the cervix's movement could stimulate peritoneal stretching. However, inflammatory markers in isolated fallopian tube torsion cases do not support this hypothesis, warranting further investigation into alternative causes (12). The rich blood supply from the ovarian and uterine arteries initially causes ischemia before infarction occurs. Torsion may be intermittent or incomplete, leading to a history of intermittent abdominal pain. Contributing factors may include hydrosalpinx, hematosalpinx, prior surgery, incomplete mesosalpinx, or the presence of cysts (13).

Jeffcoate's theory suggests that a growing pregnant uterus exerts torsional pressure on the adnexa, while Sellheim's theory posits that sudden body position changes can lead to abnormal internal organ movement. The primary symptom is lower abdominal pain, often accompanied by nausea and vomiting. Reliable symptoms include acute pain radiating to the flank or thigh. Physical examination may reveal abdominal tenderness and adnexal tenderness, although specific masses are not always palpable, and laboratory findings are typically non-specific. While ultrasound can aid in diagnosing fallopian tube torsion, definitive diagnosis relies on laparoscopy or laparotomy. Reliable symptoms to diagnose this condition

include acute lower abdominal or pelvic pain with lateral spread, which may lead to nausea and vomiting if blood flow is obstructed. Ultrasound may show a cystic mass with varying septation and internal echoes, often near the uterine horn (14). Doppler ultrasound may demonstrate high-impedance flow with reversed diastolic flow in the affected tube. Due to non-specific symptoms, diagnosis and surgery are often delayed, increasing the risk of torsion and necrosis of the contralateral tube, ultimately impacting fertility (6).

In our case, the patient was a 13-year-old girl. Another report indicated that approximately 40% of isolated fallopian tube torsion cases occur in adolescents, with an average age of 26 years. This suggests that isolated fallopian tube torsion is more prevalent in pediatric populations and should be included in the differential diagnosis for lower abdominal pain in women of childbearing age. Fallopian tube torsion is a surgical emergency that should be considered in cases of acute lower abdominal pain. Doppler examination of adnexal masses can support clinical diagnosis. Early diagnosis and treatment are essential for preserving fertility; delays can lead to necrosis and loss of tubal function. While detorsion may be feasible if there are no signs of infarction, resection is often necessary (15).

CONCLUSION

Detecting isolated fallopian tube torsion is challenging due to its rarity and nonspecific clinical presentation prior to surgery. Failure to diagnose this condition in a timely manner can lead to irreversible damage to both the fallopian tube and the ovary. Timely laparotomy can often salvage the tube and maintain the potential for future pregnancy. Diagnostic laparoscopy plays a critical role in facilitating prompt diagnosis and preventing treatment delays. Despite its potential for significant morbidity, isolated fallopian tube torsion is frequently underdiagnosed. It is imperative for the medical community—especially obstetricians and gynecologists—to enhance awareness of this condition. Global reporting of similar cases can help accurately assess their prevalence and underscore the importance of including isolated fallopian tube torsion in the differential diagnosis of acute abdominal pain in women and adolescents. Increasing awareness among clinicians, particularly primary care physicians who are often the first point of contact, is essential for enabling timely diagnoses and appropriate referrals. This proactive approach can help prevent complications associated with delayed treatment.

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Statement of Ethics

This study was reviewed and approved by Research Ethics Committees of Sabzevar University of Medical Sciences, approval number IR.MEDSAB.REC.1402.112, issued on 2024-02-06. Complete written informed consent was obtained from the patient for the publication of this study and accompanying images.

Competing Interest

The authors declared no relevant conflicts of interest.

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AFMN BIOMEDICINE

RETRACTION NOTE: "CYSTIC DUCT WITH MEDIAL SPIRAL INSERTION" (VOL. 42, NO. 3, P. 417–421)

by Ilija Golubović, Aleksandar Vukadinović, Nebojša S. Ignjatović, Miroslav Stojanović

Editorial Board of the Journal *AFMN Biomedicine*

Retraction Note

This article has been retracted post-publication at the request of the corresponding author due to an unresolved authorship dispute. Concerns were raised regarding changes made to the authorship list after the initial submission, specifically the removal of an author whose name appeared in the original submission and the addition of other authors without that individual's consent.

The editorial office requested a justification for the post-submission changes to authorship, along with a mutually agreed authorship statement signed by all parties involved. As the authors were unable to provide the requested documentation, the Editor approved the retraction to safeguard the integrity of the scholarly record.

This retraction is issued solely on the basis of the authorship dispute and does not reflect any concerns regarding the scientific content, data integrity, or conclusions of the article.

The article remains accessible but is clearly marked as retracted.

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